Advances in Cowpea Research

Edited by

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The International Institute of Tropical Agriculture (IITA) was founded in 1967 as an international agricultural research institute with a mandate for specific food crops, and with ecological and regional responsibilities to develop sustainable production systems in Africa. It became the first African link in the worldwide network of agricultural research centers known as the Consultative Group on International Agricultural Research (CGIAR), formed in 1971.

IITA is governed by an international board of trustees and is staffed by approximately 150 scientists and other professionals from about 40 countries and 1,500 support staff. Funding for IITA comes from the CGIAR, and from national and private donor agencies.

IITA conducts research, training, and germplasm and information exchange activities in partnership with regional and national programs in many parts of sub-Saharan Africa. The research focuses on smallholder cropping systems in the humid and subhumid tropics of Africa and on the following food crops: cassava, maize, plantain and banana, yam, cowpea, and soybean. It addresses crop improvement, plant health, and resource and crop management issues within a farming systems framework. The overall goal is to improve the nutritional status and well-being of low-income people in the humid and subhumid tropics of sub-Saharan Africa.

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The Japan International Research Center for Agricultural Sciences (JIRCAS) is one of 29 research institutes belonging to the Ministry of Agriculture, Forestry and Fisheries (MAFF), Government of Japan. It is unique among these for its role as a research center dealing with agricultural issues from an international perspective. JIRCAS pursues collaboration with other MAFF institutes and government agencies, such as the Japan International Cooperation Agency (JICA) as well as institutes of other countries and the Consultative Group on International Agricultural Research (CGIAR).

JIRCAS was established in October 1993 to replace the Tropical Agriculture Research Center (TARC), which had played a significant role in research relating to tropical and subtropical agriculture since 1970. It has expanded its activities to all developing regions, and added forestry and fisheries to its research portfolio, thus adopting a comprehensive approach to agricultural sciences.

JIRCAS has a current staff of 163, including research scientists and administrators, 41 of whom are located at the Okinawa Subtropical Station. It also supports about 40 researchers on long-term assignments and about 150 researchers on short-term assignments annually. Research projects deal with problems relating to the utilization and improvement of biological resources, through the use of biotechnology, the preservation of the global environment, information systems, postharvest technology, etc

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Cover: *Cowpea/sorghum intercropping, one of the many ways in which cowpea is grown. Inset shows a regeneration system that has been developed, to enable integration of desirable alien genes into cowpea.* **Pictures:** B.B. Singh and A. Pellegrineschi (inset).

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Foreword

Cowpea is an important legume of the tropics, with its various uses: as grains in processed foods, as a vegetable (fresh leaves, peas, and pods), and as dry haulms and fodder. It is an inexpensive source of vegetable protein, and a hardy crop well adapted to relatively dry environments. In combination or association with cereals and other grain legumes, it contributes to the sustainability of cropping systems in marginal lands of semiarid areas, with its fixation of nitrogen, ground cover, and the soil improvement it provides from plant residues.

These features together make cowpea a particularly attractive crop for the subsistence farmers of sub-Saharan Africa, where about 70% of the world's cowpea is grown, but where the farmer still faces numerous problems in growing and harvesting the crop. Given this background, research on cowpea has rightly received a high priority at the International Institute of Tropical Agriculture (IITA), which works in collaboration with researchers in several institutions around the world.

Sensing the need for researchers to exchange information and knowledge, as well as to jointly explore problem areas, IITA convened the first World Cowpea Research Conference, at Ibadan, Nigeria in 1984. The major papers presented at that conference were published in 1985 in a book entitled, *Cowpea Research, Production and Utilization.* Consequently, there have been new initiatives in cowpea research, especially in cowpea biotechnology and in breeding varieties for intercropping and drought tolerance.

A decade has passed since then, and IITA felt the need to convene cowpea researchers again for an active exchange of information and knowledge, so that a well-focused research agenda could be developed for the future. This resulted in the Second World Cowpea Research Conference, held at Accra, Ghana during 3–7 September 1995. The meeting was cohosted by the Council for Scientific and Industrial Research (CSIR), and the Ministry of Food and Agriculture, Ghana.

This volume brings together 33 papers presented at the Accra meeting in 1995, as modified in further review and discussion among scientists, for the benefit of all who are working to improve cowpea production around the world.

We hope that this publication will complement the earlier book, serve as a valuable reference tool to all cowpea scientists, and provide the stimulus for a further synergy of efforts that would result in food self-sufficiency in the foreseeable future for sub-Saharan Africa, the region of the world that needs it the most.

L. Brader
Director General
International Institute of Tropical Agriculture

Preface

The Second World Cowpea Research Conference, held at Accra, Ghana during 3–7 September 1995, brought together 185 participants from 33 countries, to exchange information and knowledge, and to actively explore problem areas in cowpea production for various end uses. There were 54 papers presented in six technical sessions at the conference, as well as numerous poster presentations.

All 54 papers were subjected to a peer review process, during and after the conference. We drew upon the expertise of those attending, as well as other specialists worldwide, to conduct an interdisciplinary review of each of the papers. Scientists from numerous organizations participated in this process, and they are acknowledged by name overleaf. We would like to thank them collectively here for their willing participation in this endeavor.

The 33 papers included in this volume represent our selection, based on that peer review process. In addition to the technical merits of the papers themselves, the editors employed the general criteria of overall coherence and balance between subjects in arriving at their selection. Inevitably, some papers with considerable merit had to be excluded, but we are confident that those papers will find their way to more appropriate publication outlets.

In putting this volume together, we have changed the order of some papers from their position in the conference. We believe the present order reflects a better, more coherent grouping of papers for our readers. In sum, these papers offer a synoptic overview of advances in cowpea research worldwide over the last decade or more.

We hope the publication of this volume justifies all the efforts expended on it by authors, reviewers, and editors. Any imperfections that remain, however, are the responsibility of the authors and editors.

B.B. Singh, D.R. Mohan Raj, K.E. Dashiell, and L.E.N. Jackai
April 1997

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In addition to IITA, the following sponsors provided financial assistance for the Second World Cowpea Research Conference, held at Accra, Ghana during 3–7 September 1995: the Government of Italy; the Overseas Development Administration (UK); and the Food and Agriculture Organization of the United Nations (FAO). The attendance of some participants was enabled by the support of the Coarse Grains and Food Legume Network (Asia, UNDP); the Technical Centre for Agricultural and Rural Cooperation (CTA); On-Farm Adaptive Research for West Africa (EU); Protection Ecologiquement Durable du Niébé (Swiss Development Cooperation); and Réseau Niébé de l'Afrique centrale et occidentale (RENACO). Our thanks are due to all of them.

We thank all the authors included in this volume and the reviewers we consulted for their contributions. The reviewers included G.S. Abawi, A.E. Akingbohungbe, M.O. Akoroda, A.M. Alghali, K. Ampong-Nyarko, R. Asiedu, J.N. Ayertey, D.K. Berner, S.F. Blade, H. Bottenberg, M. Bokanga, K.F. Cardwell, R.J. Carsky, O.L. Chambliss, P.Q. Craufurd, G. Dahal, M.C. Dike, A.G.O. Dixon, J.A. Duke, I.J. Ekanayake, A.M. Emechebe, M.I. Ezueh, I. Fasidi, C.A. Fatokun, B. Fawole, S. Fernandez, R.S.B. Ferris, R.L. Fery, E. Filippone, D.A. Florini, K.R. Green, A.E. Hall, L. Halos-Kim, R.I. Hamilton, R.O. Hampton, C.J. Hodgson, J.d'A. Hughes, A.A. Idowu, I. Ingelbrecht, L.E.N. Jackai, S.S. Jagtap, R.L. Jarret, S.K. Kim, L.W. Kitch, J.G. Kling, G. Konate, A.K. Kononowicz, S.T.O. Lagoke, J.A. Lane, R. Maréchal, H.D. Mignouna, G.I. Mink, W. Moar, A.J. Moule, L.M. Monti, L.L. Murdock, N.Q. Ng, S.Y.C. Ng, S.S. Nielsen, K.S. Nokoe, B.R. N'tare, G. Ntoukam, F.I. Nweke, T.I. Ofuya, G. Ogbadu, E.O. Ogunwolu, O.O. Olufajo, J.O. Olukosi, O.G. Omitogun, R. Ortiz, S.M. Osho, M. Owusu-Akyaw, P. Oyekan, A. Pellegrineschi, R.D. Phillips, R.C. Pratt, F.M. Quin, Rosa Rao, F. Saccardo, A.B. Salifu, J.A. Saunders, A.M.C. Schilder, F. Schulthess, K.V. Seshu Reddy, S.V.R. Shetty, S.A. Shoyinka, D.D. Shukla, B.B. Singh, N.K. Singh, R.J. Singh, S. Sithanantham, C.W. Ssekabembe, J.B. Suh, R.J. Summerfield, R. Tabo, S.A. Tarawali, T. Terao, G. Thottappilly, A.K. Vidaver, D. Vuylsteke, M.C. Whalen, C.T. Williams, and N.D. Young.

The editors of this volume thank D.A. Florini and G. Thottappilly for expertise provided in papers relating to pathogens and biotechnological applications, respectively, and M.O. Akoroda for his overall editorial help.

A special word of thanks should go to IITA's publications staff: Fatai Agboola kept track of disk files as papers went through several rounds of revisions; Peter Ochonogor and Simeon Ughekhai provided help with the graphics; and Jide Ojurongbe helped design the cover, as well as provided inputs to the overall book design.

Lastly, we are grateful to IITA for the staff time and resources it invested in the conference—and in this volume resulting from it—and to JIRCAS for cosponsoring this publication.

K.E. Dashiell
for Organizing Committee, Second World Cowpea Research Conference

Introduction

F.M. Quin[1]

Reliable production data for cowpea worldwide are generally difficult to obtain. Steele (1976) noted that cowpeas are not important in world trade, with the result that production data are scarce. Duke (1990) noted that FAO provided no recent statistics on cowpea, and he turned to data from around 1970 to give some assessment of the global status of this crop. The figures given were 3.1 million ha production area, 1.1 million t grain production, and 0.40 t/ha as average grain yield. At the Second World Cowpea Research Conference, held in Accra, Ghana in 1995, the same problem of lack of statistics for cowpea was discussed. Noting that not one paper had given details of crop statistics, the conference recommended that ways be explored to overcome this problem. Put another way, the conference considered cowpea of sufficient importance to warrant more reliable statistics.

With the advent of Internet, web sites, and home pages, previously unpublished FAO statistics have become available. Whereas the FAO Production Yearbook (1996, printed version) provides only combined data for several dry pulses, the FAO web site contains disaggregated data, including current figures for cowpea. For 1996, the production area is given as 5.6 million ha, of which at least 90% is in West and Central Africa, and annual world grain production is estimated at 2.7 million t. Relative to the 1970s (i.e., some 25 years later), these data indicate a near doubling (× 1.81) of production area and more than doubling (× 2.45) of grain production. Average grain yield is given as 0.48 t/ha, a figure so similar to that of the 1970s that it raises several questions. Are the crop statistics accurate? Or has there been very little impact of new technologies at the farm level, to improve the production of this crop? Or again, are the problems of production so severe that solutions to them, and subsequent transfer of these to farmers, are yet to come?

There is some evidence that the recent FAO statistics underestimate the production area. Estimates arrived at by exchange of information between cowpea scientists indicate a much larger global production area of 12.5 million ha, with 8 million ha (64% of the world total) in West and Central Africa, and an annual world grain production of 3 million t (see Singh et al. 1997, this volume). Outside Africa, the main producing areas are in Central and South America and in Asia, with several smaller areas spread over southern Europe, southern USA, and Oceania. Among countries, the main producers are Nigeria, Niger, and Brazil.

Taking these gross world estimates of production, the average world grain yield is only 0.24 t/ha, exactly one half of the FAO 1996 figure. FAO (1996) gives the average grain yield in the USA as 0.90 t/ha from 2130 ha, and in Australia as 0.40 t/ha from 7000 ha. With the yields from these two minor, but relatively 'high tech', production areas as a gauge,

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combined with the extensive production of cowpea in some of the world's poorer countries, this global estimate of 0.24 t/ha grain yield for cowpea has some credibility. The production levels in West and Central Africa obviously have a large influence on estimates of the global average grain yield. The figure of 0.24 t/ha accords with available figures for this region: for Senegal, recorded yields were 0.12–0.55 t/ha for 1983–95; for Niger, 0.05–0.18 t/ha (1986–95); for Nigeria, 0.47 t/ha (1995); and for Ghana, 0.60 t/ha (1989).

Even if the production area is some 12 million ha, this is only 45% of the area recorded for dry beans (*Phaseolus* spp., FAO 1996). Thus cowpea, on the world stage, is a minor crop, with generally low grain yields, which are 25–50% below what is achieved in some "advanced" production systems. Nevertheless, production areas are spread all around the world, and in this sense cowpea is a world crop. It is also evident from this geographic spread that cowpea fits into diverse production systems, rainfed and irrigated, and notably in marginal areas with poor soils and limited rainfall (e.g., the Sahel).

The apparent lack of improvement in farm level grain yields over a period of about 25 years may reflect the marginal environments in which the crop is grown, with the concomitant and persistent high risks of yield erosion. But it may equally well indicate that for crops such as cowpea, which are closely associated with resource-poor systems of farming, the problems are complex; the process of change in long-established practices is slow, mainly because they have evolved to minimize risk; and the whole task of generating and transferring appropriate, adoptable technologies is demanding, requiring serious institutional commitment at national and international levels.

In the following chapters of this book, the scope of current research on cowpea, the achievements to date, and future challenges and needs are presented. In combination, they demonstrate two main things: (1) the crop is important for those who produce it and, in turn, it is important for the farming systems of which it is a component; (2) the production problems are indeed complex and difficult, but excellent progress is being made despite this, with evidence of considerable progress so far and expectation of similar advances in future years.

The importance of cowpea

Cowpea is of major importance to the livelihoods of millions of relatively poor people in less developed countries of the tropics. From production of this crop, rural families variously derive food, animal feed, and cash, together with spillover benefits to their farmlands through, for example, in situ decay of root residues, use of animal manures, and ground cover from cowpea's spreading and low growth habit. In addition, because the grain is widely traded out of the major production areas, it provides a cheap and nutritious food for relatively poor urban communities.

In fresh form, the young leaves, immature pods, and peas are used as vegetables, while several snacks and main meal dishes are prepared from the grain. All the plant parts that are used for food are nutritious, providing protein, vitamins, and minerals. Cowpea grain contains, on average, 23–25% protein and 50–67% starch. Petty trading of fresh produce

and processed foods provides both rural and urban opportunities for earning cash, particularly by women.

The aboveground plant parts of cowpea, excepting pods, are harvested for fodder. In some areas, trading is these residues (haulms) can be highly remunerative. In West and Central Africa, farmers who cut and store cowpea fodder, for subsequent sale at the peak of the dry season, have been found to obtain as much as 25% of their annual income by this means. Fodder yields of 0.5 t/ha (air dry haulms) are commonly obtained in northern Nigeria. Yields as high as 2–4 t/ha can be obtained.

Going beyond its importance for food and feed, cowpea can arguably be regarded as the fulcrum of sustainable farming in semiarid lands. This is especially so for West and Central Africa. In this region, the area of cowpea production extends westerly from Cameroon through to Senegal, lying mainly between 10 °N and 15 °N, covering the dry savanna (northern Guinea and Sudan savannas) and Sahelian zones. There are a few additional pockets of production at more southerly latitudes, where the dry savanna agroecology penetrates closer to the West African coast, as in Ghana and Benin.

All of these agricultural lands are characterized by systems for farming that make limited use of purchased inputs, except for some crops such as cotton and maize. The upland soils generally have a relatively light, sandy loam texture, with moderate to low natural fertility. Production in the main cropping season is entirely dependent on rainfall, which ranges from 900 mm, at roughly 10 °N, to as low as 350 mm at the northern limit (15 °N). Intercropping of coarse grain crops (sorghum and millet) with cowpea, or cowpea-groundnut mixed as the lower storey crop(s), is widely practiced.

In these production systems, the spreading indeterminate or semideterminate bushy growth of cowpea provides ground cover, thus suppressing weeds and providing some protection against soil erosion. In addition, some cowpea varieties cause suicidal germination of the seeds of *Striga hermonthica*, a parasitic plant which may infest the cereals, often with devastating effects.

After harvest of the main upper storey cereal crop, late-season varieties of cowpea respond to improved light, and grow out to cover the land. When this crop in turn is harvested (either fodder only or grain and fodder), the root residues decay in situ, contributing some organic matter and associated nutrients to the soil. With awareness of the benefits of crop rotation, farmers will use the cowpea rows of one season for the cereal rows of the following season. Hence the cereal is well placed to benefit from the improved soil conditions that resulted from cowpea.

Another important feature of cowpea is that it fixes atmospheric nitrogen through symbiosis with nodule bacteria (*Bradyrhizobium* spp.). In so doing, the crop does not deplete the natural (and low) reserves of soil nitrogen, and many experimental findings illustrate that soil N levels increase, following cowpea. A contribution of 40–80 kg N/ha is the commonly obtained range, while the total amount of nitrogen fixation is 70–350 kg/ha.

In addition, cowpea is drought hardy, and it is able to maintain some growth or at least survive under dry soils conditions This trait is in part explained by the deep rooting habit of some varieties, and it accounts for the crop's ability to grow and yield under the semidesert conditions of the African Sahel and northeastern Brazil.

The off-take of cowpea fodder makes an important contribution to feed supplies for large and small ruminants. The spillover benefits are that traction animals maintain reasonable health status during the dry season, enabling timely land preparation when the wet season moves in. Also, return of animal manures to the land by cartage from corrals or in situ grazing contributes to soil fertility.

In West and Central Africa, with the development of irrigation schemes in some areas, and the general increased use of wetlands (inland valley systems), cowpea has found a niche in dry-season cropping. This is based mainly on use of residual soil moisture, and it is somewhat similar to the production of cowpea in rice-based cropping systems in Asia. Based on current observations, this relatively new production system is popular and expanding. As with rainfed production, both grain and fodder are produced. Interestingly, in situ grazing is common, with the farmer selling off the fodder to pastoralists who move their herd onto the field to graze. The farmer benefits from manure returns as well as in cash.

In sum, therefore, cowpea is pivotal to sustaining cereal and animal production in semiarid lands, and there is no evidence that the presence and importance of this crop in these agropastoral systems will diminish in the foreseeable future.

Cowpea research at IITA

Since its inception in 1967, the International Institute of Tropical Agriculture (IITA) has been given the world mandate for cowpea research within the Consultative Group for International Agriculture Research (CGIAR). The work on cowpea started in 1970 and, through the combined efforts of scientists at IITA and from national programs, there has been significant progress.

During 1970–75, IITA scientists began a major effort to collect world cowpea germplasm, screen them for sources of disease and insect resistance, and identify other desirable traits. In this continuing effort, IITA has at present a collection of more than 15,000 germplasm lines from all over the world. From 1970 to 1980, the cowpea breeding program concentrated on combining multiple disease resistance with a determinate, erect plant type. The focus was to develop varieties for sole cropping, which would respond to added inputs like fertilizer and insecticides. Considerable progress was made, and several varieties were developed and have been released in many countries in Asia and South America (see Singh et al. 1997, this volume). However, all these varieties had a smooth seed coat and tan or red seed color, and they were not accepted in West and Central Africa.

From 1980 to 1987, the thrust was to develop cowpea varieties with diverse seed types—white, brown, tan, red, black—and smooth and rough testa, combined with extra early maturity (60–70 days), medium maturity (70–80 days), and insect resistance. This approach

was very successful and varieties were developed that combined resistance to several diseases and aphid, bruchid, and thrips. Appropriate production and pest control strategies suitable for these varieties were also developed. Several countries, including those in West and Central Africa, have released these varieties.

By 1987, it was evident that, with some exceptions, relatively few cowpea farmers in West and Central Africa were actually growing the improved cowpea varieties in monocrops with appropriate insecticide spraying. In general, the vast majority of farmers were still growing their traditional varieties in intercrops with millet and/or sorghum, with no insecticide sprays. In response to this, IITA made a radical change to its research approach. The cowpea breeding project began to focus on developing varieties appropriate for intercropping that would produce reasonable yields of grain and fodder, with no insecticide sprays. To complement the conventional breeding program, there are other research activities that focus on controlling three postflowering insect pests. The major strategies which are pursued at present include biological control, botanical insecticides, cultural practices, and introducing foreign genes for insect resistance into cowpea, using wide crossing and biotechnology. Progress in these areas is reported in later chapters of this book.

Much of IITA's cowpea research is currently conducted in collaboration with the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and the International Livestock Research Institute (ILRI). ICRISAT is the CGIAR institute with the mandate for millet and sorghum and cropping systems work in the semiarid tropics, where cowpea is most important, and ILRI is interested in maximizing the benefits that cowpea can provide to ruminant animals, both large and small.

Cowpea research conferences

The first World Cowpea Research Conference was held in November 1984 at IITA's main headquarters in Ibadan, Nigeria with 203 participants representing 34 countries from Africa, Asia, North and South America, and Europe. Based on papers from that conference, a book was subsequently published (Singh and Rachie 1985). The Second World Cowpea Research Conference followed 11 years later, and it was held in Accra, Ghana in October 1995. The Ghanaian Council for Scientific and Industrial Research (CSIR) of the Ministry of Environment, Science and Technology, and the Ministry of Food and Agriculture joined with IITA as co-hosts of the conference. As with the first conference, the participants (185 in all) were drawn from many countries (33) worldwide, encompassing both cowpea producing countries and countries where advanced laboratories had active research programs for cowpea, e.g., Italy. All regions of sub-Saharan Africa were represented with, understandably, the strongest attendance from national program scientists from West and Central Africa. In this latter case, many scientists were valued colleagues of IITA, reflecting relationships built up over many years, through regional projects and networks, and direct links with national program projects.

It was especially gratifying to note that the West and Central Africa Cowpea Network (RENACO), which had commenced in 1978 within the framework of the Semi-Arid Food Grain Research and Development (SAFGRAD) Program was still operational, assisted by

the generous support of its original donor (USAID). Regarding direct links with national programs, Ghana provided an example which was particularly relevant to the conference. Through the (Canadian) CIDA funded Ghana Grains Development Project (GGDP), IITA had enjoyed close links with Ghana's cowpea program since 1985, particularly for improvement of cowpea germplasm.

Comparison of the programs for each of the conferences provides evidence that cowpea scientists are using new scientific methods to tackle some of the persistent problems of cowpea production. At the second conference, papers dealt with some research areas that had not featured in the first conference. For example, in 1995, there was a whole session on biotechnology, which did not receive any attention in 1984. Genetic transformation, DNA analysis (mapping and DNA marker-assisted selection), and use of foreign genes were all new topics. Wide crossing was mentioned in 1984 only in the context of elucidating taxonomic relationships, whereas in 1995 the use of wide crosses for genetic enhancement of cowpea was reported. The potential importance of one wild species, *Vigna vexillata*, in breeding (particularly for insect pest resistance) was described, whereas in 1984 this species did not receive any mention. These contrasts confirm that cowpea research is keeping abreast of new techniques and seeks to benefit from the opportunities they offer for achieving advances.

In arriving at the recommendations of the 1995 conference, all scientists, including those whose work concentrates more on field production, gave their full support to the investment of research funds in biotechnology for cowpea improvement. There was high expectation that there would be significant progress in this aspect in the years ahead. The challenge passed on to those scientists who are practitioners in this area is obvious.

The future

At present, cowpea is predominantly a crop of drier areas. However, as further advances are made in crop improvement, there will be opportunities for production in longer season, wetter agroecologies. Key areas of improvement that could enable this expansion are reliable reduction in the severity of pest and disease problems, more efficient manipulation of crop duration and continued development of multipurpose (grain and fodder) varieties. In West and Central Africa, much of the urban demand for cowpea grain is located in wetter areas. There is, therefore, good reason to expect that if suitably adapted improved varieties of cowpea are available, the crop's production area will expand accordingly. Even now, in Nigeria for example, cowpea production is more common in the forest-savanna transition zone than previously. This is an indication of future potential, not only for increases in grain production but also for fodder, and the additional contribution to soil conservation and improvement.

A recurring problem for improving natural resource management of farmed lands in more intensive systems in the humid tropics is that recommended practices (which include planting improved fallow species) essentially take land out of economic production. While they favor sustained crop production in the longer term, they require that a parcel of land is not used to deliver usable primary products in the short to medium term. This reduces the

adoptability of the practices. On the evidence of cowpea's contribution to farming systems in the dry savannas, it appears to be a good candidate for incorporation into farming systems of wetter areas by reason of its potential for use as a green manure, together with providing the primary products of grain and fodder. Research conducted in the 1980s has shown that sources of tolerance to aquic acid soils (cowpea germplasm and the associated symbionts) are available.

Undoubtedly, research advances that can reduce the severity of insect pests and diseases are central to this vision of crop expansion. Nevertheless, tailoring crop duration to rainfall patterns of wetter areas will also be a very important requirement, together with selection for plant ontogeny which favors multiple end uses, such as grain delivery, followed by one cut of vegetative growth for fodder, followed by regrowth for subsequent incorporation as green manure. In meeting this challenge, DNA markers for key physiological traits (e.g., thermophotoperiodicity) and for genes that can contribute to improving host-plant resistance will surely be valuable tools, enabling efficient and more rapid progress.

Increased urbanization opens up new opportunities for food products. Recommendations at the Accra conference stressed the need to give more attention to the use of cowpea leaves. Could a small-scale agroindustrial process be developed for cowpea leaves that would provide a nonperishable (or longer shelf-life) product for this protein-rich food? The product could benefit both urban consumers and cowpea producers, who would have more market opportunities for their crop. Storable leaf-based food products have been developed for other crops such as cassava, so there is a basis for similarly pursuing this for cowpea.

Considering the pressing need to improve the welfare of rural and urban poor in the tropics, issues relating to better food supply and greater opportunities for income generation are of paramount importance. Cowpea clearly contributes to each of these areas, and future research and development work should continue to have these emphases. Nevertheless, the spillover benefits from cowpea to natural resources management are of considerable importance, well justifying continued attention to this aspect of the crop. Optimization of cowpea's contribution to sustaining the natural resource base for crop production in the tropics should remain as an essential component of future work on this crop.

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1

Origin, taxonomy, and morphology of *Vigna unguiculata* (L.) Walp.

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Abstract

Cowpea (*V. unguiculata*) represents the main food legume in tropical Africa. Germplasm collecting missions launched over the past 10 years have provided genebanks with a wide array of variability within the cultivated and wild taxa of the species. Based on detailed studies on morphological diversity of live materials along with extensive survey of materials in major *Vigna* herbaria and ecogeographical information, a new intraspecific classification recognizing 13 varieties of wild cowpea has been proposed and described. The study points out that the southernmost region of Africa is most probably the center of origin for the species *V. unguiculata*, while its domestication might have taken place in West Africa.

Introduction

Considerable progress has been made during the past 10 years on germplasm collection, characterization, evaluation, ecogeographic studies, and taxonomy of cowpea and its wild relatives. These efforts have greatly contributed towards a better understanding of species diversity, ecogeographical distribution, and evolution of *Vigna unguiculata*. Germplasm collection activities have broadened the genetic materials available in genebanks for use in crop improvement and related research.

Taxonomy

Cowpea is a *Dycotyledonea* belonging to the order *Fabales*, family *Fabaceae*, subfamily *Faboideae*, tribe *Phaseoleae*, subtribe *Phaseolinae*, genus *Vigna,* and section *Catiang* (Verdcourt 1970; Maréchal et al. 1978). *Vigna* is a pantropical genus with several species, whose exact number varies according to authors: 184 (Phillips 1951), 170 (Faris 1965), between 170 and 150 (Summerfield and Roberts 1985), 150 (Verdcourt 1970), 154 (Steele 1976), and about 84 (of which some 50 species are indigenous to Africa) (Maréchal et al. 1978).

In their revision of the genus *Vigna*, Maréchal et al. (1978) subdivided the genus described earlier by Verdcourt (1970) into seven subgenera. In this classification, *V. unguiculata* (L.) Walpers and *V. nervosa* Markotter constitute the section *Catiang*, one of the six sections of the subgenus *Vigna*. Species of the section *Catiang* are characterized by spurred stipules below the attachment point of the leaf stalks and canoe-shaped keel with beak. The

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surface of their pollen grains are reticulate with raised exine (De Leonardis et al. 1993). Interspecific crosses made between the two species have not been successful (Mithen 1987; Ng and Apeji 1988; Ng 1995). On the basis of a study on isoenzyme variation in the genera *Phaseolus* and *Vigna,* Jaaska and Jaaska (1988) proposed to raise the section *Catiang* to the rank of a subgenus.

All cultivated cowpeas are grouped under *V. unguiculata* subspecies *unguiculata,* which is subdivided into four cultigroups, namely Unguiculata, Biflora, Sesquipedalis, and Textilis (Westphal 1974; Maréchal et al. 1978; Ng and Maréchal 1985). There has been no major contention on this classification, since its adoption over 10 years ago.

The classification and nomenclature of the wild taxa within *V. unguiculata*, however, is complicated, and could sometimes be confusing. More than 20 epithet names have been used in the past to designate wild taxa within *V. unguiculata* species complex. An extensive work on characterization of over 400 wild *V. unguiculata* accessions was conducted at IITA (Ng and Padulosi 1991; Padulosi 1993). This work, coupled with surveys of live materials in the field and specimens in major herbaria in Europe and Africa, as well as cytological studies, has led to the description of new taxa, and a change of nomenclature of some species (Padulosi 1993; Ng 1995). Parallel work on taxonomy of wild species within section *Catiang* was also conducted elsewhere (Piennaar and Wyk 1992; Pasquet 1993a).

Table 1. Classification and nomenclature of the wild *Vigna unguiculata* species complex.

Maréchal et al. (1978)	Pienaar (1992)	Pasquet (1993a)	Padulosi (1993)
V. unguiculata	*V. unguiculata*	*V. unguiculata* ssp. *unguiculata* var. *spontanea*	*V. unguiculata*
ssp. *dekindtiana* var. *dekindtiana*	ssp. *dekindtiana* var. *dekindtiana* var. *huliensis*	ssp. *dekindtiana* var. *dekindtiana*	ssp. *dekindtiana* var. *dekindtiana* var. *huliensis* var. *congolensis* var. *grandiflora*
var. *mensensis*	ssp. *mensensis*	ssp. *letouzeyi* ssp. *burundiensis* ssp. *baoulensis*	var. *ciliolata*
var. *protracta*	ssp. *protracta*	ssp. *stenophylla*	ssp. *protracta* var. *protracta* var. *kgalagadiensis* var. *rhomboidea*
var. *pubescens*	ssp. *protracta*	ssp. *pubescens*	ssp. *pubescens*
ssp. *stenophylla* ssp. *tenuis*	ssp. *stenophylla* ssp. *tenuis* var. *tenuis* var. *ovata*	ssp. *stenophylla* ssp. *tenuis*	ssp. *stenophylla* ssp. *tenuis* var. *tenuis* var. *oblonga* var. *parviflora*

For clarity, the synonyms of the various wild *V. unguiculata* species and their classification system proposed by different researchers are presented in Table 1. In our present discussion, we use the nomenclature and classification system proposed by Padulosi (1993). In this classification system, the three subspecies *dekindtiana*, *tenuis,* and *stenophylla* as recognized by Maréchal et al. (1978) were retained, but var. *protracta* and var. *pubescens* were raised to the level of two distinct subspecies, because of their very distinctive hairy characteristics in pods and other plant parts, morphology of their flowers, pollen, grains, and leaves, as well as their root systems.

Within subspecies *protracta*, three varieties, namely var. *protracta*, var. *rhomboidea,* and var. *kgalagadiensis*, were distinguished. Similarly, three varieties *tenuis*, *oblonga*, and *parviflora* were recognized within the subspecies *tenuis*, while four new varieties, namely var. *huillensis*, var. *congolensis*, var. *ciliolata,* and var. *grandiflora*, have also been proposed and added to the subspecies *dekindtiana.*

Ng (1995) proposed to reinstate var. *rhomboidea* to a species ranking in its own right, because of its strong incompatibility with other taxa within *V. unguiculata.* Pasquet (1993a) proposed that the name subspecies *unguiculata* var. *spontanea* be used to describe all the weedy forms and the intermediates between truly wild var. *dekindtiana* and cultivated cowpea. The subspecies *burundiensis* (Pasquet 1993a) is a variant of var. *ciliolata.* It is found in mid-altitudes in Zaïre, Burundi, Kenya, and Uganda.

Morphology of wild cowpea

Great variability in plant morphology has been observed in wild cowpea. Considerable variation in protein and molecular marker electrophoretic band patterns has also been detected (Vaillancourt and Weeden 1992; Vaillancourt et al. 1993; Panella et al. 1993; Pasquet 1993b). Tables 2 and 3 show the variation of some vegetative and reproductive organs of wild cowpea, and plant growth habit. These traits are useful to discriminate the various subspecies and varieties of the species. Figures 1 and 2 depict the general morphology of plants of a typical variety of each of the five subspecies described. Figure 3 shows the detailed morphology of the stigmas of the different subspecies. Most subspecies, except var. *dekindtiana* and var. *ciliolata* of the subsp. *dekindtiana*, and var. *kgalagadiensis* of the subsp. *protracta*, have the tendency to live for longer than a year (biennial or perennial).

Subsp. *pubescens* and *protracta* are pubescent, with their stems, leaves, and pods covered with hairs. Vestiture of the former subspecies is sericeous, with its hairs generally longer and denser than those of the latter species. The hairs are silky, straight, soft, and appressed to the surface of the stems and pods. On the other hand, the hair type of the subsp. *protracta* is hispid. The hairs are bristly, erect, straight, and harshly stiff. They are especially pronounced in var. *rhomboidea*, a taxon with typical rhombic leaves ranging from 4 to 15 cm long and 1.7 to 5 cm wide. This taxon has thick root stock and its stigmas are strongly bearded and thus easily recognizable from all other taxa. The varieties *protracta* and *kgalagadiensis* can be distinguished from one another by the shape and size of leaves, as well as by length of rachis and peduncle. Variety *protracta* is an annual or a perennial herb up to 2 m long, with a prostate growth habit. Its inflorescence rachis is shorter than 0.7 cm and peduncle about 7 (4–15) cm long. Its lateral leaflet is oblique, slightly to deeply lobed on the inside only, up to 7 cm long and 6 cm wide; terminal leaflet

Table 2. Summary of range of variation of some plant parts and growth habit within wild cowpea taxa†.

Taxa	Growth habit	Stem/ branch rooting	Stem width	Stipule size (mm)§ length	Stipule size (mm)§ width	Leaf texture	Stem hairiness type and intensity	Raised nervation on leaf blade
ssp. *dekindtiana* var. *dekindtiana*	an–rarely pe	absent	med–thick	11(5–23)	5(3–10)	mem–thick	glab–sparsely	scarce–med
ssp. *dekindtiana* var. *ciliolata*	an	absent	med–thick	11.5(7–15)	5(3.5–6)	med	glab	scarce
ssp. *dekindtiana* var. *congolensis*	an–pe	absent	tiny–med	10(5–16)	4.5(3–16)	med–thick	glab	scarce
ssp. *dekindtiana* var. *grandiflora*	an–pe	absent	med–thick	11(7–20)	4.5(3.5–7.5)	med	glab	scarce
ssp. *dekindtiana* var. *hulliensis*	pe	absent	med–thick	10–15	4–6	le	glab	very high
ssp. *pubescens*	an/biennial	absent	thick	11.5(6–20)	5.5(3.5–8.5)	med–thick	int–pubescent	med–high
ssp. *protracta* var. *protracta*	an–pe	absent	med	11(9–16)	4–5	med–thick	med–int bristly hairiness	med–high
ssp. *protracta* var. *kgalagadiensis*	an	absent	tiny–med	11(7–16)	5(3–7)	med–thick	scattered bristly hairiness	med–high
ssp. *protracta* var. *rhomboidea*	pe	absent	med–thick	12(7.5–16)	5(4–7)	thick–le	int–bristly hairiness	med–high
ssp. *tenuis* var. *tenuis*	pe	present	tiny	10(6–13)	4(3–6)	med	glab	scarce
ssp. *tenuis* var. *oblonga*	an-pe	present	tiny	9(6–10)	4(3–6)	med	glab	scarce
ssp. *tenuis* var. *parviflora*	pe	present	tiny	6(6–10)	4–4.5	med	glab–sparsely hairy	scarce
ssp. *stenophylla*	an–pe	absent	tiny–med	11(7.2–14)	4(3.2–5)	med–thick	glab	scarce

† an = annual; glab = glabrous; int = intensely, le = leathery; med = medium; mem = membraneceous; pe = perennial.
§ Figures in parentheses are ranges.

Table 3. Summary of range of variation within wild cowpea taxa of some plant parts†.

Taxa	Peduncle length§ (cm)¶	Rachis length (cm)	Standard color	Standard blotch	Standard length (mm)¶	Standard width (mm)¶	Calyx lobes length (mm)¶	Stigma bearding
ssp. *dekindtiana* var. *dekindtiana*	15 (2–40)	7–8‡	white–light mauve purple	narrow	19 (17–34)	27 (15–44)	3 (1–8)	low–med
ssp. *dekindtiana* var *ciliolata*	15 (7–25)	6–8‡	deep purple	flame-like	30 (25–33)	31 (17–36)	9 (6–13)	low–med
ssp. *dekindtiana* var. *congolensis*	15 (5–30)	< 2.5	purple	narrow	29 (22–39)	34 (25–40)	3 (1.7–5.5)	low
ssp. *dekindtiana* var. *grandiflora*	12 (4–23)	6–7‡	pale mauve	narrow	40 (24–47)	43 (36–49)	3 (1.2–6)	low
ssp. *dekindtiana* var. *huillensis*	20 (8–27)	4–5‡	purple	–	30–35	35–40	< 4–6	low
ssp. *pubescens*	20 (4–41)	20–25‡	deep mauve	flame-like	25 (22–39)	30 (24–38)	4 (2–6.5)	low
ssp. *protracta* var. *protracta*	7 (4–15)	< 0.7	deep purple	narrow	29 (26–33)	33 (30–36)	7 (4–10)	med–int
ssp. *protracta* var. *kgalagadiensis*	9.5 (5–20)	3–4	mauve	narrow	24 (22–27)	26 (21–30)	4 (2.2–7)	low-int
ssp. *protracta* var. *rhomboidea*	16 (7.5–30)	< 1	deep purple	narrow	26 (25–27)	40 (24–41)	4–5.5	int
ssp. *tenuis* var. *tenuis*	8 (3–23)	< 0.7	deep purple	narrow	27 (20–32)	31 (25–43)	4 (2.5–6)	low
ssp. *tenuis* var. *oblong*	8 (3–13)	< 1	deep purple	narrow	25 (19–30)	23 (18–29)	3.7 (2.7–5)	low
ssp. *tenuis* var. *parviflora*	6 (4–8)	< 0.8	purple	narrow	20 (17–25)	25 (21–28)	3.5 (2–4.5)	med–int
ssp. *stenophylla*	12 (5–20)	< 1.5	mauve–lilac pale	narrow	23 (20–26)	26 (25–31)	4 (2.5–5)	low

† Int = intense; med = medium. Keel shape (how beaked?) was only markedly beaked in spp. *dekindtiana* var. *huillensis* and scarely so in others. Pollen exine reticulation (how raised?) was slightly raised for all except for spp. *dekindtiana* var. *huillensis,* spp. *protracta* var. *protacta* var. *kgalagadiensis,* and var. *rhomboidea* with markedly raised reticulation.

§ Measured at flowering stage.

¶ Figures in parentheses are ranges.

‡ Values up to the range mentioned.

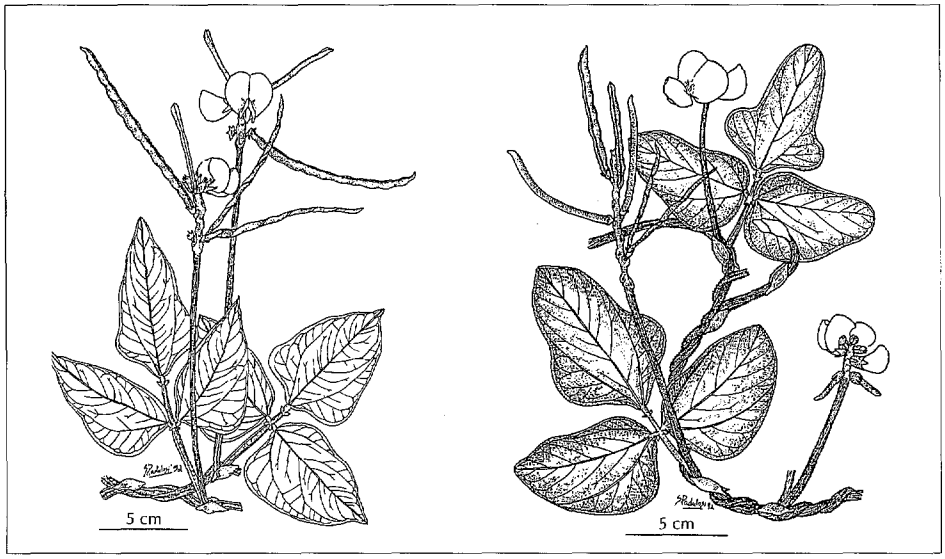

Figure 1. ***Vigna unguiculata*** **ssp.** ***dekindtiana*** **var.** ***dekindtiana*** **(left), and ssp.** ***pubescens*** **(right).**

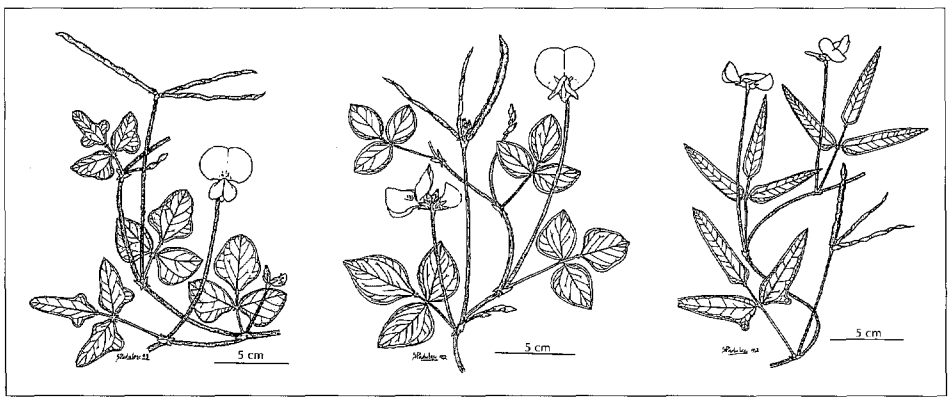

Figure 2. ***Vigna unguiculata*** **ssp.** ***protracta*** **var.** ***protracta*** **(left), ssp.** ***tenuis*** **var.** ***tenuis*** **(centre), and ssp.** ***stenophylla*** **(right).**

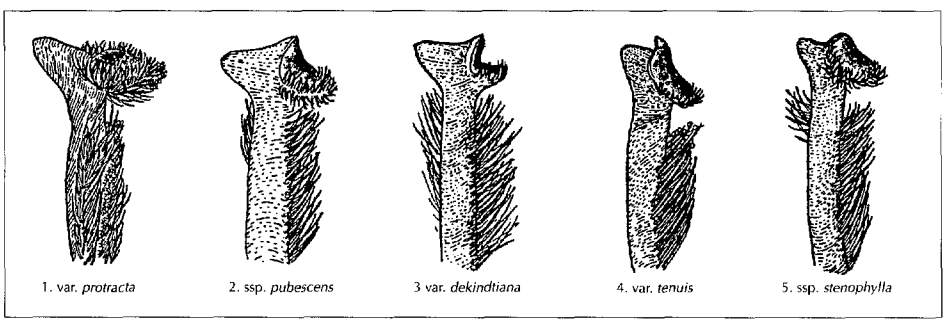

Figure 3. Stigmata of the indicated subspecies of ***Vigna unguiculata*** **(L.) Walp.**

ovate to subhastate or hastate, 5 (3–8) cm long and 3 (2–6) cm wide. Leaflets of var. *protracta* are wider than var. *kgalagadiensis*, whose lateral leaflet is up to 3 cm wide and terminal leaflet 2 cm wide. Inflorescence rachis of var. *kgalagadiensis* is 3–4 cm long and peduncle 9.5 (5–20) cm long. This taxon is an annual herb up to 1.5 m long, with a prostate growth habit. Subsp. *pubescens* has the longest peduncles and rachis, and thickest stems, as compared to other taxa within wild *V. unguiculata*. It has a deep mauve flower.

Plants of the subsp. *tenuis* are small, delicate, and tender. They produce small fleshy tuberous roots. Occasionally, adventitious rooting occurs from nodes of creeping branches. Their peduncles and rachis, similar to those in var. *protracta*, are shortest among the wild *V. unguiculata*. Three varieties are recognized in this subspecies, namely var. *tenuis* (with ovate-shaped leaves), var. *oblonga* (with oblong leaves), and var. *parviflora* (with small flowers).

Subspecies *stenophylla* has very narrow (lanceolate) and sometimes, hastate terminal leaflets, 6 (3–10) cm long and 1 (0.3–2) cm wide. Its lateral leaflets are oblique, slightly lobed on the inside, up to 7 cm long and 3 cm wide. It also produces small tuberous root. Its peduncle is intermediate in length (12 cm). Rachis is shorter than 1.5 cm. Its flower is small, pale, and mauve.

Subspecies *dekindtiana* consists of a very diverse group of varieties, represented by five taxa. Variety *grandiflora* has the largest flower in the species and is easily distinguished from all others by the size of its flowers. The standard color of the flower is pale mauve. Variety *congolensis* has small leaves; terminal leaflet is ovate-lanceolate to subhastate, 5 (3–8) cm long and 2.5 (1.2–7) cm wide; lateral leaflets are oblique, up to 6 cm long and 3 cm wide. This variety from Congo is quite similar to subsp. *tenuis*. Variety *huillensis* has a very long peduncle, with an average of 20 (8–27) cm. It has a large purple flower, with its keel markedly beaked. Its leaves are rather leathery. It is a pyrophytic species. It produces abundant flowers from peduncles originated directly from its woody rootstock, soon after bush fires occur in the savanna. It also produced flowers without bushfires, during growing seasons in Ibadan, Nigeria. Variety *ciliolata,* on the other hand, is an annual plant which is distinguishable from others by its long calyx lobes (over 9 cm long); otherwise it is very similar to var. *dekindtiana*. The calyx lobe length seems to be stable, across the different environments in Ibadan and in East Africa. The general morphology and growth habit of var. *dekindtiana* is very similar to cultivated cowpea landraces, except that its mature pods are usually black, scabrous, and much smaller than the cultivated cowpea. The pods which shatter at maturity contain tiny, dark speckled or solid black seeds, similar to other varieties of the wild species. Variation in the seed size of this variety is greater than others, and the average size (2 g/100 seeds) is also bigger.

Center of origin

The precise location of the center of origin of a species is rather difficult to determine. Previous speculation on the origin and domestication of cowpea had been based on botanical and cytological evidence, information on its geographical distribution and cultural practices, and historical records (Faris 1965; Steel and Mehra 1980; Ng and Maréchal 1985; Ng 1995).

De Candolle (1886) thought that the origin of a cultivated plant could be found where it grows wild. This procedure of locating the place of origin of a crop is correct to a certain

degree, but too often it produces erroneous interpretation. The wild plant may have been common in one area but domestication may have taken place in another, such as in the case of African cottons and the Peruvian tomato (Hawkes 1967).

A detailed study of the variation of a crop, both morphological and genetical, in relation to the geographical distribution of such variation could help in speculating on the origin of cultivated plants. Vavilov (1926) postulated that an area with intensive variation was one where the crop must have been cultivated for a long time, since in that area there would have been time for large numbers of mutations and gene recombinations to take place, as a result of interbreeding among different varieties. It is generally observed that a very large number of varieties or high variation of the species is found towards the center of the distribution area of the crop, and this is accompanied by a corresponding thinning out of the variability towards the periphery.

Based on our present investigation, the range of variation and number of varieties found in wild cowpea, as well as their primitive characteristics, such as perenniality, hairiness, small size of the pods and seeds, pod shattering, with pronounced exine on the surface of pollen, outbreeding, and bearded stigma, the highest genetic diversity and most primitive of the wild *V. unguiculata* occur in southern Africa in the region encompassing Namibia from the west, across Botswana, Zambia, Zimbabwe, and Mozambique to the east, and the Republic of South Africa and Swaziland to the south. Probably, the Transvaal region of the Republic of South Africa was the center of speciation of *V. unguiculata*, due to the presence of most primitive wild varieties, var. *rhomboidea*, var. *protracta*, var. *tenuis*, and var. *stenophylla*. Variety *rhomboidea* has a very narrow geographical distribution in the Transvaal, stretching approximately from 20 to 27 °S and 26 to 32 °E, with an isolated occurrence in Cape Town. It is found growing in the mid-altitude region. It is very commonly found in Swaziland, especially in the northwest region of the Highveld (Padulosi et al. 1990). This taxon shows a relatively high degree of variability among populations found in the region. It overlaps in geographic distribution with var. *protracta,* while the latter taxon has a wider range of geographical distribution stretching from Republic of South Africa and Swaziland to Mozambique and Zimbabwe (Padulosi et al. 1991). The var. *protracta* thrives well in a range of geographical regions and in a wide range of altitudes (from sea level up to 1800 masl). This might suggest that var. *rhomboidea* represents a sort of relic species, which has undergone a speciation process of its own, or it could well be the ancestral form of other varieties of the species *V. unguiculata*. There exists a strong genetic barrier for gene flow between var. *rhomboidea* and other taxa (Ng and Apeji, unpublished), and it was pointed out earlier that this taxon may well be a distinct species.

Continuing on our speculation on the possible evolution of *V. unguiculata*, we further hypothesize that from the Transvaal, the species moved northward to Mozambique and Tanzania where it evolved into subspecies *pubescens*. The two glabrous subspecies, *tenuis* and *stenophylla*, have high morphological similarities, and they share some similar ecogeographical distribution from South Africa to Zimbabwe and Mozambique. The taxa are found in woodland and savanna ecologies, on sandy soils. Genetically, they are probably closer to one another than to other wild taxa. They probably evolved in the Natal-Transvaal region of South Africa, from where they radiated outwards to the coastal regions in South Africa and Mozambique, and to the west in Namibia and Angola.

Variety *congolensis* closely resembles ssp. *tenuis* and it also shows some similar characteristics with ssp. *stenophylla*. It is a perennial plant with a tuberous root. It is found in the Congo Basin. This suggests that a process of natural selection must have taken place in the Zaïrean and Congo region, operating on materials naturally distributed there in the early history of the evolution of *V. unguiculata.*

Variety *huillensis*, var. *dekindtiana*, var. *ciliolata,* and var. *grandiflora* of the subspecies *dekindtiana* represent the latest varieties in the evolutionary line of *V. unguiculata.* Var. *huillensis* is found in the savanna ecology in Angola and Zambia, and in woodland/savanna regions across Namibia and Miombo vegetation in South Africa. It was found at different altitudes, but with a higher frequency in the mid-altitude region. It is quite similar to var. *dekindtiana*, but it has a perennial growth habit, with a thick woody/tuberous root system. This is a pyrophytic species. It may represent the most primitive variety among the subspecies *dekindtiana.*

Variety *ciliolata* is found in the forest ecologies in Burundi, Malawi, Zambia, Zimbabwe, southwestern Cape Flora in South Africa, and in the eastern Kivu region in Zaïre. It is found growing in places of a medium to high altitude (600–1800 masl). Except for its long calyx tubes, it resembles var. *dekindtiana.* Variety *grandiflora* is occasionally found in parts of East and West Africa. Except for its large flower size, var. *grandiflora* resembles var. *dekindtiana* and var. *ciliolata.*

Taxa within the subspecies *dekindtiana* are closely related. Variety *dekindtiana* is a pantropical variety, which is distributed throughout Africa, south of the Sahara, including Madagascar. This taxon has a wide range of morphological variation and ecological tolerance. It has the largest seeds, while the smallest seeds are those of subspecies *pubescens*, subspecies *tenuis* and subspecies *stenophylla.* Variety *dekindtiana* is believed to be the probable progenitor of the cultivated cowpea (Rawal 1975; Lush 1979; Steele and Mehra 1980; Ng and Maréchal 1985). However, it is not certain to what extent the other wild varieties or subspecies of *V. unguiculata* have contributed to the origin and diversity of cowpea.

Domestication and diffusion

Ng (1995) postulated that during the process of evolution of *V. unguiculata*, there was a change of growth habit, from perennial to annual breeding and from predominantly outbreeding to inbreeding, while cultivated cowpea (subsp. *unguiculata*) evolved through domestication and selection of the annual wild cowpea (var. *dekindtiana*). During the process of domestication and after the species was brought under cultivation through selection, there was a loss in seed dormancy and pod dehiscence, corresponding with an increase in seed and pod size. The precise location or region where cowpea was first domesticated is still under speculation. The wide geographical distribution of var. *dekindtiana* throughout sub-Saharan Africa suggests that the species could have been brought under cultivation in any part of the region. However, the center of maximum diversity of cultivated cowpea is found in West Africa, in an area encompassing the savanna region of Nigeria, southern Niger, part of Burkina Faso, northern Benin, Togo, and the northwestern part of Cameroon (Ng and Maréchal 1985; Ng 1995).

In this region, many weedy forms of var. *dekindtiana*, intermediate between truly wild forms and those very small-seeded cultivated cowpeas are found (Rawal 1975). Carbon

dating of cowpea (or wild cowpea) remains from the Kimtampo rock shelter in central Ghana has been carried out (Flight 1976), and this is the oldest archaeological evidence of cowpea found in Africa. This shows the existence of gathering (if not cultivation) of cowpea by African hunters or food gatherers as early as c. 1500 BC.

In most African countries which produce cowpea today, landraces are cultivated as a component of mixed cropping systems, particularly in millet and sorghum-based farming systems in the semiarid and subhumid tropics in Africa. The haulm is gathered to feed cattle, particularly in northern Nigeria, Niger, Mali, Burkina Faso, and northern Cameroon, as well as in Senegal. It is equally important as a pulse in these regions.

Both flowers and mature pods can be found at the same time on wild and weedy var. *dekindtiana*. Under natural conditions, very few pods can be found on a plant at a given time; however, the plant continues to produce flowers and pods over a long period. The low seed set per plant and low population density of the wild species suggest, therefore, that in preagricultural times, wild cowpea seeds could not have constituted a major portion of the human diet. At present, African farmers collect cowpea haulm by uprooting the whole plant, while it still carries green leaves and both mature and immature pods. It could be assumed that earlier African farmers similarly gathered wild cowpea plants to feed their cattle. In following this practice of gathering wild cowpea plants to feed cattle, it is probable that some seeds of the earliest mature pods, which could already have dehisced and ejected their seeds before or during the harvest, were missed, and this would have resulted in the selection of types with less shattering, while at the same time leaving behind the dehiscent wild type. Archaeological findings indicate the existence of cattle in West Africa as far back as 3000 BC (Clutton-Brock 1989). Ng (1995) postulated that cowpea cultigroup Unguiculata was, in the first place, domesticated in West Africa through this process of selection c. 2000 BC. Later, the selection for types with very long peduncles for fiber resulted in the cultigroup Textillis (Ng and Maréchal 1985). The crop was brought to Europe probably through northeastern Africa around 300 BC and to India about 200 BC. The cowpea underwent further diversification in India and Southeast Asia, producing the cultigroup Sesquipedalis with its long pods used as a vegetable and the cultigroup Biflora for its grain (Steele and Mehra 1980). The crop was introduced from Africa to the tropical Americas in the 17th century by the Spanish in the course of the slave trade. It has been grown in southern USA since the early 18th century.

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2

Cowpea genetics: a review of the recent literature

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Abstract

In the decade since the literature on the genetics of cowpea was last reviewed by Fery (1985), researchers have published numerous cowpea genetics studies, especially on economically important traits. Both qualitative and quantitative procedures have been utilized to study these traits, and considerable effort made to increase our knowledge of cowpea cytogenetics, heterosis, and problems associated with crossing cowpea with other *Vigna* species. Many inheritance studies have addressed flower traits and earliness parameters, durations of specific developmental stages, pigmentation, nitrogen fixation, mycorrhizal colonization, seedling vigor traits, plant habit and root traits, leaf traits, pod traits, seed traits and grain quality, yield and yield components, fodder quality, heat and drought tolerances, resistance to bacterial, fungal and viral diseases, resistance to root-knot nematodes, resistance to insects, and resistance to parasitic weeds such as *Striga gesnerioides* and *Alectra vogelii*.

Introduction

The literature on cowpea (*Vigna unguiculata* [L.] Walp.) genetics was last reviewed by Fery (1985). That review covered all of the pertinent literature on cytologic, qualitative, and quantitative genetics, and included an updated list of genes and a set of rules for the gene nomenclature of *Vigna*. Our objective is to review the research on cowpea genetics in more recent literature and thus complement the earlier review.

Cytogenetics and interspecific hybridization

Three recent publications (Barone and Saccardo 1990; Pignone et al. 1990; Saccardo et al. 1992) contain detailed descriptions of the cowpea karotype. Barone and Saccardo (1990) used pachytene bivalents to develop their karotype. Pignone et al. (1990) developed a banded karotype by using cells in mitotic prometaphase. Saccardo et al. (1992) used both conventional techniques and an automatic image analysis system in their work with pachytene and mitotic prometaphase and metaphase chromosomes.

Ghosh (1978) observed induced cowpea tetraploids, and noted that chromosome doubling affects many plant traits, e.g., percentage germination of seeds, nature of germination, seedling and plant survival, rate of growth, leaflet shape and color, stomata size, time and duration of flowering, flower size, pollen grain viability, number of shriveled seed per pod, and seed size.

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Fatokun and Singh (1987) successfully crossed cultivated cowpea with a hairy wild relative (*Vigna pubescence*). They used tissue culture techniques to rescue the hybrid embryos, which would otherwise have been shriveled and degenerated. The F_1 plants were vigorous in growth but were partially sterile, with only about 32% viable pollen. Cytological investigations of F_1 plants showed some meiotic abnormalities in the pollen mother cells. These abnormalities included a few univalents and quadrivalents, suggesting some structural differentiation in the chromosomes.

Barone and Ng (1990) could not obtain an interspecific cross between *V. unguiculata* and *V. vexillata*. They concluded that the following were barriers to crossability: lack of fertilization and collapse of fertilized ovules 5–8 days after pollination. All attempts by Fatokun (1991) to cross *V. vexillata* with various cultivated and noncultivated cowpeas were also unsuccessful.

Considerable progress has been made in recent years in developing innovative biotechnologies for cowpea. The focus of much of this research is on increasing our understanding of the cowpea genome and the development of techniques to insert exotic genes into the cultivated cowpea (see later in this volume: Monti et al. 1997; Fatokun et al. 1997a,b; Kononowicz et al. 1997).

Heterosis

Several studies show that cowpea hybrids can exhibit considerable heterosis for many traits. Heterosis was demonstrated for time of flowering (Adu-Dapaah et al. 1988; Lodhi et al. 1990), time to maturity (Adu-Dapaah et al. 1988), vine length (Lodhi et al. 1990), stem girth (Lodhi et al. 1990), number of pods per plant (Teofilo et al. 1984; Patil and Shete 1987; Adu-Dapaah et al. 1988), number of clusters per plant (Patil and Shete 1987), pod length (Patil and Shete 1987), number of branches per plant (Adu-Dapaah et al. 1988; Lodhi et al. 1990), leaf length (Lodhi et al. 1990), leaf breadth (Lodhi et al. 1990), plant height (Adu-Dapaah et al. 1988), number of seeds per pod (Patil and Shete 1987; Adu-Dapaah et al. 1988), grain yield per plant (Teofilo et al. 1984; Patil and Shete 1987; Adu-Dapaah et al. 1988), seed weight (Patil and Shete 1987; Adu-Dapaah et al. 1988), seed length (Patil and Shete 1987), green fodder yield (Lodhi et al. 1990), dry-matter yield (Lodhi et al. 1990), protein content of seed (Emebiri 1991), protein content of forage (Jain et al. 1980; Lodhi et al. 1990), and in vitro dry-matter digestibility of forage (Jain et al. 1980; Lodhi et al. 1990). Sherif et al. (1991) reported that F_1 hybrids displayed significantly greater resistance to drought than resistant parental cultivars.

Flower traits and earliness

Emebiri (1989b) reported that both flower size and style length are heritable, with narrow-sense heritability (Hn) estimates, calculated using variance components, of 72% and 47%, respectively. The Hn estimates for flower size and style length, calculated by regressing the mean values of F_1 plants on their mid-parent values, were 77% and 69%, respectively. Apte et al. (1987) estimated a broad-sense heritability (Hb) of 16% for number of inflorescences per plant. Brantley and Kuhn (1983) noted that plants homozygous for the *pbs* gene conditioning proliferated leaf buds were sterile (Table 1). They concluded that the sterility was caused by failure of the style to elongate. In most instances, the stigma is enclosed by united stamens.

Table 1. Cowpea gene index.

Preferred symbol	Synonym	Character†	Reference
Bgs§	*Bg*	Big seed	Karkannavar et al. (1991)
cd§		Chlorophyll deficiency	Kirchhoff et al. (1989)
cpi		Ineffective nodulation	Pemberton et al. (1990)
gc		Green cotyledon	Fery and Dukes (1994)
Hbs		Heat-induced browning in seed coat	Patel and Hall (1988)
ims		Res. to cowpea severe mosaic virus	Jimenez et al. (1989)
pbs		Proliferated buds	Brantley and Kuhn (1983)
Pm-1	Pm_1	Miniature plant	Singh (1980)
Pm-2	Pm_2	Miniature plant	Singh (1980)
pt		Nonpetiolate leaf	Fawole (1988)
pt-2		Nonpetiolate leaf-2	Fawole (1990)
Rac	Ac_1	Res. to *Aphis craccivora*	Bata et al. (1987); Ombakho et al. (1987); Pathak (1988)
Rac-2	Ac_2	Res. to *Aphis craccivora*-2	Ombakho et al. (1987); Pathak (1988)
Rav-1		Res. to *Alectra vogelii*	Singh et al. (1993)
Rav-2		Res. to *Alectra vogelii*	Singh et al. (1993)
Rav-3		Res. to *Alectra vogelii*	Atokple et al. (1995)
rcc		Res. to *Colletotrichum capsici*	Abadassi et al. (1987)
rcm-1		Res. to *Callosobruchus maculatus*-1	Adjadi et al. (1985)
rcm-2		Res. to *Callosobruchus maculatus*-2	Adjadi et al. (1985)
Rsg-1		Res. to *Striga gesnerioides*	Singh and Emechebe (1990)
Rsg-2		Res. to *Striga gesnerioides*	Atokple et al. (1995)
Rsg-3		Res. to *Striga gesnerioides*	Atokple et al. (1995)
rss		Res. to *Sphaceloma* sp.	Abadassi et al. (1987)
Rsv-1		Res. to *Septoria vignae*-1	Abadassi et al. (1987)
Rsv-2		Res. to *Septoria vignae*-2	Abadassi et al. (1987)
sbc-1		Res. to southern bean mosaic virus-1	Melton et al. (1987)
sbc-2		Res. to southern bean mosaic virus-2	Melton et al. (1987)
Spg-1§	*Pp-1*	Stem pigmentation-1	Karkannavar et al. (1991)
Spg-2§	*Pp-2*	Stem pigmentation-2	Karkannavar et al. (1991)
Sti		Stipule color; red dominant over green	Karkannavar et al. (1991)
Vv-1		*Uromyces vignae* res.-1	Chen and Heath (1993)
Vv-2		*Uromyces vignae* res.-2	Chen and Heath (1993)

† Res. = resistance.
§ Proposed new symbol.

Earliness is an important agronomic trait. Typically, it is measured by such criteria as days to flowering or days to maturity. A number of quantitative studies of the genetics of earliness parameters have been published in recent years (Table 2); the Hb estimates

Table 2. Estimates of broad-sense heritability (%) for earliness (days to flowering and days to pod maturity).

Reference	Days to flowering	Days to pod maturity
Apte et al. (1987)	58.1†	65.0
	60.8§	–
Dumbre et al. (1983)	90.0	–
Jana et al. (1982)	93.0	–
Mishra et al. (1987)	52.7	–
Pandita et al. (1982)	95.6	–
Patil and Baviskar (1987)	65.3	82.9
Radhakrishnan and Jebara (1982)	–	87.8
Roquib and Patnaik (1990a)	91.0	60.0
Senanayake and Wijerathne (1988)	67.3	–
Sharma and Singhania (1992)	95.8	–
Sharma et al. (1988)	98.0	–
Siddique and Gupta (1991)	97.8	95.5
Sreekumar et al. (1979)	69.2	–
Thiyagarajan (1989)	89.0	81.0
Vaid and Singh (1983)	56.1¶	–
	16.5‡	–

† Days to initial flowering.
§ Days to 50% flowering.
¶ Derived from F_3 populations.
‡ Derived from F_4 populations.

average 75% for days to flowering and 79% for days to pod maturity. Adu-Dapaah et al. (1988) observed a tendency for dominance of early flowering and pod maturity.

Duration of specific developmental stages

Emebiri and Obisesan (1991) observed that a plant's life cycle consists of a succession of relatively distinct phases that comprise a developmental pattern, and that seed yields of crops are often influenced by the developmental pattern. They speculated that certain phases of the developmental pattern are potentially important criteria for selecting higher yields. Emebiri and Obisesan (1991) reported Hn for the following developmental stages (first estimate calculated from genetic variance components; second estimate calculated from regression of F_2 on F_1): duration of vegetative period (days), 57% and 41%; days to pod maturity, 66% and 64%; duration of pod filling (days), 42% and 52%; growth rate of pods (mg per day), 75% and 20%; and days to leaf senescence, 57% and 57%. Emebiri and Obisesan (1991) noted that all of the phases were controlled by genes with both additive and nonadditive effects. Sreekumar et al. (1979) reported an Hb estimate of 49% for the total duration of the harvest period. Dumbre et al. (1983) reported an Hb of 40% for the duration of the maturity period.

Pigmentation

Several recently published studies address the inheritance of color in cowpea. Fery and Dukes (1994) reported that a recessive gene, *gc*, conditions the green cotyledon trait. They

noted that the *gc* gene is not allelic to nor linked with the *gt* gene that conditions the green testa trait. Patel and Hall (1988) noted that a dominant gene, *Hbs*, conditions heat-induced browning in the seed coat of the heat tolerant line TVu 4552. They did not observe any close linkages between the *Hbs* gene and genes controlling heat tolerance during floral bud development or normal brown seed coat pigmentation. Karkannavar et al. (1991) reported that a single dominant gene, *Sti*, conditions stipule color. They noted that red color is dominant over green. Karkannavar et al. (1991) also reported that two duplicate genes condition stem pigmentation. They proposed the symbols *Pp-1* and *Pp-2* for these genes, but as *Pp-1* and *Pp-2* are the symbols for purple plant pigmentation genes (Fery 1985), we propose the symbols *Spg-1* and *Spg-2*. Kirchhoff et al. (1989) demonstrated that a single recessive gene governs the inheritance of a mutant chlorophyll-deficiency trait. Since Kirchhoff et al. (1989) did not propose a symbol for this gene, we propose the symbol *cd*.

Nitrogen fixation and mycorrhizal colonization

Several researchers have studied traits influencing nitrogen fixation and mycorrhizal colonization. Miller et al. (1986) investigated traits influencing nitrogen fixation efficiency. They reported the following Hn estimates: nodule number, 55%; nitrogenase activity, 62%; nodule weight, 39%; and top dry weight, 17%. They also demonstrated that additive gene action was important for nodule number and nitrogenase activity, and that dominance and interallelic gene action was important for nodule weight and top dry weight. Dayap and Rasco (1988) published the following Hb estimates: nitrogenase activity, 24%; secondary root nodule weight, 38%; and secondary root nodule number, 9.5%. They noted that additive gene action was important for all three traits. Roquib and Patnaik (1990a) studied the inheritance of effective root nodules at 30 and 65 days after planting (DAP). They reported the following Hb estimates for effective root nodules: main root at 30 DAP, 6%; branch root at 30 DAP, 21%; main root at 65 DAP, 36%; and branch root at 65 DAP, 11%. Pemberton et al. (1990) reported that a single recessive gene, *cpi*, conditioned the inheritance of an ineffective nodulation trait. Mercy et al. (1990) demonstrated that endomycorrhizal colonization in cowpea is heritable, with an Hb of 46%.

Seedling vigor traits

Ogunbodede (1988) found considerable genetic variability in cowpea for several seedling vigor traits. He reported Hb estimates for the following traits: emergence percentage, 89%; emergence index, 46%; emergence rate index, 46%; growth rate (9–13 DAP), 7%; growth rate (13–17 DAP), 29%; growth rate (17–21 DAP), 52%; and growth rate (21–25 DAP), 57%. Ogunbodede (1988) noted that there have been reports of positive associations between seedling vigor and yield in several crops, and suggested that specific seedling vigor traits might be useful selection criteria for yield in cowpea.

Plant habit and root traits

Singh (1980) assigned the symbols *Pm-1* and *Pm-2* to two genes conditioning miniature plant habit. Karkannavar et al. (1991) studied the tendrillar and nontendrillar plant habits, and concluded that plant habit has a trigenic mode of inheritance. Uguru and Uzo (1991) studied decumbent, climbing, and bushy plant habits, and concluded that two allelic pairs

Table 3. Estimates of broad-sense heritability (%) for growth-habit traits.

Reference	Plant height	Branches/ plant	Nodes on main stem	Stem diameter	Leaves/ plant	Leaf area	Root length
Apte et al. (1987)	27.4	34.9	–	–	–	–	–
Araujo and Nunes (1983)	–	22.9	8.3	0.0	14.0	90.0	–
Dumbre et al. (1983)	78.0	–	–	–	–	–	–
Jana et al. (1982)	–	68.8	–	–	–	–	–
Pandita et al. (1982)	15.7	–	–	–	–	–	73.7
Radhakrishnan and Jebara (1982)	97.9	96.5	–	–	–	–	–
Roquib and Patnaik (1990a)	94.0	–	5.0	–	–	–	–
	–	–	7.0†	–	–	–	–
Roquib and Patnaik (1990b)	89.0§	–	–	–	–	74.0¶	–
	86.0‡	–	–	–	–	56.0††	–
Senanayake and Wijerathne (1988)	44.5	55.8	–	–	–	–	–
Sharma and Singhania (1992)	90.6	–	–	–	57.1	96.1	–
Siddique and Gupta (1991)	92.3	–	–	–	–	–	–
Thaware et al. (1991)	43.4	24.2	–	–	38.3	–	–
Thiyagarajan (1989)	67.8	42.6	–	–	–	–	–
Thiyagarajan et al. (1989)	97.6	96.2	–	–	–	–	–
Vaid and Singh (1983)	–	60.1§§	–	–	–	–	–
	–	67.0¶¶	–	–	–	–	–

† Secondary branch.
§ Early growth stage.
¶ Terminal leaflets.
‡ Rapid leaf formation stage.
†† Lateral leaflets.
§§ Derived from F_3 populations.
¶¶ Derived from F_4 populations.

govern plant habit. The works of Karkannavar et al. (1991) and Uguru and Uzo (1991) confirm conclusions drawn by earlier researchers (Fery 1985). In recent years, many researchers have used quantitative procedures to study the inheritance of growth-habit traits such as plant height, branch number, node number, stem diameter, leaf number, leaf area, and root length, and over 30 heritability estimates have been published (Table 3). Although these studies vary, their results indicate that most growth-habit traits are at least moderately heritable. For example, the average Hb estimates for plant height and branch number were 71% and 57%, respectively.

Leaf traits

Brantley and Kuhn (1983) assigned the symbol *pbs* to a recessive gene conditioning proliferated leaf buds. They observed that plants homozygous for the *pbs* gene exhibit elongated and distorted leaflets with irregular margins and abnormal vein curvature, and male sterility caused by the failure of the style to elongate. Fawole (1988) assigned the symbol *pt* to a recessive gene that governs a nonpetiolate leaf mutant. In a subsequent paper, Fawole (1990) assigned the symbol *pt-2* to a second recessive gene conditioning the nonpetiolate phenotype, and demonstrated that the *pt* and *pt-2* genes are neither allelic nor

linked. Fawole (1990) also studied the relationship between the *pt* and *pt-2* genes and the *un* gene that conditions the unifoliolate leaf trait. He found that the absence of the petiole in the unifoliolate mutant is not a pleiotropy effect of the *un* gene, as suggested by Rawal et al. (1976), but is due to a mutation of one of the genes conditioning petiole development. Fawole (1990) observed that the *un* gene exhibited both incomplete penetrance and variable expressivity, and he concluded that the gene is closely linked to one of those controlling petiole development.

Pod traits

Several recently published studies demonstrate that pod length is moderately to highly heritable; and the Hb estimates average 70% (Table 4). Additive gene effects were more important than dominance effects (Ogunbodede and Fatunla 1985). The number of seeds per pod is moderately to highly heritable; the Hb estimates average 64%. Drabo et al. (1985) observed that additive, dominance, and epistatic gene effects were of equal importance in conditioning the trait. Roquib and Patnaik (1990a) reported an Hb of 62%

Table 4. Estimates of broad-sense heritability (%) for cowpea pod and seed traits.

Reference	Pod length	Pod breadth	Seeds/ pod	100-seed weight	Seed protein content
Apte et al. (1987)	62.4	–	65.8	82.5	–
Araujo and Nunes (1983)	98.0	–	68.1	95.0	–
Drabo et al. (1984)	–	–	–	85.1	–
	–	–	–	75.4†	–
Drabo et al. (1985)	–	–	52.2§	76.0§	–
Dumbre et al. (1983)	–	–	81.0	–	–
Emebiri (1991)	–	–	–	–	70.0
	–	–	–	–	78.0¶
Gowda et al. (1991)	–	–	70.2	86.0	–
Jana et al. (1982)	85.5	–	78.6	97.9	–
Nielsen et al. (1993)	–	–	–	–	95.0
Pandita et al. (1982)	32.2	–	–	–	–
Patil and Baviskar (1987)	70.3	–	33.3	90.9	–
Radhakrishnan and Jebara (1982)	91.0	–	94.5	99.6	–
Roquib and Patnaik (1990a)	76.0	62.0	–	22.0	–
Senanayake and Wijerathne (1988)	82.1	–	45.1	96.2	92.1
Siddique and Gupta (1991)	84.1	–	75.3	94.4	–
Sreekumar et al. (1979)	–	–	40.6	96.5	–
Thiyagarajan (1989)	70.8	–	71.2	83.4	–
Thiyagarajan et al. (1989)	98.6	–	99.6	–	–
Vaid and Singh (1983)	23.8‡	–	44.5‡	15.1	–
	34.0††	–	37.5††	–	–

† Narrow-sense heritability estimate.
§ Average of 12 estimates.
¶ Derived from a different cross.
‡ Derived from F_3 populations.
†† Derived from F_4 populations.

for pod breadth. Ogunbodebe and Fatunla (1985) demonstrated that additive gene effects are usually more important than dominance gene effects in controlling seed crowding within the pod. However, they noted that dominance gene effects can be important in some crosses.

Seed traits and grain quality

Karkannavar et al. (1991) identified a dominant gene that governs big seed. They proposed the symbol *Bg* for this gene, but *Bg* is the symbol for brown grain (Fery 1985). Therefore, we propose *Bgs* for the symbol. Ogunbodede and Fatunla (1985) proposed a digenic epistatic model for seed size. Most recently published heritability estimates of seed size, usually measured as 100-seed weight, indicate that the trait is highly heritable; Hb estimates average 79.7% (Table 4). Drabo et al. (1984) concluded that the gene action controlling seed size is predominantly additive, but they also noted that additive × additive epistatic effects are significant, and estimated that the minimum number of effective factors conditioning seed size is eight.

Drabo et al. (1988) examined segregating populations for eye pattern. Their results generally support the findings reported by earlier researchers (Fery 1985). However, they noted that incomplete dominance of several seed coat pattern genes might make classification rather difficult in progeny segregating for the holstein, watson, small eye, and hilum ring traits.

Published Hb estimates for protein content in seed average 80% (Table 4). Emebiri (1989a) concluded that protein content is controlled by nuclear genes, but he could not demonstrate that extra-nuclear determinants were important. In a paper published subsequently, he reported that inheritance of protein content in seed involved both additive and nonadditive gene effects, and that cytoplasmic factors might influence the trait (Emebiri 1991).

Nielsen et al. (1993) studied various aspects of grain quality in cowpea, and reported genetic variability for protein, fat, ash, carbohydrate, and cooking time. The Hb estimates were 76% for cooking time, 95% for protein, 72% for fat, 83% for ash, and 79% for carbohydrate. Protein content was negatively correlated with fat (–0.22) and carbohydrate (–0.98), and positively correlated with ash content (+0.35). Larger seeds and seeds with smooth seed coats took relatively longer times to cook. In view of the available genetic variability, it is possible to develop cowpea varieties with higher protein content that cook relatively quickly.

Yield

The results of many recent studies indicate that the yields of both the reproductive and the vegetative portions of the cowpea plant are moderately to highly heritable under most environmental conditions (Table 5). For example, heritability estimates for cluster number, pod number, seed yield, and fresh fodder yield average 71%, 62%, 62%, and 55%, respectively. Jatasra (1979, 1980) reported that most of the genes governing seed and green fodder yields act additively. However, he observed that nonadditive gene action was more important in conditioning dry fodder yield (Jatasra 1979). Siddique and Gupta (1991) demonstrated that additive gene effects were important in conditioning both seed yield and number of pods per plant.

Table 5. Estimates of broad-sense heritability (%) for various yield parameters.

Reference	Clusters/ plant	Pods/ cluster	Pods/ plant	Green pod wt/plant	Seeds/ plant	Seed wt/ plant	Harvest index	Fresh fodder yield	Dry fodder yield
Apte et al. 1987	–	–	14.0	–	–	17.0	20.5	–	–
Araujo and Nunes 1983	–	–	67.0	–	–	52.0	–	–	–
Dumbre et al. 1983	–	–	64.0	–	–	57.0	–	–	–
Gowda et al. 1991	–	–	80.3	–	–	85.9	–	–	–
Imrie 1986	–	–	–	–	–	63.0†	67.0†	–	–
	–	–	–	–	–	25.0§	54.0§	–	–
Jana et al. 1982	–	–	96.8	94.5	–	–	–	–	–
Mishra et al. 1987	–	–	–	–	–	27.9¶	–	–	–
Pandita et al. 1982	–	–	–	–	–	91.7	–	–	–
Patil and Baviskar 1987	63.1	69.2	68.7	–	–	51.6	–	–	–
Radhakrishnan and Jebara 1982	94.1	–	98.9	–	–	99.8	–	–	–
Roquib and Patnaik 1990a	–	–	–	–	83.0	74.0	–	–	–
Roquib and Patnaik 1990b	–	–	–	–	–	–	–	14.0	24.0
Senanayake and Wijerathne 1988	–	–	31.6	–	–	90.1	–	–	–
Sharma and Singhania 1992	–	–	–	–	–	–	–	84.4	62.0
Sharma et al. 1988	–	–	–	46.9	–	–	–	–	–
Siddique and Gupta 1991	79.3	–	92.8	–	–	94.7	–	–	–
Sreekumar et al. 1979	–	–	–	–	–	43.4	–	–	74.0‡
Thaware et al. 1991	–	–	–	–	–	–	–	66.6	–
Thiyagarajan 1989	33.0	–	25.9	–	–	30.2	–	–	–
Thiyagarajan et al. 1989	98.0	–	98.6	–	–	99.8	–	–	–
Vaid and Singh 1983	51.0††	–	38.7††	–	–	54.5††	–	–	–
	78.7§§	–	27.4§§	–	–	57.8§§	–	–	–

† Single-row plots used to estimate yield.
§ Hill plots used to estimate yield.
¶ Narrow-sense heritability estimate.
‡ Haulms.
†† Derived from F_3 populations.
§§ Derived from F_4 populations.

Fodder quality

Several researchers have investigated the genetic nature of traits important to fodder quality. Sharma and Singhania (1992) reported Hb estimates for dry-matter content and crude protein content of 82% and 86%, respectively. Jain et al. (1980) demonstrated a preponderance of nonadditive gene action for total protein and in vitro dry-matter digestibility. Sharma and Singhania (1992) reported a Hb estimate for stem-leaf ratio of 96%, but Roquib and Patnaik's (1990b) estimate for the same trait was only 9%. Thaware

et al. (1991) demonstrated that Hb estimates for the components of green fodder yield, i.e., leaf yield (55%) and stem yield (62%), are smaller than the Hb estimate for green fodder yield itself (67%). Their Hb estimate for the leaf yield index [(leaf weight/weight total green forage) × 100] was only 15%.

Tolerance to heat and drought

Marfo and Hall (1992) used qualitative procedures to study the inheritance of heat tolerance during pod set, and their results suggest that heat tolerance is conditioned by a single dominant gene. However, they noted substantial environmental influence on the expression of the trait, but results of additional inheritance studies using quantitative procedures indicated that heritability is low. The Hn estimates were 24–27%, while realized heritabilities were 24–29%.

Hall et al. (1990) noted that measurements of the carbon isotope composition of plant parts can be used to estimate water-use efficiency (total biomass/transpiration) of plants, and they conducted heritability studies of carbon isotope discrimination by cowpea plants. They observed that genotypic differences were readily detected in leaves, and calculated an Hb of 76%. Ismail and Hall (1993) demonstrated that water-use efficiency and carbon isotope discrimination were strongly and negatively correlated. Using data from reciprocal crosses, they showed that both water-use efficiency and carbon isotope discrimination are controlled by nuclear genes. Both high water-use efficiency and low carbon isotope discrimination exhibited partial dominance in pot experiments. However, Ismail and Hall (1993) noted that high carbon isotope discrimination exhibited partial dominance in plants grown under natural soil conditions in a field environment.

Resistance to bacterial and fungal diseases

Development of cultivars with resistances to diseases incited by bacterial and fungal pathogens has been a major goal of most cowpea breeding programs since the early part of this century. In the past 10 years, studies on the inheritance of resistance have been published on the following diseases: bacterial blight, brown blotch, Fusarium wilt, Phytophthora, rust, scab, and Septoria leaf spot.

Prakash and Shivashankar (1984) reported that resistance to bacterial blight [*Xanthomonas campestris* pv. *vignicola* (Burk.)] is recessive, and probably inherited quantitatively, with an Hb estimate that ranged from 30 to 80% and averaged 55%. They also estimated that the minimum number of effective factors conditioning resistance was small, probably between two and four.

Abadassi et al. (1987) reported that a single recessive gene, *rcc*, governs resistance to brown blotch (*Colletotrichum capsici* [Syd] Butler and Bisby). They observed partial dominance of susceptibility over resistance. They also reported that a recessive gene, *rss*, governs resistance to scab (*Sphaceloma* sp.), and duplicate dominant genes, symbolized by *Rsv-1* and *Rsv-2*, govern resistance to Septoria leaf spot (*Septoria vignae* P. Henn).

Rigert and Foster (1987) studied the inheritance of resistances to Fusarium wilt incited by race 2 and race 3 of *Fusarium oxysporum* f. sp. *tracheiphilum* (E. F. Sm.) Synder and Hansen. They found that the cultivar California Blackeye 3 possesses both a single dominant gene that conditions resistance to race 3 and a single incompletely dominant gene that conditions resistance to race 2. Conversely, they found that the breeding line 7964

possesses both a single dominant gene that conditions resistance to race 2 and a single incompletely dominant gene that conditions resistance to race 3. Rigert and Foster (1987) decided not to propose symbols for the resistance genes in California Blackeye 3 and 7964 because the nature of the relationship between the genes was not clear. Fang and Hwang (1987) studied resistance to Fusarium wilt in yardlong bean, and concluded that the resistance is likely governed by a single recessive gene.

Bateman et al. (1989) investigated the nature of inheritance of resistance to stem and root rot incited by race 2 of *Phytophthora vigna* Purss. They demonstrated that resistance was conditioned by a single dominant gene. The relationship between this gene and the *Sr* gene that conditions resistance to stem rot is unclear (Fery 1985).

Chen and Heath (1993) reported that two genes, *Uv-1* and *Uv-2*, are responsible for the rust (*Uromyces vignae* Barclay) resistance exhibited by the cultivar Dixie Cream. Resistance is only partially dominant, but is effective against both the monokaryon and dikaryon forms of the fungus.

Resistance to viral diseases

Plant resistance is often the only feasible method of controlling virus diseases in cowpea. Since the review by Fery (1985), studies on the inheritance of resistance have been published for the following viruses (see also, later in this volume, Hampton et al. 1997): blackeye cowpea mosaic virus, cowpea aphid-borne mosaic virus, cowpea mosaic virus, cowpea severe mosaic virus, tobacco ringspot virus, and southern bean mosaic virus.

Two reports (Melton et al. 1987; Ouattara and Chambliss 1991) concluded that resistance to blackeye cowpea mosaic virus is conditioned by a single dominant gene. These results confirm earlier published work (Fery 1985).

Patel et al. (1982) reported on preliminary studies of the inheritance of both immunity and resistance to a strain of cowpea aphid-borne mosaic virus from Tanzania. They concluded that immunity was likely conditioned by a single recessive gene and several modifier genes. The resistance was shown to be partially dominant over susceptibility.

Data published in three reports (Eastwell et al. 1983; Bruening et al. 1987; Ponz et al. 1988) suggest that resistance to cowpea mosaic virus is conditioned by a single dominant gene.

Jimenez et al. (1989) reported that a single recessive gene, *ims*, conditions resistance to cowpea severe mosaic virus. Umaharan (1990) found that resistance to a Trinidad isolate of the virus is expressed as immunity, tolerance, and resistance. He concluded that the trait was conditioned by three major genes acting in a dosage-dependent manner.

Two reports (Bruening et al. 1987; Ponz et al. 1988) concluded that resistance to tobacco ringspot virus is governed by a single dominant gene. These findings confirm results published earlier by others (Fery 1985).

Melton et al. (1987) reported the resistance to southern bean mosaic virus–cowpea strain is conditioned by two recessive genes, *sbc-1* and *sbc-2*. Hobbs et al. (1987) studied three sources of resistance to this virus. Their data suggest that a partially dominant gene conditions the moderate nonnecrotic resistance exhibited by the cultivar Early Pinkeye, three or more genes with incomplete dominance condition the nonnecrotic resistance exhibited by the cultivar Iron, and a partially dominant gene with modifiers conditions the extreme nonnecrotic resistance exhibited by PI 186465.

Resistance to root-knot nematodes

Singh and Reddy (1986) reported that resistance to the southern root-knot nematode (*Meloidogyne incognita* [Kofoid & White] Chitwood) is conditioned by a single dominant gene, confirming the results that had been published earlier by others (Fery 1985). Fery et al. (1994) characterized several new sources of resistance to root-knot nematodes. They suggested that the allele at the *Rk* locus in these lines may not be the *Rk* allele for root-knot nematode resistance, but another allele that conditions an enhanced, dominant type resistance.

Resistance to insects

Resistance to insects is potentially a valuable means of control, either as a sole control measure or as an adjunct to other control measures. Recent publications report studies on the inheritance of resistance to the following insect pests: aphids, cowpea seed beetles (bruchids), and lygus bugs.

Three publications (Bata et al. 1987; Ombakho et al. 1987; Pathak 1988) report the results of inheritance studies of aphid (*Aphis craccivora* Koch) resistance in germplasm developed at the International Institute of Tropical Agriculture. Each publication reported that resistance is conditioned by a single dominant gene. Bata et al. (1987) and Pathak (1988) proposed that this gene be designated *Rac*, but Ombakho et al. (1987) proposed the symbol Ac_1. Since the Bata et al. (1987) manuscript was the earliest to be submitted for publication, we propose that the *Rac* symbol be used. Ombakho et al. (1987) and Pathak (1988) also reported the identification of a second dominant gene for aphid resistance that was the result of an induced mutation in a susceptible cultivar. Ombakho et al. (1987) proposed that the second gene be symbolized Ac_2, but Pathak (1988) proposed the symbol *Rac-2*. Since the Pathak (1988) manuscript was actually the earliest submitted for publication, we proposed that the *Rac-2* symbol be used. Both Ombakho et al. (1987) and Pathak (1988) concluded that the *Rac* and *Rac-2* genes are neither allelic nor linked.

Redden (1983) studied the inheritance of the seed resistance factor to cowpea seed beetles or bruchids (*Callosobruchus maculatus* [F.]) and concluded that the trait is inherited in a recessive manner. He found evidence for both digenic control and monogenic control with one or more modifier genes. Redden et al. (1983) reported that the seed resistance factor is mainly determined by the maternal genotype, that cytoplasmic effects are not important, that resistance is conditioned by major genes with presence of modifiers, and that trypsin inhibitors are associated with the resistance. Adjadi et al. (1985) found that the seed resistance factor is controlled by two recessive genes. They proposed the symbols *rcm-1* and *rcm-2* for the genes, and confirmed that the genotype of the maternal plant, not the genotype of the seed, controls resistance. Fatunla and Badaru (1983) studied the inheritance of the pod resistance factor to bruchids. They concluded that there is a cytoplasmic aspect to pod resistance, and that the chromosomal factors had both additive and dominance components. Rusoke and Fatunla (1987) investigated the mode of inheritance of both the seed resistance and pod resistance factors. They concluded that the seed resistance factor is controlled by cytoplasmic factors and two unlinked recessive genes, that the pod resistance factor is controlled by cytoplasmic factors and a partially dominant gene, and that the nuclear genes conditioning the two types of resistances are independently inherited.

Bosque-Perez et al. (1987) conducted inheritance studies on two types of resistance to the western plant bug (*Lygus hesperus* Knight), i.e., inhibition of nymphal growth (antibiosis) and resistance to seed damage. The Hb estimates for the antibiosis factor ranged from 4% to 43%, and averaged 29%, and those for resistance to seed damage ranged from 49% to 75%, and averaged 63%.

Resistance to parasitic weeds

Singh and Emechebe (1990) reported that a single dominant gene, designated *Rsg*, conditions resistance to *Striga* (*Striga gesnerioides* [Willd.] Vatke). Singh et al. (1993) found that duplicate dominant genes, designated *Rav-1* and *Rav-2*, control resistance to *Alectra* (*Alectra vogelii* Benth). Atokple et al. (1993) demonstrated that the genes conditioning the resistances to *Striga* and *Alectra* are neither allelic nor linked. Atokple et al. (1995) reported the results of extensive allelism tests among cowpea lines resistant to *Striga* and *Alectra*. This work revealed that different genes are responsible for the *Striga* resistances exhibited by B301, IT82D-849, and Suvita-2. Atokple et al. (1995) also reported that a single dominant gene conditioning *Alectra* resistance in IT81D-994 is not one of the two duplicate dominant genes conditioning resistance in B301. They proposed the symbols *Rsg-1*, *Rsg-2*, and *Rsg-3* for the genes conditioning resistance to *Striga gesnerioides* in B301, IT82D-849, and Suvita-2, respectively. They proposed the symbols *Rav-1* and *Rav-2* for the genes conditioning resistance to *Alectra vogelii* in B301, and the symbol *Rav-3* for the gene conditioning the resistance in IT81D-994.

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3

Recent advances in cowpea breeding

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Abstract

Cowpea is an important grain legume throughout the tropics and subtropics, covering Asia, Africa, and Central and South America, as well as parts of southern Europe and the United States of America. The use patterns, seed preferences, and cropping systems vary from region to region. Insect pests, diseases, nematodes, parasitic weeds, and drought are major production constraints. Early maturity is preferred everywhere so that cowpeas can be grown in the niches of cereal-based cropping systems, but medium- and late-maturing varieties, with and without photosensitivity, are also required in some regions, to suit the prevalent cropping systems and meet grain and fodder needs. Considerable progress has been made during the past decade in cowpea breeding, and a range of varieties has been developed, combining diverse plant type and maturity with resistance to several diseases, insect pests, and parasitic weeds. Improved varieties have also been developed for grain and fodder and for intercropping with maize, cassava, yam, millet, and sorghum for the benefit of smallholder farmers who practice intercropping and use little or no inputs.

Introduction

Cowpea, *Vigna unguiculata* [L.] Walp., is an important food legume and a versatile crop cultivated between 35 °N to 30 °S of the equator, covering Asia and Oceania, the Middle East, southern Europe, Africa, southern USA, and Central and South America (Fery 1985, 1990; Mishra et al. 1985; Singh and N'tare 1985; Watt et al. 1985; Heij 1987; Hadjichristodoulou 1991a,b; Perrino et al. 1992, 1993). However, being a drought-tolerant crop with better growth in warm climates, cowpea is most popular in the semiarid regions of the tropics, where other food legumes do not perform as well. Cowpea has the unique ability to fix nitrogen even in very poor soils (pH range 4.5–9.0, organic matter < 0.2%, and a sand content of > 85%). Also, it is shade-tolerant and, therefore, compatible as an intercrop with a number of cereals and root crops, as well as with cotton, sugarcane, and several plantation crops. Coupled with these attributes, its quick growth and rapid ground cover have made cowpea an essential component of sustainable subsistence agriculture in marginal lands and drier regions of the tropics, where rainfall is scanty and soils are sandy with little organic matter. At the same time, if early-maturing erect/semi-erect varieties are grown as a pure crop with required inputs, cowpea has the potential of yielding as high as cereals on a productivity per day basis (Singh and Sharma 1996).

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World cowpea production

It is rather difficult to obtain reliable statistics on cowpea area and production because most countries do not maintain separate records on cowpea. Probably because of these difficulties, the Food and Agriculture Organization (FAO) suspended formal publication of cowpea production data several years ago. However, based on information available from FAO and via correspondence with scientists in several countries, it can be estimated that cowpea is now cultivated on at least 12.5 million hectares, with an annual production of over 3 million tonnes worldwide. Cowpea is widely distributed throughout the tropics, but Central and West Africa accounts for over 64% of the area (with about 8 million hectares, followed by about 2.4 million hectares in Central and South America, 1.3 million hectares in Asia, and about 0.8 million hectares in East and southern Africa). Some cowpea is also cultivated in the Middle East and southern Europe. The important cowpea growing countries are Nigeria, Niger Republic, Mali, Burkina Faso, Senegal, Ghana, Togo, Benin, Cameroon, and Chad in Central and West Africa; Sudan, Somalia, Kenya, Malawi, Uganda, Tanzania, Zambia, Zimbabwe, Botswana, and Mozambique in East and southern Africa; India, Bangladesh, Nepal, Myanmar, Sri Lanka, Indonesia, China, and Philippines in Asia; and Brazil, Cuba, Haiti, USA, and the West Indies in Central and South America. However, a substantial part of the cowpea production comes from the drier regions of northern Nigeria (about 4 million ha, with 1.7 million tonnes), southern Niger Republic (about 3 million ha, with 0.3 million tonnes) and northeastern Brazil (about 1.9 million ha, with 0.7 million tonnes). With availability of new varieties, cowpea cultivation is increasing in Brazil, Cuba, Ghana, Mozambique, Nigeria, Sri Lanka, Sudan, Zambia, and Zimbabwe.

Diverse variety requirements

Cowpea is a single crop species, but the varietal requirements in terms of plant type, seed type, maturity, and use pattern are extremely diverse from region to region, making breeding programs for cowpea more complex than for other crops. The seed color preference and use patterns differ from region to region, and the maturity, growth habit, and photosensitivity requirements depend upon the cropping systems (Barrett 1987; Paul et al. 1988; Timsina 1989; Akundabweni et al. 1990; da Silva 1990; Tian and Xu 1993). Cowpea is also an important fodder crop (Kohli 1990; Nandanwar and Patil 1990; Tarawali et al. 1997). Thus, no single variety can be suitable for all conditions. There is a need to develop varieties with different attributes and resistance to major biotic and abiotic constraints, to suit the specific needs of different regions and cropping systems.

Cowpea breeding programs and progress

The advances made after 1984 are the focus of this paper, since earlier developments have been well covered by papers already cited here.

IITA'S program

The International Institute of Tropical Agriculture (IITA) develops and distributes improved cowpea materials and new germplasm lines to over 60 countries, and many national programs depend solely upon IITA to the generate breeding materials for development of new cowpea varieties suitable for their regions. The general objectives and

strategies to meet these requirements were described by Singh and N'tare (1985), but these have now been enlarged. Prior to 1987, IITA devoted most of its efforts towards developing cowpea varieties for sole cropping. Since then, the objectives have been diversified, to include breeding for intercropping as an important component of IITA's overall cowpea improvement program, as the bulk of cowpea in West and Central Africa is still grown as an intercrop (Singh 1993). IITA's cowpea breeding program currently focuses on developing the following types of varieties:

1. Extra-early maturing (60–70 days) nonphotosensitive grain type, for use as sole crop in multiple cropping systems and short rainy seasons.
2. Medium-maturing (75–90 days) nonphotosensitive grain type, for use as sole crop and intercrop.
3. Late-maturing (85–120 days) nonphotosensitive dual-purpose (grain + leaf) types, for use as sole crop and intercrop.
4. Photosensitive early-maturing (70–80 days) grain types, for intercropping.
5. Photosensitive medium-maturing (75–90 days) dual purpose (grain + fodder) types, for intercropping.
6 Photosensitive late-maturing (85–120 days) fodder type, for intercropping.
7. High-yielding, bush-type vegetable varieties.
8. Desirable seed types and seed colors, with high protein content and low cooking time.
9. Resistance to major diseases, insect pests, and parasitic weeds.
10. Tolerance to drought, low pH, and adaptation to sandy soils and low fertility.

Breeding for sole cropping. Cowpea has a great potential for increasing food legume production, if grown as a sole crop. With the advent of input-responsive, high-yielding varieties of wheat, rice, and hybrid varieties of maize and sorghum, the cultivation of food legumes has been marginalized everywhere, causing serious protein malnutrition among populations of the tropics and subtropics who derive the bulk of their dietary protein from food legumes. There is an urgent need, therefore, to enhance food legume production by breeding varieties that fit into existing niches in cereal-based cropping systems.

60–70 day cowpea varieties. The ideal cowpea variety for sole crop was conceived to have erect/semi-erect growth habit, with medium leaves and short basal branches to avoid lodging and 60–70 day crop duration with near synchronous maturity, long peduncles, and pods over the canopy for easy harvesting by manual or mechanical means.

A breeding program to develop extra-early cowpea varieties was initiated in 1979 (Singh 1982), and tests of promising varieties at several locations have led to identification and release of some of these varieties for general cultivation in many countries (Table 1). Most of the varieties developed earlier had seeds with a smooth coat and were, thus, not well accepted in parts of West Africa. Therefore, concerted efforts were made to develop early-maturing cowpea varieties with a range of seed types acceptable to different regions. Performance of selected new varieties ranged from 2 t/ha to 2.8 t/ha (Table 2). These varieties have been distributed to several national programs. Thus, various early-maturing varieties with erect/semi-erect growth habit, which yield > 2 t/ha in 60–69 days are now available. These varieties have opened the possibility of successful sole cropping in areas

Table 1. Extra-early (60–70 day) cowpea varieties released in different countries, as of 1996.

Country	Varieties released/identified for cultivation
Benin Republic	IT82E-32
Bolivia	IT83D-442, IT82D-889
Botswana	ER-7
Colombia	IT83S-841
Cuba	IT84D-449 (Titan), IT84D-666 (Cubinata-666), IT86D-314 (Mulatina-314), IT86D-386 (IITA-Peroz),IT86D-782 (Tropico-782), IT86D-792 (Yarey-792), IT88S-574-3 (OR574-3)
Ghana	IT82E-16, IT83S-728-13, IT83S-818
Guinea	IT85F-867-5
Guyana	ER-7
Liberia	IT82D-889
Mozambique	IT82E-18
Nepal	IT82D-889, IT82D-752
Nigeria	IT84E-124, IT82E-60, IT82D-716, IT84E-1-108, IT84S-2246-4, IT86D-721, IT86D-719, IT90K-76
Philippines	IT82D-889
Sri Lanka	IT82D-789, IT82D-889
Suriname	IT82D-889, IT82D-789
Swaziland	IT82E-18, IT82E-32, IT82E-71
Tanzania	IT82D-889
Thailand	IT82D-889
Uganda	IT82E-60
Yemen	IT82D-789
Zaïre	IT82E-18, IT82E-32
Zimbabwe	IT82D-889

Table 2. Performance of early-maturing varieties at Kano, Nigeria, with 2 sprays of insecticides, 1993.

Variety	Days to maturity	Grain yield (kg/ha)	Seed type
IT87D-879-1	70	2868	white rough
IT86D-1010	71	2750	white blackeye
IT90K-284-2	67	2611	tan smooth
IT87D-829-5	70	2595	white rough
IT86D-719	68	2318	white rough
IT87D-697-2	68	2232	brown rough
IT87D-611-3	68	2221	cream smooth
IT87D-941-1	68	1948	brown rough
Dan 'Ila (local)	79	1657	white rough
LSD (5%)	4	328	

with a short rainy season, double/triple cropping in rice- and/or wheat-based systems, relay cropping in areas with relatively longer rainfall after millet, sorghum, or maize, as well as parallel multiple cropping with cassava, yam, and cotton (Singh 1986, 1987a).

Dry-season cowpeas. Several countries in Asia and Africa have developed irrigation facilities and 'Fadamas' (river beds) with residual moisture, where cowpea can be grown

in the dry season (IITA 1984; Parameswaran et al. 1988; Sharanappa et al. 1991; Blade and Singh 1994). Cowpea fits very well as a rotation/alternate crop during the dry season, as it requires a moderate amount of water and matures within 60–80 days.

The major constraints during the dry season are viruses, leaf thrips, nematodes, and aphids. Several cowpea varieties developed at IITA with combined resistance to viruses, thrips, nematodes, aphids, bruchids, and *Striga* were evaluated at Wudil and Kadawa with irrigation and in the Nguru wetland area of Nigeria (with farmer participation) from 1991 to 1994 (Singh 1993; Blade and Singh 1994). As data in Table 3 indicate, these varieties had yields of 1–1.5 t/ha when planted at the end of January. They are harvested near the end of April, when prices of cowpea grain as well as fodder are high. A few selected varieties were again tested in 1993 and 1994. Their grain yields were > 1 t/ha, with fodder yields of 4–10 t/ha (Table 4).

On-farm evaluation of selected varieties, using a farmer participatory approach at several locations in northern Nigeria, confirmed on-station results, and farmers are adopting the cultivation of these improved cowpea varieties in the dry season. It is thus

Table 3. Mean grain yield (kg/ha) of some cowpea varieties at indicated planting dates in the dry season at Wudil and Kadawa, Nigeria.

	Wudil			Kadawa	Reaction to†				
Variety	19-1-91	31-1-91	31-1-92	31-1-92	Ap	Br	Tr	St	Nt
T86D-715	405	1104		–	S	S	R	S	S
IAR-48	573	1042		–	S	S	S	S	S
Local (Dan 'Ila)	1524	1119	398	851	S	S	S	S	S
IT84S-2246-4	1524	1980	1148	1638	R	R	R	S	R
IT86D-719	1146	1269		–	MR	S	R	S	S
IT90K-76	–	–	1776	1570	R	R	R	R	R
IT90K-59	–	–	1518	1148	R	R	R	R	R
IT90K-101	–	–	1033	1705	R	R	R	R	R
IT89K-288	–	–	–	1087	R	R	R	MR	R
IT89KD-374			711	1104	R	S	R	MR	R
LSD (5%)	693	682	378	491					
CV (%)	24	19	27	29					

† Ap = aphid; Br = bruchid; Tr = thrips; St = *Striga;* Nt = nematode; MR = moderately resistant; R = resistant; S = susceptible.

Table 4. Grain and fodder yield of cowpea varieties in the dry season at Kadawa, Nigeria.

	1993		1994	
Variety	Grain (kg/ha)	Fodder (t/ha)	Grain (kg/ha)	Fodder (t/ha)
IT87D-941-1	1773	4.1	1206	10.8
IT84S-2246-4	1293	6.5	925	9.9
IT90K-76	–	–	1009	9.2
Local check (Dan 'Ila)	1495	1.9	333	4.9
LSD (5%)	ns	1.5	443	2.9

expected that dry-season cowpea will gain popularity in Nigeria. Irrigated cowpeas in rice fallows are already popular in Sri Lanka and southern India.

Medium-maturing varieties. Some varieties have been developed which mature in 75–85 days (Singh 1994a). These are suitable for cultivation in areas where a full-season cowpea variety is required to fit the prevalent cropping system, soil type, and rainfall pattern. These varieties combine multiple disease and insect resistance and performed well in the subhumid and semiarid zones (see Table 5 for performance of a few promising varieties). Several varieties yielded 1.5–2 t/ha even at semiarid locations like Gumel (Nigeria) and Maradi (Niger). These varieties, along with others, have been distributed to various national programs.

Table 5. Performance of promising medium-maturing cowpea varieties (with 2 insecticide sprays) at several locations in West Africa, 1993.

	Grain yield (kg/ha)				Days to	Reaction to†		
Cowpea variety	Kano, Nigeria	Gumel, Nigeria	Maroua, Cameroon	Maradi, Niger	maturity (Kano)	BB	CABMV	Aphid
IT90K-372-1-2	1871	1791	1491	1867	78	R	R	R
IT90K-277-2	2371	1432	1829	1843	75	R	R	R
IT88DM-363	1824	1617	1737	1988	80	R	R	S
IT89KD-374-8	2045	1494	1812	1910	81	R	R	R
IT89KD-374-57	1592	1249	1525	1808	76	R	R	R
IT90K-109	1693	1496	1387	1691	77	R	MS	R
IT89KD-349	943	1059	1358	1746	78	R	R	R
IT88D-867-11	1303	982	1271	1281	80	R	S	R
IT89KD-391	727	565	1054	1095	87	R	R	R
IT88DM-400	287	766	1571	1148	92	MR	MR	S
IT90K-319	409	395	1408	1117	87	MR	MR	R
Dan 'Ila (local)	53	487	1275	955	90	MR	S	S
LSD (5%)	664	462	292	405	2.4			
CV(%)	29	29	15	19	2.8			

† BB = bacterial blight; CABMV = cowpea aphid-borne mosaic virus; R = resistant; MR = moderately resistant; MS = moderately susceptible; S = susceptible.

Bush-type vegetable cowpea. Several countries grow cowpea as a vegetable crop. The most preferred types are the yardlong cowpeas with fleshy tender pods, but these varieties need staking to keep pods from touching the ground and rotting, which involves extra cost and thus restricts the area under cultivation. Bush-type vegetable varieties with 30-cm long succulent pods have been developed, such as IT81D-1228-10, IT81D-1228-14, IT81D-1228-15, and IT86D-880, which yield up to 18 t/ha green pods with 3–4 pickings starting at 45 days after planting. These varieties have semi-erect growth habit with extra-long peduncles (40–50 cm long), protruding well over the canopy and holding the pods above the ground. Picking green pods periodically reduces the weight on peduncles and they remain upright all the time. Frequent picking also stimulates further flowering and podding on the same peduncles, which ensures a continuous supply of green pods for a 6–7 week period after the start of picking, provided soil moisture is not limiting. These varieties have

been distributed to several national programs. Some of these varieties have been found promising in China, Nepal, Sri Lanka, Philippines, the West Indies, and Nigeria (Timsina 1989; Tian and Xu 1993).

Breeding cowpea varieties for intercropping. Traditional cowpea is grown as an intercrop because farmers want cereals for home use and cowpea and groundnut as cash crops. Although growing sole crops of improved high-yielding cowpea varieties can be highly profitable when 2–3 sprays of insecticide are applied, the majority of smallholder farmers are unable to adopt such sole cropping; they thus practice intercropping. IITA established a station in 1990 at Kano, in northern Nigeria, to develop cowpea varieties for intercropping without insecticide sprays. One of the predominant systems in northern Nigeria involves millet and cowpea, in which farmers grow two types of cowpea varieties —an early-maturing type for grain, and a late-maturing type for fodder—often in the same field, planted in alternate rows as intercrops in millet and/or sorghum (Singh 1993).

Two approaches are being followed at IITA for developing varieties for intercropping: (1) the improvement of existing local varieties by incorporating resistance to aphid, thrips, bruchid, *Striga*, *Alectra*, and relevant diseases by partial backcrossing; and (2) the development of a range of new varieties with higher yield potential for both grain and fodder under intercropping.

The new breeding lines are evaluated in four systems, so that relative performance of new varieties can be assessed and the best selected for each system (Singh 1993): (1) sole crop, with 2–3 sprays of insecticide; (2) sole crop, without sprays; (3) intercrop with millet, without sprays; and (4) farmer participatory evaluation.

Improved local varieties. A number of selected local cowpea varieties (grown under intercropping) were crossed to an improved early variety, IT84S-2246-4, which has combined resistance to aphid, bruchid, thrips, and several diseases (Singh and Singh 1990). The F_1 plants were backcrossed to the respective local varieties. From the backcross populations, promising lines have been developed which resemble local varieties but combine resistance to aphid, thrips, and, in some cases, bruchids also. The most promising lines are listed below.

IT88D-867-11, derived from the cross IT84S-2246-4 × Jan Wake. It resembles 'Jan Wake', but has resistance to aphid and thrips. Its performance has been very good in the drier regions like Niamey, Maradi, and Gumel, where 'Jan Wake' (TN5-78) comes from. It has been released in Nigeria.

IT89KD-374-57, derived from the cross Dan 'Ila × IT84S-2246-4. It is similar to Dan 'Ila, but combines resistance to aphid and thrips, as well as several diseases, including viruses. Its performance has been very good at several locations in the semiarid region, and it has been released in Nigeria.

IT89KD-319, derived from the cross Kaokin Local × IT84S-2246-4. Resembles Kaokin Local and combines resistance to aphid and thrips and several diseases.

IT89KD-245, derived from the cross IT87F-1772-2 (Kanannado selection) × IT84S-2246-4. Resembles Kanannado but combines resistance to aphid, bruchid, thrips, and matures ~ 2 weeks earlier than Kanannado. It has done well as a dual-purpose variety.

IT89KD-288, derived from the cross IT87F-1772 (Kanannado selection) × IT84S-2246-4. It is as late as Kanannado, combines resistance to aphid, bruchid, and thrips, and yields more fodder and grain than Kanannado. It is becoming popular in the dry season.

Efforts are now under way to incorporate resistance to *Striga* and *Alectra* into these varieties.

New varieties. A number of new varieties for intercropping have been developed combining resistance to several diseases and insects (Singh 1991, 1993, 1994a). When the performance of new varieties was examined in pure crop and in intercrop in 1993 (Table 6), the early- and medium-maturing varieties had as good or higher grain yield potential

Table 6. Yield (kg/ha) of promising early, medium, and late-maturing cowpea varieties, with and without insecticide sprays, in different cropping systems at Kano, Nigeria, 1993.

	Pure crop (2 sprays)		Pure crop (no spray)		Intercrop (no spray)	
	Seed	Fodder	Seed	Fodder	Seed	Fodder
Early-maturing varieties (photo-insensitive)						
IT90K-284-2	2453	–	815	2166	252	595
IT90K-56	1949	–	715	1166	525	838
IT88D-643-1	1780	–	625	1666	241	353
IT89KD-389	1750	–	848	1166	328	434
IT91K-93-10	1628	–	541	1916	408	550
IT90K-59-4	1872	–	883	1250	495	431
IT84S-2246-4	1159	–	594	1000	284	483
LSD (5%)	511		257	ns	201	ns
Medium-maturing varieties (photo-sensitive)						
IT90K-277-2†	2371	–	1082	3250	452	625
IT90K-372-1-2	1871	–	600	1416	211	152
IT88DM-363†	1824	–	366	2500	569	542
IT89KD-374-8	2045	–	539	1583	279	308
IT89KD-374-57	1592	–	461	1083	196	148
IAR 48	1575	–	383	2833	494	681
Dan 'Ila	353§	–	68	1666	307	1484
LSD (5%)	664		316	1498	316	478
Late-maturing varieties (photo-sensitive)						
IT81D-985	1258	4900	470	3300	118	1042
IT89KD-252	630	5800	201	6900	102	833
IT89KD-260	617	5100	104	3100	74	646
IT89KD-288	302	5700	129	3700	19	1250
Kanannado	205	4700	0	3900	55	2083
Borno Local	204	4800	0	4300	0	2292
LSD (5%)	309	2033	450	3326	ns	1319

† Photo-insensitive.
§ Severe virus infection.

than the local varieties in both sole crops and intercrops and with or without sprays. Similar results were also obtained by Blade et al. (1992), using a different set of varieties. While the traditional varieties do not yield as much grain even with good management, they do give higher fodder yield. Thus, there is a need for dual-purpose varieties which will give reasonable grain and fodder yield. IT81D-985 and IT89KD-252 are improved dual-purpose varieties, which have higher grain yield than local varieties and similar fodder yield.

Farmer participatory evaluation. A 200 g seed sample of improved varieties for intercropping was given to selected farmers (one variety to 4–6 farmers) for evaluation. The crop was planted by farmers in their traditional systems, and totally managed by them. However, yield estimates were made by technical staff, using a 10×10 m sample plot on each farm. The results varied from field to field, as expected, but some varieties such as IT89KD-374-57, IT89KD-319, and IT88DM-867-11 consistently yielded higher than other test varieties and Dan 'Ila, the local variety (Singh 1993), indicating the adequacy of this method to detect promising varieties for intercropping in farmers' field situations. Farmers are saving the seed of promising varieties and looking forward to more materials each year.

Breeding method for intercropping. In developing improved cowpea varieties for intercropping, should the segregating populations be grown under intercrop or can they be grown and selected in sole crop up to the F_5–F_6 generations before testing under intercropping? Significant positive correlations between sole cropped and intercropped cowpeas have been reported (N'tare 1989; Blade et al. 1992; Ehlers 1994), but the results are not consistent when an insecticide is not applied. An experiment on breeding methodology is in progress at IITA Kano Station to help clarify this issue.

Breeding for disease resistance. Cowpea is attacked by over 35 major diseases caused by viruses, bacteria, fungi, and nematodes (Thottappilly and Rossel 1985; Emechebe and Shoyinka 1985; Mew et al. 1985; Lin and Rios 1985; Patel 1985). The occurrence, severity, and yield loss due to each disease and mixed infections vary from place to place, but some diseases occur and cause significant damage across the cowpea growing regions of the world (see chapters in this volume: Hampton et al. 1997; Emechebe and Florini 1997; Florini 1997). Considerable success has been achieved in breeding for resistance to major diseases.

Viral diseases. Several improved cowpea varieties combining resistances to multiple viruses have been developed at IITA (Singh et al. 1987; Thottappilly et al. 1988) and distributed to various national programs. Cowpea varieties IT82D-889, IT83S-818, IT83D-442, and IT85F-867-5 are resistant to CPMV, CAMV, CGMV, CMV, and SBMV. For a recent review of efforts elsewhere, see Hampton et al. (1997) later in this book.

Bacterial diseases. Two bacterial diseases, bacterial pustule (*Xanthomonas* spp.) and bacterial blight (*Xanthomonas vignicola*), cause severe damage to cowpeas worldwide. Several improved breeding lines have been developed at IITA which combine resistance to

these two diseases: notably TVx 1850-01E, IT90K-284-2, IT90K-277-2, IT86D-715, IT86D-719, and IT81D-1228-14 (Singh et al. 1984; Singh 1993).

Fungal diseases. Sources of genetic resistance to several fungal diseases have been reported, and resistant varieties have been developed. These include resistance to anthracnose (N'tare et al. 1984), Cercospora leafspot (Singh et al. 1984), Verticillium wilt (Moore 1974), Phytophthora stem rot (Singh et al. 1984; Bateman et al. 1989), Septoria leaf spot (Abadassi et al. 1987), brown blotch (Abadassi et al. 1987), scab (Abadassi et al. 1987), Uromyces rust (Chen and Heath 1993), and leaf smut (Singh 1993). In all cases, resistance is simply inherited (one or two gene pairs) and easy to breed for. In some cases, such as Fusarium wilt and Phytophthora stem rot, considerable strain variation exists and strain-specific resistance needs to be combined to acquire broad protection. Varieties from the USA, such as 'Iron', have been used extensively as a source of resistance to Fusarium wilt and charcoal rot (Hare and Thompson 1990). Many other sources of resistance were also identified from IITA's world collection of cowpea germplasm (Singh et al. 1984). Using these sources in systematic crossing and evaluation of segregating progenies at sites known to have high levels of these diseases, IITA has developed many varieties which combine resistance to several major diseases (Singh 1993, 1994a).

Nematodes. About 55 species of nematodes have been reported on cowpea (Caveness and Ogunfowora 1985) but the most damaging and widespread species is *Meloidogyne incognita*. Extensive work has been done on developing varieties that are resistant to nematodes in the USA (Fery et al. 1994; Roberts et al. 1997), as well as in Africa (Singh 1993) and Asia (Singh and Reddy 1986). As a single dominant gene controls the resistance of *M. incognita*, it has been possible to develop a number of resistant cowpea varieties. Scientists at IITA have combined resistance to root-knot nematode, aphid, and bruchid in a number of varieties, such as IT84S-2246-4, IT89KD-288, IT90K-59, and IT90K-76 (Singh 1993).

Breeding for insect resistance. At least 85 insect species have been identified which attack cowpea (Booker 1965), but only some of them cause widespread damage (Chalfant 1985; Daoust et al. 1985; Singh 1985; Singh and Jackai 1985). For a review of pest management practices in cowpea, see Jackai et al. (1997), in this volume.

IITA has developed a number of varieties such as IT84S-2246-4 which combine resistance to aphid, thrips, and bruchids (Adjadi et al. 1985; Bata et al. 1987; Singh and Singh 1990; Singh 1993, 1994a). Despite the extensive germplasm screening, effective sources of resistance to *Maruca vitrata* and pod-sucking bugs have not been identified among cultivated varieties of cowpea. Controlling these pests necessitates 2–3 sprays during the flowering and pod development stages. This is a problem for small-scale farmers because insecticides are beyond their reach. Therefore, three mutually compatible approaches are being followed at IITA to develop cowpea varieties which give reasonable grain yield (500–1000 kg/ha) without sprays (Singh 1993). These are as follows:

1. Incorporating the best available level of resistance to aphid, thrips, and bruchid in all of the new breeding lines.

Table 7. Grain yield (kg/ha) of indicated cowpea varieties, without insecticide protection, at different dates of planting at Minjibir, Nigeria, 1993†.

Variety	Date of planting			Maturity	Reaction to			
	9 Jun	9 Jul	9 Aug		Viruses	Aphid	Thrips	Bruchid
IT90K-277-2	1356	1277	318	M	R	R	MR	R
IT88DM-345	340	502	128	EE	R	S	MR	S
ITIT89KD-455	532	921	192	EE	R	S	MR	S
IT89KD-374-57	831	915	346	E	R	R	MR	S
IT90K-59-2	976	891	586	E	R	R	MR	R
IT90K-391	742	616	289	L	R	R	S	R
IT89KD-457	1498	851	304	M	R	R	MR	R
IT84D-666	823	668	271	E	S	S	MR	S
IT90K-261-3	1126	295	414	E	R	R	MR	R
Dan 'Ila (local)	212	0	76	L	S	S	S	S
LSD (5%)	224	219	89					

† EE = extra early, E = early, M = medium, L = late, R = resistant, MR = moderately resistant, S = susceptible.

2. Screening breeding lines, as well as germplasm accessions, to identify those which suffer less damage than others in the field from attacks of *M. vitrata* and pod-sucking bugs, and initiating a recurrent selection program to raise the level of resistance in improved lines.
3. Breeding for extra early-maturing varieties (45–55 days) with vigorous growth and acceptable seed type, which can escape insect damage.

Significant progress is being made on several fronts, and except for the two difficult pests already named, selected breeding lines have shown good field performance without insecticide sprays (Table 7). Some of these lines have been distributed to national programs for further testing.

Breeding for resistance to *Striga* and *Alectra*. Cowpea is attacked by two parasitic weeds, *Striga gesnerioides* [Wild] Vatke and *Alectra vogelii* [Benth.], particularly in the semiarid regions of West and Central Africa. Sources of resistance have been identified and the genetics of resistance to *Striga* and *Alectra* have been studied (Aggarwal 1985, 1991; Singh and Emechebe 1990; Singh et al. 1993; Atokple et al. 1993, 1995). Some improved cowpea varieties with resistance to *Striga,* such as IT88D-867-11, IT90K-59, IT90K-76, and IT90K-82-2, have been developed and distributed to national programs (Singh and Emechebe 1991; Singh 1994b; Berner et al. 1995). A more detailed review is presented later in this volume (Singh and Emechebe 1997).

Pyramiding genes for disease and insect resistance. Systematic work on breeding cowpea varieties for multiple disease and insect resistance was initiated at IITA in 1980, and significant progress has been made (Singh et al. 1984; Singh and Singh 1990; Singh 1993, 1994a). Initially, individual crosses were made involving multiple disease resistant parents, on the one hand, and germplasm lines with thrips, aphid, and bruchid resistance, on the other. By growing 4 generations in a year, it was possible to select F_6 lines with

Table 8. Progress in pyramiding genes for resistance in cowpea†.

Pest/disease factor	Variety					
	Ife Brown (1973)	TVx 3236 (1978)	IT82D-716 (1982)	IT84S-2246 (1984)	IT90K-59 (1990)	IT90K-76 (1990)
Anthracnose	S	R	R	R	R	R
Cercospora	S	R	R	MR	R	R
Brown blotch	S	R	R	MR	R	R
Bacterial pustule	S	R	R	R	R	R
Bacterial blight	MR	MR	MR	MR	R	MR
Septoria	S	S	S	S	S	S
Scab	S	MR	MR	MR	MR	R
Web blight	S	MR	MR	MR	MR	R
Yellow mosaic	S	S	R	R	R	R
Aphid-borne mosaic	S	S	R	R	R	R
Golden mosaic	R	R	R	R	R	R
Aphid	S	S	S	R	R	R
Thrips	S	MR	MR	MR	MR	R
Bruchid	S	S	R	R	R	R
Striga	S	S	S	S	R	R
Alectra	S	S	S	S	R	R
Nematode	S	S	S	R	R	R

† The earlier variety is one parent of the next variety. See dates in parenthesis after each variety. R = resistant; MR = moderately resistant; S = susceptible.

disease and insect resistance within 2 years, and recombine them again for another cycle. Several breeding lines have been developed with multiple resistance (Table 8), by segregating backcross populations and combining resistances.

Breeding for drought tolerance. Early-maturing cowpea varieties escape terminal drought (Singh 1987b), but perform poorly if exposed to intermittent drought during the vegetative stages. A simple technique, using wooden boxes, was developed to screen cowpea germplasm lines at the seedling stage, and to test their field performance at a mature stage under conditions of water deficit (Singh 1993). This work was expanded, in collaboration with the Japan International Research Center for Agricultural Sciences (JIRCAS), at the IITA Kano Station. Significant progress has been made and TVu 11979, TVu 11986, TVu 12349, Dan 'Ila, and IT90k-59-2 have been identified to be drought tolerant (Watanabe et al. 1997). The drought-tolerant lines are of two types: (1) lines such as TVu 11979 and TVu 11986 stop growth as soon as drought stress is imposed, probably to conserve moisture and survive for 2–3 weeks; whereas (2) Dan 'Ila and IT90K-59-2 mobilize moisture from lower leaves and remain alive for a longer time, while the lower leaves die one by one. Consequently, these varieties have a better regeneration potential than others. Genetic studies are in progress and suitable crosses have been made to incorporate these traits in improved varieties. The use of carbon isotope discrimination method (see Hall et al. 1997 in this volume, and other references cited therein) and assessment of other physiological parameters are too expensive for use in a breeding program. The wooden box technique is more appropriate for breeding programs in

developing countries. Efforts are also being made to combine deep root systems with drought tolerance, to enhance adaptation of cowpeas to low rainfall areas (Singh 1993).

Breeding for seed type, nutritional quality, and short cooking time. Efforts are also being made to develop varieties with a range of seed types with high protein content and short cooking time. Improved lines showed significant genetic variability for these traits (Omueti and Singh 1987; Baker et al. 1989; Nielsen et al. 1993). Among 100 lines evaluated, protein content ranged from 22.9% to 32.5% and cooking time from 21.1 min to 61.9 min, indicating the possibility of enhancing protein content and shortening cooking time by genetic improvement. Seed color was not correlated with protein content or cooking time, but seeds with rough coat cooked faster than seeds with smooth coat.

Regional and national programs in Africa

Burkina Faso, Cameroon, Ghana, Nigeria, Niger Republic, and Senegal in West and Central Africa, and Botswana, Kenya, Mozambique, Tanzania, and Zambia in East and southern Africa have active cowpea improvement programs. Regional programs such as Semi-Arid Food Grains Research and Development Project (SAFGRAD), Bean/Cowpea Collaborative Research Support Program (CRSP), and the Southern Africa Development Community (SADC) have strengthened cowpea research, training, and development activities in several countries. However, the major focus of cowpea research in these countries is to develop varieties for sole cropping. Using improved materials from IITA, with those from national programs, several varieties have been released in West Africa: IAR-48, IT84S-2246-4, IT86D-719, IT86D-721, IT90K-76, IT89KD-374, and IT89KD-867-11 in Nigeria; Mouride and Melakh in Senegal; IT82E-16 (Asantem), IT83S-818 (Bengpla), and IT83S-728-13 (Ayiyi) in Ghana; IT81D-985 (BR-1), IT81D-994 (BR-2), and IT90K-277-2 in Cameroon; TN5-78, TN88-63, TN-27-80 in Niger Republic; IT85F-867-5 (Pkoku Togboi) in Guinea; and IT82E-32, IT81D-1137 in Benin Republic.

The traditional varieties in East and southern Africa are grown as intercrops or as sole crops for leaves as well as grain (dual purpose), and they are medium- and late-maturing. However, the more recent focus has been to develop early-maturing grain-type varieties with virus resistance. One such variety, IT82D-889, has done very well and has been released for cultivation in several countries, such as Tanzania (Vuli-1), Zambia, Zimbabwe, and Swaziland (Untilane) (Mligo 1989; Natarajan and Naik 1992). The SADC/EEC/IITA Cowpea Project has organized regional breeding and testing programs involving 10 countries of southern Africa, in order to develop cowpea varieties suitable for sole cropping and intercropping, as well as dual purpose varieties.

United States and Latin American programs

United States of America. Cowpea breeding in the United States of America has enjoyed more progress in this decade. There are 11 plant breeders in public breeding programs, 6 in public institutions, and at least 3 in private companies working on cowpea in the USA. Much work is being done at the University of California, Riverside, on breeding for heat and drought tolerance (Hall 1990; Marfo and Hall 1992). The potential of cowpea as an intercrop with citrus has been shown in southern Florida (Stoffella et al. 1986), but no efforts are being made in the USA to develop varieties for intercropping.

Cowpea breeders in the USA have released about 23 improved varieties over the past 10 years (Fery and Dukes 1988; Hare and Thompson 1988; Morelock et al. 1989; Fery and Dukes 1990a,b,c; Helms et al. 1991a,b; Fery and Dukes 1992; Morelock et al. 1992; Fery and Dukes 1993). The characteristics of these varieties, most of which are "horticultural" types rather than grain types, reflect the effort of breeders to serve the three segments of the industry: dry seed, vegetable, and processors (both canning and freezing). Most varieties released have resistance to one or more diseases, and four of them have resistance to cowpea curculio, the major insect pest in southern USA.

The discovery of a new gene for persistent green seed due to green cotyledon, *gc,* non-allelic to the gene for green testa, *gt,* represents a significant improvement in consumer appeal of the frozen product. The incorporation of these genes into currently popular horticultural types, especially for the frozen food trade, and for the fresh market as well, is expected to have an impact on varieties being developed for freezer/packers, and eventually also for dry packers.

Breeding for resistance to mosaic viruses, Fusarium wilt, and root-knot nematodes are major objectives. The release of 'Mississippi Pinkeye' marked the culmination of a long and successful breeding program at Mississippi State University, which resulted in improved varieties of the major horticultural types; blackeyes, crowders, creams, and pinkeyes, all of which have resistance to the three known races of *Fusarium oxysporum,* three root-knot nematode species (*Meloidogyne incognita, M. javanica,* and *M. arenaria*), and tolerance or resistance to mosaic viruses.

The only known releases made in the USA with resistance to insect pests are those with resistance to cowpea curculio, the most serious pest of cowpea in southeastern USA. Four recently released varieties are resistant to cowpea curculio, using as the source of resistance, Ala. 963.8 and/or its derivatives, CR 17-1-13, CR 18-13-1, and CR22-2-21. Curculio resistant varieties are AUBe, Bettergreen, Bettergro, and Carolina Cream. They have pod characteristics which interfere with the ability of the adult insects to damage the seed in the process of feeding and oviposition or they are less attractive to adult curculios, resulting in reduced seed damage.

The National Aeronautical and Space Agency (NASA) of the USA is funding a project at Purdue University to select suitable crop species and varieties for cultivation by 'future space colonies'. The studies have shown that cowpea is a good candidate crop (Ohler 1994). Two varieties from IITA, IT84E-124 and IT87D-941-1, both erect and early-maturing, have shown great promise from the standpoint of dry-matter production, protein content, and a versatility of uses.

Latin America. Cowpea is widely cultivated in Latin America, and a number of countries such as Brazil, Colombia, Guyana, Jamaica, Nicaragua, Panama, Trinidad, and Venezuela have made varietal releases. Brazil has about 1.9 million hectares under cowpea production, and since 1984 it has released seven improved varieties. The major production constraints in the region are drought and diseases, particularly the viruses (Watt et al. 1985). Cowpea is grown both as a sole crop (Ferreira and Silva 1987) and as an intercrop with maize and cotton (Beltroa et al. 1986). Except for Brazil, most countries in the region do not have comprehensive breeding programs, but they evaluate materials from IITA, as well as from USA and other sources, to select varieties. A number of varieties have been

found promising from such evaluations in Belize (VITA-3), Brazil (VITA-7), Colombia (IT83S-841), Costa Rica (VITA-1, VITA-3, VITA-6), El Savador (VITA-3, VITA-5), Guyana (VITA-3), Guatemala (VITA-3), Haiti (VITA-3, IT87D-885), Jamaica (VITA-3, ER-7), Nicaragua (VITA-3), Panama (VITA-3), Peru (VITA-7), Surinam (IT82D-889, IT82D-789), and Venezuela (VITA-3). Cuba has identified several lines from IITA (Table 1) which are suitable for sole cropping and intercropping under sugarcane-based systems as a substitute for *Phaseolus* beans.

Asian programs

Half of about 1.3 million hectares under different forms of cowpea in Asia are in India alone. Other important cowpea growing countries include Pakistan, Nepal, Bangladesh, Sri Lanka, Myanmar, Malaysia, Thailand, Indonesia, Philippines, and China (Van der Massen and Somaatmadja 1990).

Cowpea is very important, but it is a minor legume in the cropping systems under which it is grown. In India, the largest country of the region, accurate data on cowpea area and production are not available. Sharma and Joshi (1993) have presented a good review of cowpea research in India, which has a comprehensive cowpea breeding program with a major emphasis on fodder type (Pal 1988; Kohli 1990; Lodhi et al. 1990; Sharma and Singhania 1992). However, sole cropping in arid regions, and in rice fallows, and in the spring/summer season is now becoming popular (Henry and Daulay 1988; Parameswaran et al. 1988). Several early- and medium-maturing varieties such as 'Amba', 'Rambha', and 'Shveta' have been developed, and are used for both green pods and dry grains.

The cowpea variety development programs in India aimed at transferring disease resistance, better grain quality, or earliness but paid less attention to developing an efficient plant type for intensive cultivation. Some varieties, such as V 16 (Amba) and V 38, were semi-spreading. V 38 has long peduncles and the pods are held above the crop canopy. The truly upright nontrailing varieties are still not available in Southeast Asia. However, a number of extra-early maturing varieties from IITA have shown great promise (Verma and Mishra 1989; Thiyagrajan et al. 1989). Several countries in Asia have identified promising grain-type varieties from IITA and released them for general cultivation: VITA-4 (Yezin 1) in India, Myanmar, and Pakistan; IT82D-889 (Prakash) and IT82D-752 (Aakash) in Nepal; IT82D-889 in Philippines and Thailand; and IT82D-789 (Wijaya) and IT82D-889 (Waruni) in Sri Lanka. Vegetable cowpeas, both yardlong and bush types, are most important in China, Indonesia, Korea, and the Philippines and several new varieties have been developed (Sunarjono et al. 1989; Zhang 1991; Tian and Xu 1993).

Conclusions and looking ahead

Thus, significant progress has been made in cowpea breeding in the past decade. Breeding for multiple disease and insect resistance, with acceptable seed quality, initiated in the early 1980s, has progressed well and should continue to be the major focus. IITA's decision in the late 1980s to include breeding for intercropping was timely and relevant to the needs of smallholder farmers in West and Central Africa. Advances in breeding for resistance to *Striga gesnerioides* and *Alectra vogelii* will have a major impact on cowpea cultivation in the dry savanna. Also, the current focus on developing varieties with differing plant type and maturity periods will enable the intensification of cropping

systems in the tropics. Pyramiding genes for resistance to aphid, bruchid, thrips, and *Striga*, as well as field resistance to *Maruca* pod borer and pod bugs, should be pursued, so as to minimize or eliminate the need for insecticide protection. Cowpea breeders should also seek to increase the genetic potential of the plant for higher grain and fodder yield, to enhance the role of cowpea in sustainable (crop/livestock) farming systems in the tropics.

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4

Wide crossing in African *Vigna* species

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Abstract

The genus *Vigna* comprises seven subgenera and sixteen sections. Cowpea, *Vigna unguiculata* (L.) Walp., is an important grain legume crop in sub-Saharan Africa and belongs to the subgenus *Vigna*, section *Catiang*. Morphologically, cowpea genotypes appear very variable. However, a high level of genome homology exists among the varieties and this, probably, is because genomes of cowpea's wild relatives have not been exploited while these varieties were being developed. Cowpea varieties are susceptible to some insect pests, especially the pod borer, *Maruca vitrata,* and a pod-sucking bug complex, both of which can cause high seed yield losses. Accessions of some wild *Vigna* species, e.g. *V. oblongifolia* and *V. vexillata,* are known to be resistant to these pests, and efforts continue to be made, through wide crossing, to transfer the resistance genes from these species to cultivated cowpea. So far cowpea has been successfully crossed only to genotypes belonging in section *Catiang*. Among the noncultivated African *Vigna* species, there have been successful crosses between *V. oblongifolia* and two others, *V. ambacensis* and *V. luteola,* although the hybrids are only partially fertile. Embryo rescue was used to recover an F_1 interspecific hybrid between *V. oblongifolia* and *V. luteola.* All three species belong to section *Vigna* of the subgenus *Vigna.* A successful cross has also been carried out between *V. davyi* and *V. vexillata,* both members of the subgenus *Plectotropis.*

Introduction

The genus *Vigna* comprises some important pulse crops that are commonly grown in the tropics. Among these is cowpea (*Vigna unguiculata* [L.] Walp.), which is grown mainly in the drier parts of sub-Saharan Africa for various uses: its grains are used as food, fresh pods of some varieties as a vegetable, leaves as spinach, and haulms as fodder. Cowpea belongs to section *Catiang*, subgenus *Vigna*. Among the several subspecies and varieties in the section *Catiang*, four cultigroups, *biflora, sesquipedalis, textilis*, and *unguiculata*, have been identified in the cultigen *unguiculata* (Baudoin and Marechal 1985).

Several cowpea varieties have been developed and adopted by farmers for planting. The progenitors of these improved varieties appear to be mainly members of the cultigroup *unguiculata*. Exploitation of the genetic potential of wild and close relatives of cowpea for enhancing cowpea productivity has not been well documented. A high level of relationship has been detected among several cowpea genotypes following the evaluation of variability in seed proteins among them (D'Urzo et al. 1990), and this may be partly attributable to the low level or nonexploitation of the crop's wild relatives. The high level of relationship reported among cowpea varieties may also be due to its being a self-pollinated crop.

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The germplasm of wild cowpea relatives distributed in southern Africa, a region now known to contain a high diversity of wild cowpea, began to be collected only recently, and many of the accessions are yet to be evaluated for their potential usefulness. These recent collections have added to the variability in the gene bank of the different subspecies and varieties of the section *Catiang*. Some of the freshly collected germplasm appear to be new taxa, which had not been previously characterized. These include *V. unguiculata* ssp. *rhomboidea* and *V. unguiculata* ssp. *protracta* var. *kgalagadiensis*, among others. Many of these new additions may share the same primary gene pool with cowpea, and it should not be difficult, therefore, to transfer any desirable traits found in any of them to cultivated cowpea. The closest relative of cowpea (*V. unguiculata*) is *V. nervosa* Markotter, which also belongs to the section *Catiang* (Baudoin and Maréchal 1985). Crossability studies between *V. unguiculata* and *V. nervosa* have yet to be reported.

A large proportion of the available germplasm lines in the primary gene pool of cowpea have been tested for their reactions to the major insect pests, and these have been mostly susceptible, especially to the *Maruca* pod borer and pod-sucking bugs. A few accessions with low resistance to these insect pests have also been identified, especially among cowpea wild relatives (*V. unguiculata* ssp. *dekindtiana*). However, all tested accessions of *Vigna vexillata*, and some of *V. luteola* and *V. oblongifolia,* show high resistance to all insect pests of cowpea. These three *Vigna* species are members of different sections and/or subgenera from cowpea. While *V. vexillata* belongs to section *Plectotropis, V. oblongifolia* and *V. luteola* belong to subgenus *Vigna* (Maréchal et al. 1978). As high resistance to insect pests has been identified among some other *Vigna* species outside of section *Catiang*, a resort to wide hybridization between cowpea and other *Vigna* species is justified.

Therefore, attempts continue to be made to transfer the genes conferring resistance to the insect pests in these *Vigna* species to cowpea.

Wide hybridization

Crosses between species or genera are usually made when it is necessary to transfer one or a few genes controlling desirable traits from one species to another, and also when a new trait absent in either parent is needed. For example, Dana and Karmakar (1990) cited the work of Rangaswamy, in which a bruchid-resistant genotype was detected among the segregating population following a cross between *V. radiata* and *V. mungo*. This trait was not expressed in either of the parents used in the cross.

Interspecific crosses are usually difficult to make because of a number of attendant problems, such as incompatibility and hybrid sterility or failure/breakdown (Table 1). Linkage drag, a situation wherein a transferred gene is flanked by the introgressed segment of DNA from the donor parent, is another problem associated with making wide crosses in crops. The DNA segment may differ in length and affect traits other than the one transferred. With the conventional backcross method of breeding, many backcrosses are required to significantly reduce the amount of linkage drag. Breaking such tight linkages may also require the growing of very large plant populations or, where feasible, DNA markers can be used to effectively select against such undesirable linkage drag (Young and Tanksley 1989). These authors suggested that monitoring recombination around the gene(s) under transfer with DNA markers would quickly and efficiently reduce the amount of linkage drag.

In view of the problems associated with making wide crosses, it is necessary that breeders should first explore variation within the species, i.e., within the crop's primary gene pool, before initiating interspecific hybridization. In grain legumes, varying levels of success have been obtained from crosses between species.

Wide crossing in the genus Vigna

Interspecific crosses have been attempted in the genus, but with limited success. There are several causes of failure in wide crosses between *Vigna* species: pollen may be unable to germinate on stigma or pollen tubes may be unable to penetrate stigma and style (Chowdhury and Chowdhury 1977); pollen tubes may be distorted and grow slowly in the style (Barone et al. 1992); young embryos may abort (Ahn and Hartmann 1978a; Fatokun 1991); and F_1 interspecific hybrids may be completely sterile (Chen et al. 1983) (Table 1).

Recent advances in tissue culture techniques, such as embryo rescue and protoplast fusion, have increased the chances of successful interspecific crosses in plants. The embryo rescue technique has been used to enhance successful interspecific hybridization between *V. radiata* and *V. angularis* (Ahn and Hartmann 1978a), and between *V. mungo* and *V. umbellata* (Chen et al. 1983). However, regeneration of plants from protoplasts of grain legumes has not been generally successful; hence, protoplast fusion as a means of bringing about interspecific hybridization is not yet routine in these crops.

Within the genus *Vigna,* some successful crosses have been reported between species, especially those in the same subgenus or section. Crosses among some species within the following subgenera have been successful: *Ceratotropis, Vigna,* and *Plectotropis.*

Interspecific crosses in the subgenus Ceratotropis

F_1 interspecific hybrids have been obtained from crosses between some of the Asiatic grams: *V. radiata* (mung bean), *V. mungo* (black gram), *V. umbellata* (rice bean), *V. angularis* (adzuki bean), and *V. aconitifolia* (moth bean) (Table 2). Mung bean (*V. radiata*) is

Table 1. Problems identified with wide crosses among *Vigna* species.

Cross	Description of barrier	Reference
Vigna umbellata × *V. radiata*	Failure of pollen tube to penetrate stigma of other species	Chen et al. (1983)
V. vexillata × *V. unguiculata*	Low frequency of pollen germination on stigma of the other species	Barone et al. (1992)
V. vexillata × *V. unguiculata* *V. umbellata* × *V. radiata*	Slow rate of pollen tube elongation in the sylar tissue and/or distorted pollen tubes	Barone et al. (1992) Chowdhury and Chowdhury (1983)
V. vexillata × *V. unguiculata* *V. mungo* × *V. radiata* *V. aconitifolia* × *V. trilobata*	Hybrid embryo fails to develop fully/or complete seed sterility	Fatokun (1991) Barone et al. (1992) Chen et al. (1983) Biswas and Dana (1976)
V. radiata × *V. angularis*	Complete sterility of F_1 plants	Ahn and Hartmann (1978a)

Table 2. Interspecific hybridization among Asiatic *Vigna* species.

Cross	11 bivalents	Pollen fertility (%)	Reference
Interspecific hybridization			
V. angularis × *V. umbellata*	100	76	Ahn and Hartmann (1978b)
V. mungo × *V. radiata*	61	46	Gosal and Bajaj (1983)
V. radiata × *V. trilobata*	42	31	Dana (1966b)
V. radiata × *V. glabrescens*	51	19	Biswas (1973)
V. glabrescens × *V. umbellata*	82	8	Dana (1964)
V. radiata × *V. umbellata*	73	3	Chowdhury and Chowdhury (1983)
Intergeneric hybridization			
Vigna mungo × *Phaseolus calcaratus*			Chowdhury and Chowdhury (1977)

probably the most widely grown Asiatic gram; successful crosses have been made among several of its accessions and some other species (*V. mungo, V. glabrescens, V. macroptilium, V. umbellata,* and *V. trilobata*). The F_1 interspecific hybrids resulting from crossing *V. radiata* with *V. mungo, V. macroptilium lathyroides,* and *V. trilobata* were partially fertile, indicating their close relationship and the possibility of gene exchange among them (Dana 1966a; Biswas and Dana 1975). On the other hand, F_1 interspecific hybrids from crossing *V. radiata* with *V. angularis, V. umbellata,* and *V. glabrescens* were completely sterile (Dana and Karmakar 1990). Crosses between other species such as *V. aconitifolia* × *V. trilobata, V. mungo* × *V. trilobata, V. mungo* × *V. umbellata,* and *V. mungo* × *V. angularis* resulted in completely seed sterile hybrids. The most successful interspecific hybridization in subgenus *Ceratotropis* is the *V. angluaris* × *V. umbellata* cross (Ahn and Hartmann 1978b), as well as *V. radiata* × *V. mungo*, as F_1 hybrids, in both cases, were highly fertile.

A numerical taxonomy of 44 accessions belonging to several *Vigna* species and subspecies, using data from restriction fragment length polymorphism (RFLP) analysis, revealed that *V. radiata* has the closest relationship with *V. mungo,* and *V. angularis* was closest to *V. umbellata* (Fatokun et al. 1993). Also, taxonomy based on the gene pool concept showed that *V. radiata* and *V. mungo* are closely related, since they share the same primary gene pool (Dana 1980). The successful cross between both species is, therefore, to be expected, although the vigorous F_1 hybrid plants were partially fertile. Also, *V. angularis* and *V. umbellata* share the same primary gene pool.

Failures of interspecific hybridization involving members of the subgenus *Ceratotropis* are due mainly to postfertilization events. The isolating barriers reported from interspecific hybridization within this subgenus are the delay or absence of divisions in endosperm and/or failure of embryo to divide (Dana and Karmakar 1990). The consequences of these events are the formation of empty shriveled hybrid seeds, with reduced germination. The death of F_1 interspecific hybrid plants at critical stages of development has also been observed. According to Dana and Karmakar (1990), unidirectional success is a common occurrence in interspecific hybridization in the subgenus *Ceratotropis*. While the cross between *V. radiata* as female and *V. umbellata* as male was successful, the reciprocal cross was not (Chen et al. 1983; Chowdhury and Chowdhury 1983). This unidirectional success was attributed to differential nucleocytoplasmic interactions in reciprocal combinations. In the cross between *V. radiata* and *V. angularis*,

weak F_1 plants were produced, and these were also characterized by irregular meiosis (Ahn and Hartmann 1978a), causing them to be sterile. Chromosome pairing at metaphase I in the hybrid plants ranged from 0 to 4 bivalents, with a mean of 2.39II + 17.22I.

An intergeneric cross between *Vigna mungo* and *Phaseolus calcaratus* was attempted by Chowdhury and Chowdhury (1977). Pods were formed and remained for only 12 days before drying and dropping when *V. mungo* was used as female parent. Endosperm tissue around the embryos soon degenerated, and the nondevelopment of the embryos led to the pods collapsing. In the reciprocal cross, pollen tubes could not penetrate the stigma.

Interspecific crosses in the subgenus Plectotropis

There are four species in this subgenus: *V. vexillata* with six identified varieties, *V. davyi, V. kirkii,* and *V. hundtii* (Maréchal et al. 1978). None of these species is cultivated. Accessions of *V. vexillata* have been evaluated for potentially useful genes lacking in cowpea and its wild relatives, and this has led to the identification of *V. vexillata* accessions with high resistance to insects. Crosses have been attempted between cowpea and *V. vexillata*, with the aim of transferring to the former the gene(s) for insect resistance.

A numerical taxonomic study, based on RFLP analysis, showed that *V. vexillata* is intermediate between the Asiatic grams and African *Vigna* species. When some data from RFLP analysis were subjected to an algorithm that determines nearest neighbor, an accession of *V. unguiculata* ssp. *dekindtiana* var. *pubescens* was closest to the outlier among the *V. vexillata* accessions. Interestingly, both *V. vexillata* and *V. unguiculata* var. *pubescens* are hairy, although the hairs are long and bristly in the former, but short and velvety in the latter. No hybrid has been obtained from crosses between accessions of both *V. vexillata* and *V. unguiculata*. There is no report of a successful cross between *V. vexillata* and any other *Vigna* species. The high resistance to insect pests exhibited by *V. vexillata* accessions calls for concerted efforts at identifying possible species that can be used as bridges for moving resistance genes from *V. vexillata* to *V. unguiculata*.

***Vigna vexillata* × *V. davyi*:** A cross has been made between *V. vexillata* and *V. davyi,* both of which are members of the subgenus *Plectotropis*. The F_1 interspecific hybrid is partially fertile and produces few viable seeds. The degree of fertility of the F_1 hybrid depends on the parents that are crossed. Pollen fertility, as measured by acetocarmine staining, was 47% when the cross involved TVNu 1335 (*V. davyi*) and TVNu 381 (*V. vexillata* var. *angustifolia*), and 59% when TVNu 1335 was crossed to TVNu 72 (*V. vexillata* var. *vexillata*). Pollen fertility of the parents was > 95% (C.A. Fatokun, unpublished data). In the former cross, pod set by F_1 plants was very low and the pods contained fewer seeds. Chromosome pairing in the hybrids at metaphase I of meiosis was generally normal, i.e., there were 11 bivalents in most pollen mother cells observed. At a very low frequency, however, there was precocious separation of chromosomes during anaphase I. Abnormal chromosome behavior was more frequently detected in the second stage of meiosis such that, at telophase II, chromosomes were not uniformly distributed to the tetrads. Micronuclei were formed and consequently pollen grains of variable sizes characterized the hybrids. In view of the partial fertility that was observed in the hybrid between *V. davyi* and *V. vexillata*, gene exchange is feasible between them. Genome relationship between the two species at DNA level was found to be ~ 76% (Fatokun et al. 1993). The relationship

between *V. vexillata* and *V. davyi* at the genome level is similar to that between *V. radiata* and *V. mungo*. Crosses between the latter pair result in hybrids that are also partially fertile. Pollen fertility of the F_1 *V. radiata* x *V. mungo* hybrid was 31–46%, and the abnormality observed during meiosis was a reduced frequency of bivalents (~ 70%) in metaphase I (Dana and Karmakar 1990). The F_1 interspecific hybrids between *V. davyi* and *V. vexillata* are now being crossed with cowpea accessions, but no hybrid has been obtained.

Interspecific hybridization in the subgenus Vigna

This subgenus is divided into six sections (Maréchal et al. 1978), and it is perhaps the most complex because it contains very diverse types. This subgenus contains the geocarpic bambara groundnut (*V. subterranea*, formerly known as *Voandzeia subterranea*), cowpea (*V. unguiculata*, along with the several subspecies in section *Catiang*), and many other noncultivated species; these include *V. luteola, V. ambacensis, V. frutescens, V. oblongifolia, V. venulosa, V. marina*, and *V. reticulata*. Crosses have been attempted between some of these species with the primary aim of identifying any that could serve as a bridge for transferring some useful genes from noncultivated to cultivated cowpea.

***Vigna oblongifolia* × *V. luteola*.** Some accessions of these species have been identified as resistant to insect pests, especially the *Maruca* pod borer and pod-sucking bugs, both of which cause high yield losses in cowpea. A cross was successfully made between these two species with the aid of in vitro culture of the hybrid embryo (S.R. Schnapp, Purdue University, USA, personal communication). The F_1 interspecific hybrid grew vigorously, though it was only partially fertile. It produced viable seeds, which were advanced to the F_2 generation. Attempts were made to cross the more fertile F_2 plants with *V. unguiculata* accessions, but this has not yet resulted in any hybrid. It has not been possible, therefore, to transfer the resistance genes from any of these *Vigna* species to cowpea.

Crosses among members of the section *Catiang*. It has been reported by many cowpea researchers that members of section *Catiang* are cross compatible and gene exchange should, therefore, not be difficult to accomplish. Experience has shown, however, that crosses between some members of this section are not easy to make and at times may result in hybrids which show partial fertility. For example, in crosses between an improved cowpea variety (IT84S-2246-4) and a genotype of *V. unguiculata* ssp. *dekindtiana* var. *pubescens* (TVNu 110-3A), using the latter as pollen parent, pods along with seeds in them collapsed after ~ 12 days; to recover most of the hybrids embryo rescue was needed (Fatokun and Singh 1987). The F_1 plants grew vigorously, but were only partially fertile. The purpose of the cross was to transfer hairiness, a characteristic of var. *pubescens*, to cowpea, in the hope that this trait may confer some degree of insect resistance on cowpea.

The F_1 hybrids of a cross between *V. unguiculata* and *V. unguicualta* ssp. *rhomboidea* were partially fertile, with pollen stainability of ~ 70%. Under greenhouse conditions, the F_1 plants flowered profusely but these flowers dropped after anthesis, thus producing no pods. During August and September, when ambient humidity was high and temperature low due to cloud overcast, the F_1 plants produced pods at a higher frequency. The few pods produced contained, on average, three seeds each. Among the F_2 plants, up to 30% set no pods, although all plants flowered (C.A. Fatokun, unpublished).

Table 3. Morphological attributes of *Vigna unguiculata* ssp. *sesquipedalis*, *V. unguiculata* ssp. *tenuis*, and their F_1 hybrid.

	V. unguiculata ssp. *sesquipedalis*	F_1 hybrid	*V. unguiculata* ssp. *tenuis*
Petiole length (cm)	8	9	5
Terminal leaf length (cm)	12	10	3
Terminal leaf width (cm)	8	7	2
Standard petal width (cm)	3	3	3
Pod length (cm)	38	9	6
Seed number/pod	13	4	11
100-seed weight (g)	17	3	1
Pollen stainability (%)	95	60	96
Peduncle length (cm)	21	36	17

These observations suggest the existence of some barrier to gene flow between cowpea and *V. unguiculata* ssp. *rhomboidea*. An examination of pollen mother cells of F_1 plants showed a high level of homology between chromosomes of both parents as 11 bivalents were commonly observed. Unequal distribution of chromosomes to the microspores at late telophase II was observed (C.A. Fatokun, unpublished), and this probably explains the presence of small, unstained pollen grains. *Vigna unguiculata* ssp. *rhomboidea* plants do not grow vigorously at Ibadan, where they flower, however, but produce few pods. As the plants are pubescent, they may be useful in developing insect-resistant cowpea varieties.

A cross between yard-long bean (*V. unguiculata* ssp. *sesquipedalis*) and *V. unguiculata* ssp. *tenuis* resulted in F_1 plants that were vigorous in growth. They were intermediate in several characters between the two parents. They showed hybrid vigor for petiole and peduncle length. Pollen stainability in the F_1 plants was 60.4%, and the pods had very few seeds (Table 3). The fertility level of hybrid plants between these two genotypes was less than that of the F_1 interspecific hybrid between *V. umbellata* and *V. angularis* (Ahn and Hartmann 1978b). Causes of the relatively low fertility of F_1 plants from a cross between ssp. *sesquipedalis* and ssp. *tenuis* are being investigated.

Chromosomal behavior during meiosis is normal when there is complete homology between the parents that were crossed to obtain the hybrid. In such cases, pollen grains are normal in size and shape and generally highly fertile. The fertility levels of some of the F_1 hybrids from crosses among members of section *Catiang* suggest a lack of complete homology. These observations call for a closer examination of the classification of accessions in the section *Catiang*. Apart from *V. nervosa* Markotter, all other members of this section belong to *V. unguiculata* and its various subspecies. Already, Smartt (1985) had opined that the present genus *Vigna* does not seem to constitute a natural group, and he further indicated that some genus as known now might be dismembered in the near future, while some subgenera might be raised to the generic rank. When this happens, many members of the section *Catiang* will be likely distributed into more species. Crosses between some members of section *Catiang* will then be regarded as true wide crosses. Crossability studies and DNA analysis will help in placing the different members of the section into their respective genomic groups. Such a grouping should have a positive impact on the exploitation of genetic potential available among the different genotypes.

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5

Isozyme markers and taxonomic relationships among *Vigna* species

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Abstract

Isozyme markers are commonly used to study populations, taxonomy, and the genetics of crop species. In the genus *Vigna*, these markers have been investigated mainly to assess genetic diversity in the *V. unguiculata* complex. The aim of this study was to extend isozyme analysis to other sections of the genus *Vigna*, in order to evaluate their taxonomic relationships. Nine species of the sections *Catiang*, *Vigna*, and *Plectotropis* were tested. Interest in the selected species is high since they carry important traits, such as resistance to biotic and abiotic stresses, which are useful in cowpea breeding programs. For each species, several accessions of different geographical origin were analyzed. Isozyme data were statistically analyzed. Similarity was evaluated and a UPGMA dendrogram was constructed. Very low levels of similarity were revealed among species, whereas variability among accessions differed within each species. In the section *Vigna*, *V. luteola* and *V. marina* were shown to be closely related, whereas the other species had a lower similarity. The *V. unguiculata* complex, which belongs to the section *Catiang*, formed a separate group when compared to the other species, although it was closest to *V. vexillata*.

Introduction

The evaluation of the diversity existing in germplasm is essential for understanding and fully utilizing its potential value. A simple and precise technique for measuring the overall genetic diversity of a crop is not yet available, and no single approach can be considered the best for measuring diversity. In fact, the classification of wild relatives of crop plants and the determination of their interrelationships require studies based on conventional methods (morphological traits, resistance to pests and disease, etc.) together with sophisticated analyses (isozymes, RAPDs, RFLPs, etc.)

The taxonomy of *Vigna*, a large genus grouping about 100 species, most of which are indigenous to Africa (Maréchal et al. 1978), needs more investigations to clarify some aspects. A better knowledge of the relationships between cowpea (*Vigna unguiculata*) and other entities of the same species is essential to select the most appropriate species that should be involved in breeding programs.

Recent studies on the relationships among *Vigna* species have been based on cytogenetic (Galasso et al. 1993), seed globulin fraction (Paino D'Urzo et al. 1990), and RFLP (Fatokun et al. 1993) analyses. In addition, isozymes have had many useful applications in

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Table 1. List of the material analyzed in the present study.

Number		*Vigna* sp.†	ssp.§	BV¶	Origin‡	Number		*Vigna* sp.†	BV¶	Origin‡
1	MG 103236	*ung*	*ung*		IITA	39	NI 326	*lut*		BRA
2	MG 103264	*ung*	*ung*			40	TVNu 172	*lut*		
3	MG 103442	*ung*	*ung*			41	TVNu 254	*lut*		CAF
4	MG 103464	*ung*	*ung*		ETE	42	TVNu 500	*lut*		BWA
5	MG 106811	*ung*	*ung*		GRC	43	TVNu 1174	*mar*		GAB
6	MG 106819	*ung*	*ung*			44	TVNu 1179	*mar*		GAB
7	MG 110844	*ung*	*ung*		EGY	45	TVNu 1386	*mar*		GAB
8	MG 110845	*ung*	*ung*			46	TVNu 1441	*mar*		MOZ
9	MG 110846	*ung*	*ung*			47	NI 440	*amb*	amb	ZAR
10	IT 82D716	*ung*	*ung*		NGA	48	NI 464	*amb*	amb	ZAR
11	IT 81D994	*ung*	*ung*			49	NI 997	*amb*	pg	NGA
12	IT 81D1137	*ung*	*ung*			50	NI 1371	*amb*	amb	CMR
13	IT 81D1151	*ung*	*ung*			51	TVNu 11	*amb*		ZAR
14	TVu 2027	*ung*	*ung*			52	TVNu 147	*amb*		GHA
15	MG 112920	*ung*	*ung*		ETE	53	NI 282	*oblo*	parv	TZA
16	MG 113016	*ung*	*ung*			54	NI 123	*oblo*	oblo	KEN
17	MG 113017	*ung*	*ung*			55	NI 335	*oblo*	oblo	CRI
18	MG 113018	*ung*	*ung*			56	NI 387	*oblo*	parv	RWA
19	MG 113107	*ung*	*ung*		NGA	57	NI 389	*oblo*	parv	RWA
20	MG 112989	*ung*	*dek*	pb	TZA	58	NI 461	*oblo*	oblo	ZAR
21	MG 116102	*ung*	*dek*	pb		59	NI 777	*oblo*	oblo	NGA
22	MG 116103	*ung*	*dek*	pb		60	NI 954	*oblo*	oblo	ZMB
23	MG 116105	*ung*	*dek*	pro		61	NI 1173	*grac*		CMR
24	MG 116106	*ung*	*dek*			62	TVNu 173	*grac*		RWA
25	MG 116108	*ung*	*dek*	men		63	TVNu 1120	*grac*		COG
26	NI 339	*vex*		mac	CRI	64	TVNu 1180	*grac*		GAB
27	NI 336	*vex*		vex	CRI	65	NI 239	*race*	race	ZAR
28	NI 557	*vex*		vex	ZAF	66	NI 447	*race*	race	ZAR
29	NI 620	*vex*		ang	AUS	67	NI 815	*race*	race	NGA
30	NI 827	*vex*		vex	BRA	68	NI 995	*race*	race	NGA
31	NI 932	*vex*		ang	PAN	69	NI 996	*race*	race	NGA
32	TVNu 240	*vex*			CAF	70	NI 1245	*race*	race	BDI
33	TVNu 292	*vex*			TZA	71	NI 1250	race	race	BDI
34	TVNu 593	*vex*			NER	72	NI 1254	*race*	race	BDI
35	TVNu 635	*vex*			COG	73	NI 1446	*race*	race	CMR
36	TVNu 719	*vex*			BWA	74	NI 122	*het*		KEN
37	TVNu 1358	*vex*			ZAF	75	TVNu 19	*het*		
38	NI 200	*lut*			TCH					

† *amb = ambacensis; ang = angustifolia; dek = dekindtiana; grac = gracilis; het = heterophylla; lut = luteola; mac = macrosperma; mar = marina; men = mensensis; oblo = oblongifolia; parv = parviflora; pg = pubigera; pro = protracta; pb = pubescens; race = racemosa; ung = unguiculata; vex = vexillata.*

§ ssp. = subspecies.

¶ BV = botanical variety.

‡ Country abbreviations from FAO/IBPGR (1973).

taxonomic and phylogenetic fields (Mowrey and Werner 1990). The interest in isozymes in *Vigna* has been mainly devoted to the study of *Vigna unguiculata* and the wild forms of this species (Panella and Gepts 1992; Pasquet 1993; Vaillancourt et al. 1993), whereas two studies have considered other *Vigna* species (Jaaska and Jaaska 1988; Vaillancourt and Weeden 1993).

The aim of the present investigation was the evaluation, based on 19 isozyme loci, of the relationships within and among *Vigna unguiculata*, *V. vexillata*, and seven species belonging to the section *Vigna*. The species examined were chosen because they carry traits for resistance to biotic and abiotic stresses; thus, they could be sources of useful genes for cowpea breeding programs (Ng 1990; Padulosi and Ng 1993).

Materials and methods

Plant material. The material analyzed (Table 1) was obtained from IITA (Ibadan, Nigeria), the University of Gembloux (Belgium), and the Germplasm Institute (Bari, Italy). The study evaluated 25 accessions of *V. unguiculata* (section *Catiang*, subgenus *Vigna*), among which 6 are wild (subsp. *dekindtiana*), and the following species of section *Vigna*, subgenus *Vigna*: *V. racemosa* (9 accessions), *V. oblongifolia* (8 accessions), *V. ambacensis* (6 accessions), *V. luteola* (5 accessions), *V. marina* (4 accessions), *V. gracilis* (4 accessions), *V. heterophylla* (2 accessions), as well as 12 accessions of *V. vexillata* (section *Plectotropis*, subgenus *Plectotropis*).

Isozyme analysis. For isozyme analysis, seeds were germinated in petri dishes at 24 °C, and transplanted to pots in a greenhouse. After 4–6 weeks, young leaves were collected and processed for isozyme analysis according to Bringhurst et al. (1981). The electrophoretic run was carried out as reported by Panella and Gepts (1992). The 10 enzyme systems tested (Table 2) were stained according to Wendel and Weeden (1989) and Panella and Gepts (1992.

Table 2. Enzyme systems assayed.

AAT	Aspartate amino-transferase	E.C. 2.6.1.1
DIA	Diaphorase	E.C. 1.6.4.3.
G6PD	Glucose-6-phosphate dehydrogenase	E.C. 1.1.1.49
IDH	Isocitrate dehydrogenase	E.C. 1.1.1.41
LAP	Leucine amino peptidase	E.C. 3.4.11.1
MDH	Malate dehydrogenase	E.C. 1.1.1.37
ME	Malic enzyme	E.C. 1.1.1.40
PRX	Peroxidase	E.C. 1.11.1.7
SKD	Shikimate dehydrogenase	E.C. 1.1.1.25
SOD	Superoxide dismutase	E.C. 1.15.1.1

Data analysis. The stained bands were scored and the DICE index of similarity (Sokal and Sneath 1963) was then computed to compare the different electrophoretic types (ETs). This index was calculated as follows: $F = 2n_{ab}/n_a + n_b$, where F is the proportion of alleles shared by two ETs (n_{ab}) among the sum of alleles that ETs a and b express (n_a and n_b).

The UPGMA clustering method (Sneath and Sokal 1973) was used to group ETs on the basis of DICE index.

Results

One region of activity was observed for the enzymes IDH, ME, and SKD; two zones of activity were visualized for the enzymes AAT, DIA, G6PD, LAP, and PRX; and three regions of activity were detected for MDH and SOD. Most isozymes were very diverse among species, but Sod-3 showed the same band for all the species and in Mdh-3 only one polymorphism was observed for *V. marina. V. luteola* and *V. marina* shared the highest number of alleles. Monomorphism for all the loci analyzed was observed within *V. heterophylla;* conversely, *V. gracilis* was polymorphic for 11 loci.

An example of the electrophoretic patterns obtained for AAT and IDH is shown in Figure 1.

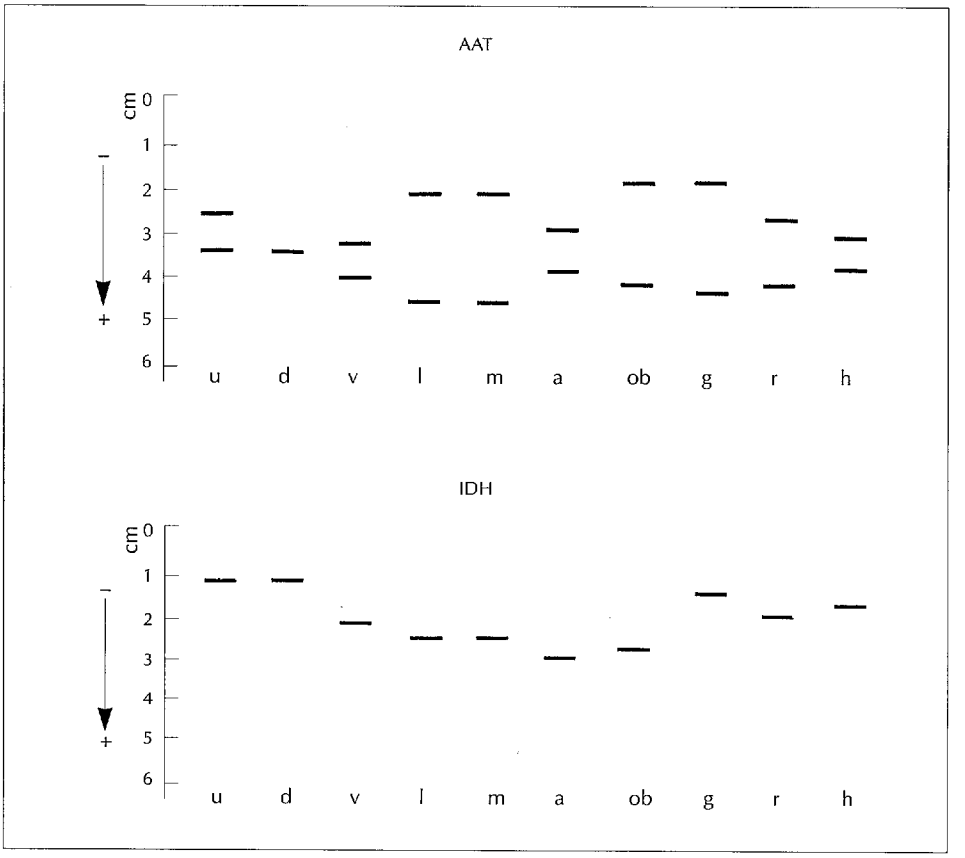

Figure 1. Zymograms of AAT and IDH in the species analyzed. For each species, the more frequent pattern is reported. (u = *V. unguiculata* ssp. *unguiculata;* d = *V. unguiculata* ssp. *dekindtiana;* v = *V. vexillata;* l = *V. luteola;* m = *V. marina;* a = *V. ambacensis;* ob = *V. oblongifolia;* g = *V. gracilis;* r = *V. racemosa;* h = *V. heterophylla*).

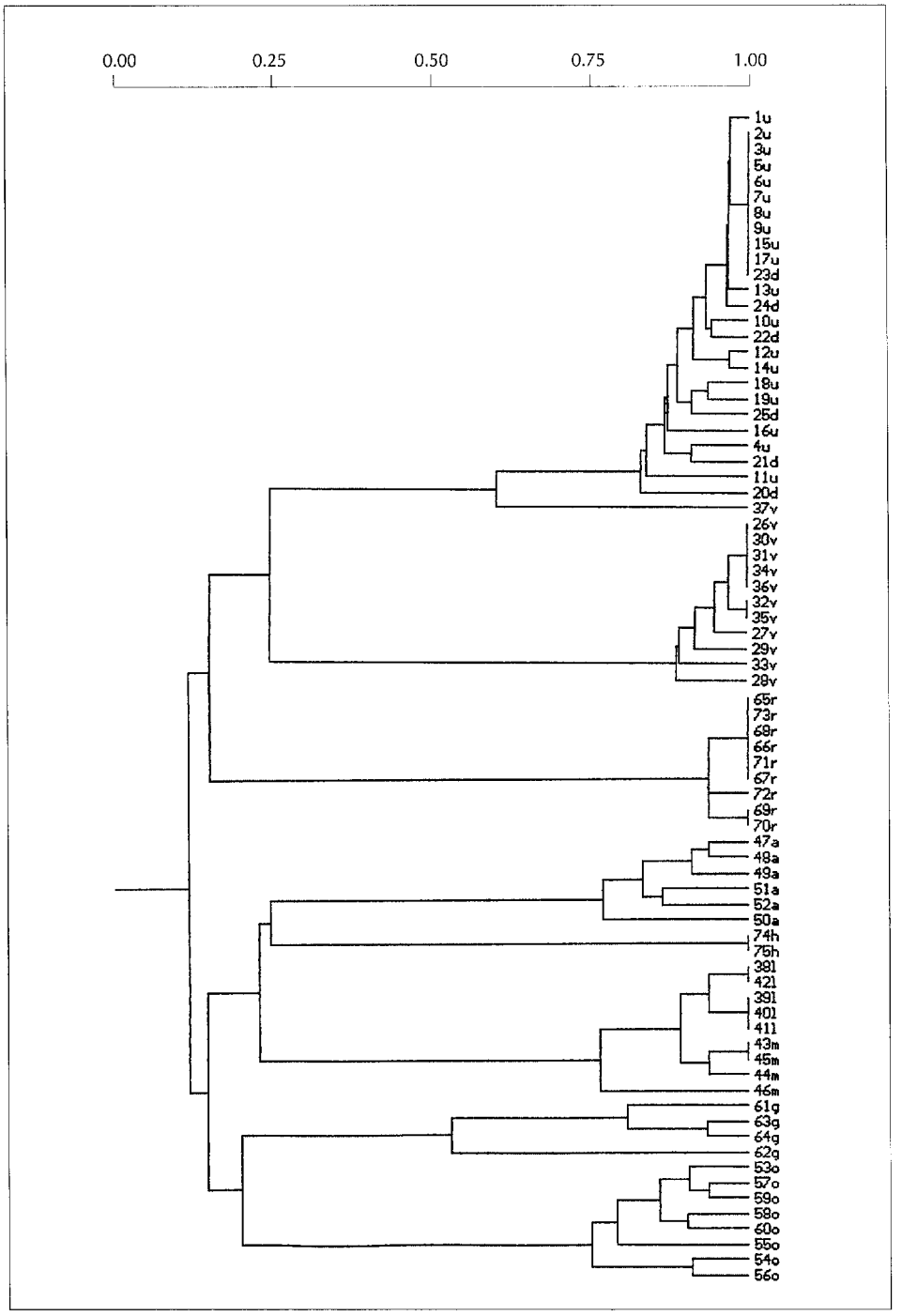

Figure 2. UPGMA dendrogram, based on DICE similarity index. Abbreviations are as given in Figure 1. Accession numbers are as in Table 1.

Intraspecific variability. In general, no correspondence was observed between the geographical origin of accessions and the degree of intraspecific similarity found.

For the *V. unguiculata* complex (subsp. *unguiculata* and subsp. *dekindtiana*), very high levels of similarity were detected between accessions (> 0.91); however, some accessions showed a lower index of similarity, but still > 0.80 (Fig. 2). The *V. vexillata* group showed a similarity index range of 0.89–1.00. *V. vexillata*, TVNu 1358 (37v in Fig. 2), clustered separately from its cospecific accessions and was revealed to be closer to the *V. unguiculata* group.

Within the species *V. racemosa* and *V. luteola,* similarity was high among the accessions (> 0.95). The *V. marina* group clustered above the similarity index of 0.92, except for TVNu 1441 (46m in Fig. 2). Intraspecific variability for *V. oblongifolia* and *V. ambacensis* was comparable (> 0.78). The highest variability was seen in the *V. gracilis* group, whereas the tw o accessions of *V. heterophylla* were identical.

Interspecific variability. The UPGMA dendrogram clustered the different species in separate groups, which showed a low similarity among them (< 0.29).

V. vexillata was found to be the most similar to *V. unguiculata*. Among the species of subgenus *Vigna, V. racemosa* was the closest to cowpea. The other species of section *Vigna* were grouped in another cluster of the dendrogram. *V. luteola* and *V. marina* were very similar; in fact their DICE index (0.77) was comparable to the values observed for intraspecific variability within the other *Vigna* species.

This high degree of similarity agreed with other evidence. *V. luteola* and *V. marina* are known to be very close morphologically and can be hardly distinguished (Padulosi and Ng 1993). Among the species that were analyzed, *V. ambacensis* was closer to *V. heterophylla, V. luteola,* and *V. marina*, whereas *V. oblongifolia* was closer to *V. gracilis*.

Discussion

A preliminary examination of the dendrogram shows no perfect correspondence with the morphological classification of the species analyzed. In fact, the cluster of *V. unguiculata* was found to be closest to *V. vexillata* (subgenus *Plectotropis*), although cowpea belongs to the subgenus *Vigna* just as the other 7 species studied (*V. ambacensis, V. gracilis, V. heterophylla, V. luteola, V. marina, V. oblongifolia,* and *V. racemosa*).

These results agree with the clustering pattern based on RFLP analysis (Fatokun et al. 1993). However, Paino D'Urzo et al. (1990) found a lower similarity between cowpea and *V. vexillata* when they were comparing seed globulin fraction of 13 species of *Vigna* belonging to four subgenera. The different conclusions could be attributed to the different markers used. The relatively higher similarity between *V. unguiculata* and *V. vexillata* suggests that attempts could be made to transfer genes from the latter species to the former. Such a transfer would be of great interest, since high levels of resistance to some cowpea insect pests have been observed in several accessions of *V. vexillata* (Ng 1990). However, since direct conventional crosses have failed, Fatokun et al. (1993) have suggested the possibility of using *V. unguiculata* subsp. *dekindtiana*, var. "*pubescens*" as a link. In our UPGMA dendrogram, one accession of this wild variety (20d) is revealed as being fairly close to *V. vexillata* 37v (Fig. 2). This result, if confirmed by further studies, could enable gene transfer in cowpea.

When the species of section *Vigna* were considered, *V. racemosa* separated from the others, with a similarity index of 0.16 and a slightly higher similarity with *V. unguiculata* and *V. vexillata* (0.19). A similar result was obtained by Paino D'Urzo et al. (1990).

Only for *V. marina* was it possible to relate the geographical origin of the accessions to their position in the cluster. In fact, two groups were observed for this species (43m, 44m, 45m, and 46m, respectively, in Fig. 2), the former containing 3 accessions from West Africa and the latter with one accession from East Africa (Table 1). Major differences between accessions from these geographical regions were also observed at morphological levels. Therefore, our results seem to confirm the existence of two subspecies, as proposed by Padulosi and Ng (1993).

Conclusions

The relatively low isozyme similarities observed for the species that were analyzed suggest that these entities share a very low common genetic basis. This could explain the difficulties in transferring genes from wild *Vigna* species into cowpea. On the basis of this isozyme study, the major divergence within the present taxonomy, based on morphological traits, concerns the relatively high similarity between *V. vexillata* and *V. unguiculata*, although they belong to different subgenera. The very high level of similarity revealed between *V. luteola* and *V. marina* suggests that the relationship between them should be further investigated.

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6

Flavonoid HPLC fingerprints of wild *Vigna* species

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Abstract

Thirty-two wild species and varieties of *Vigna* of different origin were screened for their flavonoid content. The compounds detected were utilized to assess both inter- and intraspecific relationships. Flavonoid HPLC fingerprints support evidence for the existence of different flavonoid chemotypes, which may reflect differences in geographic origin. As far as interspecific relationships are concerned, species belonging to sections *Vigna, Plectotropis,* and *Ceratotropis* do not show flavonoid glycosides in common with cultivated lines of *Vigna*. By contrast, some relationships have been found between cultivated lines and wild species of section *Catiang*. A greater variability in flavonoid aglycone class and glycosylation pattern has been observed in cultivars of *V. unguiculata* (L.) Walp., compared to the wild species. The taxonomic and ecological significance of these findings is discussed. Finally, the existence of a positive relationship between resistance/susceptibility characteristics against aphids and qualitative and/or quantitative flavonoid content is also discussed.

Introduction

The genus *Vigna* (Leguminosae) contains ~160 species, of which several are economically important crops in the agricultural ecosystem of tropical regions. Although frequently revised by taxonomists, the genus has been divided into 7 subgenera (Maréchal et al. 1978). Two subgenera (*Sigmoidotropis* and *Lasiospron*) are endemic to America, and five subgenera (*Vigna, Haydonia, Plectotropis, Macrorhyncha*, and *Ceratotropis*) are distributed in Africa and Asia (Ng and Maréchal 1985). Due to the presence of several centers of origin and the large morphological diversity, it is difficult to draw intrageneric relationships within *Vigna;* consequently, chemical markers have been used to help in establishing generic relationships (Birch et al. 1986; Rao et al. 1992; Panella et al. 1993; Vaillancourt and Weeden 1993; Zallocchi and Pomilio 1994). As a useful tool for the characterization and classification of higher plants, the importance of flavonoids as chemical markers in plant taxonomy is well documented (Bate-Smith 1966; Harborne 1971; Harborne and Turner 1984; Van Sumere et al. 1985; Bohm 1987; Perrino et al. 1989; Hegnauer and Grayer-Barkmeijer 1993).

In order to evaluate the taxonomic significance of flavonoid occurrence, it is essential to identify, at least partly, the various compounds present. In some cases, it may be sufficient to establish which classes of flavonoids are represented (i.e., the presence or

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absence of flavonols, flavones, or C-glycosylflavones) and differences in hydroxylation, O-methylation, and glycosylation (Bohm 1987). Variations in taxonomic characters may be induced or influenced by environmental factors. However, when changes do occur they can involve level and position of glycosylation, with no alteration of flavonoid aglycone (Bohm 1987); qualitative variability will probably be due to genetic variation, while the quantitative variability will reflect environmental factors.

Flavonoid HPLC fingerprints of *Vigna* leaves have shown considerable promise: there are qualitative and quantitative differences in flavonoid patterns between species and/or accessions (Lattanzio et al. 1990; Lattanzio et al. 1992). In addition, chemical characterization of *Vigna* species is of particular relevance because wild species represent a reservoir of useful genes that could be used in cowpea improvement. Investigation on the levels of resistance of wild species of *Vigna* to pests and diseases showed good levels of resistance, offering promise for their potential use in cowpea breeding (Padulosi and Ng 1990).

In the present study, 32 *Vigna* species and/or accessions were analyzed by HPLC for their leaf flavonoid contents. The flavonoid glycosidic patterns were used to draw intra- and interpecific relationships. The ecological significance of these findings is discussed.

Materials and methods

Most *Vigna* species used in this study (Table 1) were collected in Africa. All seed samples were supplied by the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

All species were grown from seed in a growth chamber at 25 °C (70% RH), with a 12h:12h light-dark photoperiod up to emission of the sixth leaf. Second and third leaves from the tip were collected from each plant (5–10 g) and analyzed for phenolic compounds.

For qualitative determination of the flavonoids, the plant material was refluxed with hot methanol-ethanol-water (MeOH-EtOH-H_2O) (4:4:2) for 1 h. After centrifugation, the solution was concentrated under vacuum and partitioned with petroleum ether (bp 40–70 °C). The aqueous fraction was analyzed for flavonoid HPLC fingerprint. For flavonoid aglycone analysis, the same fractions were hydrolyzed under nitrogen with 0.3 M HCl and then extracted with diethyl ether (Et_2O). Finally, the Et_2O extracts were concentrated under vacuum and redissolved in MeOH. The latter were analyzed by HPLC, using a Perkin Elmer Series 4 liquid chromatograph, equipped with a computer-aided spectrophotometric photodiode array detector 1040 Hewlett Packard, following the method of Lattanzio and Van Sumere (1987). In all cases, flavonoids were subjected to UV spectroscopy and chromatographic comparison against authentic samples, by means of a computer program.

From chromatograms and using an HP-K3 software postrun analysis coupled with PE-Chromatographics 2 software, a flavonoid fingerprint of the different *Vigna* species was obtained, based on the retention times of the flavonoid glycosides, knowledge of aglycone, and spectral data.

Results and discussion

Cultivated lines of V. unguiculata *(L.) Walp.*

Flavonoid HPLC analyses clearly showed that cultivated lines of *Vigna unguiculata* (L.) Walp. always contained three flavonoid aglycones: quercetin (the most abundant), kaempferol, and isorhamnetin (Table 1). The flavonoid glycoside patterns of the different

Table 1. Distribution of flavonoid aglycones in *Vigna* species and/or accessions of different origin.

Subgenera/sections/taxa†	Origin	K§	Q	I	A
Vigna					
Catiang					
V. unguiculata (L.) Walp.					
ssp. *unguiculata* cg. *unguiculata* Westphal (c)	Nigeria	+	+++	+	–
ssp. *dekindtiana* var. *dekindtiana* MG 112997 (w)	Nigeria	+	+++	+	–
ssp. *dekindtiana* var. *dekindtiana* TVNu 413 (w)	Nigeria	(t)	+++	–	–
ssp. *dekindtiana* var. *mensensis* (Schweinf.) M.,M.&S.(1) TVNu 862 (w)	Zimbabwe	+++	(t)	–	(t)
ssp. *dekindtiana* var. *protracta* (Wilczek) M.,M.&S. TVNu 965 (w)	Swaziland	+++	+	+	–
ssp. *dekindtiana* var. *pubescens* (Wilczek) M.,M.&S TVNu 110 (w)	Tanzania	+++	(t)	–	–
ssp. *stenophylla* (Harv.) M.,M. and S. TVNu 714 (w)	South Africa	+++	+	–	–
ssp. *tenuis* TVNu 661 (E. Mey.) M.,M.&S. var. *tenuis* TVNu 661 (w)	Congo	+++	(t)	–	–
Vigna					
V. ambacensis Baker var. *ambacensis* TVNu 755 (w)	Central Africa Rep.	+++	+	–	–
V. gracilis Hooker fil var. *gracilis* TVNu 18 (w)	Ivory Coast	+++	(t)	–	–
V. heterophylla A. Richard TVNu 19 (w)	Kenya	+	(t)	+++	–
V. luteola (Jacq.) Bentham TVNu 475 (w)	Kenya	–	+++	–	–
V. luteola (Jacq.) Bentham TVNu 172 (w)	Brazil	+++	(t)	–	–
V. luteola (Jacq.) Bentham TVNu 905 (w)	Botswana	+++	–	–	–
V. marina (Burm.) Merrill var. *oblonga* TVNu 1174 (w)	Gabon	(t)	–	+++	–
V. marina (Burm.) Merrill var. *marina* TVNu 717 (w)	Mozambique	+++	–	–	–
V. oblongifolia A. Richard var. *oblongifolia* TVNu 88 (w)	Nigeria	–	+++	–	–
V. oblongifolia A. Richard var. *oblongifolia* TVNu 40 (w)	Rwanda	–	+++	–	–
V. oblongifolia A. Richard var. *oblongifolia* TVNu 37 (w)	Costa Rica	–	+++	–	–
V. oblongifolia A. Richard var. *oblongifolia* TVNu 135 (w)	Nigeria	–	+++	–	–
V. racemosa Hutch & Dalziel TVNu 181 (w)	Nigeria	+	+++	(t)	–
V. racemosa Hutch & Dalziel TVNu 260 (w)	Central Africa Rep.	+	+++	–	–
V. racemosa Hutch & Dalziel TVNu 96 (w)	Nigeria	+++	+	–	–
V. racemosa Hutch & Dalziel TVNu 45 (w)	Zaire	–	+++	–	–

Table 1. continued.

Subgenera/sections/taxa†	Origin	K§	Q	I	A
Plectotropis					
V. kirki (Baker) Gilloet TVNu 364 (w)	Malawi	+++	+	–	–
V. kirki (Baker) Gilloet TVNu 865 (w)	Tanzania	+	+++	–	–
V. vexillata A. Richard var. *vexillata* TVNu 74 (w)	Rwanda	–	+++	–	–
V. vexillata A. Richard var. *macrosperma* TVNu 64 (w)	Australia	–	+++	–	–
V. vexillata A. Richard var. *vexillata* TVNu 72 (w)	Costa Rica	–	+++	–	–
Ceratotropis					
V. radiata (L.) R. Wilczek TVau 67 (w)	Indonesia	+	+++	(t)	(t)
V. radiata (L.) R. Wilczek TVau 58 (w)	Indonesia	+	+++	–	–

† c = cultivated; w = wild; M., M.&S. = Maréchal, Mascherpa et Stainier.

§ Relative amounts: K = kaempferol; Q = quercetin; I = isorhamnetin; A = apigenin; (+++) major flavonoid in the extract; (+) present; (t) present as trace; (–) absent.

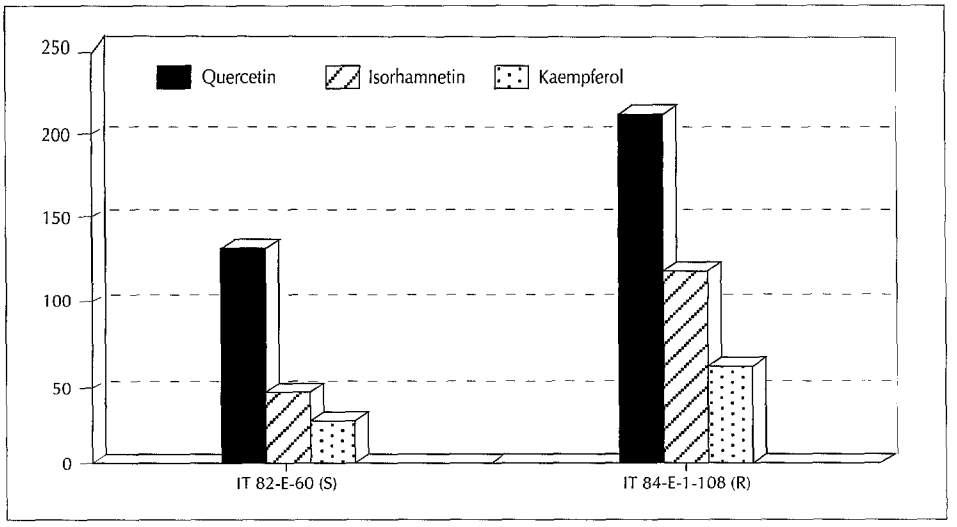

Figure 1. Flavonoid content (mg/100 g dry weight) in near-isogenic lines of *Vigna unguiculata* (L.) Walp. (R = resistant, S = susceptible).

analyzed lines were similar and showed 10 different glycosides; among them, 2 p-coumaroylglycosides of kaempferol and 5 of quercetin were found. Generally, wild species of *Vigna* lacked these more complex glycosides; only ssp. *dekindtiana* var. *dekindtiana* MG 112997 and *protracta* TVNu 965, ssp. *stenophylla* TVNu 714, and ssp. *tenuis* var. *tenuis* TVNu 661 contained one or two flavonol p-coumaroyl glycosides. These latter wild taxa, according to the classification scheme of Maréchal et al. (1978), revised by Ng and Maréchal (1985), are considered as subspecies of *V. unguiculata*, section *Catiang*. As regards the flavonoid glycoside content in cultivated lines of *V. unguiculata*, quantitative differences were found among the assayed lines. A positive relationship between resistance/susceptibility against aphids and flavonoid glycoside amount was also found. The resistant lines showed a flavonoid content higher than the susceptible ones. When the flavonoid aglycone content of two near-isogenic lines of *V. unguiculata* was considered (Fig. 1), the level in IT 84-E-1-108 (resistant) was twice that in IT 82-E-60 (susceptible).

There seems to be no reason to believe that resistance relationships found in domesticated plants do not occur also in wild ones. Thus, chemotaxonomic data on flavonoids in *Vigna* may also contribute to research in the field of plant ecology and crop protection. Besides these theoretical aspects of resistance/flavonoid relationships, additional information has been obtained from in vitro bioassays. In feeding experiments, 0.1 mM of some phenolics, in particular quercetin among the *Vigna* flavonoids, showed significant antifeedant activity on *Aphis fabae* (Scopoli) (Lattanzio et al. 1990; Lattanzio et al. 1992).

Wild species of Vigna

The wild species or subspecies of *Vigna* showed one, two, or rarely three flavonoid aglycones, and their flavonoid glycoside HPLC fingerprints were simpler than those of cultivated lines. The number of flavonoid glycosides ranged between two (*V. unguiculata* ssp. *dekindtiana* var. *pubescens* TVNu 110) and seven (*V. kirki* TVNu 865). None of the

Table 2. Flavonoid glycosides identified in some *Vigna* accessions.

Taxa	Flavonoid glycosides
V. unguiculata ssp. *dekindtiana* var. *dekindtiana* TVNu 413	Hyperoside (Quercetin-3-galactoside)
V. unguiculata ssp. *dekindtiana* var. *mensensis* TVNu 862	Robinin (Kaempferol-3-robinoside-7-rhamnoside)
V. unguiculata ssp. *dekindtiana* var. *protracta* TVNu 965	Robinin
V. unguiculata ssp. *stenophylla* TVNu 714	Kaempferol-3-rutinoside
V. ambacensis var. *ambacensis* TVNu 755	Kaempferol-3-rutinoside
V. gracilis var. *gracilis* TVNu 18	Robinin
V. heterophylla TVNu 19	Kaempferol-3-rutinoside, Isorhamnetin-3-rutinoside
V. marina var. *marina* TVNu 717	Robinin, Kaempferol-3-rutinoside
V. marina var. *oblonga* TVNu 1174	Isorhamnetin-3-rutinoside, Kaempferol-3-rutinoside
V. oblongifolia var. *oblongifolia* TVNu 135 and TVNu 37	Rutin (Quercetin-3-rutinoside)
V. racemosa TVNu 181	Rutin
V. racemosa TVNu 96 and TVNu 260	Rutin, Robinin
V. luteola TVNu 475	Rutin
V. luteola TVNu 172 and TVNu 905	Robinin
V. kirki TVNu 364 and TVNu 865	Hyperoside, Kaempferol-3-rutinoside
V. vexillata var. *vexillata* TVNu 72 and TVNu 74	Rutin
V. vexillata var. *macrosperma* TVNu 64	Rutin
V. radiata TVau 58 and TVau 67	Rutin, Kaempferol-3-rutinoside

flavonoid glycosides was found in the cultivated lines (Table 2). All compounds identified in the leaf extracts of *Vigna* were flavonol-3-O-glycosides, except for the less common 3,7-diglycosylation occurring in robinin (kaempferol-3-robinoside-7-rhamnoside). These glycosides contain the usual flavonoid sugar moieties such as glucose, rhamnose, and rutinose, besides the uncommon galactose and robinose (rhamnosylgalactose). The only compound detected in the three subgenera considered was Kaempferol-3-rutinosided.

With regard to the number of flavonoid aglycones in the leaf extracts (Table 1), there were three clear groups of species. The first group included species containing one aglycone: quercetin (*V. vexillata*, *V. oblongifolia*, *V. luteola* TVNu 475, and *V. racemosa* TVNu 45) or kaempferol (*V. luteola* TVNu 905 and *V. marina* TVNu 717). Other species showed only one aglycone present in detectable amounts plus traces of a second flavonoid aglycone (ssp. *dekindtiana*, var. *dekindtiana* TVNu 413, var. *mensensis* TVNu 862, and var. *pubescens* TVNu 110; ssp. *tenuis* var. *tenuis* TVNu 661; *V. gracilis* TVNu 18; *V. marina* TVNu 1174; and *V. luteola* TVNu 172). The second group included species containing two aglycones as well as, in some cases, traces of a third aglycone (ssp. *stenophylla* TVNu 714; *V. ambacensis* TVNu 755; *V. heterophylla* TVNu 19; *V. racemosa* TVNu 181; *V. racemosa* TVNu 260; *V. racemosa* TVNu 96; *V. kirki*; and *V. radiata*). Finally, the third group included subspecies of *V. unguiculata* (L.) Walp. (ssp. *unguiculata* and ssp. *dekindtiana* var. *dekindtiana* MG 112997 and var. *protracta* TVNu 965) belonging to section *Catiang*, containing three aglycones.

Two taxa of *Vigna* (ssp. *dekindtiana* var. *mensensis* TVNu 862 and *V. radiata* TVNu 67) showed traces of apigenin. In evolutionary terms, flavones are generally considered to be more advanced characters than flavonols by loss of 3-hydroxyl group (Harborne 1971; Williams et al. 1993; Zallocchi and Pomilio 1994). As only traces of apigenin were present, it would not be correct to speculate on the evolutionary significance of this flavone. However, further studies on ecogeographical distribution of apigenin in species of *Vigna* and other related genera suggests the process of diversification at work among and within legume species.

Intra- and interspecific relationships

Within the taxa analyzed, there was evidence of both intra- and interspecific chemical variation. Chemical analyses reflected the wide morphological variation in the genus *Vigna*. In addition, chromatographic data supported evidence for the existence of different flavonoid chemotypes in some of the species (*V. marina, V. unguiculata* ssp. *dekindtiana*, and *V. luteola),* which probably reflected the difference in geographic origin. There was extensive flavonoid glycoside variability encountered in this study.

The analyzed accessions of *V. marina* showed two completely different flavonoid patterns. Two chemotypes were identified, based upon a combination of aglycone structure and glycosylation pattern. One (TVNu 1174) contained two isorhamnetin glycosides and traces of two kaempferol glycosides, while *V. marina* TVNu 717 contained only kaempferol glycosides. Kaempferol-3-O-rutinoside was the only glycoside found in both accessions. These differences in flavonoid HPLC fingerprints are related to the wide morphological variation between the accessions. The two accessions of ssp. *dekindtiana* var. *dekindtiana* also showed flavonoid HPLC fingerprints as regards qualitative and quantitative aspects, MG 112997 being similar to the cultivated lines with regard to the number and the relative abundance of flavonoid aglycones, and the presence of one p-coumaroyl glycoside of quercetin. Otherwise, TVNu 413 contained only three quercetin glycosides. *V. luteola* accessions also showed two different chemotypes. The accession TVNu 475 contained only quercetin (rutin and a second quercetin glycoside), while the other two accessions, the kaempferol chemotypes TVNu 172 and TVNu 905, were similar qualitatively with some quantitative differences in their flavonoid glycoside pattern; both contained robinin.

No chemotypes were distinguishable among the accessions of *V. racemosa, V. vexillata, V. oblongifolia, V. kirki,* and *V. radiata*, collected from different geographical zones.

As regards interspecific relationships, species of sections *Vigna*, *Plectotropis*, and *Ceratotropis* did not show flavonoid glycosides similar to those in cultivated lines of *Vigna*. In contrast, some relationships have been found between cultivated lines and wild species of the section *Catiang*. The flavonoid HPLC fingerprint of ssp. *dekindtiana* var. *dekindtiana* MG 112997 and ssp. *dekindtiana* var. *protracta* TVNu 965 was very similar to the cultivated lines, having four flavonoid glycosides, including the rare acyl glycosides. The occurrence of similar flavonoid patterns in morphologically distinct taxa suggests they had a common ancestor and that climate and habitat changes could have caused them to adapt morphologically, while retaining, in part, their original leaf flavonoid pattern (Panella et al. 1993; Williams et al. 1993). Minor relationships were found between cultivated lines and the other subspecies of the section *Catiang*: ssp. *stenophylla* TVNu 714 and ssp. *tenuis* TVNu 661 had two glycosides, while ssp. *dekindtiana* var. *mensensis* TVNu 862 and ssp.

dekindtiana var. *pubescens* TVNu 110 had only one glycoside in common with ssp. *unguiculata*.

In the section *Vigna*, some relationships were observed among *V. ambacensis* TVNu 755, *V. gracilis* TVNu 18, *V. marina* TVNu 717, and *V. racemosa* TVNu 96 (kaempferol chemotypes of this section based upon a combination of aglycone class [Table 1] and glycosylation pattern, because two kaempferol glycosides in common were found in these species). The quercetin chemotypes of section *Vigna*—*V. luteola* TVNu 475, *V. oblongifolia* TVNu 37, and *V. oblongifolia* TVNu 135—also showed a great similarity to one another. This agrees with the results obtained by Vaillancourt and Weeden (1993) using molecular markers. Overall, the isorhamnetin chemotypes of this section—*V. marina* TVNu 1174 and *V. heterophylla* TVNu 19, both containing kaempferol-3-rutinoside and isorhamnetin-3-rutinoside—were remarkably similar to one another. The presence of 3'-O-methylation in the B-ring of quercetin (isorhamnetin) could be considered a fairly advanced character, absent in other wild species containing quercetin and/or kaempferol (Zallocchi and Pomilio 1994). The common flavonoids in this section seem to be robinin (*V. gracilis, V. marina, V. racemosa*, and *V. luteola*), rutin (*V. oblongifolia, V. racemosa*, and *V. luteola*), kaempferol-3-rutinoside (*V. ambacensis, V. heterophylla*, and *V. marina*), and an unidentified (tR = 29.56 min) kaempferol glycoside (*V. ambacensis, V. gracilis, V. marina, V. racemosa*, and *V. luteola*). Rutin and kaempferol-3-rutinoside were also found in *V. radiata*, section *Ceratotropis*. Finally, in the section *Plectotropis, V. vexillata* containing rutin, and *V. kirki* containing kaempferol-3-rutinoside and hyperoside, represent two different flavonoid chemotypes according to their aglycone structure and glycosylation pattern.

In conclusion, flavonoid HPLC fingerprints together with other biochemical markers and/or morphological data can provide useful characters for defining species in the *Vigna* genus. From Table 1, it is evident that *Vigna* species produce essentially flavonol structures that are usually 3-O-glycosides. The most characteristic feature of these compounds in *Vigna* is the presence of flavonoid p-coumaroyl glycosides in cultivated lines, while the wild species are generally devoid of these substances, with the exception of four wild species in the section *Catiang*, and are all classified as subspecies of *V. unguiculata*. A greater variability in flavonoid aglycone class and glycosylation pattern occurs in cultivars of *V. unguiculata* ssp. *unguiculata* compared to the wild species. This observation seems to confirm that cultivation and/or domestication may cause or increase species diversification. The large differences between cowpea and wild species of *Vigna* may indicate that the cowpea has been isolated from other species for a very long time, and thus accumulated a large genetic diversity. This ancient genetic divergence may, in part, explain the lack of success in hybridization between cowpea and other species of this genus (Fatokun 1991; Vaillancourt and Weeden 1992).

Furthermore, as regards the role of endogenous flavonoids in the resistance mechanism against aphids, it has been frequently pointed out that these compounds which taxonomists use to separate species could hardly have had enough adaptive value for survival through natural selection. In fact, when the resistance characteristics to aphids in different accessions of the same species of *Vigna* have been considered, it became evident that quercetin chemotypes show a higher level of resistance compared to the kaempferol ones. These results provide useful information to further explore the gene expression of the resistance factors.

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7

Photoperiod, temperature, and the growth and development of cowpea

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Abstract

The effects of photoperiod and of temperature within the sub- and supraoptimal ranges on seed germination, seedling emergence, leaf appearance, and days from sowing to first flowering of cowpea are described. The cardinal temperatures (i.e., the base, optimum, and ceiling values) for each of these processes and events are defined. A base temperature of 8–11 °C is appropriate for all developmental processes, and the optimum temperature for most rapid reproductive development is close to 28 °C. There is considerable variation among genotypes in the responsiveness of flowering to photoperiod. Models and methods to characterize the cowpea germplasm for responses to temperature and photoperiod are described.

Introduction

Cowpeas (*Vigna unguiculata* [L.] Walp.) are grown for fodder (haulms) and food (leaves, immature pods, and seeds) in a wide range of environments, from 40 °N to 30 °S and in lowland and highland ecologies, principally in West Africa, but also in Asia, Latin America, and North America (Rachie 1985). They are especially important in the subhumid and semiarid lowlands of West Africa, between latitudes 7 and 14 °N.

Cultivars adapt to these diverse environments through considerable plasticity in phenology (i.e., time from sowing to maturity) and morphology (growth habit), the main determinants of which are responses to temperature and photoperiod (Summerfield et al. 1974; Wien and Summerfield 1980). Given the overriding importance of phenology, both in general adaptation to different ecologies and regions and specifically to duration of growing season and resource capture and use (Shorter et al. 1991; Richards 1993), this paper focuses on responses to temperature and photoperiod. Methods and models to characterize these responses in diverse genotypes will be described. The effects of photoperiod and temperature, particularly in the supraoptimal range, on the initiation and survival of reproductive structures will also be briefly considered.

Phenological development

Effects of temperature

The concept of thermal time to describe responses to temperature has several advantages (Squire 1990); its principles are illustrated here using data for seed germination (Fig. 1a).

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The rate of seed germination (expressed as the reciprocal of time to 50% germination [0.5_g, d]) increases linearly as temperature increases from the base temperature, T_b (that temperature at and below which rate of development is zero), to the optimum temperature, T_o, where rate of germination is most rapid (minimum time to germinate). As temperature continues to increase above T_o, rate of seed germination decreases linearly until the ceiling (or maximum) temperature, T_{ce} (that temperature at and above which the rate is zero), is reached. Hence, in the suboptimal range (i.e., $T_b < \bar{T} < T_o$), rate of progress towards 50% germination is given by the thermal time relation:

$$1/0.5_g = a + b\bar{T} \quad [1]$$

where T is mean temperature and a and b are genotype-specific constants. The base temperature is given by

$$T_b = -a/b \quad [2]$$

and the thermal time, θ, for seeds to germinate in the suboptimal range is

$$\theta = 1/b \quad [3]$$

Equation 1 can also be expressed as

$$1/0.5_g = (\bar{T} - T_b)b \quad [4]$$

When temperature is in the supraoptimal range (i.e., $T_o < \bar{T} < T_{ce}$), the same thermal time equations apply (Garcia-Huidobro et al. 1982), and Equation 4 becomes

$$1/0.5_g = (\bar{T} - T_o)b' \quad [5]$$

where the value of b' is negative.

These thermal time relations describe the responsiveness to temperature over the entire temperature range (i.e., $T_b < \bar{T} < T_{ce}$) for seed germination, seedling emergence, leaf appearance, and reproductive development (appearance of flower buds, open flowers, and mature pods). The responses of a range of lines and cultivars, mostly from Nigeria, are summarized in Table 1.

The developmental processes of seed germination, seedling emergence, and the appearance in sequence of flower buds, open flowers, and mature pods all have base temperatures between 8 °C and 11 °C. Among cultivars and cultivated landraces from West Africa, there were no significant differences in T_b for seed germination (Craufurd et al. 1996a), or for time from sowing to first flowering (Craufurd et al. 1996b,c), and a common T_b of 8 °C could be fitted. In contrast, within germplasm lines collected from a wide range of latitudes (0–30 °N and S) and ecologies (lowland forest to Sahel), there were significant differences in T_b for seed germination (7–14 °C), which could, in part, be related to latitude of origin (lines collected close to the equator had warmer values of T_b).

Reported values of T_b for leaf appearance range between 7–10 °C and 16 °C (Table 1). The cooler values for T_b, which agree with the values for other developmental processes,

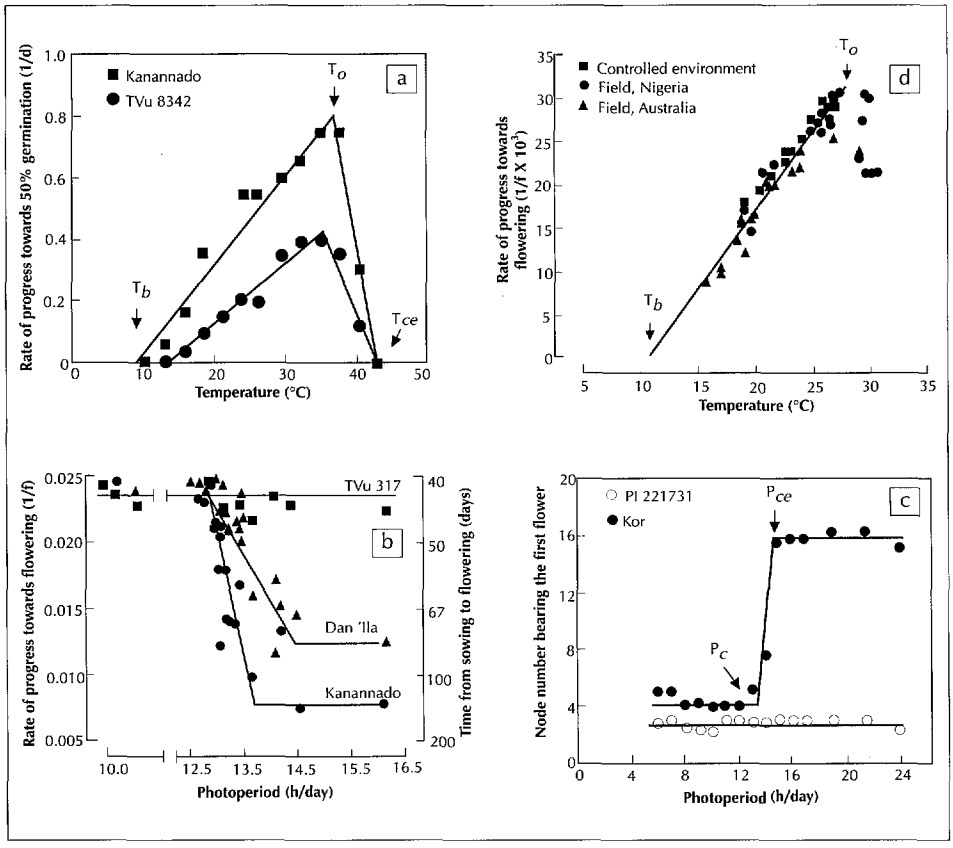

Figure 1. (a) Relation between rate of progress towards 50% germination ($1/0.5_g$, d) and temperature in cowpea cv. Kanannado and TVu 8342. T_b base temperature; T_o optimum temperature; T_{ce} ceiling temperature. (b) Effect of photoperiod (hours per day) on rate of progress from sowing to first flowering ($1/f$, d) in four genotypes of cowpea grown at mean temperatures of 25–27 °C in the field in Nigeria: TVu 317, Dan 'Ila, and Kanannado (from Craufurd et al. 1995b,c). (c) Effect of photoperiod (hours per day) on the number of the node bearing the first flower in two genotypes grown in controlled environments: PI 221731 and cv. Kor (replotted from Wienk 1963); P_c and P_{ce} are the critical and ceiling photoperiods, respectively. (d) Relation between rate of progress from sowing to first flowering ($1/f$) and mean pre-flowering temperature in the photoperiod-insensitive accession TVu 946 grown in controlled environments and in the field in Nigeria and Australia.

were determined in controlled environments over a wide range of temperatures (15–33 °C). In contrast, the value for T_b of 16 °C was obtained from field data for a single genotype grown in a narrow range of temperatures (Littleton et al. 1979) and is probably incorrect (Craufurd et al. 1997).

The optimum and ceiling temperatures for germination (35 °C and 43 °C, respectively) hardly varied among genotypes (Craufurd et al. 1996a). Those genotypes originating from arid or semiarid environments and/or known to have some heat tolerance (e.g., TVu 4552 and TVx 3236: Patel and Hall 1990; Ntare 1992) did not have warmer values of T_o or T_{ce}.

Table 1. The cardinal temperatures (base T_b, optimum T_o, and ceiling T_{ce}) and thermal time (q) for developmental processes and events in cowpea.

Process/event	Cardinal temp. (°C) T_b	T_o	T_{ce}	Thermal time (q; °Cd)	No. of genotypes	Reference
Seed germination	8–11	35	43	35	1–23	Covell et al. 1986; Craufurd et al. 1996a; Ndunguru and Summerfield 1975
Seedling emergence	11	–	–	43	5	Craufurd et al. 1996a; Angus et al. 1981
Leaf appearance	7–10, 16	≥ 28	–	30–60	1–6	Littleton et al. 1979; Craufurd et al. 1997
First visible bud	8–10	27–29	–	350–580	8–10	Hadley et al. 1983; Dow el-Madina and Hall 1986
First open flower	8–10	27–29	36	550–830	6–28	Hadley et al. 1983; Dow el-Madina and Hall 1986; Ellis et al. 1994; Craufurd et al. 1996b,c
First ripe pod	8	≥ 28	–	940–1130	10	Hadley et al. 1983

However, Covell et al. (1986) have shown that a small proportion (30%) within a population of seeds of TVx 3236 had a T_o closer to 40 °C. It is clear then that cowpea can successfully germinate in the hot soils, which characterize the semiarid tropics.

The optimum and ceiling temperatures for reproductive development (28 °C and 36 °C, respectively) were cooler than for seed germination, with no significant differences between genotypes in the studies reported to date. Given that mean temperatures during the growing season in West Africa are between 25 °C and 29 °C (Kowal and Knabe 1972; Wien and Summerfield 1980), these findings point to a thermal regime close to optimal for cowpea.

It seems reasonable to conclude that a T_b of 8–11 °C is appropriate for development in cultivated cowpea. Ong (1987) and Ong and Monteith (1985) have shown that a base temperature of 10 °C is also appropriate for all developmental processes in groundnut (*Arachis hypogaea*) and pearl millet (*Pennisetum glaucum*).

Effects of photoperiod

Photoperiod has little or no direct effect on leaf appearance (Wienk 1963; Craufurd et al. 1997), and its effects on other aspects of vegetative growth (e.g., branching and internode elongation) have not been reported. In contrast, photoperiod can have great effects on reproductive development, although some genotypes are insensitive (Ellis et al. 1994).

Cowpea responds to photoperiod in a manner typical of quantitative short-day plants (SDP), i.e., photoperiods longer than a critical value delay, but do not prevent, flowering (Njoku 1958; Wienk 1963; Lush et al. 1980; Wien and Summerfield 1980; Hadley et al. 1983; Dow el-Madina and Hall 1986; Patel and Hall 1990). Obligate or facultative SDP, where flowering will only occur in short days, have not yet been reported. Relatively photoperiod-insensitive or so-called "day-neutral types" have also been found and are widely used in breeding programs (Singh and Ntare 1985).

The appearance of flower buds, open flowers, and mature pods all respond to photoperiod in a quantitatively similar manner (Hadley et al. 1983; Dow el-Madina and Hall 1986). The initiation of floral buds and their subsequent development into flowers may require different numbers of inductive short days (Lush and Evans 1980) and/or may have different critical photoperiods (Wien and Summerfield 1980). Either way, as photoperiods shorten towards the end of the rainy season (September–October in West Africa; February–March in southern Africa), these adaptive features ensure timely flowering (Wien and Summerfield 1980).

The effects of photoperiod (when temperature is more or less constant) on days from sowing to first flower in four genotypes grown in Nigeria (Fig. 1b), and on the number of the node bearing the first flower in two genotypes grown in controlled environments (Fig. 1c), combine to illustrate several concepts on the topic commonly and uncritically referred to as "photoperiod sensitivity."

It is clear that TVu 317 (Fig. 1b) and PI 221731 (Fig. 1c) are insensitive to photoperiod; rate of progress towards flowering ($1/f$) in TVu 317 is constant (i.e., flowering occurs about 40 days from sowing, irrespective of photoperiod), while in PI 221731 the first flower appears at the same node in all photoperiods between 10 h and 16 h per day. However, photoperiod delays flowering ($1/f$ is reduced) in Dan 'Ila, TVu 1188, and Kanannado (Fig. 1b), and changes the nodal position at which the first flower opens in cv. Kor (Fig. 1c), but only when photoperiod is longer than a critical value. That critical photoperiod, P_c, above which longer photoperiods delay flowering, can vary; for example, between 12 h 15 min and 13 h 20 min at 25–27 °C as in Fig. 1b, or over a much wider range (11–16 h per day) in other genotypes at different temperatures (Njoku 1958; Wienk 1963; Wien and Summerfield 1980; Hadley et al. 1983; Dow el-Madina and Hall 1986; Craufurd et al. 1996c). Below P_c, photoperiod has no effect on time from sowing to flowering, and so plants respond as if they are photoperiod insensitive.

There were significant differences between genotypes in their relative response to photoperiod (i.e., the slope when $P > P_c$), ranging from slightly sensitive (Dan 'Ila) to acutely sensitive (Kanannado) (Figs. 1b and 1c). The photoperiod-responsive range has an upper limit or ceiling, P_{ce}, defined as that photoperiod at and above which neither photoperiod nor temperature (Roberts and Summerfield 1987) has any further effect on days from sowing to flowering (or on the number of the node at which the first flower appears), and so days from sowing to flowering is thus a constant and maximum value. The ceiling photoperiod (at a mean temperature of 25–27 °C) is about 13.8 h per day in Kanannado and 14.7 h per day in Dan 'Ila, when the maximum periods to flowering are 132 days and 82 days, respectively. The ceiling photoperiod is a useful adaptive feature, because it ensures that those photoperiod-sensitive genotypes will eventually flower in environments that are not inductive.

In summary, in order to characterize genotypes for "photoperiod sensitivity," information is required on the value of P_c, on the effect of photoperiod on rate of development when $P_c < P < P_{ce}$, on the value of P_{ce}, on the maximum time to flower when $P \geq P_{ce}$, and on the way in which P_c and P_{ce} may vary with T.

These responses to photoperiod can be described and quantified in a manner analogous to a thermal time approach. Hence, when temperature is constant (and $T_b < T_o$) and mean photoperiod, P, is longer than P_c but shorter than P_{ce} (i.e., $P_c < P < P_{ce}$), days from sowing to flowering (f) can be described by

$$1/f = a' + c'P \qquad [6]$$

where a' and c' are genotype-specific constants. The value of the constant c' is a measure of photoperiod sensitivity and is negative for SDP (Summerfield et al. 1993; Craufurd et al. 1996c).

When $P > P_{ce}$ there is no further delay in flowering, and so

$$1/f = d' \qquad [7]$$

where d' is a genotype-specific constant.

Where responses have been quantified in this manner (Hadley et al. 1983; Ellis et al. 1994; Craufurd et al. 1996c), there is between genotypes about a 15-fold difference in photoperiod sensitivity, c', large differences in P_{ce} and d' but much less variation in P_c (Table 2).

The genetics of photoperiod sensitivity have not been clearly elucidated (Fery 1985), probably because the number of days from sowing to flowering (the most common data recorded to assess sensitivity) is in fact an expression of earliness (i.e., minimum time to flowering when $T = T_o$ and $P = P_c$), combined with the consequences of genes for sensitivity to temperature and photoperiod. Clearly, to assess genes for photoperiod sensitivity, it is essential that temperature be constant and near optimal, and that photoperiod lies between P_c and P_{ce}; outside these limits, it is not the effect of photoperiod *per se* on flowering that will be described.

Table 2. Variation in earliness (minimum time to flower), base temperature (T_b), temperature sensitivity ($_b$), critical (P_c) and ceiling photoperiod (P_{ce}), photoperiod sensitivity (c') and maximum time to flower ($1/d'$) among cowpea genotypes examined in Nigeria (from Craufurd et al. 1996b,c).

Value	Earliness† (DAS)	T_b (°C)	Temperature sensitivity (10^4 x b)	P_c (h/day)§	Photoperiod sensitivity (10^4 x b)	P_{ce} (h/day)§	$1/d'$ (days)
Minimum	35	7.3	12.1	12.2	–1.2	<16	86
Maximum	99	10.1	16.5	13.4	–18.2	13.8	138
Mean	50	7.6	13.0	12.8	–8.9	–	108

† Source: IITA (1974); days after sowing.

§ Values of P_c and P_{ce} when mean temperature = 27 °C.

Modeling flowering responses to temperature and photoperiod

In natural environments, both temperature and photoperiod responses affect days from sowing to flowering. After substantial work in controlled environments and in numerous field locations, simple linear-rate models incorporating responses to temperature and photoperiod have now been developed (Roberts and Summerfield 1987; Summerfield et al. 1991).

In photoperiod-insensitive genotypes and in photoperiod-sensitive genotypes when $P < P_c$, rate of progress from sowing towards flowering ($1/f$) can be quantified by the thermal time relation (Equation 1). For example, TVu 946, a photoperiod-insensitive genotype, was grown in controlled environments in Reading (mean temperature 19–27 °C), in the field in Australia (mean temperature 16–29 °C) and in the field in Nigeria (mean temperature 19–30 °C) under photoperiods of 11–16 h per day. Progress from sowing to flowering in these diverse environments is accurately described by Equation 1 (Fig. 1d), given that T_o is close to 26–27 °C.

In photoperiod-sensitive genotypes, when $P_c < P < P_{ce}$, then rate of progress towards flowering is determined by temperature (Equation 1) and photoperiod (Equation 6) in an additive manner (i.e., there is no interaction between photoperiod and temperature), and so

$$1/f = a' + b' + c' P \qquad [8]$$

where a', b' and c' are genotype-specific constants. In this so-called photothermal plane, warm suboptimal temperatures ($T_b < \bar{T} < T_o$) hasten flowering (b' is positive) and longer photoperiods delay flowering (c' is negative). However, the response to P is often so dramatic that the value of b' tends to zero (i.e., the temperature response is completely masked) (Fig. 2a).

The critical photoperiod, defined earlier, is the intersection of the thermal and photothermal plane, and it can vary with temperature (Roberts and Summerfield 1987). In practice, photoperiod may well transgress both P_c and P_{ce} at various times during the growing season, and so models which incorporate the thermal, photothermal, and plane of maximum delay are required. Such a three-plane model was evolved for TVu 1188, a moderately photoperiod-sensitive genotype, derived from data collected in controlled environments at Reading and in the field in Australia and Nigeria (Fig. 2a).

Experience with these models in Australia (Summerfield et al. 1993; Ellis et al. 1994) and Nigeria (Craufurd et al. 1996c) has confirmed their value and utility for characterizing genotypes for flowering responsiveness to temperature and photoperiod. Studies with the maturity isolines of soybean (*Glycine max* cv. Clark) have confirmed that the constants derived from the models (i.e., a, b, a', b', c', and d') do indeed describe unambiguous genetic effects (Upadhyay et al. 1994).

Screening for photothermal responses

There are two key factors in screening for responses of flowering to temperature and photoperiod: (1) in order to describe the response to temperature in photoperiod-sensitive genotypes, short days (i.e., $P < P_c$) must be used to remove any confounding with photoperiod; and (2) in order to describe responses to photoperiod, a range of photoperiods, shorter and longer than P_c and P_{ce}, respectively, must be used, preferably at a

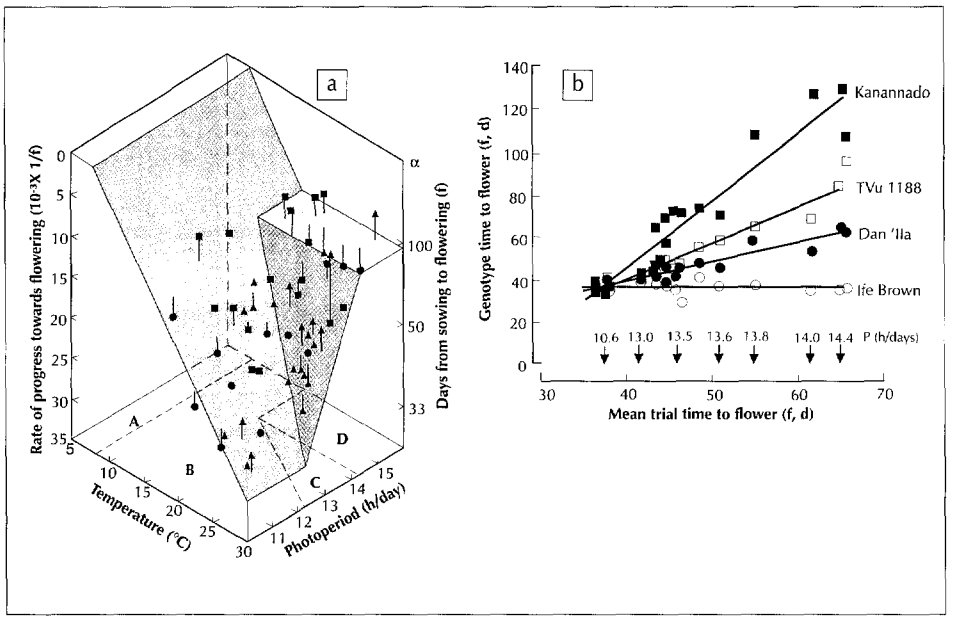

Figure 2. (a) Photothermal flowering response of cowpea accession TVu 1188 grown in controlled environments and in the field in Nigeria and Australia. The model fitted (Equations 1, 7, and 8) gave: a = – 0.0109 (SE 0.00326), b = 0.0014 (0.00014)***, T_b = 7.9 °C (thermal plane, projected onto Sector B); a′ = 0.1413 (0.02208)***, b′ = 0.0001 (0.00037)^{NS}, c′ = – 0.0092 (0.00136)*** (photothermal plane, projected onto Sector C); and d′ = 0.0106 (0.00078)*** (plane of maximum delay, projected onto Sector D). R^2 = 80.9%; n = 55. The broken line between Sectors A and B is the base temperature. The distances of the experimental points from the fitted response surfaces are shown by vertical lines extending above or below the symbols. (b) Analysis of photoperiod sensitivity among four genotypes of cowpea: fitted relations between genotype time to flower *(f,* d) and mean trial time to flower (*f,* d) when mean photoperiod varies from 10 to 14.5 h/day and mean temperature is 25–28 °C in Ife Brown, Dan 'Ila, TVu 1188 and Kanannado. Mean trial *f* is determined from observations on 21 genotypes. Arrows show the mean photoperiod for particular cohorts of data points.**

temperature close to the optimum to minimize confounding effects. A minimum cohort of six or seven environments are thus required (Table 3).

The environments listed in Table 3 can be created easily in the field, often at just one or two locations, by using different sowing dates and the natural consequent variations in temperature and photoperiod, combined with simple light-excluding coverings or photoperiod-extension treatments (e.g., Craufurd et al. 1996b,c). Alternatively, a network of locations exhibiting the target seasonal variations in temperature and photoperiod can be used.

We have also shown (Craufurd et al. 1996d) that by using an *a priori* knowledge of P_c, and thereby classifying locations into those screening for temperature or photoperiod, a stability or sensitivity analysis (Finlay and Wilkinson 1963) of days from sowing to flowering can also be used to screen for (relative) photoperiod sensitivity (Fig. 2b). This approach works well in Nigeria, because mean temperatures during the growing season

Table 3. Minimum number of target photothermal environments for screening for thermal and photoperiodic flowering responses.

	Photothermal environment	
	Photoperiod (h/day)	Temperature (°C)
Temperature response	Short days (< P_c)	Cool (15–20°)
	Short days† (< P_c)	Warm days† (28°)
Photoperiod response	13.0	Warm (28°)
	13.5	Warm
	14.0	Warm
	24.0	Warm§

† Also screens for minimum time to flower, and thus the presence of any juvenile phase.
§ Screening for maximum time to flower and to confirm all genotypes to be quantitative SDPs.

vary only slightly between latitudes 7 °N and 13 °N and, therefore, variation in photoperiod is the most important environmental factor determining days from sowing to flowering. However, in other countries or regions, where there is more variation in temperature and photoperiod between locations, such a simplified approach may not be appropriate.

The effect of photoperiod and temperature on flower bud, open flower, and mature pod production

Studies in various legumes, notably bambara groundnut (*Vigna subterranea*) (Linnemann 1991; Linnemann and Craufurd 1994) and groundnut (*Arachis hypogaea*) (Flohr et al. 1990), have shown that long photoperiods during reproductive growth reduce flower and pod production and thus seed yield. Indeed, bambara groundnut genotypes that are insensitive to photoperiod with respect to the onset of flowering can then be markedly sensitive with respect to pod production. Similar studies have not been reported in cowpea, though it seems reasonable to assume that long days affect postflowering growth and development in cowpea in a similar manner. However, given that reproductive development and podding in most cowpea growing environments (e.g., in West Africa) occur when photoperiod is shortening, these effects are probably not of major importance.

The effects of supraoptimal temperature on reproductive development in cowpea have been researched by Hall and his coworkers in California (Hall 1992). These studies have shown substantial effects of high night temperature (24–30 °C) on bud development, as well as pod and fruit set (e.g., Warrag and Hall 1984; Dow el-Madina and Hall 1986; Patel and Hall 1990). Their work has also shown marked interactions between supraoptimal temperature and photoperiod: hot, long days (> 14 h per day) can suppress floral bud development and reduce pod set compared with that on hot, short days (Warrag and Hall 1984; Dow el-Madina and Hall 1986; Mutters et al. 1989). These studies suggest that while mean daily temperature has proved effective in modeling phenology, there may be circumstances where high night temperature can have specific effects on phenology that need to be taken into account. Three putative genes (*pt1*, *pt2*, and *pt3*) have been identified which

confer heat tolerance under hot long-day conditions (Hall 1992); a fourth gene (*Ha*) confers heat tolerance during later periods of pod set (Marfo and Hall 1992). Significant differences in the ability of genotypes to set pods under high temperatures have been observed in California (Patel and Hall 1990) and in Niger (Ntare 1992).

Utilizing photothermal responses in breeding programs

Given that flowering dates are routinely recorded in breeding trials and national and international yield trials, and that many scientists are familiar with regression techniques, we believe that the approaches outlined here can be easily and simply applied without recourse to additional trials to characterize genotypes. The careful selection of sites for their contrasting photothermal environments, similar to the manner in which locations are chosen for disease or insect pressures, should contribute substantially to an improved understanding of phenological adaptation.

There is obviously considerable variability within the cowpea germplasm for photoperiod sensitivity, even though most of the genotypes examined to date have originated within West Africa. There appears to be much less variation in response to temperature these materials, and there is an urgent need to evaluate genotypes from other regions. There is also considerable variability in the germplasm for inherent earliness (Table 2), and so the development of photoperiod-insensitive, medium- to long-duration genotypes (i.e., akin to the long-juvenile trait in soybean: Sinclair and Hinson 1992) would be feasible and worthwhile.

The selection of appropriate strategies for phenological adaptation in different cropping environments, in terms of inherent earliness and sensitivity of progress towards flowering to temperature and photoperiod, can now be evaluated, using long-term weather databases and the phenology models presented in this paper.

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8

Water-use efficiency and drought adaptation of cowpea

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Abstract

Adaptation of cowpea to water-limited conditions was examined using a model, which assumes that grain yield is dependent on the sum of transpiration per day × water-use efficiency (W) × partitioning of carbohydrate to grain, between first flowering and maturity. Measurements of leaf stable carbon isotope composition were shown to be useful for selecting for differences in W in cowpea. In selection experiments, genotypic mean grain yields in both adequately irrigated and dry environments were positively correlated with carbon isotope discrimination, indicating negative correlations with W and genotypic variation in stomatal conductance. Consequently, general adaptation may be associated with low W. It is not clear, however, whether smaller W would be adaptive in all dry environments, because theory suggests that high W should be adaptive in very dry environments, where genotypes have the same limited supply of water. Genes conferring contrasting W appeared to differ between tropical Senegal and subtropical California. Delayed leaf senescence (DLS) was shown to enhance adaptation to drought by increasing the plasticity of the duration between first flowering and maturity. Plants with early flowering and DLS resist midseason drought by having the ability to recover in response to late-season irrigation. Genes responsible for DLS were consistently expressed in Senegal and California.

Introduction

Progress in breeding cultivars for dry environments has been slow, and achieved mainly by yield testing advanced lines over several locations and years. An example of this approach for cowpea (*Vigna unguiculata* [L.] Walp.) is the breeding of cultivars "Mouride" (Cisse et al. 1995) and "Melakh" (Cisse et al. 1997) in Senegal. This empirical approach is slow because of the need to assess the yield of large numbers of lines across several locations and over several years, and the substantial variation from the effects of environment, error, and genotype × environment interactions. Physiological, morphological, or phenological criteria that could be selected in early generations would complement empirical breeding methods, based upon yield evaluation of advanced lines. Selection for phenological traits,

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such as date of flowering, and for morphological traits, such as the presence of awns in wheat, has been useful in some cases, but there has been little use of physiological criteria in breeding for improved adaptation to dry environments (Blum 1988).

Several physiological processes have been suggested as being involved in plant adaptation to dry environments, including osmotic adjustment and desiccation tolerance (Basnayake et al. 1993). Cowpea, however, has exhibited little osmotic adjustment in leaves (Shackel and Hall 1983, 1984), and no significant genotypic differences in osmotic potential of leaves were detected in earlier research (Hall and Patel 1985). Some genotypic differences have been reported in the ability of cowpea to survive drought imposed during the vegetative stage under field conditions (Watanabe et al. 1997). Studies using grafting and different pot volumes demonstrated that ability to survive drought depended on shoot characteristics when the root zone was restricted, and the combination of shoot and root characteristics when long pots were used (Watanabe et al. 1997). However, cowpea cultivars already have substantial ability to survive vegetative stage drought (Turk et al. 1980) associated with maintenance of high leaf water potentials (Turk and Hall 1980; Petrie and Hall 1992a). For breeding purposes, metrical traits, thought to confer adaptation to drought, should be evaluated in terms of their influence on grain yield and, in some cases, forage yield. Mathematical models provide a way to relate yield to the levels of expression of different metrical traits.

Model for plant yield in water-limited environments

Studies have been conducted in which cowpea was subjected to drought during the vegetative stage (prior to floral buds reaching the macroscopic stage, where they are visible) and the reproductive stage (Ziska and Hall 1983; Ziska et al. 1985). These studies demonstrated that grain yield of cowpea is strongly dependent upon the water supply during the reproductive stage, with relatively little influence of vegetative-stage drought on grain yield. A simplified model is proposed, based upon these observations.

$$Y = \sum_{i=o}^{i=n} T_i \times W_i \times HI_i$$

where grain yield/area (Y) depends upon the duration in days from the first appearance of macroscopic floral buds (i = o) to physiological maturity of the latest-maturing pods (n), transpiration per day (T_i), water-use efficiency per day (W_i is plant dry biomass production per day/T_i), and harvest index per day (overall HI is defined as grain biomass/total plant dry biomass, and it often is estimated based on the ratio of grain biomass to total shoot biomass). A more complete model, which includes evaporation from the soil, has been discussed by Hall et al. (1994a). These models would be very useful in plant breeding if the various components (n, T_i, W_i, and HI_i) were relatively independent or positively associated, so that increases in any component would increase yield. Unfortunately, this appears not to be the case. The value of T_i depends upon the evaporative demand, extent of canopy coverage of the ground surface, and canopy conductance to water vapor. The magnitude of W_i depends upon the leaf internal [CO_2], which is influenced by both the leaf conductance to water vapor and the photosynthetic capacity of the mesophyll cells.

The magnitude of HI_i depends upon the extent of partitioning of dry matter to grain, leaves, stems, roots, and nodules. The size of n depends upon the factors influencing the duration of pod production, which are complex and poorly understood. An example follows of possible interactions among these components.

Assume that a genotype is developed with deeper roots than a local cultivar and consider whether it would have greater grain yield in a water-limited environment. The deeper roots would enable the genotype to access more soil water and this would have the following benefits. The genotype could have a longer reproductive period (n) and, through indirect effects on canopy conductance and canopy growth, greater daily transpiration (T_i) and photosynthesis, and possibly greater nitrogen fixation than the local cultivar. The deeper roots also could have negative effects on yield. Any greater partitioning of carbohydrate to roots during the reproductive stage would reduce the amount of carbohydrate available for partitioning to grain (HI_i), compared with the local cultivar. Also, where greater root access to soil moisture results in more open stomata and greater leaf conductance to water vapor, it will tend to decrease daily water-use efficiency (W_i). Apparently, the highest grain yields in water-limited environments will be achieved by cultivars that have optimal, intermediate expressions of n, T_i, W_i, and HI_i (Hall 1981). The specific intermediate level of expression of a trait that is adaptive will depend upon the environmental conditions. For example, roots of a particular depth would only be adaptive if they access adequate soil moisture at times when the water considerably influences grain yield (Passioura 1972). Since rainfall varies widely from year to year in semiarid climatic zones, any useful variation in trait expression would increase the extent of adaptation. For example, deeper roots would be more adaptive in years where adequate moisture is available deeper in the profile. In a dry year, where only the upper layers of the soil profile are moist, a more superficial root system would be adaptive.

The last implicit component of the model is the date of first flowering. The level of this trait that is adaptive is governed by the same rules as the other traits in the model. Intermediate expression is needed with the specific date of flowering that is optimal, depending upon the specific environmental conditions. Useful plasticity in the duration of the reproductive phase from first flowering to maturity would increase the extent of adaptation, as will be shown in the discussion of delayed leaf senescence.

Experimental selection and evaluation of model traits

Four factors determine the usefulness of a trait in plant breeding: (1) the ability to screen for the trait and discover accessions with differences in trait expression that can be used as parents; (2) the ability to transfer the trait from an exotic parent into a desirable genetic background; (3) the influence of the level of trait expression on crop yield and adaptation in different environments; and (4) the presence of correlations between the levels of expression of a trait and those of other traits, which have either negative or positive consequences for adaptation. These factors will be considered when discussing experimental studies with cowpea of the various model traits.

Transpiration

Depending upon the environmental circumstances, adaptation to water-limited environments may be enhanced by selecting for either greater or smaller T_i. Sensitive stomatal

closure in response to soil drying would reduce T_i and enable plants to survive severe drought and more efficiently use water (higher W_i). Apparently, cowpea cultivars already have very sensitive stomatal responses to soil drying (Bates and Hall 1981; Shackel and Hall 1983; Petrie and Hall 1992a) that result in higher W_i (Hall and Schulze 1980; Kirchhoff et al. 1989; Hall et al. 1992; Ismail and Hall 1993a,b). Cultivars with deep rooting, which access more soil moisture, would have greater T_i than those with shallow rooting, which lack adequate water in their root zone. Accessions can be evaluated for depth of rooting by growing them in tubes (Watanabe et al. 1997). Alternatively, rooting can be evaluated under natural soil conditions by placing an herbicide deep in the soil and scoring plant response as an indicator of the time taken for the roots to reach the herbicide (Hall and Patel 1985; Robertson et al. 1985). The influence of rooting on plant water relations is complex, however, and the uniformity of root distribution may have a greater impact on the maintenance of plant water status than the density of roots (Petrie and Hall 1992a,b,c; Petrie et al. 1992).

Water-use efficiency and harvest index

Research conducted at the beginning of this century with plants in pots established that genotypic differences were present in W, the ratio of seasonal plant biomass production to seasonal transpiration (Briggs and Shantz 1914). From that time until the 1980s, few evaluations were conducted of genotypic differences in W. Pots are needed to facilitate measurements of transpiration and root biomass, and the studies require much labor and the soil environment is artificial. The value of W can be estimated by instantaneous gas exchange measurements of the ratio of CO_2 uptake to transpiration. However, gas exchange measurements have not been effective in detecting genotypic differences in cowpea (Hall et al. 1992). A major breakthrough occurred in the early 1980s when Farquhar et al. (1982) demonstrated that, according to theory for C_3 plants, W should be related to the extent that plants discriminate against the heavy stable isotope ^{13}C compared with the more abundant isotope ^{12}C (Δ) in photosynthetic uptake of CO_2. The negative correlation between W and Δ is due to their mutual dependence upon the $[CO_2]$ inside leaves. Empirical studies have now shown for many C_3 species that for plants growing in the same aerial environment, there is a strong negative correlation between W and Δ, as expected based on theory (Hall et al. 1994a). The value of this correlation to breeding is that Δ can be measured on plants in field nurseries, whereas W cannot. In principle, large numbers of genotypes could be evaluated, since it is simply necessary to take leaf samples (preferably at the same nodal position), dry them, grind them, and measure Δ using an isotope ratioing mass spectrometer. Unfortunately, such measurements are expensive (about US $15/sample), although methods are available for reducing costs (Hall et al. 1994a).

For cowpea, genotypic differences in Δ have been described under field conditions (Hall et al. 1990, 1992, 1994b). The genotypic differences in Δ are negatively correlated with W, as expected (Ismail and Hall 1992). Studies with reciprocal crosses indicated that inheritance of Δ and W are determined by nuclear genes (Ismail and Hall 1993a). Realized heritabilities were low for Δ (0.25 and 0.31), and not as high as for days to flowering (0.89 and 0.96) (Menéndez and Hall 1995). The data indicated that selection for earliness could be effective in the F_2 generation, whereas selection for Δ should be conducted with

advanced families. Genetic correlations were observed between Δ and days to flowering, which were negative and small to intermediate in magnitude (–0.14 to –0.66) (Menéndez and Hall 1995). This indicates that selection for earliness in the F_2 generation could result in some indirect selection for high Δ and, therefore, low W. In addition, in one cross, genetic correlations were observed between Δ and HI (0.38 and 0.43) (Menéndez and Hall 1996). This indicates that selection for low Δ could result in some undesirable, indirect selection for low HI. However, by simultaneously imposing selection pressure for these traits, it should be possible to obtain plants with appropriate levels of earliness, HI, and Δ. What is not clear is whether breeders should select for low Δ (and high W) or high Δ (and low W).

Theory and the model suggest that for dry environments, where genotypes have the same limited supply of water, one should select for low Δ to attain high W. However, studies in two water-limited environments in northern Senegal indicate a tendency for well-adapted local cultivars to have high Δ and poorly adapted exotics to have low Δ (Hall et al. 1994b). Also, well adapted cultivars and advanced lines selected for high grain yield under irrigated conditions in California have high Δ (Hall et al. 1993). In addition, substantial genotype × environment interaction was observed for Δ, when comparing the same set of genotypes in a tropical zone (northern Senegal) and in two subtropical zones (southern California and the high plains of Texas) (Hall et al. 1994b). Comparisons of Δ values between the tropical zone and the two subtropical zones indicated no consistency in genotypic ranking, and correlation coefficients for genotypic comparisons were small. The genotype × environment interaction for Δ would not necessarily constrain cowpea breeding programs in developing improved cultivars for specific target production regions. In fact, genotypic rankings for Δ were quite consistent between wetter and drier environments, and different years, within the study sites in Senegal, Texas, or California. However, cowpea performance with respect to Δ and W does not appear to be transferable to radically different production zones. Apparently, attainment of high Δ requires different sets of genes in radically different production zones. This is consistent with the observation that cowpea cultivars developed for specific production zones often have low yields in other production zones.

Selection studies also indicate that high Δ may be associated with high yields and local adaptation. A breeding program was conducted in which three parents with low Δ were crossed and progeny were selected for earliness, high grain yield, and high shoot biomass production under dry conditions (imposed by providing only moisture stored in the soil after the early vegetative stage) in the F_2 and F_5 generations (Hall et al. 1993). In addition, two parents with high Δ were crossed and progeny were selected for heat tolerance, high harvest index, and high grain yield under adequately irrigated conditions in the F_2 and F_4 generations (Hall et al. 1993). Then yield trials were conducted under both adequately irrigated and dry conditions (at the University of California, Riverside) with nine selections from the low Δ parent progeny, six selections from the high Δ parent progeny, and three well-adapted lines that had been bred by selection for yield under adequately irrigated conditions.

Under dry stored-soil-moisture conditions, Δ of the genotypes was positively correlated with grain yield (Fig. 1a) and shoot biomass (Fig. 1b). Even the subset of genotypes from the progeny of the low Δ parent cross, which had been selected for yield under dry

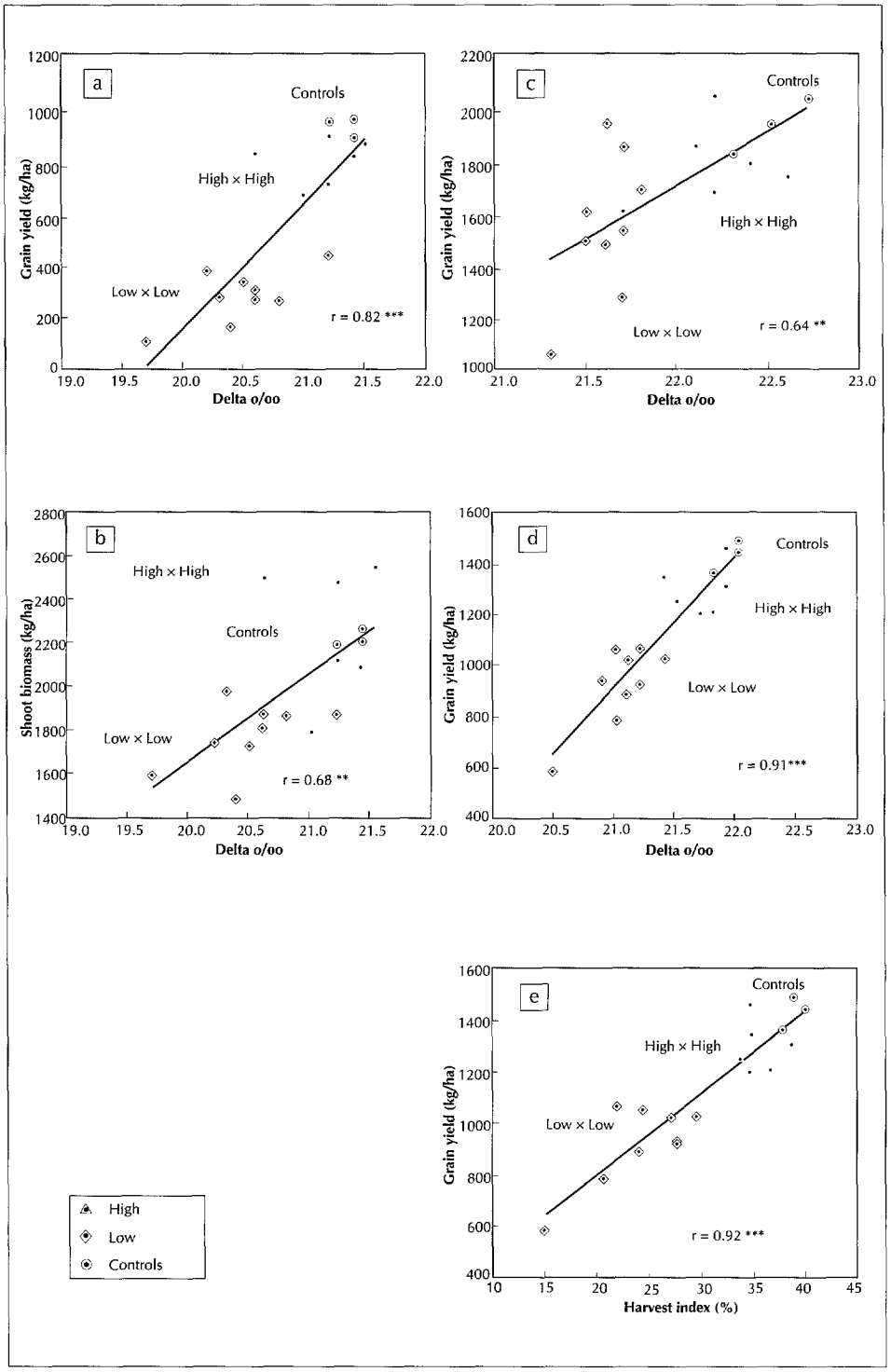
a
Controls
High × High
Low × Low
r = 0.82 ***
Grain yield (kg/ha)
Delta o/oo
c
Controls
High × High
Low × Low
r = 0.64 **
Grain yield (kg/ha)
Delta o/oo
b
High × High
Controls
Low × Low
r = 0.68 **
Shoot biomass (kg/ha)
Delta o/oo
d
Controls
High × High
Low × Low
r = 0.91***
Grain yield (kg/ha)
Delta o/oo
e
Controls
High × High
Low × Low
r = 0.92 ***
Grain yield (kg/ha)
Harvest index (%)
High
Low
Controls

Figure 1. (opposite) (a) Correlations between leaf carbon isotope discrimination (delta is Δ in parts per thousand) and grain yield of cowpea genotypes grown under dry stored-soil-moisture conditions at Riverside, California, in 1992. Low x low are F_6 progeny selected under dry conditions from crosses among three parents with low Δ. High x high are F_5 progeny selected under wet conditions from a cross between two parents with high Δ. Controls are well-adapted lines bred by selection for yield under adequately irrigated conditions. (b) Correlations between leaf carbon isotope discrimination and total shoot biomass of cowpea genotypes grown under dry stored-soil-moisture conditions at Riverside, California, in 1992. (c) Correlations between leaf carbon isotope discrimination and grain yield of cowpea genotypes grown under adequately irrigated conditions at Riverside, California, in 1992. (d) Correlations between leaf carbon isotope discrimination and grain yield of cowpea genotypes grown under adequately irrigated and dry stored-soil-moisture conditions at Riverside, California, in 1992. (e) Correlations between harvest index, measured as grain yield/total shoot biomass, and grain yield of cowpea genotypes grown under adequately irrigated and dry stored-soil-moisture conditions at Riverside, California, in 1992. For (a), (b) and (c), values are genotypic means averaged across four plots in randomized blocks. For (d) and (e), values are genotypic means averaged across both the adequately irrigated and the dry experiments.

conditions, exhibited positive correlations between Δ and grain yield ($r = 0.67$; $n = 9$) and shoot biomass ($r = 0.50$; $n = 9$). Plants with low Δ, which probably had high W, may have had low shoot biomass under conditions of severe water limitation, because they partitioned substantial biomass to roots. But we were unable to test this hypothesis.

Under adequately irrigated conditions also, grain yields were positively correlated with Δ (Fig. 1c). In addition, a very strong positive correlation was observed between average grain yield across adequately irrigated and dry environments, and Δ for all genotypes (Fig. 1d) and the progeny from the low Δ parent cross ($r = 0.79$; $n = 9$). The genotypic variation in average grain yield was mainly attributable to variation in harvest index for all genotypes (Fig. 1e) and the progeny from the low Δ parent cross ($r = 0.75$; $n = 9$). Apparently, general adaptation with respect to grain yield is associated with high Δ, and presumably low W.

The extent to which the positive correlation between grain yield and Δ is due to physiological processes or genetic linkage is not known. If the genotypes varied in stomatal conductance, as has been observed for some cowpea genotypes (Ismail and Hall 1993b), those with higher conductance would have higher Δ. Under well-watered conditions, they also could have greater seasonal photosynthesis, and more grain yield. Similar results were observed for wheat genotypes under well-watered conditions by Condon et al. (1987). For plants under dry conditions, the results are more difficult to interpret, because higher conductance would only result in greater seasonal photosynthesis if these genotypes also had access to more soil water, so that they maintain higher transpiration rates over longer periods than the genotypes with lower stomatal conductance. It should be noted that the progeny from the low Δ crosses were subjected to selection for large total shoot biomass under stored-soil-moisture conditions, which could have resulted in indirect selection for deeper and more effective root systems. As to genetic linkage, selection increased Δ in the progeny from the low Δ crosses, but it may have been caused by the selection for earliness and high harvest index, which Menéndez and Hall (1995, 1996) showed to have genetic correlations with Δ. A very strong positive correlation was observed between average HI and leaf Δ for all genotypes ($r = 0.93$; $n = 18$) and the progeny from the low Δ parent cross

($r = 0.76$; $n = 9$). Genetic correlations between HI and leaf Δ were observed also for another cowpea cross (Menéndez and Hall 1996). The physiological basis of this correlation is not known.

Genotypic means for 20 cowpea accessions averaged over 17 trials in Kenya exhibited positive correlations between grain yield and Δ measured in grain (R.B. Austin 1991, personal communication). Studies with other C_3 species also showed positive correlations between grain yield and Δ under well-watered conditions (Condon et al. 1987; Morgan et al. 1993) and dry conditions (Craufurd et al. 1991). We speculate that selection for high Δ may be useful in enhancing general adaptation and yield potential of cowpea. But for cowpea grown in very dry environments, where genotypes have the same limited supply of water, it is not clear whether one should select for low, high, or intermediate Δ. The higher W associated with lower Δ should be adaptive in these conditions. Also, sensitive drought-induced stomatal closure could enhance the ability of plants to survive vegetative-stage drought (Watanabe et al. 1997), and would result in lower Δ.

Complex responses have been observed with F_1 hybrids of cowpea compared with their parents (Ismail et al. 1994). The hybrids had high Δ (and low W), similar to the high Δ parent, in adequately irrigated conditions with unrestricted rooting, and low Δ (and high W), similar to the low Δ parent, in dry, restricted-rooting conditions. This suggests a mechanism whereby hybrids may be more broadly adapted than parents if high Δ is advantageous in adequately irrigated conditions, and low Δ in very dry conditions. The different response of the hybrids to soil conditions, compared with the parents, may be from differences in root signaling because, under dry conditions, the hybrids had more abscisic acid (ABA) in their xylem sap than did their parents (Ismail et al. 1994). The higher concentration of ABA could result in greater drought-induced stomatal closure in the hybrids, which would result in higher W and lower Δ, compared with midparent means, which were observed.

Selection for high HI may be useful in some conditions. The HI was positively correlated with grain yield (Fig. 1e) and Δ, and it is cheaper to measure than Δ. In field nurseries, HI can be estimated by the ratio of grain yield to total shoot biomass. Yield potential of cowpea at high plant densities was positively correlated with HI measured at low plant densities (Kwapata and Hall 1990). This indicates that the yield potential could be raised by selecting for high HI with widely spaced plants in the F_2 generation. This possibility is supported by the observation that realized heritabilities of HI for two cowpea crosses were moderate (0.14 and 0.42) (Menéndez and Hall 1996). Early-generation selection for HI is probably most useful within populations from crosses involving at least one exotic parent with low yields. Selection for HI may not be effective with populations from crosses among elite parents that already have relatively high HI.

Duration of flowering and podding

Early erect cowpea cultivars, which begin flowering about 30 days after sowing in the tropics, have proved to be useful in some dry environments and years because of their ability to escape drought (Hall and Patel 1985). Unfortunately, these cowpea cultivars may be more sensitive to midseason drought than medium-cycle spreading cultivars (Thiaw et al. 1993). Grain yield of cowpea is more sensitive to soil water deficits during flowering and pod-filling than during the vegetative stage (Turk et al. 1980; Ziska and Hall 1983;

Ziska et al. 1985). Indeterminate cowpeas have been discovered that begin flowering early, but have delayed leaf senescence (DLS) after producing the first flush of pods, which enables them to produce a second flush of pods (Gwathmey et al. 1992a). The DLS was shown to enhance adaptation to midseason drought by permitting substantial recovery in pod production after the drought (Gwathmey and Hall 1992). The combination of early flowering and DLS may increase adaptation of cowpea to environments which frequently experience either terminal or midseason droughts. An early cultivar would be effective when the rainy season is short, but without droughts at midseason, and the DLS would enhance adaptation when midseason droughts occur, and increase forage production and quality. The DLS results from a higher proportion of plants surviving after the production of the first flush of pods and probably results from the maintenance of root viability (Gwathmey et al. 1992b), which could enhance nitrogen fixation, which is very sensitive to drought (Elowad and Hall 1987).

Field nurseries, where the seasonal supply of water through irrigation or rainfall is adequately long and pests were controlled, were shown to be effective in screening for DLS in Riverside, California, and Bambey, Senegal. An adequate supply of soil water is necessary, because all genotypes will senesce if they are subjected to an extended drought during pod maturation. In making selections, DLS must be combined with early flowering and substantial production of pods during the first pod set. Where few or no pods are produced during the first flush, due to attacks by insects or male sterility or pod picking or other factors, a "false" DLS is induced which has limited agronomic value. Consistency of genotypic ranking for DLS of cultivars and some progeny from crosses over two years in both a subtropical and a tropical environment (Table 1) indicates that DLS may have a heritability high enough to permit breeding, and it is not substantially affected by genotype × environment interactions. In subtropical conditions in Riverside, California, with moderate night-time temperatures, the first flush of pods is fully mature about 100 days after planting, and obtaining the second flush of pods requires a season of about 140 days

Table 1. Expression of delayed leaf senescence in four cultivars (CB5, Melakh, Mouride, and Ndiambour), an advanced breeding line (#8517), and five progeny lines at the two indicated locations over 2 years, with adequate irrigation or rainfall.

Genotype	Origin	Riverside, California		Bambey, Senegal	
		1992	1993	1992	1993
CB5	California	low	low	low	low–mod.
Melakh	Senegal	low	low	low	low
Mouride	Senegal	low	low	low	low
Ndiambour	Senegal	mod.	high†	mod.	mod.
8517	California	high	high	high	high
9-3-2	Mouride × 8517	high	high	high	high
9-5-2	Mouride × 8517	high	high	high	high
9-6-4	Mouride × 8517	high	high	high	high
26-1-2	Mouride × 8517	high	high	low	low
26-3-1	Mouride × 8517	high	high	low	low

† This is a "false" DLS because Ndiambour had low pod set.

(Gwathmey et al. 1992a). Under optimal conditions in California, cowpea has yielded 5 t/ha of grain in the first flush, and an additional 2 t/ha in the second flush. In tropical conditions in Bambey, Senegal, with warmer night-time temperatures (Thiaw et al. 1993) and adequate rain, the same early flowering genotypes (e.g., #8517) mature their first flush of pods in about 65 days after planting, producing 2 t/ha of grain, and the second flush of pods is mature in about 95 days, producing an additional 1 t/ha (S. Thiaw, unpublished data). Consequently, early-flowering cultivars with DLS should be adapted to the broad range of conditions that can occur in the Sahelian zone of Africa, where the annual rainfall may vary from 200 mm, with a short growing season of less than 60 days, to 400 mm in wetter years, with a growing season as long as 90 days, but with the possibility of midseason droughts (Dancette and Hall 1979; Khalfaoui 1991).

Conclusions

Breeding programs developing cowpea cultivars for water-limited environments should continue to rely mainly on evaluating yield of advanced lines over several locations and years. Attempting to increase yields by selecting for increased water-use efficiency, by indirect selection for low carbon isotope discrimination, is not recommended at this time. It should be possible to increase water-use efficiency using this method, but empirical studies indicate that general adaptability may be associated with high carbon isotope discrimination. Adaptation to some dry environments may be enhanced by early generation selection for very early flowering, coupled with delayed leaf senescence and ability to recover from midseason droughts, and produce a second flush of pods. Delayed leaf senescence may also enhance nitrogen fixation, as well as forage production and quality, in cultivars bred mainly for grain production. Advanced lines with early flowering and delayed leaf senescence should be further evaluated in multilocational yield trials, to determine their extent of adaptation in the Sahelian and Sudan Savanna zones of Africa.

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Cowpea in traditional cropping systems

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Abstract

The production of cowpea in Africa and America is reviewed briefly. In traditional cropping systems in West Africa, a diversity of both systems and varieties is observed. The contribution of cowpea to overall productivity is small (on average about a tenth of grain yields, averaging 1.5 t/ha), and this fact, together with drought and pest vulnerability, creates a paradox in view of its widespread popularity. The answer to this paradox may lie in cowpea's labor complementarity with the major grain crops, and its value for human diets, livestock fodder, and soil nutrient interactions. Farming systems in this region are changing and are being driven by four sets of factors: population growth (increasing land scarcities), market integration (urban demand), technological change (new cultivars and production methods), and intensification (adaptive change in soil fertilization regimes). Cowpea plays an important role in nutrient cycling (N) in the high-intensity system of the Kano close-settled zone in northern Nigeria, while genetic diversity is exploited to minimize risk in the drier areas. Research and extension agencies should recognize the systemic linkages of cowpea in maintaining sustainable farming systems, and the need to support diversity and indigenous technology.

Introduction

Cowpea originated in Africa and it became an integral part of traditional cropping systems throughout Africa, particularly in the semiarid region of West African savanna (Steele 1972). Cowpea moved to Asia much earlier than America, but it has been entrenched in the cropping systems of both continents, even if it is less important than in Africa (Ng and Marechal 1985). Of the world total of about 8 million ha, Africa accounts for 6 million ha. Cowpea is adapted to warm weather and requires less rainfall than most crops; therefore, it is primarily cultivated in the semiarid regions of lowland tropics and subtropics, where soils are poor and rainfall is limited. The crop is often grown without inputs. Consequently, the yields are poor. Whereas the way cowpea is cultivated in different continents depends upon the agroclimatic conditions, its use has depended upon the socioeconomic conditions, ethnic culture, and traditions of the people who grow it. This paper briefly reviews cowpea cultivation in different continents and focuses on the use and role of cowpea in smallholder farming systems in sub-Saharan Africa with special reference to the dry savanna in Nigeria, which represents one of the major cowpea growing areas of West Africa. Finally, the key constraints in cowpea productivity are analyzed, and their implications in strategies for cowpea improvement are discussed.

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Cowpea production and utilization in different continents

Asia and Oceania. Cowpea is widely grown in India, Nepal, China, Pakistan, Bangladesh, Sri Lanka, Burma, Indonesia, Philippines, Korea, Thailand, Vietnam, Cambodia, Laos, and Australia (Mishra et al. 1985). The cultivated cowpeas in this region are of three types—grain, vegetable (yard-long bean and bush sitao), and fodder. The grain and fodder cowpeas are more prevalent in the Indian subcontinent and vegetable types are common in China, Korea, and other far eastern countries. Grain-type cowpeas range in maturity and plant type from early-erect to late-spreading and indeterminate types. The early-erect types are grown as a pure crop in different niches with cereals, particularly in rice or wheat fallows (Pandey and Ngarm 1985). The late-spreading and indeterminate types are invariably grown in mixture with maize, sorghum, millet, etc., with very low density. Immature pods are often picked at 4- to 6-day intervals throughout the growing season for use as a vegetable, called "Bori" in parts of India, Bangladesh, and Nepal; and the dry grains are used like other pulses in diverse forms. The yard-long bean is a very popular vegetable in China, Korea, Indonesia, Philippines, and Thailand, where it is grown as a pure crop in wide rows with trellis support. The bush-type vegetable cowpea (bush sitao) is relatively early maturing, and it has a semierect plant type and long peduncles, with 25–30 cm long pods that are thick, fleshy, and succulent. These are often grown as a pure crop without any trellis support. Fodder-type cowpeas are grown pure or in intercrop with maize and sorghum in high densities, and harvested along with the cereals at the time of flowering for green fodder.

Europe and northern America. Both fodder and grain type varieties are grown in southern Turkey, Greece, Italy, Bulgaria, Spain, and in southern USA, mostly as a pure crop. Large-seeded blackeye/browneye cowpeas are commercially grown in the states of Georgia, California, Texas, Mississippi, Arkansas, and Tennessee and most of the cultivation is mechanized (Fery 1990). A limited amount of vegetable cowpeas, both yard-long bean and bush sitao, are also grown.

Central and South America. Cowpea is an important crop in several countries of Central and South America, particularly in the drier regions of Brazil, Venezuela, Peru, Panama, El Salvador, Haiti, Ecuador, Guyana, and Suriname (Watt et al. 1985). Cowpea is mostly cultivated as a pure crop but is also grown as an intercrop with maize (Mafra and Cardoso 1988). Both grain-type and vegetable-type varieties are grown. Climbing-type indeterminate cowpeas are grown on a small scale in the wetter regions, with trellis support. There is a great deal of diversity in preference for seed color in this region. Therefore, varieties with different seed colors such as white, red, brown, black, cream, and speckled are found in the region.

East and southern Africa. Cowpea is a very important legume crop in the coastal savanna covering Somalia, Kenya, Tanzania, Mozambique, and the dry savanna of Tanzania, Kenya, Zambia, Zimbabwe, and Botswana (Amable and Rugambisa 1992). Cowpea is also cultivated in the drier low hills of Tanzania, Kenya, Uganda, Rwanda, and Burundi. It is normally planted as a pure crop in the coastal regions, and as a mixed crop with maize in other regions. It is consumed both as grain and as a vegetable, but unlike in Asia where

green pods are eaten, in East and southern Africa, the tender leaves are regularly picked and eaten as spinach. Actually, cowpea leaves are a more important source of food than cowpea grain in this region. Almost every household has a few rows of late-maturing, drought-tolerant spreading type cowpea exclusively for leaf picking, and the same varieties are also grown as an intercrop with maize at very low densities to produce grains. The planting pattern differs widely from farmer to farmer. Cowpea may be planted in alternating rows with maize in good rainfall regions, or as one row of cowpea for 2–3 rows of maize in drier regions.

Central and West Africa. Cowpea is the major food legume in Central and West Africa, where more than 60% of the world's cowpea is cultivated. The bulk of the crop is grown in the northern savannas as an intercrop with sorghum and millet, but cowpea is also planted in the humid zone as an intercrop with yam, cassava, and maize. Cowpea is used primarily as a food grain, except for a few pockets such as Benin Republic and eastern Nigeria, where the tender leaves and green pods are used as a vegetable. In the savanna areas, cowpea fodder is equally important because of the large number of cattle and the long dry season when there is not much to graze upon. In these areas the farmers grow two types of varieties in the same field: (1) a grain type which matures earlier; and (2) a fodder type which matures late and is normally harvested at the end of the rainy season, just before permanent wilting sets in.

Traditional intercropping systems in West Africa

Diversity in cropping systems

Cowpea is grown throughout West Africa from wet to dry zones in a variety of crop mixtures, but the importance of cowpea as a component crop is greater towards the northern areas, where rainfall is less and soils are poor. A general survey of cropping systems in West and Central Africa from 1988 to 1990 (Singh 1993) covering Nigeria, Benin Republic, Niger Republic, Togo, Cameroon, and Burkina Faso identified 15 major cropping systems (Table 1), in addition to several others which vary from farmer to farmer. In the forest and Guinea savanna zones, cowpea is intercropped primarily with maize, cassava, yam, and groundnut. The cowpea density is very low (1000–5000 hills/ha) and interspersed among the companion crops. In the northern Guinea savanna, cowpea is intercropped with groundnut and/or sorghum. Planting of component crops is normally done in rows with systematic intercropping patterns, which may vary from alternate row intercropping to within-row intercropping, with varying distances giving a grid of groundnut or sorghum rows crossed by the cowpea rows every 2–3 m. The cowpea population is low, but individual plants spread over a 2–3 m radius.

Cowpea is intercropped with millet and/or sorghum, with or without groundnut, in the Sudan savanna, in several diverse and complex patterns with varying interplant distances and planting sequences of component crops. In a commonly practiced cropping system in Minjibir and Gezawa local government areas of Kano state, Nigeria (Fig. 1), millet is planted first in wide rows (1.5–3 m apart) at the onset of the rains (May–June), with a 1 m hill-to-hill distance within rows, reaching 4000–6000 hills/ha. Grain-type early cowpea varieties are planted between alternate millet rows at a hill-to-hill distance of 1 m, when the rains become more stable towards the end of June. Fodder-type, late-maturing cowpea is

Table 1. Cowpea in the major cropping systems of West Africa.

A. Forest and southern Guinea savanna
   1. Cassava-cowpea
   2. Maize-cassava-cowpea
   3. Maize-cowpea
   4. Maize-cowpea, relay or double crop in second rainy season
   5. Maize-groundnut-cowpea

B. Northern Guinea savanna
   6. Maize-cowpea-relay
   7. Groundnut-cowpea
   8. Groundnut-sorghum-cowpea with or without millet
   9. Sorghum-cowpea

C. Sudan savanna
   10. Sorghum-groundnut-cowpea
   11. Millet-sorghum-cowpea, relay with or without groundnut
   12. Millet-sorghum-cowpea-groundnut
   13. Millet-groundnut-cowpea

D. Sahelian zone
   14. Millet-cowpea
   15. Millet or cowpea

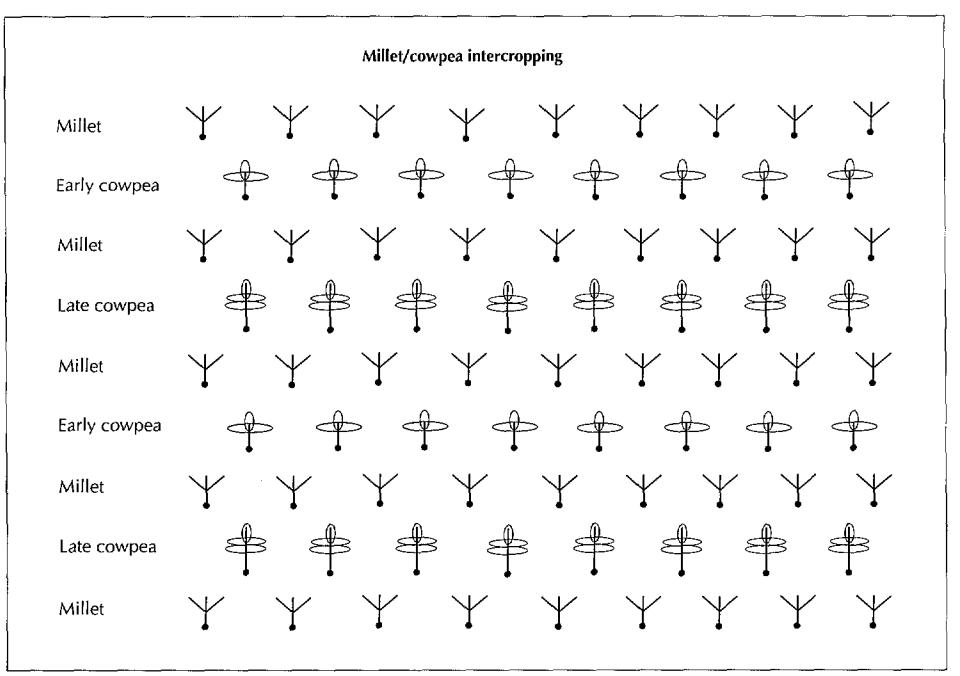

Figure 1. Traditional intercropping systems involving relay with early and late cowpeas in millet fields.

planted late, in mid-July, in the remaining rows. Thus, the pattern of "millet-early cowpea and millet-late cowpea" is repeated. Sorghum and groundnut can either replace or supplement millet and early cowpea in the rows, resulting in a more complex mixture. The early cowpea and millet are harvested at the end of August end or the beginning of September, and the late cowpea/sorghum are left in the field until the onset of the dry season (October–November). The farmers wait until the cowpea leaves show signs of wilting and then they cut cowpea plants from the base and roll the plants into bundles with all the leaves still intact. These bundles are kept on roof tops or in tree forks for drying, to be sold/used in the peak dry season (March–May) when fodder prices are up. If there are rains in October–November or if residual moisture is sufficient, both fodder cowpea and sorghum produce a reasonable amount of grain and fodder. Thus, the cropping system practiced in northern Kano state utilizes rainfall from May to October quite effectively, and the emphasis is on both grain and fodder.

Diversity in crop varieties

Within the two types of cowpea varieties—grain type and fodder type—there is wide variation for seed type, seed size, seed color, hilum color, and plant type. Individual samples from 36 farmers' fields have shown from 1 to 11 seed types in cowpeas of early grain-type and from 3 to 7 seed types in the late fodder-type cowpeas. The varieties have local names that often describe their characteristics. The genetic diversity within each group of varieties is probably maintained by the farmers, to ensure stability under the harsh environmental conditions in which cowpea is grown.

Contribution of the component crops in overall productivity

A detailed study to quantify the contribution of each component crop in the mixed cropping systems revealed major constraints, as well as overall productivity, of different systems (Singh 1993). The study covered 14 farmers' fields in Minjibir and Gezawa local government areas of Kano state, Nigeria, which is the heart of the cowpea growing region in West and Central Africa. A 20 x 20 m block was studied in each field and detailed notes were taken on field, history, land preparation, crops and varieties planted, dates of planting, planting patterns, diseases, insects, maturity, harvesting, and yields of grain and fodder. Yields of component crops were estimated on each farmer's plot (Table 2). The average grain yield of cowpea was 110 kg/ha, with about 1200 kg/ha of millet or 937 kg/ha of sorghum, with large variation in the fodder yield. The total biomass ranged from 2.35 t/ha to 14.52 t/ha (thickly planted late sorghum). The principal constraints for cowpea production were insects (primarily *Maruca*), low plant density, drought stress for late cowpea, and competition with cereals.

Though the findings of a study of this type may differ from year to year because of variation in weather, pests, and other factors, the data gathered indicated that the average cowpea grain yield under traditional intercropping systems is < 100 kg/ha in the Kano area, and that the millet/sorghum yields are about 1 t/ha. Thus, the average grain productivity of traditional cropping systems in this part of Kano state is being sustained at < 1.5 t/ha. The challenge is to find ways of improving this productivity without using additional inputs, which are not presently available. Varietal improvement and modified cropping systems may be the answers.

Table 2. Yields of component crops in different cropping systems (Minjibir and Gezawa local government areas, Kano state, Nigeria, 1991)†.

Cropping system	Grain yield (kg/ha)					Fodder yield (kg/ha)				Total biomass (kg/ha)
	Ecp	Mlt	Srg	Gnt	Total	Lcp	Mlt	Srg	Total	
Mlt+Ecp+Lcp	123	1455	–	–	1578	1643	2300	–	3943	5521
Ecp+Srg+Gnt	138	–	2150	168	2456	–	–	3270	3270	5726
Ecp+Srg	405§	–	905	–	1310	–	–	7083	7083	8393
Ecp+Srg+Lcp	128	–	945	–	1073	–	–	4710	7673	8746
Ecp+Srg	173	–	280	–	453	–	–	14065	14065	14518
Mlt+Ecp+Lcp	83	1858	–	–	1941	2365	3060	–	5425	7366
Ecp+Mlt+Gnt+Lcp	60	1663	–	43	1666	1598	3075	–	673	6339
Ecp+Srg+Gnt	75	–	1505	38	1718	–	7060	–	7060	8778
Ecp+Mlt+Srg+Lcp	160	693	628	–	1481	1365	1602	965	3932	5413
Ecp+Mlt+Srg+Lcp	23	750	1318	–	2091	775	1105	5030	6910	9001
Mlt+Lcp	–	1363	–	–	1363	1920	4208	–	6128	7491
Mlt+Ecp+Srg	148	700	105	–	953	–	2283	830	3113	4066
Mlt+Ecp+Lcp	125	1348	–	–	1473	748	3815	–	563	6036
Ecp+Srg+Gnt+Lcp	85	–	503	270	858	850	–	640	1493	2351
Mean	132	1216	937	129	1458	1580	3056	4575	5667	7124

† Mlt = millet, Ecp = early cowpea, Srg = sorghum, Gnt = groundnut, Lcp = late cowpea.
§ Sprayed twice with cymbush.

Role of cowpea in sustainability of traditional farming systems

Three paradoxical characteristics of the cowpea component in rainfed farming systems in the dry savanna should be noted.

1. *Generally low yields.* Yields of < 100 kg/ha are not uncommon (Table 2), and such yields are not regarded by farmers as evidence of failure. When considered as a return to invested labor and capital, however, they seem marginal in conventional terms.

2. *Vulnerability to drought.* Although cowpea is considered to be a drought-resistant crop, rainfall failure is a frequent cause of shortfalls in output. The increased incidence of drought since the mid-1960s has driven a shift to early-maturing varieties brought (according to farmers' own perceptions) from farther north.

3. *Vulnerability to pest attack.* In most systems in question, the high probability of heavy yield loss from pest infestation is fully recognized, and many of the predators and their life cycles are known. However, no economically viable control measures seem to be available.

Yet, notwithstanding these disincentives, cowpea is an extremely widespread and highly valued component, not merely of the cropping system, but of the farming system as a whole, in drought- and pest-prone environments with soils of low intrinsic fertility. This paradox can be understood, in socioeconomic terms, by reference to four complementarities observed:

1. *Labor complementarity.* (a) cowpea planting follows that of cereals, minimizing competition with them for scarce labor; (b) intercropping cowpea with cereals on land that has to be cleared or ridged anyway gives cowpea almost a 'free ride' on account of the low labor input; (c) the spreading habit of the crop minimizes weeding for the whole mixture (on the other hand, weeding may require more skill); (d) harvesting is spread out over time, allowing flexible use of labor; and (e) female labor is normally used extensively in harvesting cowpea, whereas cereal harvesting is mainly men's work, thereby maximizing the use of the labor force.

2. *Dietary complementarity.* Although much cowpea is sold, it has a most important subsistence role in household diets, complementing nutritional deficiencies of cereals.

3. *Livestock interactions.* Cowpea residues are highly valued as fodder in systems where every adult, male or female, and some children own or aspire to own small ruminants or cattle.

4. *Soil nutrient interactions.* Although little data has been adduced to show the quantitative impact of nitrogen fixation in cowpea mixtures on farmers' fields, the beneficial interaction of cowpea with cereals is usually recognized by farmers, either in intercropping or in rotation systems.

These considerations imply that to look at cowpea in conventional field-crop terms (or to assume that output maximization is the producer's main objective) is inappropriate. Of course, it has long been acknowledged, principally in connection with cereals such as sorghum and millet, that (1) security of food output is more important than its maximization, and (2) intercropping two or more crops actually does maximize output per hectare in many dry rainfed systems, vis-à-vis monocropping. But here, we are looking at something else. Cowpea production is essential for the viability of the farming system as a whole, yet alongside the cereal crops, its importance, measured in economic terms, is secondary. There is little doubt that output maximization, consistent with security goals, drives cereal production. The complexity of the cowpea component in the system is, however, of a different order. Its success or failure does not by itself determine the food sufficiency of households, because, in any case, all households grow it.

The dynamics of farming systems

It is essential to understand dry savanna farming systems, not merely in terms of factor ratios as we find them today, but in a longitudinal perspective. Their evolution in West Africa is being driven by (1) population growth; (2) market integration; (3) technological change responding to, among other things, significantly increased aridity since the mid-1960s; and (4) adaptive change, which is dominated by an intensification imperative, where the land supply can support long bush fallow regimes.

Population growth. Population growth in the West African Sahel has been estimated to be in the order of 2.2% per year (IUCN 1989), and the average annual growth rate in northern Nigeria between 1962 and the recently published census of 1991 was 2.2% per year. Such

a rate is considerably lower than the rates of over 3% commonly estimated for African countries in recent years (Kenya, for example, maintained 3.76% between 1969 and 1979). Also, it needs to be recognized that in the Sahel, outmigration (and exceptionally high infant and child mortality) produces localized anomalies, where population stagnates or declines.

Increasing population density increases both the demand for food and the labor available to produce it; it lowers interaction and education costs, facilitates the growth and diversification of markets, and drives technological change. A new study of dryland management in Machakos district, Kenya, over the past six decades traces a dramatic turnaround in conservation to these factors and argues a "Boserupian" theory of agricultural change (Boserup 1965; Tiffen et al. 1994; English et al. 1994). Density-driven intensification has been argued for the Kano close-settled zone, Nigeria (Mortimore 1993b).

Market integration. The West African dry savanna farming systems were incorporated into the world market historically through the agency of groundnut cultivation. Policy, research, and extension were concentrated on this crop. Nevertheless, the impact of groundnut cultivation on the evolution of smallholder systems is imperfectly understood and controversial. For example, the widely published interpretation of groundnut expansion as a soil degrading process in Senegal (Franke and Chasin 1980) stands in contrast to an absence of such interpretations of the equally important northern Nigerian groundnut zone (Mortimore 1989). Cowpea, however, being produced for subsistence or for local markets, was ignored by officials during the colonial period. By the 1960s and 1970s, there was a long-established cowpea trade network, linking the producing areas in northern Nigeria with the major centers of demand in the south (Mortimore 1980).

Technological change. In response to persistent African drought (Hulme and Kelly 1993), dryland farmers have adapted their technologies and management, within the constraints imposed by environmental conditions and available choices. A study conducted in the 1980s in the francophone Sahel, which compared six distinct farming systems, emphasized the shift toward more intensive use of wet sites (Boulier and Jouve 1988), and showed that farmers do search for adaptive options. In Nigeria, the range of such options has been extended since the 1960s. For example, the adoption of the fast weeding tool, "ashasha", roughly doubles the size of holding that can be effectively weeded using family labor. Early-maturing varieties of millet, cowpea, and sorghum have spread into Nigeria from farther north.

Adaptive change towards intensification. The transect represented by the three sites (Table 3) proceeds from high to low population densities and from a wetter to a drier ecology. Tumbau and Futchimiram represent stability at high and low densities, while Dagaceri represents areas with rapid conversions of natural vegetation to arable. In all three systems, cowpea features prominently.

Contrary to expectations based on the widely promoted linkage between population growth and environmental degradation, the farming system of the Kano close-settled zone, of which Tumbau forms a part, is managed sustainably on the basis of highly integrated crop, livestock, and tree components, as shown by historical and decadal-scale evidence,

Table 3. Land-use change in three farming systems.

	Location		
	Tumbau	Dagaceri	Futchimiram
Mean annual rainfall (1960–91), mm	728	427	452
Annual rainfall received (1992–95)†, mm	533	360	301
Population density/km^2	300	170	<100
Cultivated land (%)			
1950–57	77.8	35.6	22.1
1969–71	86.4	56.1	22.2
Grassland and woodland (%)			
1950–57	12.8	60.1	76.1
1969–71	6.7	36.8	73.7

† Based on 3 years (1992, 1993, 1995) for Tumbau, all 4 years for Dagaceri, and 3 years (1992, 1994, 1995) for Futchimiram.
Source: Turner (in preparation).

soil fertility indicators, and preliminary nutrient cycling measurements (Mortimore and Turner 1993a,b). Under annual cultivation, soil nutrient status held up between 1977 and 1990 (13 years) in respect of organic carbon, total nitrogen, magnesium, calcium, and pH. Physical properties remained stable.

At the other end of the transect (Futchimiram), there was no significant change in the percentage of arable area over time, or land shortage. Fallows of 8–10 years appear to restore soil nutrient status to levels comparable to land that has never entered the cultivation cycle. Fallow cycles often follow cultivation for up to 20 years or more. This system is not, therefore, under stress. Cowpeas are grown in rotation with millet, and intercropping is not practiced.

If the spatial density variations (Table 3) provide an analogue of change through time in dry savanna farming systems, fundamental changes in the ratio of land to labor, and therefore in the economics of intensification, should be expected to occur in the medium or long term. If the land-use changes—not yet begun at Futchimiram, in full swing at Dagaceri, and more or less completed at Tumbau—are also predictable, soil fertility maintenance would be the key to the sustainability of these systems. Of the strategies available to maintain soil fertility, those having the widest social distribution (lowest cost) are organic manure (mostly derived from animals) and nitrogen-fixing crops.

An association has been found, in African dryland farming systems, between population density, livestock density, and crop-livestock integation (McIntire et al. 1992; Mortimore and Turner 1993). Integration brings conservation benefits to the soil, which explains why prognoses of degradation following increased human and livestock densities have frequently been wrong (Turner et al. 1993).

Set in this context of farming system dynamics, cowpea is recognized in local practice as having beneficial interactions, both economic and nutrient. These cannot always be distinguished easily in farmers' thinking. "For a legume to contribute significantly to the

long term maintenance of soil fertility, it must leave behind in the cropping system more N than it removes from the soil" (Giller and Wilson 1991). But the economic value of the crop may lead to removals threatening its beneficial effects on soil N, as observed in Tumbau, where cowpea is in demand for straw and the beans are sold as well as eaten.

Cowpea in nutrient cycling

The Kano close-settled zone has an intensive farming system. Most of the land (86.4%) was under cultivation in 1971, with only a small amount (6.7%) remaining as grassland or woodland (Table 3). Farmers integrate the cultivation of crops with trees and livestock. Crops such as groundnut and cowpea, whose residues provide good fodder for livestock, are intercropped with cereals. Trees are grown in association with crops on farmers' fields.

As mentioned earlier, the four main crops in the system are sorghum, millet, groundnut, and cowpea. These are always intercropped, and may be planted in a variety of mixtures and densities. Yields of cowpea ranged from 19–236 kg/ha of threshed grain, and 100–400 kg/ha of cowpea fodder harvests in 1993. The wide range in yields is due, in part, to the variability in planting patterns (Table 4).

The livestock (predominantly small ruminants) are allowed to graze fields during the dry season, but are kept tethered in compounds at night and throughout the rainy season. The manure they produce is collected. Rejected feed is trampled by the animals and incorporated into the farmyard manure. Ash from cooking fires and any other waste material is also added to the farmyard manure. The practice of using crop residues and weeds as animal fodder and collecting the manure to be used as fertilizer results in a very efficient recyling of nutrients within the system.

Table 4. Crop yields in Tumbau, Kano close-settled zone, 1993.

Crop	Grain (kg/ha)		Residues (kg/ha)	
	Mean	Range	Mean	Range
Cowpea	109	19–236	337	100–400
Groundnut	232	33–396	479	108–856
Sorghum	306	52–847	985	146–2011
Millet	618	144–1128	1328	127–3060

Table 5. Nutrients (%) removed by 100 kg of grain and fodder in the harvest at Tumbau, 1993.

Nutrient	Threshed cowpeas (100 kg)†	Cowpea fodder (100 kg)
Nitrogen	2.37	1.19
Phosphorus	0.15	0.13
Potassium	2.02	1.38
Magnesium	0.58	0.33
Calcium	0.51	0.89

† Equivalent to 128 kg unthreshed.

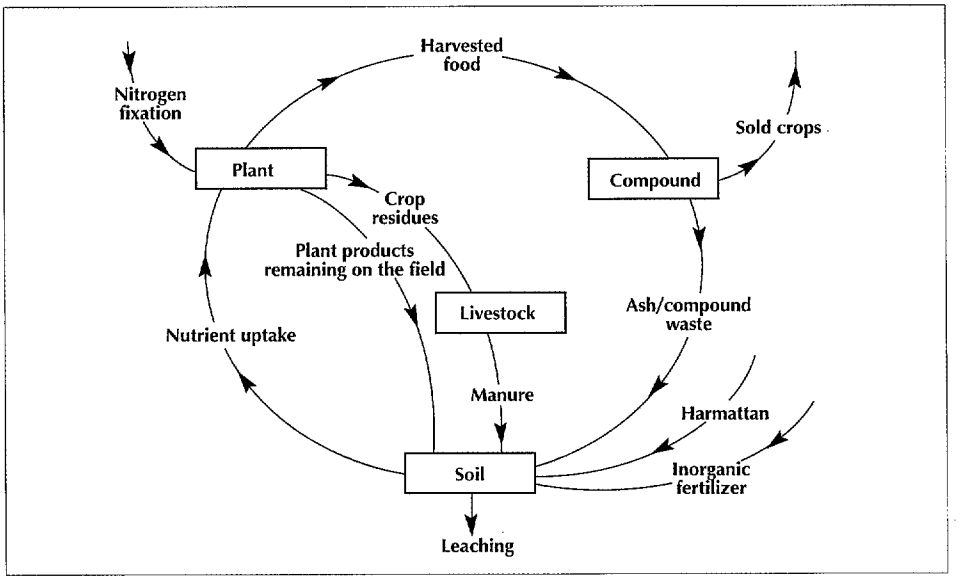

Figure 2. Nutrient cycling in the Kano close-settled zone.

A diagram of biomass produced from one farm shows that the majority of the material coming from the land is used within the farmer's compound, and the nutrients within it are eventually recycled to the soil (Fig. 2). The key to this system is the use of leguminous crops that provide not only valuable food crops for the farmer, but also valuable animal fodder. Moreover, cowpea and groundnut fix nitrogen, thereby introducing more of this nutrient into the system.

While nutrients are removed from the soil in harvesting cowpea grain and fodder (Table 5), the roots of cowpea plants are left in the soil. Most of the nitrogen in cowpea plants comes from the atmosphere via nitrogen fixation, rather than from the soil. Even though other crops growing in the same field may benefit indirectly from nitrogen fixation due to more soil nitrogen being available for nonfixing plants, the main benefit of nitrogen fixation to the soil is only seen after the nutrients within the cowpea plants have been cycled through livestock and returned to the soil as manure (Fig. 2). Reliable estimates of nitrogen fixation by cowpea within this cropping system are not available. Much of the research on nitrogen fixation in field crops has been done in trials where basal doses of fertilizer (phosphorus, but sometimes nitrogen as well) have been applied, and where the situation was simplified by monocropping (Singh and Rachie 1985; Giller and Wilson 1991; Mulongoy et al. 1992). Trials have included inoculation with appropriate strains of rhizobia (Eaglesham et al. 1982; Ofori et al. 1987). There are so many variables involved that "many measurements of nitrogen fixed in the field remain little better than informed guesses" (Giller and Wilson 1991).

Cowpea in biodiversity management

Diversification as a strategy for mitigating risk is expressed in several forms in dry savanna livelihood systems: (1) income diversification between farm, livestock, and nonfarm

Table 6. Number of cultivars in use in four villages.

Crop	Tumbau	Dagaceri	Kaska	Futchimiram
Early cowpea	5	3	5	4
Late cowpea	4	2	4	2
Pearl millet	12	7	6	3
Sorghum	22	7	3	3
Total†	51	33	34	28
Mean annual rainfall (1992–95)§, mm	533	360	326	301

† Includes groundnut, peppers, melon, bambara groundnut, beniseed, rice, and vegetables.
§ Based on 3 years (1992, 1993, 1995) for Tumbau, all 4 years for Dagaceri and Kaska, and 3 years (1992, 1994, 1995) for Futchimiram.
Source: For Tumbau, Yusuf (1996); for Dagaceri, Mohammed (1996); for Kaska, Ibrahim (1996); and for Futchimiram, Chiroma (1996).

activities; (2) subsistence diversification between crops, livestock products, and the collection of wild foods; (3) herd diversification among livestock producers; and (4) cultivar diversification. The use of several different cowpea varieties is to be expected in this context.

In four sites distributed along a gradient from wetter to drier agroecological conditions in northern Nigeria, the numbers of cowpea varieties found in use in each site were 9, 5, 9, and 6 (Table 6). This diversity is consistent with that of the food cereals and other crops.

Diversity enhances the choice in conditions of spatial and temporal variability in rainfall and pest hazards. Although one or two cultivars are most frequently used (in particular, the short-cycle "dan arba'in"), the other cultivars form a genetic reserve. In the past two or three decades, falling rainfall expectations have led farmers to prefer short-cycle varieties. Some of the late-maturing varieties which were once popular are on the verge of disappearing in the dry sites. Newly introduced, early-maturing varieties have come from the north (Niger Republic) or the east. This underlines the fact that adaptation to drier ecological conditions is taking place. Insect pressure may be a factor in the continued use of low-priced or otherwise disadvantaged varieties. Shrubby growth habit minimizes insect damage. Drought resistance was also considered important and, if absent, could be traded off against high yield or good taste and price. Fodder production was regarded highly.

Implications for research and extension

1. The importance of cowpea in dry savanna farming systems is linked to its economic and ecological interactions with the other components of the system. These interactions are imperfectly understood, and vary from system to system. Appropriate agro-economic and biochemical research methods need to be applied to representative systems. Output maximization as a sole breeding objective takes inadequate account of these interactions.

2. Farming is evolving towards more labor-intensive systems in many areas, driven by demographic and economic forces. Cowpea has a crucial role to play in the achieve-

ment of sustainability. It facilitates crop-livestock integration, which is associated, in dryland Africa, with intensification and land-conserving investments. In fixing N, it imports nutrients into the cycle. Its economic function in the system is complementary to that of cereals. It is universally known, understood, and accepted.

3. The diversity of farming systems, their temporal variability, and exposure to pest hazards, pose a major challenge to breeding. Rather than a few high-yielding varieties, whose adaptability is unknown to users, site-specific menus of diverse cultivars may be more useful. Research must be interactive with farmers. Improved pest resistance is more likely to benefit farming households than marginal yield increases.

4. The following cowpea breeding objectives at IITA (Singh 1993) are relevant to the problems encountered by smallholder farmers in the semiarid regions:

   - develop grain, fodder, and dual-purpose varieties;
   - develop varieties for intercropping, as well as pure cropping;
   - combine resistance to major insects, such as aphid, bruchid, thrips, *Maruca*, and pod bugs, along with resistance to major diseases;
   - combine drought, heat, and shade tolerance;
   - develop varieties with an inherent capacity for good growth under low fertility, i.e., for better N fixation and nutrient use.

   In addition to these objectives, however, a strategy is necessary to deal with ecological diversity (soil, rainfall), the complexity of system interactions, and the diversity of farmers' practice and objectives.

5. A majority of cowpea growers have no contact with formal extension agents. However, their interest in improved varieties (from whatever source) generates indigenous, adaptive, technological change. Formal extension systems must integrate with such autonomous technological change if the limited resources available to governments are to be effectively used.

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Recent developments in cowpea cropping systems research

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Abstract

The importance of cowpea (*Vigna unguiculata* [L.] Walp.) is underscored by its use as a component in many cropping system combinations in Africa, Asia, and tropical America. Cropping systems research over the past decade has served as a multidisciplinary approach to examine the biological superiority of specific innovations, as well as the degree to which such innovations fit existing practices and meet the needs of resource-poor farmers. The scope of cropping systems research includes physiology (the nature of intercropping competition for light, water, and nutrients; useful intercrop cowpea characteristics), agronomy (cropping combinations, patterns, and timing of operations), and plant breeding (yield stability, breeding methodology, and identification of superior lines for specific cropping systems). The overriding assumption is that complex cropping systems are more stable than sole crop arrangements, but with lower total yields. Owing to increases in population and land pressure, it is crucial that improved systems of production provide a range of alternatives to increase yield, while maintaining the natural resource base. Cowpea research in the past 10 years has improved the focus on aspects of agronomy, plant breeding, and physiology. This paper highlights our understanding of improved yields and nutrition for resource-poor farmers.

Introduction

A cropping system has been defined as the sequence of crops grown in one field, and the way in which they are managed (Davis and Woolley 1993). This simple definition hides the incredible complexity that makes up the multiple cropping systems that are prevalent in the tropics. Cropping systems research is concerned with understanding how the large numbers of components which make up the cropping system interact. Scientists have noticed that, in many cases, the typical model of research does not yield useful results. This method usually involves the testing of one or two new variables on a research farm, and then recommending the highest yielding combination to local farmers. A typical farmer has a wide range of factors to deal with, which includes a specific piece of land with physical and fertility constraints, subject to the climatic conditions within the region. This

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climate consists of certain expectations with regard to rainfall, temperature, and radiation. Farmers have to decide how and when they will till their land in anticipation of planting crops. Although these are usually low-input systems, farmers may have to decide how to use limited quantities of organic fertilizer. Planting itself presents the farmer with a range of decisions that need to be made. What combination of species should be planted, and which varieties selected from within those species? What planting arrangements should be followed, and how many plants of each component crop should be sown? Farmers also understand that staggering the planting dates of certain crops could be advantageous for some crop species.

Crops grown in a specific area are determined by a number of factors. Even with adequate precipitation and sunlight, optimum temperatures, and fertile soil, it is quite possible that other factors will influence which crops are cultivated. There may be economic concerns (commodity prices, transport costs, etc.), social factors (consumer taste preferences, religious motivation, tradition) or even political reasons (marketing boards, price controls, price stability) that determine the crop choices that a farmer makes (Steiner 1984).

Cowpea is an important grain legume in West Africa. It provides an inexpensive source of protein for the urban and rural poor of the region (Alghali 1991). Nigeria accounts for 70% of the world's cowpea production. The grain is valued for its flavor and short cooking time, and the plant is especially favored by farmers because of its ability to maintain soil fertility through its ability to fix nitrogen. Farmers are also interested in the cowpea haulms that are used to maintain livestock during the dry season. Although the sole-crop potential grain yield of the crop is high (1.5–3.0 t/ha) when insecticide is applied to the crop, the actual farm yields that are obtained in the West African region are much lower (0.025–0.100 t/ha) due to severe attack from an extensive pest complex (Rachie 1985). Another reason for yields which do not equal research station results is that 98% of the cowpea in West Africa is intercropped (Arnon 1972). This is in comparison to routine yields of 0.3–0.5 t/ha at the IITA Kano research station for intercropped cowpea with no insectide protection.

Intercropping is usually defined as growing two or more crops simultaneously on the same field (Andrews and Kassam 1976). Subsistence farmers who practice low-input farming are particularly dependent upon this form of crop production (Ntare 1990). Hildebrand (1976) noted that intercropping is common where farmers lack land and/or capital, but labor is plentiful. Although agricultural research originally focused on sole cropping, and ignored the potential of intercropping (Willey and Osiru 1972), there has been a gradual recognition of the value of this type of cropping system (Blade 1992). Intercropping reduced damage caused by pests and diseases, and also ensured greater yield stability by producing some yield, even though some of the component crops failed (Andrews 1974). Although there are some contradictory reports, intercropping has been shown to produce higher and more stable yields in a wide range of component combinations (Ofori and Stern 1987).

This paper reviews cropping systems research conducted with cowpea as one of the component crops. The biological advantages and farmer relevance of these systems will be assessed, and an overview of recent physiological, agronomic, and breeding research presented.

Biological advantages of cowpea intercropping

The partitioning of limited resources among crop plants occurs whenever plants are grown in association. This competition was originally defined as beginning when the immediate supply of a single necessary factor fell below the combined demand of the plants (Clements et al. 1929). Other terms such as hardship and interference were coined to indicate the effects that plants grown together had on one another (Harper 1961). Although many intercropping studies attempt to identify specific limiting factors, de Wit (1960) did not regard this as important, but instead defined the entire plant milieu as "space", and noted that growing plants competed for this entity. The concept of space provided a method for understanding how intercropping could produce yield advantages under certain circumstances. If sole crops consist of identical plants which have the same type and timing of resource demands, then intercrops with different plant types which possess variable requirements could use more "space" when grown in association. Huxley and Maingu (1978) referred to cereal-legume associations, calling the cereal the dominant crop and labeling the legume as the dominated species.

A common index of intercropping productivity is the land equivalent ratio (LER), which is defined as the ratio of the area needed under sole cropping to one of intercropping at the same management level to produce an equal amount of yield. LER is the sum of fractions of the yields relative to their sole crop yields (Francis 1986). An LER >1 indicates that the intercrop is more productive than the comparative monocrops. Such a situation indicates the potential for overyielding (Willey 1979). Heibsch (1980) proposed an area-time equivalency ratio, which was capable of evaluating crops on a yield per day basis. Hiebsch and McColloum (1987) suggested that the advantages of intercropping were overstated when LER was used.

The potential for overyielding indicates that resources are maximized in an intercropping system. Research has indicated that growing two or more species at the same time can have advantages in light interception, water use, and nutrient uptake.

Tsay (1985) has reported that the amount of light intercepted by crops is dependent upon the geometry and plant architecture of the component crops. The usual cowpea intercrop combines a tall cereal crop with a lower storey cowpea crop. Research on light has indicated that there is a benefit where resources are maximized when intercropping is done. Willey (1979) has suggested that the advantage may have to do not only with the amount of light intercepted at a particular time, but also with how light is intercepted during the entire growing season. The rapid establishment of a prostrate cowpea will enable more light to be used than if only a dominant crop, such as millet, is grown. Of course, this holds true when cowpea is planted with a slow-developing tuber, such as cassava. Clark and Francis (1985) have observed that if a tall crop, especially a C_4 plant, is combined with a shorter C_3 crop, there can be an enhanced use of total light. They also observed that maize-bean systems established total ground cover 1 week prior to sole crop beans and 3 weeks prior to sole crop maize. Srinivasan et al. (1990) observed that shade-tolerant cowpea performed well under *Casuarina equisetifolia.* As could be expected, Kang et al. (1985) reported that pruning leucaena increased the yield of maize and cowpea in an alley crop. Fawusi et al. (1982) reported that a maize-cowpea system intercepted 52.3% of incoming light, which is less than the 76.4% interception in a maize-cowpea system reported by Blade (1992). This difference was probably due to measurements being

taken 6–8 weeks after planting in the Fawusi study, so that the total canopy was not developed. An additional explanation is that Blade used an early-maturity cowpea line, which rapidly established ground cover.

Water utilization can be increased when cowpea is grown with other crops. Lal and Maurya (1982) reported large differences in root stratification between cowpea (upper soil levels) and maize (lower soil levels) during development. The total rooting mass of the maize/cowpea intercrop was larger than either of the monocultures, but was smaller than the combined monocrop total. Shackel and Hall (1984) noted that neither sorghum or cowpea had an advantage in soil water uptake, since osmotic potential in either crop was not substantially affected by intercropping. In a humid forest experiment, it was observed that water-use efficiency was higher in a maize/cowpea intercrop than in either sole crop when water was not limiting, but in drought conditions the water-use efficiency of sole maize was greater than that of the intercrop (Hulugalle and Lal 1986). Ofori and Stern (1987) suggested that cereal and legume intercrops use water equally, and that competition for soil water may not be a determining factor for efficiency in intercrop systems. Villegas and Morris (1990) reported that monocropped cowpea and a cowpea/sorghum intercrop were equally effective at halting the drainage of residual water through the soil profile. In northern Nigeria, when water is limiting, an early-maturity cowpea can develop rapidly and expend limited soil moisture. Rees (1986) noted that cowpea was a strong competitor for water due to its deep rooting capability. Water was diverted from sorghum to cowpea in a sorghum/cowpea intercrop under the semiarid conditions of Botswana.

The roots of intercropped species compete for finite nutrient resources. In a maize/cowpea study, Wahua (1983) reported that cowpea was severely affected by maize competing for nitrogen. Nitrogen uptake by intercrop cowpea was 64 kg/ha, but sole crop cowpea took up 88 kg/ha. Wahua also observed that maize was much more competitive for potassium in comparison to cowpea, especially at high nitrogen levels. Stoop (1986) noted that high soil phosphorus levels favored cowpea growth in cereal-cowpea associations. Both Chang and Shibles (1985) and Ofori and Stern (1986) observed that when no nitrogen was applied, there was strong competition for soil nitrogen. This was especially true between 49 and 63 days, when both crops were in their reproductive stages. Intercropping of cereals and legumes can result in a "nitrogen-sparing" effect, which results when soil nitrogen remains available to the cereal crop due to nitrogen fixation supplying some of the legume crop's nitrogen requirements. There is also the possibility that nitrogen is transferred from legumes to associated grasses during the growing season. Eaglesham et al. (1981) reported nitrogen transfer from cowpea to maize, but Ofori et al. (1987) found that ^{15}N concentrations did not differ between sole and intercropped maize grown in association with cowpea. Blade (1992) reported significant ^{15}N dilutions in intercropped maize from a field experiment, where maize was harvested one month after the cowpea had been harvested. Since ^{15}N applications were done at the late podding stage of cowpea, it appears that most of the transfer was due to decomposition of cowpea leaves, roots, and nodules. Burton et al. (1983) observed that nitrogen leaching from leaves, and the decomposition of legume leaves may also result in nitrogen transfer to the associated cereal.

What are the optimum physiological traits for cowpea? Terao et al. (1997) indicated that the ideotype for cowpea grown in the cereal-based cropping systems of the West

African savanna is a variety with a prostrate growth habit and a well-developed root system. The cowpea must also have high transpiration efficiency. This is similar to the findings of Ntare and Williams (1992). Although Ntare (1990) observed that early-maturing erect cowpea lines were useful in these systems, their appropriateness was judged by how little they affected millet yield. Terao et al. (1997) also reported that the amount of light reaching cowpea in cereal-based cropping systems varies (30–75% of ambient light). They noted that if the cereal canopy intercepts large amounts of light, cowpea growth is so limited that almost no foliage can intercept what light does pass through the cereal canopy. The local cowpea varieties are successful due to their flexibility in response to competition. Light in the early stages of development will influence the branching patterns, which will in turn determine the source and sink of the plant.

Agronomic advances in cowpea intercropping

The immense variety of permutations associated with the management of a piece of land have led to research which provides location-specific information that is often difficult to generalize for the efficiency of intercropping. Species selection, relative time of sowing, and both arrangement and spacing of constituent crops present infinite combinations that the researcher must deal with.

Cowpea is generally grown as the understorey crop in a system based on cereals or tuber crops. Cowpea is useful because it establishes rapidly, and this results in less soil erosion, a reduction in soil temperature, and lower weed pressure (Zuofa et al. 1992). Cowpea is often relay-planted into the cereal crops of the West African savanna. Farmers want to ensure that all fields have their cereals planted as early as possible, to take advantage of early rains, as well as the nitrogen flush which occurs when the onset of the rains moistens the dry soil, whose microbial activity releases plant-available nitrogen. Later planting of cowpea can reduce the competition with cereals to ensure that the high-priority cereal yields are not reduced. Following harvest of the millet, the cowpea is able to take advantage of late-season residual moisture and additional light, which influences both grain and biomass production. Such a system is indicative of a cardinal rule in intercropping: try to select crops, or use management techniques, to maximize the gap between reproductive periods. This will reduce the simultaneous demand for resources.

Remison (1982) reported no advantage when either maize or cowpea were planted early, in comparison to simultaneous planting. Ofori and Stern (1987) also noted no advantage in a maize/cowpea intercrop, although they did report that the LER followed cowpea yield trends rather than those of maize. Nangju (1979) found that late planting of cowpea in established maize resulted in cowpea grain yield decreases of 58–78%. Blade (unpublished) found that in the Sudan savanna, delaying cowpea planting by 2 or 3 weeks resulted in a cowpea grain yield reduction of over 50% in comparison to simultaneous millet/cowpea planting (Table 1). Similar results were observed in an experiment where no insecticide was applied. The rationale for these experiments was that improved cowpea lines must have the flexibility to perform well in systems where cowpea planting time can vary greatly, due to environmental and farmer constraints. However, in 1993, simultaneous millet/cowpea planting reduced grain yield (715 kg/ha), in comparison to 940 kg/ha when cowpea was planted 3 weeks after millet. Agronomists must be careful not to suggest innovations that clash with farmer objectives.

Table 1. Cowpea grain and fodder yields (kg/ha) for a 2-year millet/cowpea intercrop experiment testing a local and an improved cowpea variety using four dates of planting in 1993 and 1994 (three insecticide sprays).

Time of cowpea planting	90K–59	Dan 'Ila	Mean
Cowpea grain yield (kg/ha)			
Simultaneous	394	244	319
1 week after millet	259	167	213
2 weeks after millet	176	156	166
3 weeks after millet	131	67	99
Mean	240	159	
LSD (5%) between cowpea lines averaged over plantings = 61			
LSD (5%) between planting treatments averaged over cowpea lines = 77			
Cowpea fodder yield (kg/ha)			
Simultaneous	494	819	657
1 week after millet	430	754	592
2 weeks after millet	394	647	520
3 weeks after millet	300	538	419
Mean	405	690	
LSD (5%) between cowpea lines averaged over plantings = 217			
LSD (5%) between planting treatments averaged over cowpea lines = 155			

Agboola and Fayemi (1971) did not observe any difference in yield when maize and cowpea were planted in the same or alternate rows. Fawusi et al. (1982) reported that LER values increased as maize and cowpea density increased, and that cowpea was less competitive, since cowpea yields decreased significantly at higher maize densities. Chang and Shibles (1985) noted that the level of the maize population usually limited intercrop cowpea yield, but cowpea density had no influence on maize productivity. Ofori and Stern (1986) pointed out that even though the cereal usually produces a larger proportion of the intercrop yield, any LER advantage for a particular system is usually influenced by the legume's productivity.

The planting of strips of component crops has also been attempted. Strips of cowpea within strips of cereal rows increase the ease of weeding and the spraying of insecticide, reduce the influence of competition in comparison to alternate row planting (Cenpukdee and Fukai 1992), and take advantage of the "border effect". Baldev and Ramanujam (1980) described the "border effect" as the compensatory yield of the outer rows of the dominant crop, which can over-compensate for the reduced yields in the dominated crop. It is possible that differential competition at the interface could result in no yield loss for the understorey crop (Lai and Wen 1990). Blade (unpublished) reported that when varying the number of cowpea rows (1–4) between single millet rows, the best mean grain yield resulted with three rows of cowpea (Table 2). The traditional practice of alternating single rows of millet and cowpea resulted in the lowest cowpea yield. Dan 'Ila fodder production

Table 2. Cowpea grain and fodder yield (kg/ha) for five cowpea lines and four row arrangements in a millet/cowpea experiment (three years) at Kano (three applications of insecticide).

Cowpea line	Cowpea rows between millet rows 1	2	3	4	Mean
Cowpea grain yield (kg/ha)					
89KD-391	535	548	560	450	523
84S-2246-4	242	341	392	392	341
89KD-374-57	378	463	572	498	478
Dan 'Ila	225	268	319	288	275
89KD-288	0	0	0	0	0
Mean	283	332	372	330	
LSD (5%) between cowpea lines averaged over treatments = 76					
LSD (5%) between row arrangements averaged over cowpea lines = 57					
Cowpea fodder yield (kg/ha)					
84S-2246-4	432	545	653	874	626
89KD-374-57	619	902	1006	1272	950
89KD-391	1180	2388	1932	2150	1913
Dan 'Ila	890	1177	1326	4457	1962
89KD-288	2714	3126	3485	3715	3260
Mean	1167	1628	1680	2494	
LSD (5%) between cowpea lines averaged over treatments = 993					
LSD (5%) between row arrangements averaged over cowpea lines = 874					

was much larger in the four-row treatment; this indicated that the local check had the ability to take advantage of the extra light so that a large amount of fodder was produced.

Cropping systems are also influenced by the application of mineral fertilizers. The addition of nitrogen to a cereal/cowpea system is generally thought to favor the cereal at the expense of cowpea (Midmore 1993). Fukai et al (1990) have reported that when soil nitrogen levels are low, the legume is less affected than the cereal, but the addition of nitrogen has the effect of both decreasing the legume's nitrogen fixation and increasing the cereal's development. Such growth increases the cereal's ability to intercept light. Ofori and Stern (1987) found that LERs did not increase when nitrogen was added to the maize/cowpea system. Chang and Shibles (1985) also reported that increased nitrogen and high maize density resulted in decreased cowpea yield due to shading. Such data indicate that intercropping is most beneficial when soil fertility is low (Rachie and Rockwood 1973). One promising report was that there was cowpea cultivar variation in how cowpea cultivars respond to nitrogen when intercropped with maize (Ezumah et al. 1987). Researchers at Nyankpala Agricultural Experiment Station in Ghana have done extensive work on nitrogen balance in maize/cowpea intercropping systems, indicating the benefit of

the legume crop during the growing season and for subsequent crops. Singh (1993) estimated that cowpea contributed 46–54 kg/ha of nitrogen to the following season's wheat crop.

In a 2-year experiment in the Sudan savanna, Blade (unpublished) used four fertility treatments on an alternate-row millet/cowpea intercrop: (1) broadcast NPK at recommended rate; (2) broadcast P at recommended rate; (3) 50% of recommended P rate applied only on cowpea rows; and (4) the nonfertilized check. The NPK treatment significantly increased millet yield in comparison to the nonfertilized check, but cowpea was unaffected. Millet was not affected by the other treatments. If the recommended P rate was broadcast on the plot, cowpea grain yield increased (480 kg/ha) in comparison to the check (397 kg/ha). If 50% of the recommended P rate was applied only to the cowpea rows, the cowpea grain yield increased to 607 kg/ha. Such results indicate that simple management techniques can greatly improve overall yield. The technology was not new to farmers, since they now drop handfuls of NPK near the hills of millet along the cereal row, to maximize the impact of costly and sometimes limited stocks of inorganic fertilizer.

Intercropping research has also sought methods of management that limit the impact of weeds, pests, and insects. Matteson et al. (1984) reported that maize/cowpea systems had 42% less flower thrips (*Megalurothrips sjostedti)* than sole cowpea. However, cropping pattern had no effect on *Maruca* pod borer or pod-sucking bugs. It was also reported that early infestations of *Maruca* were equal in sole and intercropped cowpea, but 12 weeks after planting the populations were significantly higher in the sole crop plots. Alghali (1993) noted that intercropping cowpea with sorghum reduced flower thrip and pod-sucking bug populations. Tests indicated that only two sprays of insecticide in the intercrop equaled the protection provided by three applications in the sole cowpea crop. Ezueh (1991) noted that mixed cropping can protect cowpea from insect attack. Jackai et al. (1985) also indicated the appropriateness of intercropping as one component of integrated pest management.

Cowpea improvement for cropping systems

Plant breeding initially focused on the selection of genotypes that perform well in sole cropping. It was thought that superior sole crop lines could be planted in intercrops with the same results. Selection was usually done under research station conditions, which tended to eliminate many of the problems (low fertility, lack of labor, weeds) which existed on the fields of traditional farmers. This led to many cowpea improvements that could not be taken advantage of by the small-scale farmer.

Plant breeders were subsequently influenced by the objectives farmers set for their intercropping systems. Yield stability, maximum profitability, increased biological yield, or provision of a nutritionally balanced harvest may be some of the goals which the breeder must take into consideration. The primary focus breeders have had in the past 20 years when looking for genotypes that do well in intercropping systems is the existence of genotype × cropping system interactions. When studying cereal/legume combinations, several studies have indicated that significant interaction between cereal genotypes and cropping patterns does not occur (Davis and Garcia 1983; Francis et al. 1983), although Odo (1991) reported differences in the response of short and tall sorghum varieties when intercropped with cowpea.

However, Davis and Garcia (1983) and Woolley and Rodriguez (1987) both found highly significant bean genotype x cropping system interactions. In the most recent review of the literature, Smith and Zobel (1991) reported that significant genotype × cropping system interactions occur, especially in the dominated (cowpea in cereal-based systems) species. Blade et al. (1992) reported significant cowpea genotype × cropping system interactions in both forest and savanna ecologies. Variation among environments for cowpea grain yield was greater when no insecticide was applied. Singh (1993) noted that one strategy for improving cowpea for traditional cropping systems was defect elimination of selected local varieties, or development of completely new photosensitive, spreading-type varieties by standard methods, using relevant parents. He also proposed the screening of advanced breeding lines using cropping systems (and inputs) of the subsistence farmer. The lines selected from such screening are then evaluated under farmer-participatory trials.

Blade (1992) reported tremendous differences in the response of cowpea in four management systems: sole crop + insecticide, sole crop + no insecticide, intercrop + insecticide, and intercrop + no insecticide (traditional). Cowpea genotypes that performed well in intercrop + no insecticide systems across the West African savanna were identified (Table 3), and they have also performed well in farmers' fields in several West African countries, including Cameroon (Endondo 1994). Evaluation of improved cowpea in the other management systems provided useful information concerning how genotypes responded in "improved" management systems, as well as how traditional management limited the genotypic potential of the tested lines.

Yield stability is a complex product of genetic yield potential and tolerance to stress conditions (Smith and Francis 1986). Subsistence farmers require crop varieties which produce an acceptable yield under a wide range of environmental variability. Finlay and Wilkinson (1963) devised the method of using simple linear regression of genotype performance on an environmental index (usually the mean of all genotypes in each

Table 3. Cowpea grain yield (kg/ha) for ten cowpea lines intercropped at seven locations with no insecticide protection in 1993.

Cowpea line	Kano, Nigeria (642)†	Wudil, Nigeria (750)	M. Madori, Nigeria (426)	Maidugiri, Nigeria (239)	Maroua, Cameroon (947)
90K-59	648	189	391	39	238
89KD-319	379	42	77	7	175
89KD-374-57	392	68	244	38	249
89KD-261-3	279	61	161	28	124
89KD-355	330	56	221	28	177
89KD-391	470	41	116	0	186
89KD-277-2	341	162	186	10	530
89KD-867-11	349	72	289	16	122
89KD-245	188	0	9	32	38
Local check	195	14	158	17	188
LSD (5%)	158	86	135	ns	87

† Rainfall (mm).

environment). Gomez and Gomez (1983) used this method to rank the performance of soybean lines in several cropping systems. They observed that all varieties tended to have higher yields in environments with high environmental indices (as was expected), but that the varietal ranking differed from one environment to another. Blade et al. (1992) reported that yield stability of cowpea lines varied, depending on cropping system (sole or intercrop), as well as whether or not insecticide was applied. It is thus critical that plant breeding programs develop lines suited for specific cropping systems, such as the traditional (intercrop-no insecticide) management system; many promising lines may be rejected if selection is only done in high-input sole crop management systems. Singh (1993) suggested evaluation of new breeding lines under three systems: (1) pure crop with two sprays of insecticide, (2) pure crop with no insecticide, and (3) intercrop with no insecticide. This would enable the breeder to select suitable varieties for different systems, and also select varieties with low genotype × environment interaction for wide adaptation.

Cropping systems research and extension

During the 1970s, the problems associated with agricultural experimentation based at research stations evolved into a discussion on how agricultural scientists could better relate their work to farmers and their concerns. This led to the development of a framework that involved participatory research. Francis et al. (1989) documented that this transition was not always easy. It was perceived that farmers were interested in large plots where they could visually judge the effect of a new variety or fertilizer application. Farmers wanted innovations which did not cost a great deal of capital, or demand a great change in traditional farming methods, and the focus to be on changes that increased yield and profitability while reducing risk. If experimentation was done on the farm, a farmer wanted conditions to be representative of their own farm, so they could be confident that the new methods would work for them. On the other hand, scientists wanted replicated plots for statistical testing of their hypothesis with specific treatments, which could result in publishable data. Researchers wanted to generalize results of such on-farm experiments as what would happen on a larger regional basis.

Participatory research (Maguire 1987) was developed in order to provide a link between farmers and researchers. The steps in this process, along with recent cowpea research examples, can be summarized as follows:

1. *The problem must be identified.* Alghali (1991) went to farmers to understand what they saw as the major problem in cowpea production. The farmers said that insects were the biggest cause of yield losses. Low fertility, shading due to cereals, and low cowpea population were also identified as constraints.

2. *Objectives must be set.* Seventy farmers surveyed by IITA Kano researchers (Singh 1993) indicated that fodder was a key element of the cowpea crop. In some cases, farmers planted specific cowpea varieties which were known to supply either grain or fodder, so that both requirements could be met. Both breeders and farmers came to an understanding that this must be part of the selection criteria for intercropped cowpea. These observations also underscored the potential for dual-purpose (combined grain and fodder) cowpea varieties.

3. *Selection of solutions and project design must be done.* Farmers who were looking for a new crop to grow in dry season fadama areas came to IITA; they wanted to grow different improved cowpea lines on their own plots to evaluate the usefulness of the IITA materials. These materials were much more successful than local cowpea lines (Table 4) because of aphid resistance. Good lines were multiplied, and many farmers benefited when they opted for the tested cowpea to plant in their own fadama plots.

4. *Project implementation.* Farmers tested improved IITA cowpea lines in their rainy season intercrops in the Kano region. Management of the crops was done by the farmer, but data collection and overall project management were handled by the researcher. However, it was critical that the farmers' observations concerning the new lines were noted, since this provided valuable information about what criteria farmers use to judge cowpea lines.

5. *Interpretation and sharing of results.* Farmers were brought together and discussions were held concerning how improved IITA cowpea lines performed on their own fields. Farmers showed great interest in lines that produced excellent yields for both grain and fodder. They were the ones to champion specific genotypes, which should be made available to the farming public.

It is clear that such applied research must be based on a strong research base, which is capable of generating new technologies that are attractive to farmers. Shetty (1993) reviewed the approach of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) to cropping systems research. Site selection and description are the

Table 4. On-farm evaluation of dry season cowpea lines (sole crop-no insecticide application) at several locations in northern Nigeria (1994).

	Farmers (no.)	Mean yield — Grain (kg/ha)	Mean yield — Fodder (t/ha)
Cowpea line			
89KD-941-1	8	690.8	1.8
89KD-374-57	11	536.3	1.5
84S-2246-4	8	604.3	1.4
Local check	11	573.6	1.4
LSD (5%)		ns	ns
Date of planting			
1–10 Feb	13	943.3	2.2
11–20 Feb	15	477.7	1.5
21 Feb – 28 Mar	9	314.1	0.7
LSD (5%)		288.6	0.6

starting points to conduct useful and representative research. It is then necessary to identify specific systems which are important in the area, and exhibit sustainability and stability of production. Such an undertaking must also have a multidisciplinary approach, which would incorporate the observations and expertise of an entire team of agricultural scientists. Growth analysis, nutrient and moisture uptake, light interception, and nitrogen fixation could involve physiologists and soil scientists. Integrated pest management systems could be tailored by entomologists, virologists, and pathologists. Cropping system agronomists would focus on designing alternative systems on the basis of this work, with plant breeders identifying suitable genotypes which fit into the system. This research effort would involve all groups of researchers, including Consultative Group on International Agricultural Research (CGIAR) centers, national programs, universities, and research staff of local development authorities.

The future of cowpea cropping systems research

The importance of cowpea as an intercrop component has prompted a considerable volume of cropping systems research in the past decade. Researchers have made great progress since the pioneering work of the Institute for Agricultural Research, Samaru, when questions were first raised about the role of cowpea in the traditional cropping systems of the West African savanna (Andrews 1972; Norman 1974).

The farming systems now used have evolved by trial and error over a long period of time (Okigbo and Greenland 1976). These systems, to survive, must be well adapted to the environment in which they exist. However, there is a great deal of opportunity to improve these systems through changes to factors which are managed by the farmer. Owing to constant change in both the environment and the farmer's actions, cropping systems undergo a continuous cycle of changes from new crops, different pest complexes, access to inputs, or increased population density.

Cropping systems researchers, in collaboration with farmers, have increased the productivity of these complex systems, while maintaining their yield stability. The success of IITA in developing improved cowpea varieties can be measured by the interest of governments and farmers throughout West Africa in lines that perform well in all systems: both sole crop and intercrop, as well as niche opportunities, such as in dry season fadama or irrigated production. This has been accomplished by understanding the dynamics of these complex systems, and implementing innovative changes which address the socio-economic and biological constraints of farmers and their farms.

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11

Agro-physiological constraints in intercropped cowpea: an analysis

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Abstract

Factors affecting cowpea growth in millet/cowpea intercropping were investigated in northern Nigeria. Ecological studies showed that cowpea received from < 30% to > 75% of incident light inside the intercropped canopy. In these light-limited conditions, cowpea varieties with a spreading growth habit can harvest more light than those with an erect growth habit by producing more leaves, as well as expanding their leaf area. However, the local spreading type has a low yield potential because of its low harvest index and inadequate root system (compared to the shoot system). Improvement of these two points in the local spreading variety without reducing its adaptability to shade will produce a variety that is better adapted to intercropping.

The effect of shade is most serious in the branch initiation stage, about 3–4 weeks after sowing, which inhibits branching significantly. Since leaves, which become source, as well as pods, which become sink, grow on each branch and the main stem, the final grain yield in nonbranched cowpea is significantly reduced. Shade in the grain-filling stage also reduces final seed yield, but the effect is not as pronounced as shading during the branch-initiation stage.

Root competition between cowpea and millet was greatest when cowpea was planted simultaneously with millet in the low rainfall environment. In alternate row intercropping with 75 cm row width, millet roots run horizontally and turn deep under the cowpea plants, while cowpea roots are distributed under the cowpea plant itself. This creates high root competition because roots of both species share the same root zone. In these conditions, if cowpea is planted simultaneously, millet roots are reduced in the deep zone. Consequently, early onset of drought reduces millet yield because millet does not have deep roots if planted simultaneously with cowpea.

Introduction

Cowpea (*Vigna unguiculata* [L.] Walp.) is a leguminous crop, especially important in the dry savanna of Africa because of its lower water requirement (Hall and Patel 1987) and it is a superior source of protein. The dried seeds, green pods, and leaves are consumed as human food, whereas the dried haulm is important as livestock feed.

There are numerous constraints in cowpea production in this region. Insect pests, plant diseases, parasitic weeds, low soil fertility, and drought are major yield-reducing

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factors. Traditionally, the farmers in this semiarid region of West Africa practice intercropping of cowpea with millet and/or sorghum partly to circumvent these problems (Steiner 1982).

Intercropping also affects soil fertility maintenance through N_2 fixation and differential uptake of soil nutrients (Reddy et al. 1992), as well as by reducing drought stress in the early growth stage of the plants through mutual protection from direct sunlight and wind. Therefore, the traditional intercropping systems fit well in the environment of the dry savanna. However, the major weakness of intercropping is that the yields of cowpea are very low.

This paper presents our understanding of the physiological mechanisms that limit cowpea growth and seed yield in intercropping, and it proposes screening methods and breeding strategies to improve productivity in intercropping, as well as new strategies to improve cropping systems.

Light penetration and canopy structure in intercropping

When cowpea is intercropped with tall cereal crops, light is an important limiting factor. Therefore, ecological studies were conducted in 1992 and 1993 in different intercropping systems in northern Nigeria so as to evaluate the effect of light competition for light on cowpea growth.

The structure of intercropped canopy and light penetration were measured in farmers' fields near Minjibir in Kano state, Nigeria, after Monsi and Saeki (1953) (Fig. 1). Plants in 1 m^2 were sampled layer by layer from ground level (at 10 cm intervals under the legume crops and at 30 cm intervals when only cereal crops were sampled). Samples were separated according to species and separated into leaves, which are shown on the left side in each graph, and other parts (stems including leaf sheath, petioles, peduncles, and flowers, and pods or panicles) are shown on the right (Fig. 1). Fresh weight of different parts was measured and shown against the height. Light intensities in different layers were measured using Sunfleck ceptometer (Decagon Devices Inc.).

In one field, soil fertility was low and the millet grew poorly so that less than 25% of incident sunlight was utilized by millet (Fig. 1a). In these conditions, cowpea growth was not badly affected because nearly 80% of sunlight penetrated the canopy. Cowpea utilized about 50% of this light. However, 40% of sunlight was still wasted.

The canopy profile of millet-sorghum-groundnut intercropping in another farmer's field (Fig. 1b), showed that one well-tillered millet plant and four sorghum plants grown in 1 m^2 absorbed about 75% of the incident light. In such crowded conditions, it seems difficult for the legume plant to grow well (unfortunately, cowpea was not available for destructive sampling).

The canopy profile of millet intercropped with simultaneously planted cowpea variety Dan 'Ila in our experimental field at Minjibir (Fig. 1c) showed that about 60% of light was absorbed by millet. Over 50% of penetrated light (25% of incident light) was absorbed by cowpea, and only about 15% of the light was not utilized by the plants.

These results suggest that it is important to maintain proper density, i.e., the cereal canopy should not be too dense, so as to allow for the better growth of legume crops, and thus keep total utilization of light (cereal + legume) high. It seems that at least 40% of incident light is necessary to grow healthy cowpea plants.

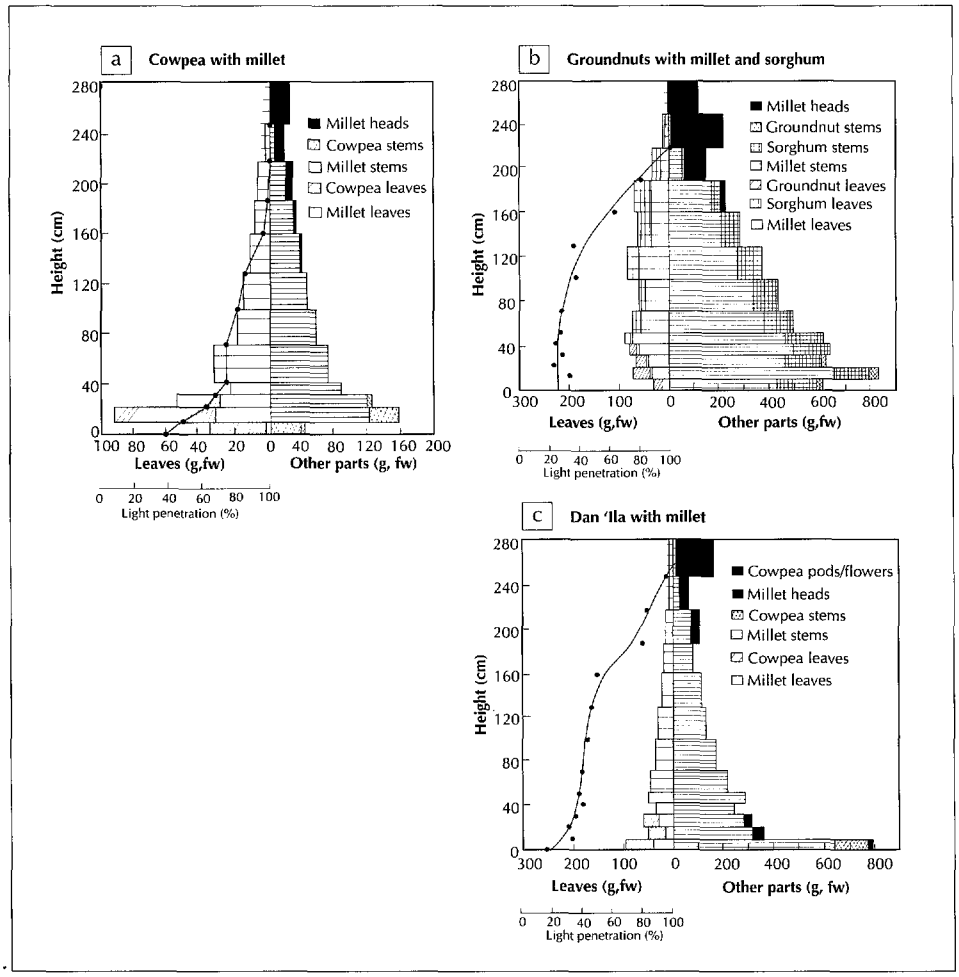

Figure 1. Light penetration curves and profiles of canopy structures in (a) a farmer's field intercropped with cowpea and millet, (b) another farmer's field intercropped with groundnuts, millet, and sorghum and (c) an experimental field intercropped with cowpea and millet.

Types of cowpea adapted to intercropping

There are several types of cowpea, which differ in growth habits. To determine the type of cowpea most adapted to intercropping, a field experiment was carried out in 1992 at Minjibir, Kano state, Nigeria. The yields of monocropped cowpea and intercropped cowpea with millet were compared among four cowpea varieties: Dan 'Ila, a local spreading type; IT82D-716, an improved erect type; IT86D-715, an improved semideterminate type; and IT89KD-374, an improved spreading type. Cowpeas were planted at two densities: (1) 20 cm from plant to plant within rows and 75 cm between rows, giving a high population density of 66,667 plants/ha; and (2) at 75 cm between rows and 50 cm between plants in the row, giving a low population density of 26,667 plants/ha. The respective densities in intercropping were half of the monocropped cowpea, because of alternate

millet rows with cowpea. A basal application of 54 kg nitrogen, 26 kg P_2O_5 and 26 kg K_2O per ha was made.

Grain yields of cowpea were then considered on a per plant basis (Fig. 2a). In the monocropped cowpea planted at high density, IT82D-716 had the highest yield, followed by IT86D-715 and IT89KD-374. The local, grain-type spreading cowpea (Dan 'Ila) had

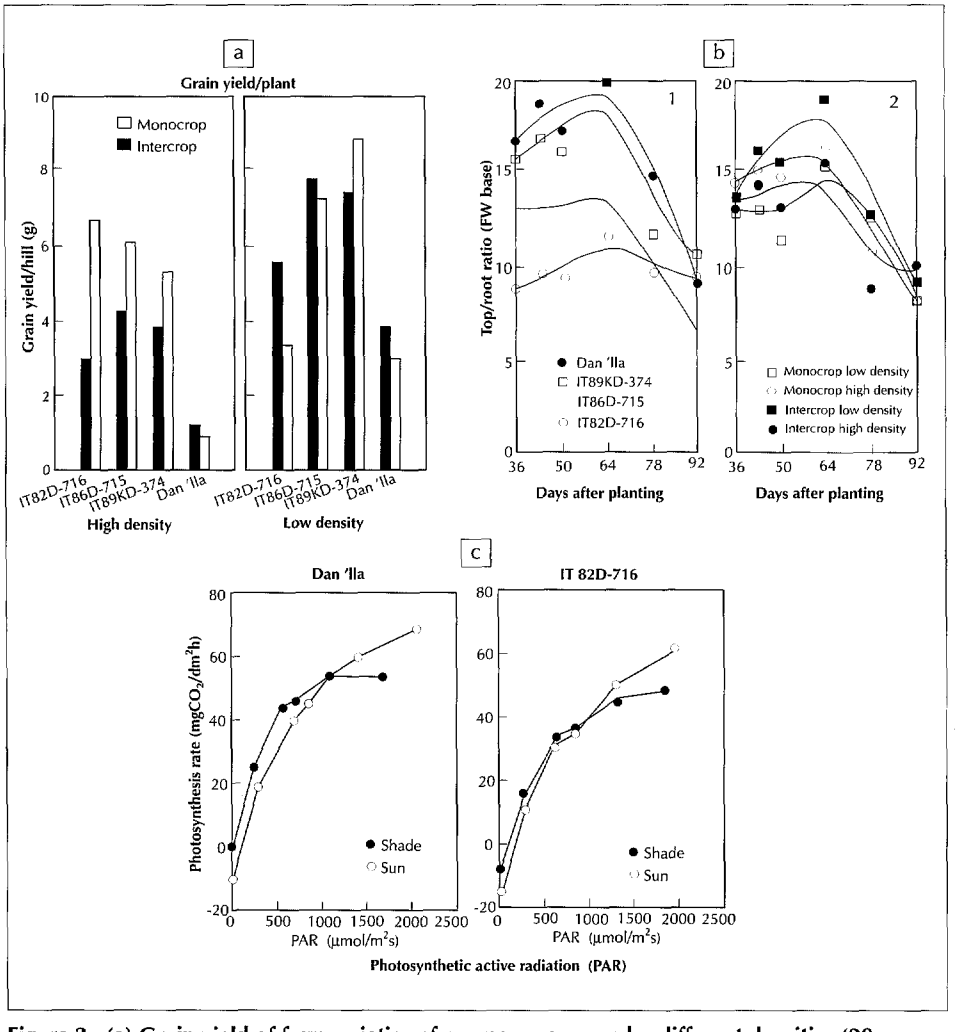

Figure 2. (a) Grain yield of four varieties of cowpea grown under different densities (20 cm between hills high density, 50 cm between hills low density) and cropped solely or intercropped with millet. Row spacing between cowpea and cowpea or between cowpea and millet was 75 cm. Millet was planted 1 m apart, within a row. (b) The dynamics of top/root ratios calculated on the basis of fresh weights in four varieties of cowpea grown at the same denisties as in (a) and under different cropping systems, showing (1) differences among varieties when all the cropping systems were averaged and (2) differences among cropping systems when varieties were included. (c) Photosynthetic light-curves of sun-grown and shade-grown cowpea varieties Dan 'Ila and IT82D-716.

the lowest yield. In low density monocropping, yields of Dan 'Ila and IT89KD-374 were increased by 230% and 65%, respectively, compared to high density, while yields of IT86D-715 increased by only 19% and IT82D-716 decreased by 50%. These data indicate that low density is favorable for spreading-type cowpea, but not for erect-type cowpea.

Intercropped yield was highest in IT86D-715 and IT89KD-374, followed by Dan 'Ila and IT82D-716. It appears that the intercrop yield depends on two different factors: (1) the potential yield of a variety in monocropping systems; and (2) its adaptability to intercropping, which is indicated by the ratio of intercropped yields to monocropped yield. IT82D-716, with the highest yield potential in monocropping, showed the lowest intercrop adaptability. IT86D-715 and IT89KD-374 showed only slightly lower yield potential in pure crop than IT82D-716, and their intercrop adaptabilities were not so low. Accordingly, these varieties showed high yields in intercropping. Dan 'Ila had the higher intercrop adaptability but a very low yield potential, thus giving poor yields in both pure and intercropping.

The low yield potential of Dan 'Ila is due to its low harvest index. This variety has good features for high intercrop adaptability. Improvement of translocation efficiency of this variety, without decreasing its intercrop adaptability, will increase its yields in intercropping. IT89KD-374, which is an improved variety derived from Dan 'Ila, showed a better harvest index with fairly high intercrop adaptability.

Dan 'Ila's high intercrop adaptability seems correlated with its ability to expand leaves. When the dynamics of top/root (T/R) ratios of four varieties was considered on the basis of fresh weights and cropping systems (Fig. 2b), highly significant differences were observed among the varieties (Fig. 2b[1]). Dan 'Ila had the highest T/R ratio, followed by IT89KD-374, IT86D-715, and IT82D-716. No significant differences were observed between cropping systems or cowpea planting densities (Fig. 2b[2]). Dan 'Ila's high T/R ratio is mainly due to its vigorous shoot growth, which gives it a high ability to expand its leaf area and thus capture more light under the cereal canopy.

The higher T/R ratio of Dan 'Ila also explains its low yield in monocropping, compared to that in intercropping. High T/R ratio may cause more water loss through transpiration than uptake, and hence reduced yield in monocropping. The high T/R ratio of Dan 'Ila therefore has the merit of efficient light capture but the demerit of excess transpiration.

Our conclusion is that the type of cowpea adapted to intercropping is the spreading-type cowpea, improved to retain substantial root system, and high translocation efficiency. This conclusion is consistent with the data of N'tare and Williams (1992). However, N'tare (1989, 1990) suggested that late-maturing cowpea is more competitive and reduces millet yield. In our experiments also, the local spreading-type cowpea affected millet yield more than the early erect type, although to a lesser extent (data not shown). Improvement of cropping systems to reduce root competition is necessary.

Adaptability of cowpea in the shade

Given that light is an important limiting factor in intercropped cowpea, the shade adaptation of cowpea was analyzed in a pot experiment, using IT82D-716 and Dan 'Ila. Pots containing 12 kg soil were prepared and basal application of fertilizer at the rate of 0.18 g each of N, P_2O_5, and K_2O per pot (corresponding to about 40 kg each/ha) was made just before planting. Three plants were grown in each pot.

Shade treatment of 40% of incident light was started 11 days after sowing. Seventeen days after the start of shade treatment, photosynthesis rates in different light intensity were measured, using an ADC LCA3 portable photosynthesis measurement apparatus. At 42 days after planting, plants were sampled and the fresh weight of leaves, petioles, peduncles, pods, stems, and roots was measured.

The ratio of fresh leaf weight/fresh root weight in Dan 'Ila increased by 116% in shade-grown plants over sun-grown plants, while that in IT82D-716 increased by only 42%. Moreover, specific leaf area (cm^2/g fresh weight) in Dan 'Ila increased 97% in shade-grown plants over sun-grown plants, while that in IT82D-716 increased by only 32%. This means that Dan 'Ila can increase its leaves in area 120% more than IT82D-716 at the same level of shading. This leaf expansion ability contributes to the efficient light capture of Dan 'Ila in intercropping.

When photosynthetic light intensity curves (Fig. 2c) are considered, the shape of the curve in sun-grown Dan 'Ila was almost the same as that of IT82D-716. Whereas the curve in shade-grown Dan 'Ila peaked around 50% of full sunlight, IT82D-716 did not peak at this light intensity. Another important feature in photosynthesis was the difference of respiration rate, which was lower in Dan 'Ila than in IT82D-716, and lower in the plants grown in shade than under sunlight. Accordingly, the highest apparent photosynthesis rate was obtained in the shade-grown Dan 'Ila when light intensity was less than 50% of full sunlight. This flexibility of Dan 'Ila to adapt in shade conditions supports its adaptability to intercropping.

Inhibition of branching in late-planted intercropped cowpea

The yield of intercropped cowpea depends on the planting time of cowpea relative to millet (N'tare 1990; N'tare and Williams 1992; N'tare et al. 1993; Reddy et al. 1992). In our experiment in 1991, cowpea planted 2 weeks later than millet grew to only 20% of monocropped cowpea planted at the same time; whereas in 1990, when cowpea was planted simultaneously with millet, intercropped cowpea growth was as good as monocropped cowpea. This was confirmed in the planting time experiment conducted in 1993 (data not shown). What factors drastically reduce growth and yields of intercropped late-planted cowpea? N'tare et al. (1993) suggested that this reduction occurs because of reduced time to develop canopy, caused by short photoperiod. However, that is not the main reason for a reduction of yield to 33% (N'tare et al. 1993) or of growth to 20% (our data in 1991, not shown), because: (1) the early nonphotosensitive variety (IT82D-716) also drastically reduced its growth in late-planted intercropping; (2) the difference became obvious even in the early stage, when photoperiod was adequate; and (3) late planting did not affect the growth of monocropped cowpea.

The main reason for yield reduction in late-plant intercropped cowpea was a lack of branching or delayed branching (Fig. 3). In the simultaneous planting, the number of branches in intercropped cowpea was 3–4, depending upon varieties, which is only slightly less than that in monocropping, which ranges from 4–6. However, the number of branches in the intercropped cowpea planted 3 weeks later decreased to 0.5–2, while the monocropped cowpea still had 4–5 branches per plant.

The mechanism of this suppression of branching is not yet clear. However, it is likely to result from lack of adequate light in the late-planted intercrops. It is known that the lack

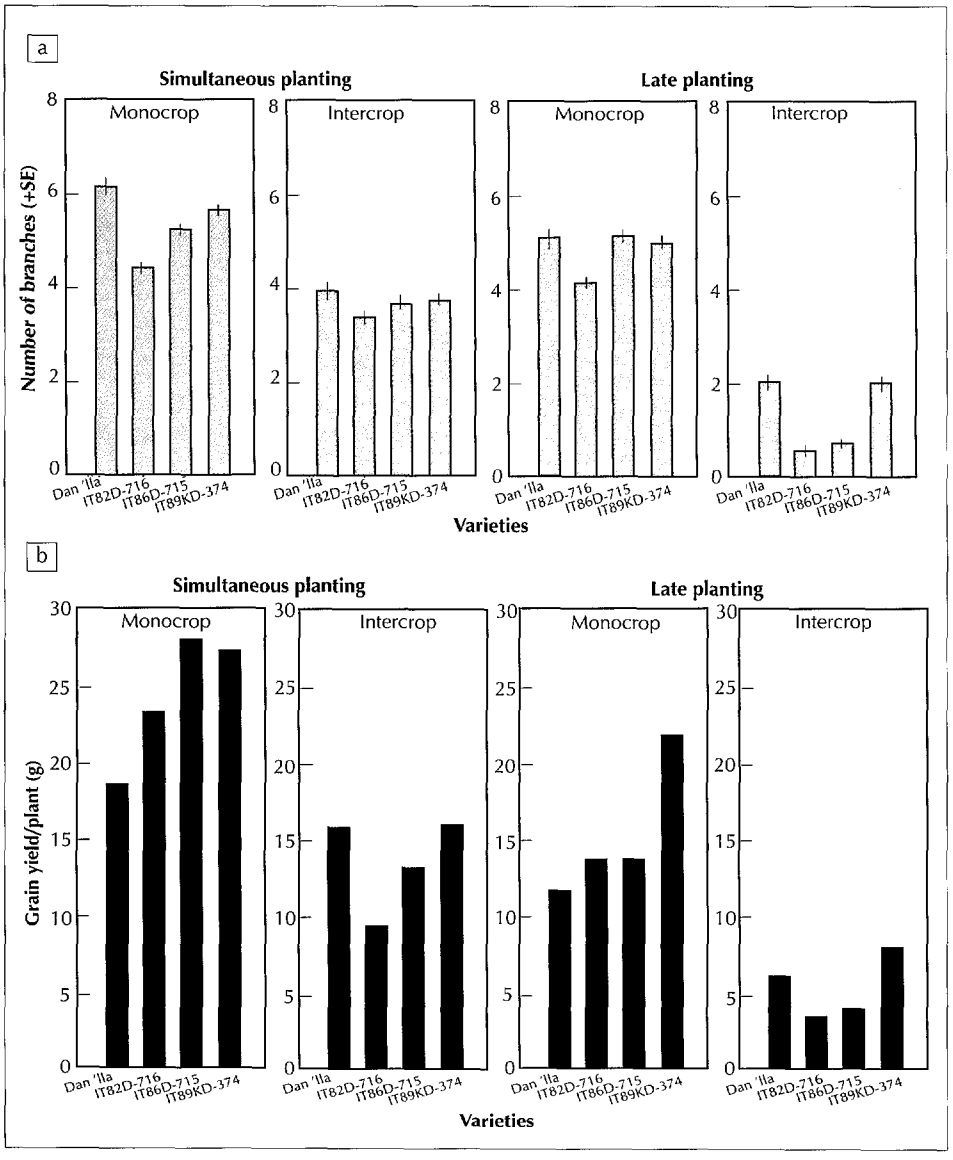

Figure 3. (a) Number of branches growing from the main stem in four varieties of cowpea, monocropped and intercropped with millet, planted simultaneously with millet, and planted 3 weeks later. Branch numbers are means of 40 plants. Branches were counted 74 days after millet planting, when late-planted cowpea had reached reproductive stage (53 days after planting). (b) Grain yield of four varieties of cowpea monocropped and intercropped with millet, planted simultaneously with millet, and planted 3 weeks later.

of light changes phytochrome to its inactive form (Pfr), which stimulates apical dominance to escape from a light-deficient condition. The lack of red light caused by the absorption of chlorophyll in millet leaves accelerates this change.

The number of branches established in early growth stage decides the plant skeleton. It limits both the number of leaves, which produce photosynthate (source), and number of pods, which become the sink. Therefore, a plant with four branches can develop in five directions and can thus yield five times more than a plant without branches, which grows in one direction.

This hypothesis was supported by yield data (Fig. 3b). The differences of yields are basically well explained by the numbers of branches, especially in intercropping. Other contributory factors are low translocation efficiency and imbalance in T/R ratio. The high yields from simultaneously planted monocropped cowpea (except Dan 'Ila) are attributable to the high rate of photosynthesis in the grain-filling stage. Also, a little better yield in simultaneous planting than late planting showed the effect of longer growth duration.

This result suggests two strategies to get high yield in intercropped cowpea. One is by improving the cropping system, either by planting cowpea simultaneously with millet and/or by widening the spacing between the rows of millet, such as strip cropping (Blade et al. 1997). The second is to breed cowpea varieties that branch well even under severe shading. Breeding for branching seems possible, because there are varietal differences in the ability to branch in the intercropped condition.

Differences in branching abilities were revealed among these four varieties. The erect-type cowpea varieties (IT82D-716, IT86D-715) almost did not branch in late-planted intercropping, whereas spreading-type varieties (Dan 'Ila, IT89KD-374) could grow two branches in the same conditions. Late-flowering, spreading-type varieties still retain their vegetative growth ability at a later stage of growth, when the millet leaves become dry and the light available to cowpea improves. These spreading varieties also have efficient light accumulation systems, as shown above. These results are consistent with the observation that spreading-type cowpea performs better than erect-type cowpea under intercropping.

Yield of millet in millet/cowpea intercropping

It has been discussed that, in intercropping, millet yield is reduced if cowpea is planted simultaneously with millet (N'tare 1990; N'tare and Williams 1992). However, no reduction of millet yield was observed when cowpea was planted one week after millet (N'tare and Williams 1992; Reddy et al. 1992). Therefore, we studied how millet yield was affected by intercropped cowpea. Yields of millet in monocropping and that intercropped with high and low densities of cowpea were measured in 1992 (data not shown). Interrow spacing between millet and millet was 1.5 m and was not filled in monocropped millet, but was filled by one row of cowpea in intercropping. Although high-density cowpea reduced millet yield, the difference was not significant.

In 1993, we compared millet yields between an intercrop with simultaneously planted cowpea and another with late-planted cowpea (Table 1). The trials were conducted at two sites: Minjibir, where total annual rainfall that year was 815 mm, and Mallam Maduri, where total rainfall in the same year was 426 mm. Grain yield of millet was significantly higher at Minjibir than at Mallam Maduri. The differences at Minjibir between millet yields in simultaneously planted cowpea and late-planted cowpea were not significant. At Mallam Maduri, however, simultaneously planted cowpea significantly reduced millet yield 16% over late-planted cowpea. This yield reduction was not caused by the reduction of head number or by head size, but by the reduction of seed size. This indicates that millet

Table 1. Grain yield, number of heads, head length, and seed size of millet intercropped with (1) simultaneously planted cowpea, and (2) cowpea planted 3 weeks later, in two locations in Nigeria.

	Grain yield (g/hill)	Number of heads (/hill)	Head length (cm)	Seed size (g/1000 seeds)
Minjibir (yearly rainfall = 815 mm)				
Simultaneous planting	300	11.96	32.15	10.27
Late-planting	314	13.15	33.28	10.01
Significance	ns	**	*	**
Mallam Maduri (yearly rainfall = 426 mm)				
Simultaneous planting	197	8.55	31.5	9.47
Late-planting	234	9.15	31.9	10.07
Significance	***	ns	ns	***
Significance between sites	***	***	*	**

*, **, *** indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

was forced to mature at the end of its growth stage in intercropping with simultaneously planted cowpea, because of insufficient water supply at the grain filling stage of millet, but there was no significant stress in tillering stage and panicle formation stage. Millet yield was thus affected by simultaneously planted cowpea, particularly when rainfall was limited, but was not affected if there was adequate soil moisture at the maturation stage.

Analysis of root distribution

Severe competition for water occurs in simultaneous planting of cowpea and millet. Therefore, we observed root distribution of millet and cowpea in intercropping. Cowpea was planted either simultaneously with millet or 3 weeks later than millet. Pits were dug to expose the rectangular plane against the crop row, and soil cores including roots were sampled from the center of each 10×10 cm^2, using a soil core sampler (38 mm diam. and 200 mm long). After roots were washed and separated, root length was measured using a root scanner (Delta T Devices Ltd) (Harris and Campbell 1989). Roots were sampled from 81 to 86 days after millet was planted. Four replications of the sampling were averaged.

The real data are not shown here, but the distributions of roots are illustrated for simultaneous planting (see Fig. 4a), and late planting (Fig. 4b). Millet root grew laterally within 0–40 cm under soil surface, meeting in the middle of two millet plants where cowpea was sown and, if cowpea was planted later, grew deeper at this point. Since millet root architecture has no tap root, millet roots did not grow deep under their own plant but invaded soil zones under cowpea plants. The density of late-planted cowpea root was low enough not to disturb the distribution of millet roots. In the simultaneous planting, dense cowpea roots prevented millet roots from penetrating the deep zone under cowpea plants. In this case, there was clear separation in which millet roots mainly spread horizontally under the soil surface, whereas cowpea roots mainly shared the deep zone. This separation will occur if there is enough rainfall towards the end of the millet harvest; millet collects surface moisture widely and cowpea uses deeply penetrated water. However, if the onset of drought is earlier, it will reduce millet yield but will not affect cowpea yield much. This

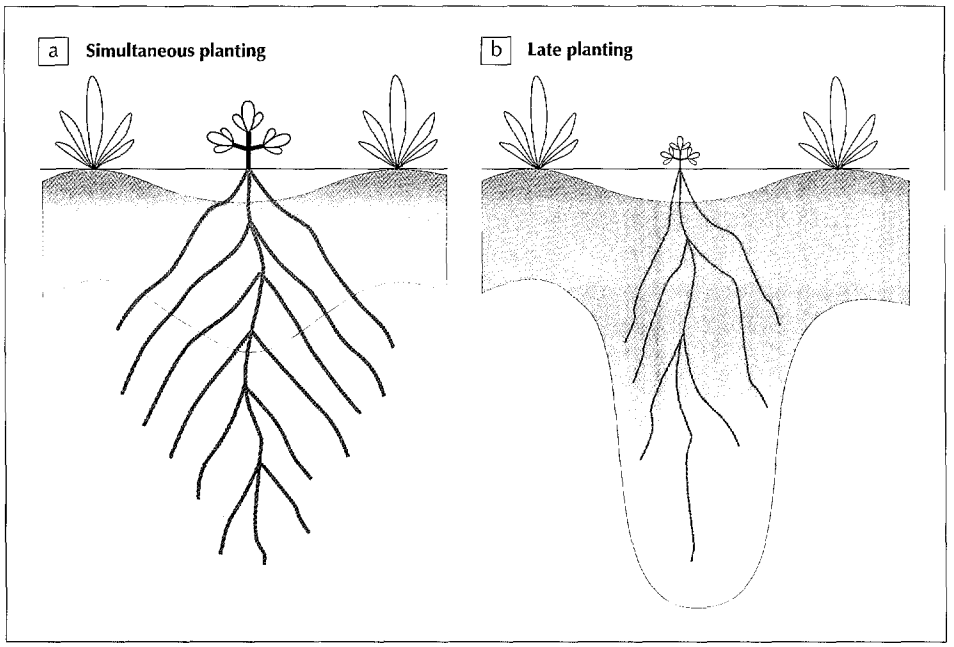

Figure 4. Root distribution of millet (shaded area) and cowpea (drawn) when (a) cowpea is planted simultaneously with millet and (b) cowpea is planted later than millet.

could explain the reduction of millet yield in simultaneous planting at Mallam Maduri (Table 1).

The root distribution indicates an important problem: the usual practice of intercropping millet and cowpea in alternate rows will create severe root competition under the cowpea row. Planting cowpea later than millet is one way to reduce millet yield losses. However, planting cowpea too late will drastically reduce cowpea yield, as mentioned above. Planting cowpea one week later than millet may be appropriate (N'tare and Williams 1992; Reddy et al. 1992).

The pattern of cowpea and millet root distribution will then help minimize the competition, and thus improve the cropping system. One suggestion is the efficient use of the root-free zone under millet. Planting two rows alternately in intercropping or strip cropping, with four rows of cowpea and two rows of millet, seems promising, because the cereal roots mutually penetrate zones under rows of the cereal plants. Planting cowpea and cereals in the same hill so that cowpea roots distribute among cereal root systems is another option for reducing root competition, but before recommending it, the problem of light competition will have to be resolved.

Conclusions

Cowpeas grow under the shade of the cereal crop canopy in intercropping. The light available to cowpea ranged from < 30% to > 75%. However, if the cereal canopy is too dense, cowpea growth seems to be inhibited and more light may be wasted. Therefore, it is important not to plant the cereal crop too densely. This will enable cowpea plants to grow

vigorously and utilize more light. At least 40% of light should reach the top of the cowpea canopy under the millet for normal cowpea growth.

Traditional spreading-type cowpea has a good feature for high intercrop adaptability, although yield potential is low. A poor root system, relative to its vigorous shoot system, and inefficient translocation from leaves to grains seem to limit its productivity. Improvement of the root system and translocation ability in local, spreading varieties will give varieties best adapted to intercropping.

The reason the local, spreading-type variety has a high adaptability in the intercrop is its flexibility of growth. This variety can spread twice more leaf area than an erect-type variety under shade through increasing top/root ratio and expanding leaf area per unit leaf weight. Also, the photosynthetic light curve of the local spreading-type variety changed under shade to a more efficient form, as the plant adjusted to collect low intensity light better than the erect-type variety did.

Cowpea requires enough sunlight in the early stage (3–5 weeks after planting) to produce vigorous branching. This will determine the extent of future growth, because it limits the sizes of both source and sink. Therefore, simultaneous planting of cowpea and millet is recommended if there is no severe competition for water. However, simultaneous planting in severe drought conditions will reduce millet growth. In this case, wider rows or strip cropping of late-planted cowpea is recommended.

Millet roots do not grow under their own plant but are distributed densely under the cowpea plant in intercropping, if there is no root competition. If there is competition, millet roots are prevented from invading the deeper zone, and so mainly share the upper horizontal layer, while cowpea roots grow vertically from upper to lower layers. This may be the main reason why simultaneously planted cowpea reduces millet yield if drought onset is early.

The development of cropping systems that use the root zone complementarily is needed.

Acknowledgments

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Evaluation methods for drought tolerance of cowpea

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Abstract

Cowpea *(Vigna unguiculata* [L.] Walp.) is primarily grown in dry regions in the tropics, particularly in sub-Saharan Africa. Drought is important among several yield-reducing factors. Nine hundred cowpea accessions from the Genetic Resources Unit of IITA were evaluated for drought tolerance in a field during the dry season at the IITA Kano Station in northern Nigeria. Some of them were retested three times in greenhouses, once in Nigeria and twice in Japan, using seedlings planted in small pots with a soil moisture level of 3% wt. Tolerance scores to drought correlated highly significantly in repeated evaluations. Two of the accessions, TVu 11979 and TVu 14914, were always highly tolerant. Merits and demerits of the two methods, a field evaluation method and a pot evaluation method, are discussed.

Introduction

Cowpea is one of the most drought-tolerant crops, and it is widely intercropped with millet or sorghum in the Sudan savanna. Owing to scarce and erratic rainfall in the area, yield of cowpea remains low and unstable. Therefore, a higher tolerance to drought is needed, to get higher and stable yields. In breeding to enhance drought tolerance, it is necessary to identify efficient methods to evaluate levels of tolerance in germplasm for crossing and for selection of segregated breeding materials.

In this study, two methods were tried: (1) a field evaluation in the dry season, and (2) a pot evaluation for seedlings.

Materials and methods

Field evaluation in Nigeria

In November 1990, at the beginning of the dry season, 900 cowpea accessions from the Genetic Resources Unit of IITA were planted without replication in a field at the IITA Kano Station, northern Nigeria. Two cultivars, IT82D-716 and Dan 'lla, were also planted at 3 replicate spots to check the uniformity of evaluation. Thirty seeds per accession were sown in 10 hills (0.1 m between hills, and 0.2 m between rows). No fertilizer was applied.

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3. International Institute of Tropical Agriculture (IITA), Kano Station, Sabo Bakin Zuwo Road, PMB 3112, Kano, Nigeria.

To ensure good germination and vigorous early growth, the field was watered as evenly as possible for 12 days after sowing, using a vinyl hose connected to the tip of a watering can. Thereafter, the field was left unwatered for about 3 months, during which no rain fell. At 9 days after germination, plants were thinned to one plant per hill.

In the middle of February 1991, 5 plants which showed different degrees of tolerance were sampled to get a set of criteria for classification of tolerance. They were classified as follows. Highly susceptible (score 1): the plant was dead and dry in the seedling stage, with one trifoliate leaf. It was about 10 cm tall. Highly tolerant (score 5): the plant had 7 green trifoliate leaves, and it was still growing. It was about 40 cm tall. The other 3 plants showed intermediate tolerance, with different degrees of defoliation, discoloration of leaves, and plant height. Scores 2, 3, and 4 were given for these samples: susceptible, intermediate, and tolerant, respectively. Each accession was evaluated after all 10 plants had been observed.

Pot evaluation

Evaluation of potted seedlings was performed three times, pot evaluation 1 in Nigeria in 1991, pot evaluation 2A and 2B in Japan in 1992.

Pot evaluation 1 (in Kano, Nigeria). From the results of the preceding field evaluation, 25 accessions were chosen, covering the range of scores from 1 to 5, with 5 accessions chosen for each score. Seeds were sown in small pots (both diameter and depth about 10 cm, filled with 600 g of sandy dry soil without fertilizer) in March 1991 in a greenhouse at the IITA Kano station. After germination, plants were thinned to one plant per pot.

In order to find the optimum level of soil moisture for discrimination of tolerance, seedlings were evaluated at 3 levels of soil moisture, with 3 replicate pots for each level. Plants were kept well watered, until they had completed the development of primary leaves. Then, soil moisture was adjusted to the following 3 levels: 2%, 3%, and 5% in weight. Every morning, pots were weighed on an electric balance and were replenished with water, so as to keep the levels of soil moisture constant.

About 2 weeks after beginning the adjustment of soil moisture levels, each plant was scored (1 to 5) as follows: 1 = the plant is dead and dry; 2 = the plant is still alive, but most leaves are fallen; 3 = leaves are yellow and/or wilting; 4 = leaves are a little yellowish or partly yellow; 5 = leaves are green. Scores of 3 replicates were averaged.

Pot evaluations 2A and 2B (at Tsukuba, Japan). To check the reproductibility of pot evaluation, the seedlings of 87 accessions and 5 cultivars (IT81D 994, IT81D 1228-4, IT82D 889, IT84S 2246, and Suvita 2) were evaluated twice (pot evaluation 2A and 2B) at the soil moisture level of 3% wt. in a greenhouse at Tsukuba, Japan in July 1992 (2A) and in August 1992 (2B). The methods were almost the same as mentioned in pot evaluation 1, except that the soil was a mixture of volcanic ash soil and sand (volume ratio 1:1) and that the number of replications was 5, instead of 3. In each evaluation, scores of the 5 replicates were averaged. Using the average of 2 evaluations, the tested lines were classified into 5 categories: (1) highy susceptible, with mean score between 1 and 2; (2) susceptible, between 2.0 and 2.6; (3) intermediate, between 2.6 and 3.6; (4) tolerant, between 3.6 and 4; and (5) highly tolerant, between 4 and 5. In evaluation 2A, germination of 2 accessions

was too slow to evaluate. Thus, the number of accessions successfully evaluated was 85, excluding the cultivars.

Results and discussion

Field evaluation

In the field evaluation, a wide range of tolerance was observed, which suggested the possibility of breeding tolerant cultivars. Some accessions died soon after watering ceased. These plants developed only 1 or 2 trifoliate leaves, while others managed to grow as tall as about 40 cm, with 7 to 8 nodes. Leaves of these plants were green and new leaves were still developing. In most cases, however, leaves of the lower few nodes were fallen. Some early and highly tolerant accessions developed 4 or 5 pods per plant, some of which were mature. This observation suggested the possibility of cultivation of cowpea in the dry season, sowing highly tolerant cultivars at the end of the rainy season.

Of the 900 accessions, 792 germinated well enough to get 5 or more hills. The remaining accessions were omitted from evaluation. There were 22 highly tolerant accessions (Table 1), and these could be useful for cowpea breeders.

This method was simple and saved labor. We could evaluate around 1,000 accessions at a time. However, we noticed that tolerance scores of 2 cultivars (IT82D-716 and Dan 'lla), planted at 3 replicate spots, were not always the same. Plants towards the edge of the alleys grew better than others in the same accession, indicating that residual soil moisture differed from place to place, and that degrees of competition for water between adjacent accessions were affecting plant growth. We also noticed that differences in maturity might be an important factor leading to misevaluation, because sometimes we had difficulty in distinguishing senescence from effect of drought. Thus, in field evaluation in the dry season, it is recommended (1) to use the flattest field possible for uniformity of residual soil moisture, (2) to prepare 3 replicated plots if possible, and (3) to evaluate materials within maturity groups if the information on maturity is available in advance.

Table 1. Cowpea accessions evaluated as highly tolerant in field evaluation (in Nigeria, 1990–91).

Accession† (TVu no.)	Origin (country)	Accession (TVu no.)	Origin (country)
91	South Africa	7866	Nigeria
111	Botswana	7929	Nigeria
617	Nigeria	8358	Tanzania
1548	Ghana	8565	Nigeria
3930	Nigeria	8713	Benin
4744	Niger	9178	Nigeria
4746	Niger	10460	Sierra Leone
4747	Niger	11414	Kenya
6914	Botswana	11979	Sudan
7320	Ghana	11984	Sudan
7841	Nigeria	14914	Niger

† Accessions of cowpea germplasm are numbered by TVu (Tropical *Vigna unguiculata*) number at the Genetic Resources Unit of IITA.

Pot evaluation

In pot evaluation 1, the optimum level of soil moisture for the discrimination of tolerance was found to be 3% (Table 2). At the soil moisture level of 5%, drought stress was not strong enough and the differences of scores between the most tolerant accessions and the most susceptible ones were not large enough. At the soil moisture level of 2%, on the other hand, drought stress was too strong to discriminate differences in tolerance properly.

In this method, degrees of wilting and discoloration were fairly uniform among plants of the same accession, and replication by 3 seemed adequate to evaluate tolerance correctly so long as germination was good and uniform. This method, however, was laborious, because we had to give water to pots one by one on a balance every morning. The maximum number of pots that could be taken care of was around 500, which allowed us to evaluate only about 100–170 lines at a time.

The optimum level of soil moisture for the discrimination of tolerance depends on the characteristics of the soil used. For instance, in pot evaluations 2A and 2B, where volcanic ash soil was used, we had to mix equal volume of sand to make the soil moisture level 3%, for optimum discrimination of tolerance, so that preliminary tests with available soil at hand are needed to identify the optimum level of soil moisture.

The tolerance scores obtained by repeated pot evaluations in 2A and 2B showed only small differences between scores of the repeated evaluation, and highly significant correlation coefficients between the scores ($r = 0.655$, ** $n = 90$). Pot evaluation was found to be reliable at a fixed level of soil moisture.

In pot evaluation 2A, germination was not uniform and in some accessions plants suffered from a kind of soilborne disease (not identified). Therefore, in pot evaluation 2B, seeds were scratched with a flat file and treated with a seed disinfectant (water-soluble

Table 2. Drought tolerance scores of cowpea seedlings evaluated by the pot evaluation method at three levels of soil moisture (pot evaluation 1, in Nigeria, 1991)†.

Accession (TVu no.)	Soil moisture (%)			Accession (TVu no.)	Soil moisture (%)		
	5	3	2		5	3	2
11982	5.0	4.7	1.0	127	5.0	2.0	1.0
14914	4.7	4.7	1.0	7878	3.0	2.0	1.0
11979	5.0	4.0	1.7	760	2.0	1.0	1.0
9167	5.0	4.0	2.0	8885	4.3	1.7	1.0
6914	4.3	3.7	1.0	7426	3.0	1.7	1.0
7841	4.5	3.0	1.0	8365	4.7	1.3	1.0
59	5.0	2.7	1.0	7778	3.7	1.3	1.0
7381	3.7	2.7	1.0	9357	3.0	1.3	1.0
8715	3.0	2.7	1.0	12355	4.7	1.0	1.0
8713	5.0	2.3	1.0	7758	3.7	1.0	1.0
433	5.0	2.3	1.0	8401	3.0	1.0	1.0
928	5.0	2.0	1.0	8048	3.0	1.0	1.0
85	5.0	2.0	1.0				

† Criteria of scores are as follows: 1 = plant is dead and dry. 2 = plant is still alive, but most leaves are fallen. 3 = leaves are yellow and/or wilting. 4 = leaves are yellowish or partly yellow. 5 = leaves are green. Scores of three replicates were averaged.

benomyl). These treatments were found to be quite effective in getting uniform and healthy seedlings.

It was noticed during this evaluation that all the cultivars tested were classified into intermediate and upward. Even though drought tolerance had not previously been a major breeding objective for cowpea at IITA, it is highly possible that relatively tolerant lines had been selected through yield trials under varying climatic conditions.

Comparison of evaluations by the two methods

In order to compare the tolerance scores evaluated in the two different methods employed, field evaluation and pot evaluation of seedlings, tolerance scores of the 23 accessions that were commonly used were listed (Table 3). In some accessions (for instance, TVu 8713, TVu 8885, and TVu 12355) a fairly large discrepancy of scores was observed among the 3 evaluations (the field evaluation and the two pot evaluations). But as a whole, the scores were correlated highly significantly. The correlation coefficients of scores between the field evaluation and pot evaluation 1, between the field evaluation and pot evaluation 2, and between pot evaluations 1 and 2 were 0.666**, 0.561**, and 0.664**, respectively. These findings suggested that pot evaluation with young seedlings might also be used for

Table 3. Drought tolerance scores of cowpea, evaluated by two different methods, field evaluation method and pot evaluation method (field evaluation, in Nigeria 1990–91; pot evaluation 1, in Nigeria 1991; pot evaluation 2, in Japan 1992).

Accession (TVu no.)	Field evaluation	Pot evaluation 1	Pot evaluation 2†	Mean
14914	5	4.7	5.0	4.9
11979	5	4.0	4.8	4.6
7841	5	3.0	4.8	4.3
11982	4	4.7	4.0	4.2
6914	5	3.7	2.7	3.8
9167	3	4.0	3.7	3.6
433	4	2.3	4.3	3.5
8713	5	2.3	2.7	3.3
928	4	2.0	3.7	3.2
8885	4	1.7	4.0	3.2
59	2	2.7	3.8	2.8
85	4	2.0	2.3	2.8
8715	3	2.7	2.5	2.7
7426	3	1.7	3.0	2.6
7878	2	2.0	3.6	2.5
127	1	2.0	3.5	2.2
7758	3	1.0	2.0	2.0
760	2	1.0	2.5	1.8
12355	1	1.0	3.5	1.8
9357	2	1.3	1.3	1.5
8401	1	1.0	1.9	1.3
7778	1	1.3	1.5	1.3
8048	1	1.0	1.2	1.1

† Average of two evaluations (pot evaluations 2A and 2B) is listed.

screening tolerant germplasm for crossing materials or for selecting segregated materials in breeding.

Many traits have been proposed for improving the performance of drought-affected crops (Ludlow and Muchow 1990). Some of them, for instance deep rooting (Hurd 1974; Hamblin and Tennant 1987; Lorens 1987; Watanabe 1993), may not be expressed in small-potted seedlings. Therefore, the highly significant correlation observed between scores evaluated by the two different methods was beyond expectation. Further tests are needed to clarify which accessions differ in the evaluation and which do not. Discrepancy or consistency of evaluation between the two methods may offer us important information on the mechanism of genetic differences in drought tolerance. At this stage of research, we conclude that both methods are available and we can choose either of them, keeping in view such factors as the objectives of the research, available fields or facilities, labor force, and climatic conditions.

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Cowpeas in rice-based cropping systems: integration of experimentation and modeling

J. Timsina[1]

Abstract

Cowpea grown in rice-based cropping systems in tropical Asia is subjected to varying moisture and water table regimes. Yield and yield components, root and shoot growth, and crop and soil water relations of cowpea cultivars differing in maturity were compared under (i) a line-source moisture gradient on an Iso-hyperthermic, clayey, Typic tropudalf, and (ii) a naturally occuring Typic tropudalf toposequence, during 1986–88 in the Philippines. In the post-rice environment, the medium-maturing cultivars (MMCs) outyielded early-maturing cultivars (EMCs), whereas in the pre-rice environment, early maturity had a distinct yield advantage. Cowpea roots concentrated more in the 0.1–0.2 m layer in the high water table and in saturated soil. The MMCs had greater root length density, stomatal conductance, and leaf water potential, compared with EMCs. A mechanistic crop growth model was calibrated and validated using the experimental data, and the model was used to study yield stability of the cultivars and finally to extrapolate yields to other locations in the Philippines. The model was used to predict best planting dates for cultivars of differing maturity in differing environments. It was concluded that experimentation and modeling should be integrated to enhance the efficiency of any research process.

Introduction

In South and Southeast Asia, where a monomodal rainfall pattern exists, rice is grown during the rainy season and legumes, including cowpeas, are grown during the pre- and postrainy seasons. In the postrainy season, the crop is established during the wet-dry transition period when waterlogged soils are a serious environmental constraint during the vegetative stage and dry soils are a constraint during the reproductive stage. On the other hand, in the prerainy season, the crop is established during the dry-wet transition period when dry soils are a constraint during the vegetative stage and waterlogged soils are a constraint during the reproductive stage of the crop (Zandstra 1982; Del Rosario and Pandey 1985; Pandey et al. 1986). Contrasting rainfall and water table regimes in the pre-rice environment (PrRE) and post-rice environment (PoRE) result in yield instability of cowpea cultivars over seasons and years.

Waterlogging and drought are two of the most important factors responsible for the low yields of cowpea in rice lands (Rachie 1985). Most previous research on cowpea response to drought and flooding, however, was on plants grown on freely drained sandy soils. In

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fine-textured soils with low saturated hydraulic conductivity, high water-holding capacity, and high capillary movement from the water table, cultivar response to excess and dry soil moisture regimes may be expected to differ from that observed on coarse textured soils (Hartman and De Boodt 1973; Summerfield and Lawn 1987). Classifying the response to alternating excess moisture and drought stress during the vegetative and reproductive stages, and identifying cultivars that can withstand such dual stress, may facilitate the development of more productive and stable cropping systems.

Hence we hypothesized that a strong maturity × niche interaction exists in cowpea, and that the interaction could be associated with differential adaptation of the cultivars differing in maturity in terms of root and shoot adaptation and plant and soil water relations. The adaptive mechanisms could differ across locations. Hence we also hypothesized that experimentation alone would be insufficient for understanding the adaptability and suitability of crop cultivars in any location and season. Extrapolation of research results from research sites to other potential growing areas is a major concern in agricultural research in the developing countries, and experimentation alone would again be insufficient to permit extrapolation. Crop simulators have a much greater potential for making extrapolative predictions (Chanter 1981).

We integrated experimental and modeling approaches to understand the adaptability and suitability of cowpea cultivars in rice-based cropping systems for different locations of the Philippines. We conducted six field experiments during dry and wet seasons (post- and pre-rice seasons) at the International Rice Research Institute (IRRI; 14° 17' N, 121° 5' E, 23 m altitude), Los Baños, Laguna, Philippines from November 1986 to May 1988. Three experiments were conducted in bunded lowland rice fields on Isohyperthermic, clayey, typic Tropudalf soils, with a fluctuating shallow to medium water table (0.52–1.32 m below the soil surface). Three other experiments were conducted in naturally occurring sloping toposequence fields, with a typic Tropudalf soil. We then modeled the different cowpea processes using the experimental data from our own experiments, validated the model, and predicted the growth and yield of the selected cultivars for a range of locations in the Philippines. The details about these experiments can be found in Timsina (1989). Eight papers were published in international journals (Timsina et al.1989; Penning de Vries et al. 1992; Timsina et al. 1993a,b,c,d; Timsina et al. 1994a,b). This paper synthesizes the work from all those publications, and it aims to demonstrate the integration of experimentation and modeling approaches for understanding the adaptability and stability of cowpea cultivars in a set of diverse environments.

Experimental approaches

Twenty-four diverse cultivars (Fig. 1) were selected, based on maturity and economic uses. Of these cultivars, 11 represented early-maturing grain and vegetable types (55–60 days) and 13 represented medium-maturing grain and dual-purpose types (65–80 days). Four cultivars, CES 41-6, LBBS No. 1 (Los Baños Bush Sitao No. 1), BS 6[(LBBS No. 1 × COI) 4-2-1-2], and All Season, originated from the University of the Philippines at Los Baños, while all the other cultivars were obtained from IITA through IRRI. During the post-rice season of 1986–87 and the pre-rice season of 1987, the cultivars were screened under a variable moisture regime using a line-source sprinkler system (Hanks et al. 1976) and under a variable water table regime along a toposequence (Mambani and Lal 1983).

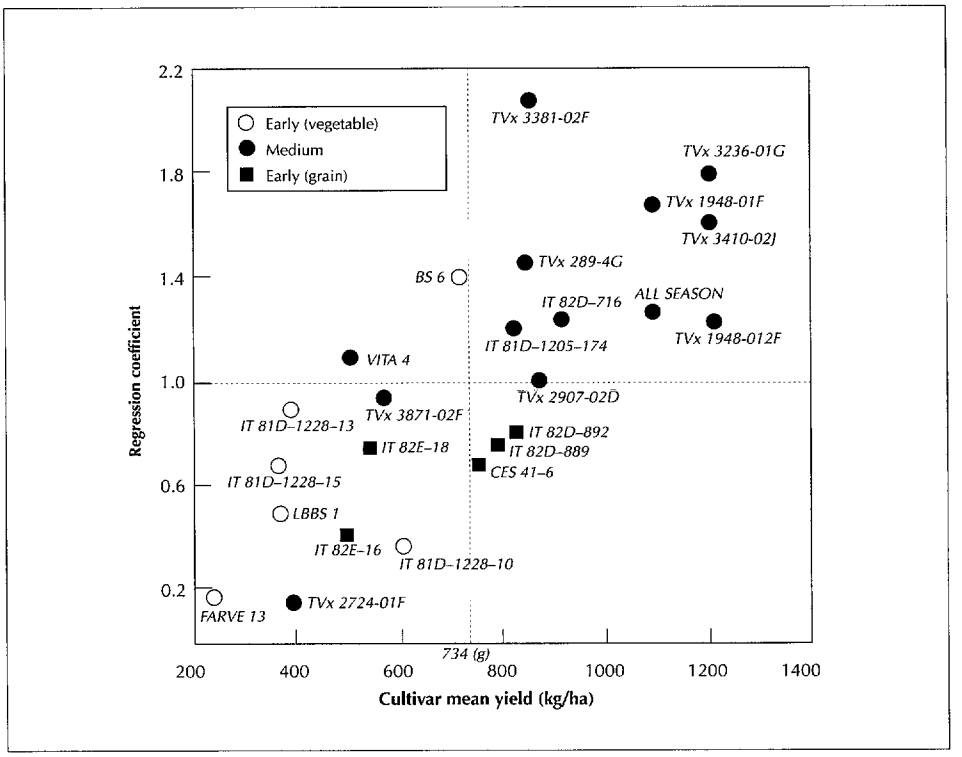

Figure 1. Relationship between cultivar adaptation and cultivar mean yield.

Stability analysis of the cultivars was performed using the models of Finlay and Wilkinson (1963) and Eberhart and Russel (1966). Based on the stability analysis, the two best medium-maturing cultivars (MMCs) and the two best early-maturing cultivars (EMCs) each from line-source experiments (LSEs) and toposequence experiments (TSEs) were selected for studying their adaptation to a range of moisture and water table regimes during the PoRE in 1987–88. Yield and yield components, shoot-dry matter partitioning, root length density (RLD), growth analysis, and plant and soil water relations were studied in both LSEs and TSEs. All the experiments were conducted in a strip plot design. Experimental details, layout, and crop environment have been discussed elsewhere (Timsina 1989; Timsina et. al. 1993a, 1994a).

Seed yield and its stability

Individual cultivar seed yields were negatively correlated with increased irrigation amount in the 1986–87 post-rice LSE, as opposed to their positive response in the 1987 pre-rice LSE. Likewise, most MMCs performed better in all water table regimes as compared to the EMCs in both PoRE and PrRE (Timsina 1989). In the 1986–87 PoRE, irrespective of moisture regimes, the MMCs as a group outyielded the EMCs, whereas in the PrRE, the EMCs (grain type) as a group had a distinct yield advantage. In the 1986–87 TSE, seed yields of the MMCs as a group were highest for the medium water table (MWT) site,

Table 1. Mean seed yield (kg/ha) of four groups of cowpea cultivars in varying moisture and water table depth regimes in various growing situations.

Moisture regime (MR)/ water table depth regime (WT)	Early-maturing cultivars		Medium-maturing cultivars	
	Grain	Vegetable	Grain	Dual
Post-rice, 1986–87				
Fully saturated	250	210	460	460
Wet	350	310	450	650
Nonirrigated	470	390	730	920
		SE (mean) ± 92		
Pre-rice, 1987				
Full evapotranspiration	1100	430	950	750
Partial evapotranspiration	840	310	700	550
Nonirrigated	580	260	509	400
		SE (mean) ± 105		
Dry season, 1986–87				
Shallow water table	770	460	1060	1280
Medium water table	1040	780	1920	2020
Deep water table	1180	1170	1750	1670
		SE (mean) ± 150		
Wet season, 1987				
Shallow water table	360	200	480	450
Medium water table	530	380	810	590
Deep water table	630	460	1030	790
		SE (mean) ± 100		

whereas those of the EMCs were highest for the deep water table (DWT) site. Irrespective of the water table level, the MMCs outyielded the EMCs. In the 1987 wet season experiment, all groups produced highest seed yields under the DWT site (Table 1). Pooled deviations as measured by stability analysis indicated that there was a high G × E interaction. Relationship between regression coefficient (cultivar adaptation) and cultivar mean yields (Fig. 1) showed that most EMCs were poorly adapted to favorable (high-yielding) environments and differed in their adaptation to unfavorable (low-yielding) environments. Most MMCs, on the other hand, were adapted to high-yielding environments, with yields in the low-yielding environments being comparable with those of EMCs, and thus were considered to be stable cultivars. During the PoRE in both LSE and TSE in 1987–88, the yield and yield component responses followed similar patterns as previous years' experiments (Timsina et al. 1993a, 1994a).

Phenology

Saturated soil and SWT significantly delayed the flowering and maturity of all cultivars in all experiments. The delay in maturity was greater than that in flowering, varying from 3 to

10 days, and the delay was more apparent in MMCs than in EMCs (Timsina et al. 1993a, 1994a). It is important to note that phenology and phenological response to important environmental factors, such as groundwater and rainfall, is critical to crop growth simulation models.

Shoot growth

Saturated soil during the PoRE adversely affected total shoot dry-matter accumulation (SDMA) and its partitioning in both LSE and TSE. The SDMA was reduced by 20% for TVx1948-012F, an MMC, and by 50% for IT82D-889, an EMC, in the fully saturated soil, compared with nonirrigated soil. IT82D-889 had a greater proportion of standing biomass in leaves (64, 59, and 76% of the total) than in stems (36, 41, and 24% of the total) during the first 31 days after emergence (DAE) under fully saturated, wet, and nonirrigated plots, respectively. This was rapidly reversed as the early cultivar matured, such that leaf biomass dropped to 7–10% of the total at 51 DAE. The medium-maturing cultivar retained more leaf biomass (18–27%) at harvest (Timsina et al. 1994b).

Total biomass and grain yields were much higher for the MMCs than the EMCs in all water table regimes in the TSE. Yet, the SDMA of all cultivars was reduced in the SWT regime. It was slowest in BS 6, an EMC, and fastest in TVx3410-02J, an MMC. The reductions in stems, leaves, pods, and total dry matter were by 82, 77, 88, and 84%, respectively, for BS 6 at maturity, while for TVx3410-02J, the reductions were only by 44, 3, 57, and 55%, respectively. The SDMA under MWT was much higher than that under SWT. There was retardation in the growth of EMCs after 43 days, suggesting that the EMCs subsequently experienced significant environmental stress. Late-season stress was not evident in MMCs, which registered their most rapid growth between 53 and 66 DAE (see Timsina et al. 1993b). The advantage in SDMA was associated with a spurt in growth by the MMCs during the late growing season, as the water table declined to levels more conducive to root health. The EMCs apparently did not experience a comparable advantage because they proceeded through the reproductive events and matured while the root environment was less favorable.

Plant heights, leaf area, and crop growth rates of all cultivars were affected by the SWT and by the saturated soil. Plant heights in the DWT sites were usually higher than in the MWT sites in contrast to biomass accumulation patterns. Saturated soil reduced the plant height by 7–10% and the leaf area index by 20–30%, as compared to the nonirrigated soil. Slow early leaf area development in the MMCs was largely compensated by more rapid canopy development later, while it was not compensated in the EMCs because of earlier initiation of reproductive events. Anaerobiosis due to SWT reduced the leaf area and crop growth rate, and delayed the timing of peak growth rate (Timsina et al. 1993b, 1994b).

Root growth

Roots of all cultivars were concentrated predominantly in the 0–10 cm depth at 30 DAE (vegetative stage) in both TSE and LSE in the dry season 1987–88. The RLD was highest in the fully saturated plots in LSE and in the SWT sites in TSE (Timsina et al. 1993b, 1994b). This observation implies that the roots tended to accumulate near the soil surface in response to oxygen deficiency under anaerobic conditions. The roots in the surface layer of the soil were spongy and thick. It is not clear whether these roots improved waterlogging

tolerance, but the formation of such roots is an adaptive mechanism for plant survival under anaerobic conditions (Jackson 1955; Russel 1977), and it needs further investigation for cowpea.

As the season progressed, water table declined, and the roots grew deeper. Root growth at deeper soil depths was greater in nonirrigated and in the DWT sites in LSE and TSE, respectively. The MMCs had greater RLD than the EMCs, especially at the 0.4–1.0 m depths, in all water table and moisture regimes, particularly in the driest regime. Soil water extraction pattern showed that the two MMCs apparently extracted water from greater depths as compared to the EMCs (Timsina et al. 1993b). These cultivars obtained a higher proportion of their transpiration requirements from capillary water rising from the water table. The MMCs maintained a relatively shallow root system during the vegetative stage and a deep root system during the reproductive stage. The shallow root systems could enhance root aeration during the period of saturated soil moisture conditions during the vegetative phase. During the reproductive phase, drainage and drought stress were associated with deeper root penetration. Thus, the MMCs exhibited a dual mechanism of adaptation to waterlogging and drought. Russel (1977) also reported that living roots are confined to the surface layers of soil under saturated conditions, and when water drains rapidly, the rate of deep root extension influences the subsequent ability of the plant to survive and yield.

Plant water relations

There was a seasonal trend of increasing canopy temperature (CT) of all cultivars, associated with a decrease in leaf water potential (LWP). LWPs were generally lower and CTs were higher in the SWT regime. On any given day, the differences in the LWP and CT values among the three water table regimes were generally lower in the MMCs than in EMCs. The MMCs (TVx3236-01G and TVx3410-02J) generally maintained a higher LWP and lower CT than the EMCs (BS 6 and IT82-892) (Timsina et al 1993b). The LWPs and diffusive conductances (DCs) were also higher in the nonirrigated than in the fully saturated sites. The LWPs and DCs were greater for TVx1948-012F, an MMC, than for IT82D-889, an EMC (Timsina et al. 1994b).

Simulation modeling approaches

Model structure, parameterization, and validation

A simulation modeling approach was adopted in understanding the yield variability/stability of the cultivars as a result of G × E interaction. Three modules (L1D, L2C, and L2SS) from MACROS-CSM (Penning de Vries et al. 1989) were selected and combined to simulate cowpea growth and seed yield on soils with impeded drainage. The model, written in Continuous System Modeling Program (CSMP), is largely explanatory, mechanistic, and process-based. The L1D module simulates crop growth and development processes, using a 1-day time interval of integration. The L2C module simulates transpiration and evaporation, and the L2SS module simulates the water balance for crop growth with partially or fully saturated soils, typical for rice-based cropping systems. Major modifications made in the original model included effect of N-redistribution, after-effects of water stress, and effects of drought and waterlogging on germination and reproductive

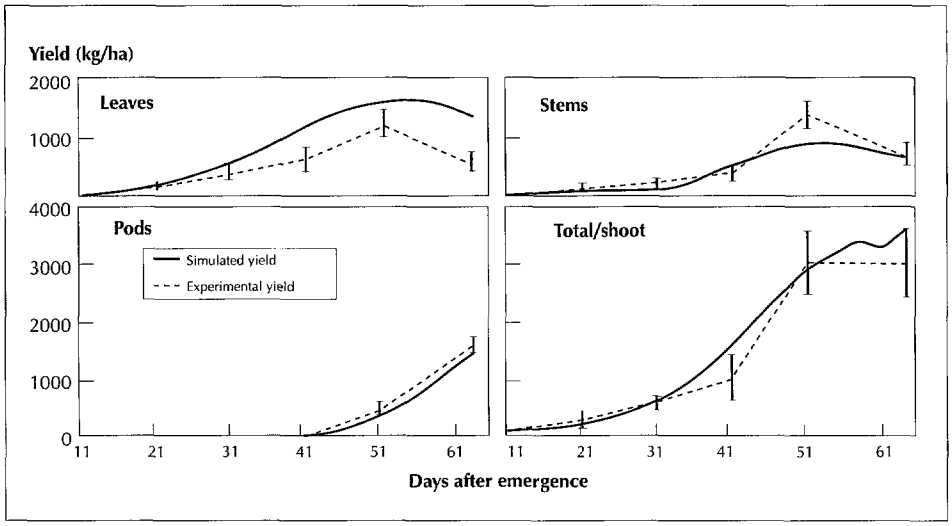

Figure 2. Simulated and experimental weights (kg/ha) of leaf, stem, pod, and total shoot of TVx1948-021F in a post-rice environment.

growth. Several realistic assumptions, conditions, and decision criteria were made during such modifications. Documentation of the model is presented in Penning de Vries et al. (1992) and Timsina et al. (1993c).

When the model was parameterized using experimental data for TVx1948-012F and IT82D-889 and run, the model results were similar to the experimental results. The model predicted weights of pods, stems, and total shoot dry matter satisfactorily, but overestimated the leaf dry weight (Fig. 2). When the model was validated using independent data sets from IRRI (Villegas 1982; Pandey et al. in press), the simulated and the observed seed yields, evapotranspiration, and soil moisture contents were somewhat similar, thus providing confidence about the application of the model.

Model application

Simulation models have great potential for making extrapolative predictions, i.e., predictions beyond the range of database (Chanter 1981), and they allow us to make use of our knowledge of specific plant processes. Our model validation was succeeded by running the model for different water table depth regimes to establish cowpea performance for a long-time series for three locations in the Philippines differing in rainfall patterns and latitudes: Los Baños (14° 17' N, 121° 5' E, 23 m altitude), Iloilo (10° 52' N, 122° 5' E, 8 m altitude), and Davao (7° 4' N, 122° 5' E, 125 m altitude). Twenty years of historical weather data were used for Los Baños, while 5 years of actual and additional 15 years of generated weather data were used for Iloilo and Davao. Simulation was carried out for a range of planting dates for two situations: first, where sufficient water is available either from groundwater, irrigation, or rain for germination of seeds and growth of seedlings until 10 DAE; and second, where water shortage or excess may occur during germination and seedling establishment. The first simulates the situation where the farmer plants after rain

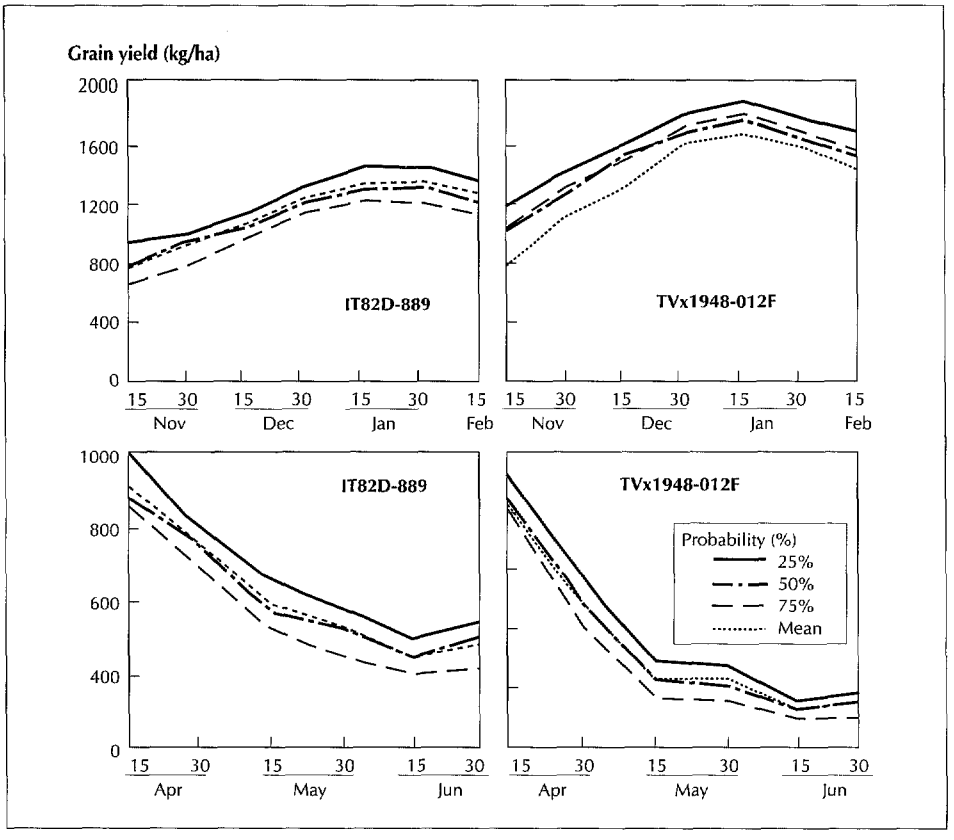

Figure 3. Effect of date planting on simulated grain yields of two cowpea cultivars for fields with a shallow water table and partial irrigation in pre- and post-rice environments at Los Baños, Philippines.

or provides initial irrigation (partial irrigation), whereas the second simulates a strictly rainfed situation. All simulations were carried out for optimal nutrients supply and situations free of insects, diseases, and weeds.

Figure 3 shows the effect of date of planting on simulated grain yields of TVx1948-012F, an MMC, and IT82D-889, an EMC, for fields with a SWT and partial irrigation during PrRE and PoRE at Los Baños. For all dates in the PoRE, mean seed and biomass yields of TVx1948-012F were higher than those of IT82D-889, while in the PrRE, the reverse was the case, thus corroborating the results from the field experiments. The patterns of simulated yields of these cultivars (IT82D-889 during PrRE and TVx1948-012F during PoRE) for fields with a SWT with partial irrigation were different for the three sites (Fig. 4). The yearly and seasonal variation in biomass yields of these cultivars during the PrRE and PoRE under exclusively rainfed and partially irrigated situations also exhibited different relationships (Timsina et al. 1993d).

Based on the simulation results, optimum planting dates for different moisture regimes during PrRE and PoRE were also determined for the three locations. The simulation results presented here have important policy implications, as they provide guidelines for

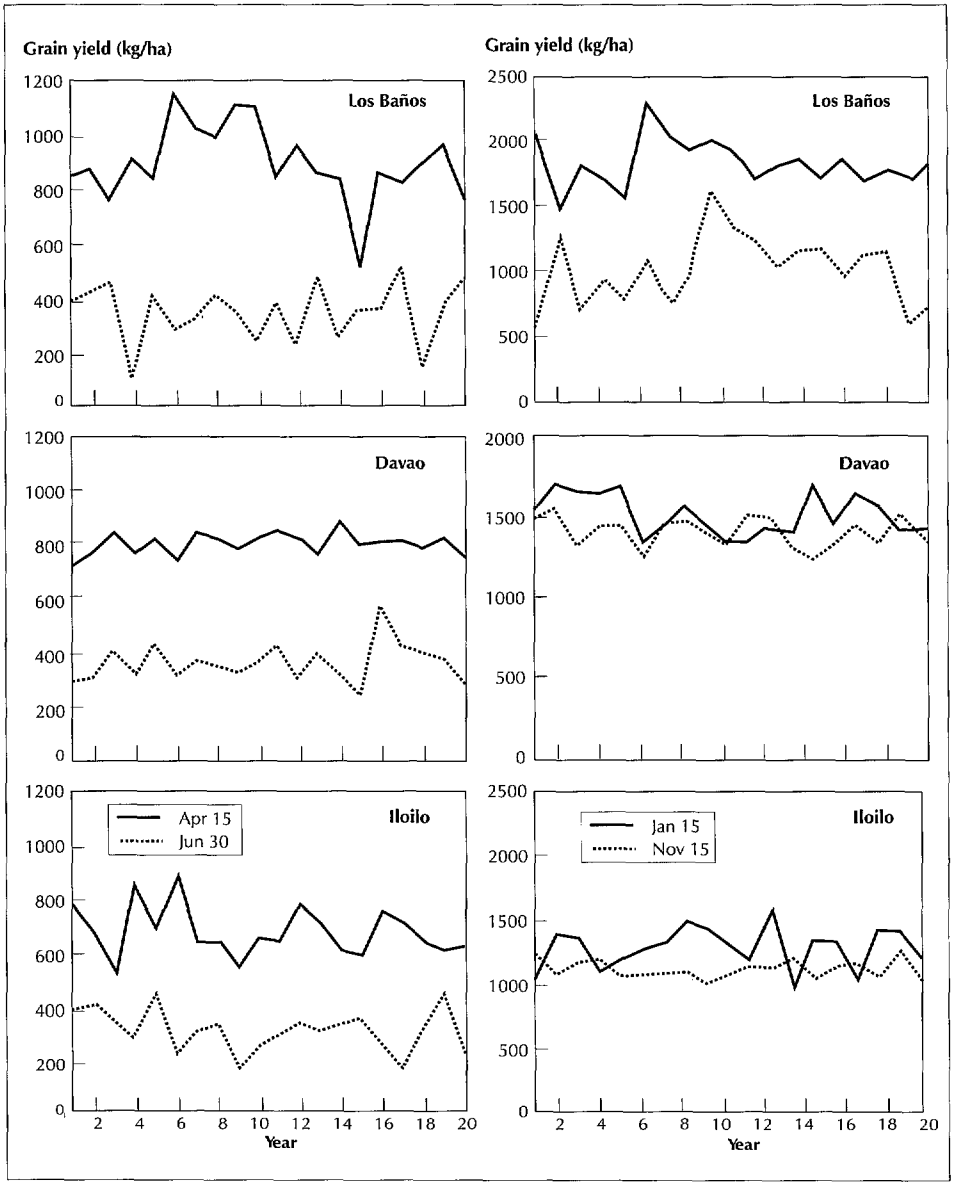

Figure 4. Year-to-year variation in simulated grain yields of two cowpea cultivars for fields with a shallow water table and partial irrigation, sown in pre- and post-rice environments at Los Baños, Davao, and Iloilo in the Philippines.

agricultural extension and planning offices for the introduction of cowpeas in new areas. Required levels of crop protection and fertilization, as well as expenses involved, are calculated for soybeans using SOYCROS (Penning de Vries et al. 1992), and can also be calculated for cowpea using this model.

Integration of experimentation and modeling

The field experiments provided a measure of the yield stability of the cultivars, and were also useful in understanding the adaptability of the selected cultivars in the rice-based cropping systems. However, as the experiments were conducted for only a few seasons at one location, the results are location-specific and will be valid only for areas with conditions similar to those of the experimental site. The growth and behavior of the cultivars differ from season to season and from year to year, as a result of seasonal and yearly variation in weather patterns, water table depth regimes, and soil types. Therefore, one cannot generalize the conclusions on yield stability for other seasons, years, and locations. To generalize the conclusions from experimentation, one has to conduct experiments over many seasons, years, and locations, which is an almost impossible task. Thus experiments are rather limited as far as measuring seasonal and yearly variability of crop yield is concerned.

Experiments and models, however, both have strengths of applications. In our study, experiments were useful to determine the response of the cultivars to moisture regimes (in PrRE and PoRE) and to water table depth regimes (during dry and wet season topo-sequences). Models and simulations have advantages for predicting overall yield responses and long-term perspective. The simulation results supported those of the field experiments, and, furthermore, allowed a multiyear analysis for estimation of the long-term variability of cultivar-specific yields. The model allowed for the investigation and identification of stable cultivars, based on multiyear weather data. The model also identified optimum planting dates of early- and medium-maturing cultivars for rice-based cropping systems in the Philippines.

Conclusions

Our studies gave some insights into the adaptability and stability of cowpea cultivars across a range of moisture and water table depth regimes for contrasting post-rice and pre-rice situations. Two approaches were examined: experimentation and mechanistic modeling. The experimental approach indicated that among the cultivars tested, representing early- and medium-maturity groups, there was a distinct maturity × season interaction. In the PoRE, irrespective of water table level, the MMCs outyielded EMCs; in the PrRE with an SWT, EMCs demonstrated a distinct yield advantage.

These observations were explored in greater depth with a cowpea simulation model. The simulation results indicated that the yields of EMCs and MMCs were distinctly different for the PoRE and the PrRE, and that the optimum planting dates differed for the three experimental sites in the Philippines. Mechanistic computer simulation models can be powerful tools for predicting and extrapolating yields for various situations, as the opportunities are limited in every developing country for conducting a series of long-range accurate field experiments and for taking intensive measurements using sophisticated instruments. Our studies demonstrated the value of integrating experimental and modeling approaches in improving our understanding of the adaptability and stability of cowpea cultivars grown in the rice-based cropping systems in the Philippines. Agricultural research systems can thus integrate experimental and modeling approaches in order to enhance the efficiency of the research process.

Acknowledgments

This contribution is based on eight published papers and one unpublished paper. Drs D.P. Garrity, F.W.T. Penning de Vries, and R.K. Pandey are coauthors in those publications.

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14

Viral diseases of cowpea and their control by resistance-conferring genes

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Abstract

Cowpea crops are susceptible to more than 20 viral diseases. Some of the most destructive viral pathogens are transmitted from one plant generation to the next through the seed, and thus are generally disseminated to most cowpea-producing regions of the world. Seedborne cowpea viruses, after establishment in plantings as seedborne inoculum, are typically spread within fields by insect vectors (either aphid or beetle species). The most effective control of cowpea viral diseases, universally, has been the development of improved genotypes with resistance to viral infection. The historic productiveness of cowpea breeder-geneticists, describing genes/resistance to almost every major virus, now provides opportunities to develop multiple resistance to diseases, insect pests, *Striga* spp., and drought. Although cowpea may lag behind other major food plants in the availability of superior new cultivars with multiple-disease/pest resistance, an extremely valuable base of germplasm exists for much greater development and utilization in the future.

Introduction

Far-reaching developments have occurred in plant virology since the First World Cowpea Research Conference in November 1984 (Thottappilly and Rossel 1985). Since that time, researchers have sequenced and mapped the genomes of many viruses, determining the genetic structure/function of important viral pathogens, and have established a meaningful taxonomic system for virus families and genera. In this system, molecular-genetic information developed for *one member* of a viral family provides essential clues to the nature of lesser-known members of that family. Indeed, strategic molecular biology research has facilitated logarithmic increases in our knowledge of the properties of viruses since 1984.

There have also been many surprises along the way, particularly in the genetic engineering of viral genes into crop species, producing transgenic plants. Whereas viral gene transfers were initially carried out somewhat simplistically, they are now viewed with increased understanding and maturity. We are now learning that very small changes in viral gene sequences (Lindbo et al. 1993b) and the points of insertion into host chromosomes

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have significant consequences for viral-gene expression and in the trans-gene antiviral function. Accordingly, successes from "viral coat protein-mediated resistance" have ranged from mediocre to superb, usually with little understanding of the disparity (Lindbo et al. 1993a). Mixed results from this approach have prompted scientists (1) to transform plants with mutant (defective) viral coat-protein genes and/or investigate the mechanisms yielding successes; (2) to explore/implement viral genes other than the coat protein gene (native or mutant); or (3) to transform plants with nonviral genes/sequences that logically might interfere with one or more steps of viral genome translation, transcription, or genome/virion movement. These and other newer lines of research promise improved understanding of viral structure and function, as well as clearer insights into mechanisms of natural resistance to viral infection.

General knowledge pertaining to *Vigna unguiculata* genetics and germplasm has also expanded. Numerous sources of disease/pest resistance have been reported during the past decade. Implementation of these resources has resulted in new, improved cowpea cultivars with multiple disease resistance, pioneered by Lima et al. (1979), Mali et al. (1981), Patel et al. (1982), and Price and Cishahayo (1986). A new cowpea cultivar, recently developed for Senegal, possesses combined resistance to two cowpea aphid-borne mosaic virus pathotypes, bacterial blight, storage weevil, *Striga*, and drought (Ndiaye et al. 1993; Cisse et al. 1995). Cultivars with multiple virus resistance were also developed in Nigeria (Singh et al. 1987) and Brazil (Santos et al. 1987, 1990). Other reported virus-resistant cowpea genotypes include 'Seoweondongbu', Korea (Kim et al. 1986), and 'Bettersnap', USA (Fery and Dukes 1995). Neither breeding approaches nor germplasm resources are any longer factors limiting such advancements. The future holds further promise in this regard, with new insights expected from biotechnology. But in 1996, conventional resistance breeding remains the most practicable measure for controlling cowpea viral diseases.

Cowpea viruses

This review complements and/or updates previous reviews by Allen (1983), Mali and Thottappilly (1986), Shoyinka et al. (1988), and Thottappilly and Rossel (1985, 1992). Of the viruses occurring in cowpea crops around the world (Table 1; the viral terms and acronyms used are consistent with those of Hull et al. [1991], wherever possible), the seedborne viruses considered most insidious and damaging include: blackeye cowpea mosaic potyvirus (BlCMV), cowpea aphid-borne mosaic potyvirus (CABMV), cucumber mosaic cucumovirus (CMV), cowpea mosaic (CPMV) and cowpea severe mosaic (CPSMV) comoviruses, southern bean mosaic sobemovirus (SBMV), and cowpea mottle carmovirus (CPMoV). Some combinations (e.g., BlCMV + CMV; BlCMV + CPSMV; and CMV + CPSMV + SBMV) can cause drastically worsened disease symptoms and crop losses (Kuhn 1990; Anderson et al. 1994). Other detected cowpea mixed-infections include CMV + CPSMV and CPSMV + SBMV (R.O. Hampton et al. 1992, unpublished results). These viruses have been disseminated to, and established in, most cowpea-producing areas of the world as infected commercial seedlots, variety trials, or germplasm.

Important nonseedborne viruses include cowpea golden mosaic geminivirus, which causes one of the most destructive cowpea diseases in the world, and cowpea chlorotic mottle bromovirus, which causes disease losses either alone or in combination with other viruses (reviewed by Kuhn 1990).

Table 1. Some properties of viruses causing principal diseases of cowpea†.

Virus	Transmission				Coat protein Mol Wt x 10^4	Genome		Key references
	Mech.	Vector	Seed (%)	Particle		Parts	Nucleotides x 10^3	
Blackeye cowpea mosaic potyvirus (BlCMV)	Yes	Aphid	3–55	Filament	3.4	One	9.5	Purcifull and Gonsalves 1985; Taiwo et al. 1982a
Cowpea aphid-borne mosaic potyvirus (CABMV)	Yes	Aphid	0–40	Filament	3.4	One	9.5	Bock and Conti 1974; Taiwo et al. 1982a
Cowpea chlorotic mottle bromovirus (CCMV)	Yes	Beetle	0	Icosahedron	2.0	Three	8.2	Kuhn 1964; Allison et al. 1989
Cowpea golden mosaic geminivirus (CGMV)	No	Whitefly	0	Duplex	31	ssDNA§ Two	5.0	Thottappilly and Rossel 1992; Bashir and Bashir 1988
Cowpea mosaic comovirus (CPMV)	Yes	Beetle	0–5	Icosahedron	2.3, 3.7	Two	9.4	Agrawal 1964; van Kammen and de Jager 1978; Lomonossoff and Shanks 1983
Cowpea mottle carmovirus (CPMoV)	Yes	Beetle	0–10	Icosahedron	4.4	One	4.4	Bozarth and Shoyinka1979; Thouvenel et al.1990
Cowpea severe mosaic comovirus (CPSMV)	Yes	Beetle	3–10	Icosahedron	2.3, 3.7	Two	9.4	de Jager 1979; Chen and Bruening 1992a,b
Cucumber mosaic cucumovirus (CMV)	Yes	Aphid	4–26	Icosahedron	2.4	Three	8.3	Francki et al. 1979; Rezaian et al. 1984
Southern bean mosaic sobemovirus (SBMV)	Yes	Beetle	4–4	Icosahedron	3.1	One	4.2	Shepherd and Fulton 1962; Wu et al. 1987

† Other viruses reported to infect cowpea include alfalfa mosaic virus (ilar-like), cowpea mild mottle carlavirus, peanut mottle potyvirus (PMV), peanut stunt cucumovirus, sunnhemp mosaic tobamovirus, tobacco ringspot nepovirus (TRSV), and tobacco streak ilarvirus. Tomato spotted wilt tospovirus is thrip-transmissible and, since 1984, has assumed almost worldwide distribution in both temperate and semitropical regions, and can potentially cause damage to food legume crops, including cowpea.

§ All other viruses listed have genomes comprising ssRNA.

Blackeye cowpea mosaic potyvirus (BlCMV). BlCMV occurs more or less worldwide and is transmitted nonpersistently by several aphid species, including *Aphis craccivora* (Purcifull and Gonsalves 1985). Particularly in combination with other viruses (Pio-Ribeiro et al. 1980; Collins et al. 1985; Kuhn 1990), it can inflict severe losses on cowpea crops. Distinct BlCMV strains exist (Bashir 1992; Bashir and Hampton 1992), but strain variants may be less decisive in BlCMV disease epidemics than is notable for CABMV.

The work of Taiwo et al. (1982a) partitioned potyviruses seedborne in cowpea into two distinct kinds. With differing results and interpretations, Dijkstra et al. (1987) distinguished two potyviruses, but recommended that both be called BlCMV. The relationship between BlCMV and CABMV was discussed at a potyvirus taxonomy workshop (Barnett 1992), with clear indications that BlCMV and CABMV were distinct potyviruses and that separate nomenclature be maintained.

Bashir (1992) biologically and serologically characterized some 140 cowpea potyvirus isolates seedborne in cowpea seedlots from various countries (Bashir 1992; Bashir and Hampton 1992, 1993), in comparison with type isolates BlCMV-Georgia (BlCMV-GA) and CABMV-Morocco (CABMV-Mor). This work clearly partitioned the two viruses, determined that CABMV-Kenya (Bock 1973; Dijkstra et al. 1987) was instead BlCMV, and verified much of the work of Taiwo et al. (1982a). Key isolates characterized by Bashir (1992) were also instrumental in definitive monoclonal antibody distinctions of the two viruses by Huguenot et al. (1993, 1994). The Florida isolate of BlCMV was considered by McKern et al. (1992) to be a strain of bean common mosaic virus.

Genetic resistance to BlCMV and CABMV in cowpea is distinct (Bashir 1992) and independently inherited (Taiwo et al. 1982b). The nucleotide sequence of the BlCMV genome has not yet been published.

Cowpea aphid-borne mosaic potyvirus (CABMV). First described by Lovisolo and Conti (1966), CABMV is endemic in Africa. It is now widely disseminated in the world through infected cowpea seedlots, and causes severe crop damage either alone (Ndiaye et al. 1993) or in combination with other viruses. Like BlCMV, it is transmitted nonpersistently by several aphid species, including *Aphis craccivora*. The virus comprises numerous distinct strains (Fischer and Lockhart 1976; Bashir 1992; Ndiaye et al. 1993), with separate cowpea genes conferring resistance to each (Bashir 1992; Ndiaye et al. 1993).

CABMV and BlCMV produce indistinguishable symptoms on cowpea genotypes susceptible to them, typically consisting of veinal chlorosis, interveinal chlorosis, or dark-green vein banding (Bock and Conti 1974; Purcifull and Gonsalves 1985). The Morocco isolate (Fischer and Lockart 1976), CABMV-Mor, has been widely used as a quasi type isolate, but it is extremely virulent and poorly representative of 80 separate seedborne CABMV isolates that were evaluated at Corvallis, Oregon, USA (Bashir 1992; Ndiaye et al. 1993).

The potyvirus designated PTY+ by Ndiaye et al. (1993) was later determined to be a distinct, virulent strain of CABMV (R.O. Hampton, unpublished results). This CABMV pathotype clearly differs from CABMV-Mor, and sources of cowpea genetic resistance were identified (Ndiaye et al. 1993). A sizeable but unknown number of pathogenic variants exist in nature, some of them responding to separate cowpea genes/alleles for resistance. The nucleotide sequences of the CABMV genome have not yet been published.

Cowpea chlorotic mottle bromovirus (CCMV). CCMV was not accepted as a distinct virus until the definitive work of Kuhn (1964a) and Bancroft et al. (1968). Kuhn (1964b) also developed differential hosts for distinguishing CCMV, SBMV, CMV, and BYMV (actually BlCMV). In susceptible cowpea cultivars, CCMV can cause severe crop damage, alone or in mixed infections. Uniquely severe disease is caused by CCMV in mixed infections with SBMV (Kuhn and Dawson 1973). Once assumed to be confined to North and South America, CCMV was more recently isolated from *Desmodium heterocarpon* and *Clitoria ternatea* in Nigeria (Thottappilly et al. 1993). The occurrence of CCMV in natural hosts outside of the Americas suggests that it may persist in native legumes of other cowpea producing regions of the world. The genomic RNA of CCMV was sequenced and compared to that of other bromoviruses by Allison et al. (1988, 1989).

Cowpea golden mosaic geminivirus (CGMV). CGMV, as a singular causal agent, has not yet been isolated, purified, and identified. Thottappilly (1992) and Thottappilly and Rossel (1992) reported the occurrence of CGMV-like diseases in at least seven countries of Africa. The agent may be similar to pathogens partially characterized as "cowpea yellow fleck" from India (Sharma and Varma 1976), as "cowpea bright yellow mosaic" from Pakistan (Ahmed 1978), and as "mungbean yellow mosaic virus" from Pakistan (Bashir and Bashir 1988). Cowpea samples from Nigeria with CGM symptoms produced weak reactions with monoclonal antibodies specific to whitefly-transmitted geminiviruses (Thottappilly and Rossel 1992), suggesting that CGM is a geminivirus.

The CGM disease, as currently recognized in Pakistan (M. Bashir, personal communication), has caused increasingly severe damage to cowpea plantings in that country since 1988. No sources of genetic resistance to CGM were identifiable in recent evaluations in Pakistan of *V. unguiculata* germplasm. According to Anno-Nyako (1980), many cowpea cultivars tested at IITA in Nigeria were resistant to CGMV, and attempts to retrieve the virus from inoculated plants were unsuccessful. The identification of resistance sources, however, is expected to depend on controlled inoculations of plant genotypes with defined virus isolates capable of reproducing typical golden mosaic symptoms in standardized cowpea genotypes. If the disease is caused by a complex of distinct viruses, cowpea resistance must then be independently tested for each component pathogen of the complex.

Cowpea mosaic comovirus (cowpea yellow mosaic) (CPMV). CPMV, originally described as cowpea yellow mosaic virus (Chant 1959), reportedly occurred in the Americas before 1964, since an isolate from Suriname was identified as CPMV (Agrawal 1964). It has since been reported from several African countries (Thottappilly and Rossel 1985). Though its identity and existence in older cowpea landraces/varieties in both West Africa (Chant 1959; Patel and Kuwite 1982) and India (Hampton et al. 1992) are generally accepted, CPMV was not detected recently in either Senegal (Ndiaye et al. 1993) or Pakistan (Bashir and Hampton 1993). Some CPMV isolates appear to be marginally seed transmissible (Gilmer et al. 1974 suspected 1–5%), but this could not be confirmed in other cowpea genotypes (Thottappilly and Rossel 1988a).

Owing to its common occurrence, epidemic potential, and pathogenicity, CPMV is one of the most important cowpea viruses in Africa. Most locally grown varieties (large, white, rough-seeded) appear highly sensitive and susceptible. The virus also occurs in pigeonpea

(Bock 1971), soybean (Thottappilly and Rossel 1992), and bambara groundnut (Thottappilly and Rossel 1997). The best and most practical method of control may be the use of resistant cultivars (Robertson 1965; Williams 1975, 1977; Singh et al. 1987).

The RNA genome of CPMV, type member of the comovirus group, has been sequenced and defined in a classic series of investigations by van Kammen and colleagues, as reviewed by Matthews (1991).

Cowpea mottle carmovirus (CPMoV). Originally isolated in Nigeria (Shoyinka et al. 1978; Bozarth and Shoyinka 1979), CPMoV readily cross-reacts with antiserum to bean mild mosaic carmovirus (Gillaspie et al. 1994) and is probably abiotically transmitted in soil for > 2 months after infected plants are removed (R.O. Hampton, unpublished results). An Ivory Coast isolate of CPMoV was characterized by Thouvenel et al. (1990), who also considered it a significant disease, since it caused a 65% reduction in yield there. In addition, the virus has been reported from the Republic of Benin (Thottappilly and Rossel 1988b), Togo (Gumedzoe et al. 1990), and Pakistan (Bashir and Hampton 1993). It has also been detected in seedlots from Botswana and Senegal (R.O. Hampton, unpublished).

The capsid protein gene of CPMoV was sequenced by Kim and Bozarth (1992), and the sequencing of the whole CPMoV genome was recently completed by You (1995) and You et al. (1995). The genomes of four other carmoviruses have been sequenced, as reviewed by Hacker et al. (1992) and Skotnicki et al. (1993), further promoting the possibility of developing viral-gene-mediated resistance to CPMoV in cowpea.

Cowpea severe mosaic comovirus (CPSMV). CPSMV was characterized by Shepherd (1964) as "Arkansas cowpea mosaic virus". Its host range was very extensive, compared to the narrow host range of cowpea mosaic, and isolates of this type, transmitted by Chrysomelid beetles, were separated from CPMV by Agrawal (1964) and named CPSMV (de Jager 1979). CPSMV-induced symptoms in some cowpea genotypes are similar to those of CPMV. Contrary to the term "severe", these symptoms may or may not be more severe than those of CPMV. Certainly, the CPSMV isolate of de Jager (1979) induced very severe symptoms on well-known cowpea cultivars. Crop losses inflicted by CPSMV can be severe (50–80%, Debrot and De Rojas 1967; Valverde et al. 1982); however, losses depend largely on specific interactions between CPSMV strains and cowpea genotypes. CPSMV is seed transmissible and also efficiently transmitted by several beetle species, including *Cerotoma ruficornis* and *C. trifurcata* (Walters and Barnett 1964; Debrot and De Rojas 1967), which can retain the infective virus for more than 7 days.

The virus may have assumed worldwide distribution via movement of infected seedlots and appears to be more common than CPMV in the cowpea cultivars of southern Europe and the Americas and less common in old world cowpea-growing regions (Bashir and Hampton 1993; Ndiaye et al. 1993).

CPSMV comprises at least nine serotypes (J.H. Hill, isolate donations to The American Type Culture Collection; Di et al. 1993) and an unknown number of pathogenic variants. No sources of CPSMV resistance are known among US cowpea cultivars, as reviewed by Kuhn (1990); however, four IITA TVu lines (612, 1460–2, 1948, and 2480) were highly resistant to all tested CPSMV variants (Fulton and Allen 1982). The nucleotide sequence of CPSMV genomic RNA was published by Chen and Bruening (1992a,b).

Cucumber mosaic cucumovirus (CMV). CMV is one of the most broadly adapted of all plant viruses (Francki et al. 1979), and is also commonly seedborne in cowpea seedlots. Despite its common and widespread occurrence, through both seed- and aphid-transmission, CMV is considered a mild cowpea pathogen, except in infection-sensitive genotypes and/or when combined with BICMV (Pio-Ribeiro et al. 1980; Anderson et al. 1994) or with other viruses (Collins et al. 1984; Kuhn 1990). The epidemiology of CMV in *Vigna* spp. has been documented by Lakshman et al. (1985).

Although the term "cowpea strain" (CMV-CP) is used in the literature, it was not included among recognized CMV strains by Gibbs and Harrison (1970) or Francki et al. (1979). The extent to which cowpea isolates differ from other legume-infecting forms is not well defined. Legume-infecting isolates CMV-Pg and CMV-Le (Hampton and Francki 1992) are distinguishable from CMV-CP biologically but have antigenic determinants in common with CMV-CP. Antisera/IgG to either CMV-Pg or CMV-Le react with, but also differentiate, CMV-CP (R.O. Hampton, unpublished results).

The tripartite RNA genome of CMV was sequenced and defined by Symons and colleagues (Gould and Symons 1982; Rezaian et al. 1984, 1985) and cloned, transcribed, and tested for infectivity by Hayes and Buck (1990). Several pathological traits have been ascribed to genomic RNA-1, 2, and 3 (Rao and Francki 1982; Edwards et al. 1983; Lakshman et al. 1985). Because of our present knowledge of the CMV genome, CMV-mediated transgenic resistance appears plausible as a CMV control measure, particularly if no natural resistance to CMV were available in *V. unguiculata.*

Southern bean mosaic sobemovirus (SBMV). The cowpea strain of SBMV (SBMV-C) was discovered as a seedborne isolate in a seedlot of 'Wilt Resistant Early Ramshorn' cowpea (Shepherd and Fulton 1962). It often occurs in mixtures with other beetle-transmissible viruses, including CCMV (Kuhn 1990) and CPSMV (R.O. Hampton, unpublished results). Like other seedborne cowpea viruses, SBMV-C is becoming distributed to most cowpea-producing regions of the world. Reports of SBMV from India and many locations in Africa since 1974 were reviewed by Thottappilly and Rossel (1992).

SBMV-C-induced symptoms are exceptionally variable among cowpea genotypes (Kuhn 1990), ranging from symptomless infection to severe mottle/mosaic with leaf deformity. Kuhn (1990) reviewed several forms of SBMV resistance in cowpea, including infection localization and inhibition of virus synthesis. Another resistance mechanism in 'Bountiful' bean, associated with the formation of abnormal SBMV-C virions, apparently prevented systemic spread of the virus to noninoculated trifoliolate leaves (Fuentes and Hamilton 1993). However, resistance to intercellular SBMV-C movement in inoculated primary bean leaves was overcome by co-infection with sunnhemp mosaic tobamovirus (Fuentes and Hamilton 1991). The molecular structure of the SBMV virion was determined by Rossman and colleagues (e.g., Silva and Rossman 1987) and has perhaps received more attention than any other plant virus, relative to virion fine-structure. Antigenic determinants of the SBMV capsid were defined with monoclonal antibodies by Tremaine et al. (1985). The SBMV RNA genome was sequenced and defined by Wu et al. (1987).

Other viruses. Viruses isolated from cowpea but of undetermined or minor significance include alfalfa mosaic virus (Jaspers and Bos 1980), cowpea mild mottle carlavirus (Brunt

and Kenton 1973; Anno-Nyako 1980), peanut mottle potyvirus (Demski et al. 1983), peanut stunt cucumovirus (Abdelbagi and Ahmed 1990), sunnhemp mosaic tobamovirus (Chant and Gbaja 1987), and tobacco ringspot nepovirus (de Zeeuw and Ballard 1959; Mali and Ganacharya 1984). Beet curly top geminivirus (Matthews 1991) has been observed and identified in cowpea, in California (R.O. Hampton and A. Hall 1990, unpublished). Tomato spotted wilt tospovirus is infectious to cowpea, has caused increasing damage to susceptible crops in temperate and semitropical regions (Brunt et al. 1996), and could become a threat to cowpea crops.

Genetics of cowpea viruses

A significant and expanding base of information on nucleotide sequences and junctions of viral genes is now available for genetic engineering. This database provides unprecedented opportunities to increase our understanding of viral gene structure and function, facilitating effective choices and applications of viral-sequences and mutant viral-sequences as transgenes. In the past, sequences from the viral coat-protein gene were used almost exclusively to produce transgenic plants. However, all viral genes are now being viewed as potential and manipulable inhibitors of virus synthesis and/or movement. This subject was reviewed expertly by Buck (1991) and, notwithstanding some confusion in resistance terminology, by Fraser (1990a,b).

Genes conferring resistance to cowpea viruses

Until genetic engineering is further refined, breeding for virus resistance remains the most practical approach for controlling viral diseases of cowpea (e.g., Rossel and Thottappilly 1988). Current concepts relating to virus-resistance breeding were thoroughly reviewed recently from three perspectives (Kyle and Provvidenti 1993; Provvidenti 1993; Scully and Federer 1993). Resistance- or tolerance-conferring cowpea genes or genetic resources were reported between 1955 and 1992 for ten viruses pathogenic to cowpea crops (Table 2). Of the resistance-conferring cowpea genes that have been reported (whether or not named), ten are recessive and eight are dominant.

It is noteworthy that resistance to BlCMV was determined to be recessive in three cases (Reeder et al. 1972; Walker and Chambliss 1981; Taiwo et al. 1982b) and dominant in two (Strniste 1987; Ouattara and Chambliss 1991). The recessive-gene sources were, respectively, PI 297562, TVu 2480, and cultivar Worthmore. The dominant-gene sources were cultivars Pinkeye Purple Hull BVR and White Acre BVR. These two genes were compared by Strniste (1987) and shown, by demonstration of independent inheritance, to be distinct. Two independent genes governing resistance were also demonstrated for a Tanzanian isolate of CABMV, one recessive and one "partially dominant" (Patel et al. 1982). Partial dominance in this case was probably attributable to lower-than-normal virulence of the virus isolate, which may have been modified (partially attenuated) after successive local-lesion passage through *Chenopodium amaranticolor*. This virus isolate was later reported to be BlCMV, rather than CABMV (Bashir 1992; P.N. Patel, personal communication, 1992). Likewise, both dominant and recessive genes govern cowpea resistance to CPMV (Patel 1982a) and SBMV (Brantley and Kuhn 1970; Hobbs et al. 1987).

Until the singularity or diversity of CGMV is clearly defined, cowpea resistance to the CGM disease cannot be expected.

Table 2. Genes or genetic sources reported for resistance to viral diseases of cowpea.

Virus†	Gene(s)	Source§	Reference
BYMV (BlCMV)¶	*By*‡	PI 297562	Reeder et al. 1972
BlCMV	*bcm*	TVu 2480	Taiwo et al.1982b
	blc	Worthmore	Walker and Chambliss 1981
	–	PEPH-BVR, WA-BVR, Corona	Kuhn et al. 1984
	1-r††	Mississippi Silver	Melton et al. 1987
	1-D§§	PEPH-BVR	Strniste 1987
	1-D§§	WA-BVR	Ouattara and Chambliss 1991
	–	TVu 2657 and 3433, Big Boy, Brown Sugar Crowder, Corona, Texas Cream #8, Serido	Bashir 1992
CABMV (BlCMV)¶¶	1-r††	TVu 612, TVu 1948	
	1-Dp§§	Tvu 408-P, TVu 410 (many others also)	Patel et al. 1982
CABMV	–	(sources unknown)‡‡	Ladipo and Allen 1979
	–	TVu 401, TVu 1582	Bashir 1992
CMV	*cc*‡	PI 255811	Rogers et al. 1973
CMV	1-D§§	'Black', Dixie Queen	Sinclair and Walker 1955
	1-D§§	Selection from 'Black'	de Zeeuw and Crum 1963
	1-D§§	'Fetriat' (tolerance)	Khalf-Allah et al. 1973
CPMV	–	Arlington, Blackeye, others	Robertson 1965
	mvs	TVu 227, TVu 345, TVu 612, and TVu 2331	Patel 1982a
	–	Arlington	Eastwell et al. 1983
	1-D§§	Arlington	Ponz et al. 1988a
CPMoV	–	TVu 3901 (tolerant)	Allen et al. 1982
CPSMV	–	TVu 612, TVu 1460-2, TVu 1948, TVu 2480, Macaido	Fulton and Allen 1982
PMV	–	Corona, Early Pinkeye, Iron, Worthmore	Bijaisoradat et al. 1988
SBMV	–	Iron, Clay, others	Kuhn and Brantley 1963
	Sbm‡	Clay	Brantley and Kuhn 1970
SBMV	–	PI 147562, PI 186465	Kuhn et al. 1986
SBMV	*sbc-l, sbc-2*	Mississippi Silver	Melton et al. 1987
	sbm-2	(PI 186465)	Hobbs et al. 1987
TRSV	*Tr*‡	California Blackeye #5	de Zeeuw and Ballard 1959
	–	(sources unknown)‡‡	Mali et al. 1981
	1-D§§	Arlington	Ponz et al. 1988a

† See Table 1 for virus names.
§ BVR = BlCMV-resistant; PEPH = Pink Eye Purple Hull; WA = White Acre.
¶ Reported as BYMV; actually BlCMV (O.L. Chambliss, personal communication).
‡ Term assigned by Fery; previously reviewed (Fery 1985).
†† Resistance apparently conferred by a single recessive gene; no term assigned.
§§ Resistance apparently conferred by a single dominant gene; no term assigned.1-Dp = partial dominance reported.
¶¶ Reported as CABMV; actually BlCMV (P.N. Patel, personal communication).
‡‡ Published resistance sources not accessible to authors.

After the evaluation of cowpea genotypes for possible resistance to CMV by Brantley et al. (1965), most cowpea researchers concluded that resistance to CMV in *Vigna unguiculata* was rare or nonexistent, despite reports to the contrary by Sinclair and Walker (1955), de Zeeuw and Crum (1963), and Khalf-Allah et al. (1973). Unfortunately, identities of the 476 cowpea genotypes tested by Brantley et al. (1965) were not published for future reference. In Kuhn's review (1990), however, it was concluded that most cowpea cultivars were tolerant to CMV, and that CMV resistance in *V. unguiculata* was unlikely. More recently, cowpea cultivar 'Pampo' has been reported as highly resistant to CMV (Da Ponte and Alves 1994). Such conflicting reports of cowpea resistance to CMV could suggest intraline heterogeneity, differences among CMV strains, and/or different inoculation methods used for resistance screening. Further investigation is still needed to determine whether resistance to CMV exists in established cowpea cultivars or in international collections of *V. unguiculata* germplasm.

Resistance to CPMV is commonplace among *V. unguiculata* cultivars. Wilson (1977) and Patel (1982b) each found a broad assortment of CPMV-tolerant and resistant cowpea genotypes, and many new cowpea lines and cultivars are CPMV-resistant (e.g., Ndiaye et al. 1993). Epistatic/hypostatic relationships among dominant genes conferring resistance to CPMV (Patel 1982a) were reviewed by Fery (1985).

Numerous pathogenic variants of some viruses, particularly CABMV and CPSMV, constrain breeding programs which attempt to incorporate genes conferring resistance either to all known pathotypes or to locally predominant pathotypes. The effects of coexisting pathogenic variants were exemplified in the work of Ndiaye et al. (1993), in which new cowpea lines bred specifically for CABMV resistance were severely attacked by a distinct indigenous strain of CABMV, and that too in the same region. Resistance breeding to the corporate indigenous strains of CPSMV has been successful in boosting cowpea production in South America (Rios and Neves 1982; Mendoza et al. 1990; and Santos et al. 1987, 1990). Fortunately, Fulton and Allen (1982) used several available CPSMV strains in screening cowpea for CPSMV resistance. By this process, three TVu lines (612, 1460–2, 1948) were determined to be uniformly resistant/immune to all tested isolates of the virus (i.e., these genotypes possessed genes/alleles conferring resistance to all available pathogenic variants). As indicated previously (see CPSMV), the pathogenic variation among CPSMV isolates is extensive and, to date, remains only meagerly defined. The genes conferring resistance to CABMV had not previously been named (Fery and Singh 1997), and additional work is required to define genes conferring tolerance to CPMoV and resistance to CPSMV.

Bruening and associates effectively integrated the knowledge of plant genetics (Eastwell et al. 1983; Sanderson et al. 1985; Bruening et al. 1987) with viral molecular genetics (Kiefer et al. 1984) and molecular mechanisms of virus resistance (Ponz et al. 1988a,b). In this classical effort, an inhibitor of CPMV polyprotein processing was found to be coinherited with immunity to CPMV in cowpea cultivar Arlington. The data showed that immunity to CPMV was conferred by a specific *V. unguiculata* proteinase inhibitor in this cultivar. Without cleavage by a CPMV-encoded proteinase, the polyprotein product CPMV RNA translation was rendered functionless and virus synthesis was thus precluded.

The large range of genotypes identified as resistance sources for BlCMV, CPMV, and SBMV particularly, allows breeders to more readily develop new virus-resistant cultivars

of different maturities, classes of plant and seed type, and market requirements. Similarly, multiple sources of virus resistance provide a broader genetic background, probably providing more stable resistance for new cultivars than could be expected from single resistance sources. The total genetic resources available to cowpea breeders compare favorably with those of other world crops, and warrant greater utilization by breeding programs of both developing and developed countries (Fery 1985).

Beyond the purposes of this chapter, a condensation and synthesis of worldwide virus-resistance sources would be beneficial to cowpea breeding programs. Otherwise, valuable bits of information tend to lie hidden for decades.

An updated, corrected list of genes described for *Vigna unguiculata* is included in another chapter of this book (Fery and Singh 1997). It should help fill the information gap, and thus pave the way for effective utilization by crop improvement scientists of the available sources of resistance.

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15

Shoot and pod diseases of cowpea induced by fungi and bacteria

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Abstract

This paper presents a global perspective on bacterial and fungal pathogens that directly infect cowpea foliage, stems, and pods. A brief outline is presented of the main symptoms, distribution, economic importance, epidemiology, and control of bacterial blight/pustule and of 11 fungal diseases: anthracnose, Ascochyta blight, black leaf spot (= leaf smut), brown blotch, brown rust, Cercospora and Pseudocercospora leaf spots, powdery mildew, Pythium soft stem rot, Septoria leaf spot, Sphaceloma scab, and web blight. Minor diseases are listed in a Table.

Introduction

This paper will focus, as the title indicates, on shoot and pod diseases of cowpea induced by fungi and bacteria. It will thus exclude bacterial and fungal diseases incited by soilborne pathogens, i.e., those which naturally infect the plant only through its underground parts, even if they induce major symptoms in any of the aerial parts of the cowpea plant. Other papers in this volume cover nematodes and other soilborne pathogens (Florini 1997; Roberts et al. 1997), the parasitic weeds *Striga* and *Alectra* (Singh and Emechebe 1997; Lane et al. 1997), and virus diseases (Hampton et al. 1997; Huguenot et al. 1997). Taken together, these papers bring us up to date and supplement information contained in an earlier volume (Aggarwal 1985; Caveness and Ogunfowora 1985; Emechebe and Shoyinka 1985; Lin and Rios 1985; Mew et al. 1985; Patel 1985; and Thottappilly and Rossel 1985) on the global range of cowpea diseases and pathogens.

Major bacterial diseases

Bacterial blight and bacterial pustule. Bacterial blight (induced by *Xanthomonas campestris* pv. *vignicola* [Burkholder] Dye) is probably the most widespread disease of cowpea, having been reported from all regions of the world in which cowpea is cultivated. By contrast, bacterial pustule has a more restricted distribution; until the recent report of its occurrence in Nepal by Dahal et al. (1992), it was considered to be limited to Africa (Patel 1981). There is still some controversy about the species of *Xanthomonas* that induces bacterial pustule. Based on differences in pathogenic behavior of the bacterial blight and the pustule pathogens, Patel and Jindal (1982) suggested that the pustule pathogen should

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be regarded as a distinct pathovar of *X. campestris,* namely *X. campestris* pv. *vignae-unguiculatae*. Emechebe and Shoyinka (1985) speculated that it could be a strain of the bacterial blight pathogen, *X. campestris* pv. *vignicola*, and preliminary characterization of 120 isolates from pustule or blight symptoms support their point of view (K. Wydra, personal communication, IITA, Cotonou, Benin). Pathogenic variability has been reported for both pathogens; Patel (1981) reported the existence of three races of the bacterial pustule pathogen, while Prakash and Shivashanker (1982) suggested that the race of the bacterial blight pathogen prevalent in India differs from that prevalent in Nigeria.

The pathogens of both diseases are seed transmitted, while secondary spread occurs by wind-driven rain (Preston 1949; COPR 1981). Insects have also been implicated in secondary spread of the bacterial blight pathogen (Kaiser and Vakili 1978). Both diseases cause premature leaf fall and water-soaked dots on the undersurface of leaves (Williams 1976). Unlike bacterial pustule, bacterial blight induces large, irregular foliar lesions with yellow margins (Patel 1982), stem cankers, and both preemergence and postemergence seedling mortality (Kishun 1989).

Total crop loss in susceptible varieties may result from seedling cankers or severe cankers of peduncles and floral cushions on older plants. Kishun (1989) working in India—where bacterial blight is considered the most destructive among all cowpea diseases (Prakash and Shivashanker 1982)—reported grain yield losses of 2.7–92.2%, depending on the susceptibility of the variety.

Apart from the work of Ekpo (1978, quoted by Allen 1983), who reported yield losses due to bacterial pustule of 1.8% and 26.6% in resistant and susceptible varieties, respectively, the only other attempt to quantify losses caused by bacterial pustule was that of Omotunde (1987) at Ibadan, Nigeria. He reported 76.8% and 2.3% losses in grain yields of susceptible (TVx 301) and resistant (TVu 43) lines, respectively.

The influence of some cultural practices on the severity of bacterial blight has received relatively little attention. Rao and Hiremath (1985) in India showed that disease severity was increased by N and P applications, but was decreased by the applications of moderate levels of K and Mo, and high doses of Ca and Mg. In Kenya, Ouko and Buruchara (1987) showed the contrasting effects of cropping system on the incidence and severity of bacterial blight in cowpea grown in the long or the short rainy season. At 40 days after inoculation during short seasons, disease incidence was 62.5% in a cowpea/maize intercrop, compared to 75% in a sole crop of cowpea and 92.3% in a cowpea-maize relay crop. By contrast, in long seasons, blight incidence was 68.7% in a cowpea-maize relay crop and 100% in both sole cropped cowpea and a cowpea/maize intercrop. In a sowing date trial in India, Kishun and Chand (1989) showed that damage by bacterial blight was lower in an early-sown crop than in a later-sown crop, and that the disease intensified with an increase in plant populations.

Emechebe and Shoyinka (1985) suggested that the incidence and severity of both diseases would decrease if farmers sowed only pathogen-free seeds. Soni and Thind (1991) showed that it was easy to obtain pathogen-free seeds from healthy pods. The effectiveness of this control measure can be enhanced by seed treatment with an antibiotic or a mixture of an antibiotic and a fungicide, such as streptocycline (100 µg/ml) plus captan (2000 µg/ml) (Jindal and Thind 1990). Suitable rotations of three consecutive cowpea-free growing seasons should also be effective against these host-specific xanthomonads.

Host plant resistance is the most viable option for the control of cowpea bacterial blight and pustule (Emechebe and Shoyinka 1985). Singh (1994) has listed many advanced breeding lines that are resistant to bacterial blight and are being used in breeding work.

Minor bacterial diseases

In their review of cowpea diseases in Latin America, Lin and Rios (1985) listed bacterial blight and two minor bacterial diseases, namely bacterial wilt (*Pseudomonas syringae* pv. *solanacearum*) and halo blight (*P. syringae* pv. *tabaci*). While halo blight was found in two states in Brazil, bacterial wilt was reported only in an irrigated area. Both diseases were thought to be of no economic importance in cowpea production in Brazil.

According to Patel (1985), cowpea in the USA is affected by two bacterial diseases: bacterial blight and bacterial leaf spot induced by *Pseudomonas syringae* pv. *syringae.* Although leaf spot has been reported from several states and the pathogen has an extensive host range, the disease is considered to be economically unimportant in the USA (Patel 1985). The same disease was recently reported in Romania (Severin and Stancescu 1990). Bacterial leaf spot has not been reported under natural conditions in Africa. In the rainforest zone of Nigeria, Oluwadare and Umechuruba (1991) recorded the effect of antibiotics on the isolation of *P. syringae* pv. *syringae* from cowpea seeds, but their report did not indicate whether or not the bacterium induced leaf spot in cowpea in the field.

Major fungal diseases

The major fungal diseases of cowpea are discussed below in the alphabetical order of their common names.

Anthracnose. Until recently, the pathogen of cowpea anthracnose was regarded as a form of *Colletotrichum lindemuthianum* [Sacc. and Magn.] Briosi and Cav., the pathogen of anthracnose on *Phaseolus* beans. However, Bailey and associates (Bailey et al. 1990; O'Connell et al. 1992; Pain et al. 1992) have raised important questions about the taxonomic status of the cowpea anthracnose pathogen. On the basis of the molecular, morphological, and antigenic differences that exist between the anthracnose pathogens of cowpea and *Phaseolus* beans, it was suggested that the cowpea anthracnose pathogen should be regarded as a species that is distinct from *C. lindemuthianum*, probably a form of *C. gloeosporioides.*

Typical anthracnose lesions (tan to brown, sunken and lenticular) on susceptible varieties enlarge rapidly and coalesce to girdle stems, peduncles, and petioles. Profuse sporulation occurs. In contrast, lesions on resistant varieties are tiny, necrotic flecks or lenticular, shiny reddish-brown lesions; the fungus does not sporulate on such lesions (Williams 1975a; Emechebe and Shoyinka 1985).

The pathogen is seed transmitted (Emechebe and McDonald 1979) and Qureshi et al. (1985) suggested that it was introduced into Pakistan from Nigeria on infected seed. Prasanna (1985) found 2–88% infected seeds in seed samples from India and showed that the germination decreased with an increase in seed infection, which resulted in seed rot and seedling mortality. Infected seed is one source of primary inoculum (Prasanna 1985), as is infected trash (Onesirosan and Sagay 1975; COPR 1981). Secondary spread is by rain splash, air currents, and contact with man and animals (COPR 1981).

Anthracnose causes economic losses in tropical regions of Africa, Latin America, and Asia where conditions are wet and humid for the main part of the growing season (Dhiman et al. 1989; Latunde-Dada 1990). In the rainforest zone of Nigeria, yield losses of up to 50% occurred in susceptible varieties in the early 1970s (Williams 1973) but anthracnose is now less important following the use of resistant commercial varieties, e.g., TVx 3236. A grain yield loss of about 43% was reported in India by Sohi and Rawal (1984), but they also found high levels of resistance in many cultivars (Sohi and Rawal 1983).

Although cowpea varieties resistant to anthracnose are readily available (Singh 1994), the pathogen is highly variable and the occurrence of five putative races has been reported on breeding lines evaluated in various parts of Nigeria (Emechebe 1986). Consequently, other control measures are usually combined with the growing of resistant varieties, such as sowing seed obtained from anthracnose-free multiplication fields. While foliar application of fungicides by low-input farmers is probably not economical, some foliar fungicides, such as benomyl and carbendazim have reduced losses from > 40% to < 5% (Sohi and Rawal 1984). However, strains of the pathogen resistant to several of the most effective fungicides (e.g., carbendazim and thiophanate-methyl) have been detected in India (Naik and Anilkumar 1991).

Ascochyta blight. Emechebe and Shoyinka (1985) listed Ascochyta blight (*Ascochyta phaseolorum* Sacc.) among the major cowpea diseases in Africa. In Latin America, it occurs more frequently in the hot, rainy season than in the dry season (Lin and Rios 1985). Kannaiyan et al. (1987) also reported that the disease is severe only in the wet season in Zambia. We did not find reports of the disease in the USA.

Ascochyta blight causes severe defoliation and lesions on stems and pods, which may result in death. Severe epidemics occur mostly at medium elevations (500–1200 m); thus, screening of germplasm lines is in progress in Plateau state of Nigeria. Primary inoculum comes from infected seed and plant debris, while secondary spread is by rain splash, air currents, and wind-driven moisture.

There are few recent reports on Ascochyta blight. Price and Cishahayo (1986) suggested that the same species attacked *Phaseolus* bean and soybean in Rwanda. In Brazil, Rios et al. (1986) showed that the cropping system affected the number of leaf lesions and necrotic leaf area but not the lesion diameter, which was a good indicator of level of susceptibility. They also found that applying benomyl to foliage, burning crop residue, or incorporating crop residue into the soil did not influence Ascochyta blight development.

Black leaf spot or leaf smut. The taxonomy of the cowpea black leaf spot pathogen is still controversial. While pathologists in Latin America regard the pathogen as a true smut (Basidiomycotina), *Entyloma vignae*, because chlamydospores germinate to produce promycelia and sporidia (Prabhu and Albuquerque 1982), pathologists in Africa and India (Allen 1983) consider the pathogen to be *Protomycopsis phaseoli*, a Hemiascomycete, because a spore-filled vesicle is produced while chlamydospores germinate (Haware and Pavgi 1976). The symptoms from samples collected in parts of Africa and in Brazil were found to be identical in all respects (Allen 1983). Thus, black leaf spot and leaf smut are regarded as synonymous, pending further taxonomic work.

A good account of the symptoms has been provided by Singh and Allen (1979). The disease occurs widely in tropical Africa, Central America, Brazil, India, and Nepal (Vakili 1978; Allen 1983; Rios 1988). In Nigeria, we have observed the disease in various agroecological zones, from the rainforest to the Sudan savanna. The disease appears early in the season: typically, sooty black leaf spots usually remain confined to lower leaves in the canopy, except on susceptible varieties, where the spots may be seen on upper leaves. Cowpea smut is one of the most important diseases of cowpea in the north and northeast of Brazil, causing up to 40% loss in grain yield there (Lin and Rios 1985).

Some *Protomycopsis* spp. survive as chlamydospores in infected plant debris on the soil surface for at least 2 years, but lose viability if the debris is incorporated into the soil (Pavgi and Haware 1969). Thus, control measures include destroying crop residue, deep plowing, or crop rotation. Several cultivars (including one of the most popular Nigerian cultivars, Ife Brown) are resistant to the pathogen in Brazil (Lin and Rios 1985). In Nigeria, some varieties found to be resistant in Brazil, including Ife Brown, were moderately susceptible under natural and augmented inoculum pressure; many varieties were resistant, but only IT88S-584-1 had no symptoms in replicated trials in Kano and Ibadan in 1995 (T.O. Adejumo, T. Ikotun, and D.A. Florini, 1995, unpublished data, IITA and University of Ibadan, Ibadan, Nigeria).

Brown blotch. Brown blotch, first described in 1981 by Emechebe (1981), is induced by two species of *Colletotrichum: C. capsici* [Syd.] Butler and Bisby and *C. truncatum* [Schw.] Andrus and Moore. Results of surveys conducted from 1984 to 1986 showed that > 90% of brown blotch specimens were infected by *C. capsici*, although mixed infections on the same plant part were observed (A.M. Emechebe, 1986, unpublished data, IAR, Zaria, Nigeria). All plant parts above soil level show symptoms of the disease, which include one or more of the following: seeds failing to germinate, seedlings damping off, stems or branches girdling, flowers aborting, immature pods mummifying, and/or pods and leaves showing lesions.

The pathogen infects all parts of the seed (Alabi 1981), and it survives the dry season in seed (Emechebe 1981) and in infected debris (Okpala 1981); secondary inoculum is disseminated by rain splash, wind-driven rain, and air currents. The optimum temperature for radial growth and sporulation in artificial culture is 25 °C (Alabi and Emechebe 1992). Seedlings aged 1–2 weeks at the time of artificial inoculation were more severely affected by brown blotch than those inoculated at 3–6 weeks of age (Alabi 1994). The incubation period on all aerial plant parts was 2–3 days, regardless of age of plant at inoculation; by contrast, the latent period varied from 5 days (on the petiole) to 16 days (on the stem) (Alabi 1994).

Emechebe (1986) described eight possible races of *C. capsici,* after studying the qualitative interactions between 120 Nigerian isolates of *C. capsici* and different cowpea lines. Four races occurred mostly in the Guinea and Sudan savanna ecologies, while the other four were obtained from the rainforest zone. The most virulent races attacked both TVx 3236 and IT82D-716, which are known for their high levels of resistance to brown blotch.

In West and Central Africa, brown blotch is particularly important in the rainforest zone, the southern Guinea savanna, and the southern part of the northern Guinea savanna.

In the northern Guinea savanna of Nigeria, yield loss due to brown blotch was 46% (Alabi 1994), but it can reach 75% in very wet years in the same area (Emechebe and Shoyinka 1985). Infected plants produce tiny and wrinkled seeds that are unmarketable. Equally important is the reduction in stand establishment from 88% (for healthy seeds) to 24% (for seeds infected by *C. capsici*) (Emechebe 1981).

There is little information about the importance of brown blotch in Asia. Although Ravi and Anilkumar (1991) indicated that they obtained a virulent culture of *C. truncatum* (used in their fungicide resistance study) from cowpea cultivar C157, they did not indicate the importance of the fungus in cowpea production in India. Earlier, Prasanna (1985) merely noted that *C. capsici* is seedborne in cowpea without indicating if the fungus induced any disease in cowpea in the field.

The tactics used for the control of anthracnose outlined above also apply to brown blotch. In addition, seed treatment with benomyl or carbendazim has been shown to be a viable option for the peasant farmer in the West African northern Guinea savanna (Emechebe et al. 1994). By contrast, although foliar-applied fungicidal sprays are effective under field conditions (Alabi and Emechebe 1992), the technology may not be economically feasible for the low-input farmer.

Brown rust. The exact name of the cowpea rust fungus has been a subject of controversy among plant pathologists. The one point of agreement is that it is a species of *Uromyces*. Many authors (Emechebe and Shoyinka 1985; Lin and Rios 1985; Patel 1985) regard it as *U. appendiculatus* [Pers. ex Pers.] Unger, while others (Chandrashekar et al. 1989) consider it as *U. phaseoli* var. *vignae*. Detailed studies by Heath and associates (Kim et al. 1985; Elmhirst and Heath 1989) have provided strong support for the designation of the cowpea rust fungus as a separate species, namely *U. vignae* Barclay. In their subsequent histopathological studies, they have consistently referred to the rust pathogen as *U. vignae* (Chen and Heath 1990; Heath 1990) and more recent authors, such as Xu and Mendgen (1991), have adopted this nomenclature.

The main symptoms of brown rust are slightly raised brown or black pustules on the leaves (COPR 1981). When leaves of young plants are covered by pustules, wilting may occur during periods of acute soil moisture deficit. Leaves on heavily infected older plants dry up and fall prematurely. Dissemination of the uredospores may be through contact with people, animals, and farm implements, but the main agents are wind and, to a much lesser extent, insects (COPR 1981). The pathogen survives the period between crops as teliospores in infected crop residue.

Cowpea rust can be regarded as a major cowpea disease in the rainforest and southern Guinea savanna zones of West Africa and in medium-elevation areas of East Africa (Emechebe and Shoyinka 1985). Moderate to high intensities of rust occur as well in the northern Guinea savanna of Burkina Faso (Konate and Ouedraogo 1988). Quantitative estimates of crop losses caused by brown rust are rare, but we have observed severe epidemics in the Jos plateau and the rainforest zone of Nigeria, causing premature defoliation and even crop failure. Similarly, Mariga et al. (1985) reported that cowpea rust occasionally causes epidemics of economic importance in Zimbabwe.

Although Patel (1985) and Lin and Rios (1985) indicated that cowpea rust is not economically important in the USA and Latin America, Stoffella et al. (1990) have shown

that brown rust is one of the two most important fungal diseases of cowpea at Fort Pierce, Florida, USA.

The only economically viable option for the control of brown rust of cowpea, apart from crop sanitation, is the growing of resistant varieties; many commercial varieties are resistant to the disease (Patel 1985; Singh 1994).

Cercospora and Pseudocercospora leaf spots. Cercospora leaf spot is induced by *Cercospora canescens*, while Pseudocercospora leaf spot is induced by *Pseudocercospora (Mycosphaerella) cruenta*, formerly *C. cruenta* (Emechebe and Shoyinka 1985). Pseudocercospora leaf spot appears as chlorotic spots on the upper leaf surface, which gradually become necrotic, with profuse masses of conidiophores and spores, appearing as downy gray to black mats on the lower leaf surface (Emechebe and Shoyinka 1985; Lin and Rios 1985; Patel 1985). Severely affected plants defoliate prematurely. Cercospora leaf spot is characterized by mostly circular, cherry red lesions. Coalescence of leaf spots results in generalized yellowing of the leaf and subsequent defoliation of severely infected plants.

Both pathogens survive the no-crop season on infected crop residue and in infected seed (Williams 1975b; Patel 1985). Sporulation is favored by humid weather, warm temperatures, and dense plant populations. Spores are dispersed by wind and rain splash. Yield losses of 18–42% have been recorded for these leaf spots in Nigeria (Williams 1975a) and the USA (Schneider 1973).

Since 1985, very little work has been done on the two diseases. Kannaiyan et al. (1987) reported that "Cercospora" leaf spots (*C. canescens* and *P. cruenta*) are severe in the wet season in Zambia and that none of the 336 cowpea entries screened was resistant to the diseases. Similarly, Zhang and Huang (1990) listed Pseudocercospora leaf spot as one of the important diseases of cowpea in China. In Zimbabwe, however, Mariga et al. (1985) did not consider Cercospora and Pseudocercospora leaf spots to be economically important. Hartmans (1988) and Emechebe (1988) reported that *P. cruenta* has become more prevalent in the Nigerian Sudan savanna, although its effect on cowpea production in this zone is yet to be determined.

Powdery mildew. Cowpea powdery mildew is induced by the oïdial phase (*Oidium* spp.) of *Erysiphe polygoni* DC and *Sphaerotheca fuliginea*. *E. polygoni* is prevalent in all cowpea growing regions, but *S. fuliginea* has been reported only from India (Jhooty et al. 1985).

The diagnostic sign of this disease is copious, white, powdery fungal growth, mainly consisting of oïdia, the repeating spores of the fungus, on the upper leaf surface. Chlorotic and then brown patches appear first on the undersurface of the leaf, and they later become distinct on the upper leaf surface. Severely mildewed leaflets fall, resulting in partial or complete defoliation of the plant.

E. polygoni has a broad host range of more than 500 species of higher plants, both annuals and perennials, especially in the family Leguminosae (Ainsworth 1971). The fungus probably perpetuates itself on these hosts from one season to another as conidia; ascospores have not been detected in the tropics. Disease development in Latin America and Zambia was favored by wet weather (Lin and Rios 1985; Kannaiyan et al. 1987). By contrast, in the Sudan savanna zone of Nigeria, we observed moderate damage by powdery

mildew during the dry period at the end of the rainfed season and greater severity in irrigated, dry-season cowpea than in rainfed cowpea of the same variety. The disease is also destructive under hot, dry conditions in the screenhouse. In India, the disease increases rapidly during the dry and cool season (Mew et al. 1985). Since there are several races of the pathogen (Lin and Rios 1985), it is reasonable to expect the differences in the above reports. Indeed, Rodríguez and Meléndez (1984) have suggested that there is a new race capable of attacking cowpea under high relative humidity and heavy rains in Puerto Rico.

Cowpea powdery mildew is important in Zambia (Kannaiyan et al. 1987), Zimbabwe (Mariga et al. 1985), Florida, USA (Stoffella et al. 1990), Puerto Rico, and other cowpea-producing countries of Latin America (Rodríguez and Meléndez 1984; Lin and Rios 1985). The disease is so important in India that fungicidal sprays have been recommended for its control (Singh and Anilkumar 1986). However, we found no estimates of yield losses due to powdery mildew in cowpea.

Two control methods have received the greatest attention: growing resistant varieties and application of fungicides. Lin and Rios (1985) noted that resistant cultivars exist in Latin America but their use is limited by the occurrence of races, presumably with matching virulence genes. In India, both highly resistant and partially resistant lines have been identified (Raju and Anilkumar 1990, 1991). In Zambia, Kannaiyan et al. (1987) found no line to be resistant out of 140 entries, although two of them were moderately resistant (scoring 2–3 on a rating scale of 1–9). Fungicides have been evaluated as seed, soil, or foliar treatments for the control of cowpea powdery mildew. Singh and Anilkumar (1986) concluded that effective protection of cowpea was obtained by seed treatment with carbendazim, followed by one foliar-applied spray of triadimefon. In Puerto Rico, Rodríguez and Meléndez (1984) obtained very effective control of powdery mildew with dinocap in the dry season but not in the rainy season. Biweekly application of 0.26 kg/ha of benomyl also protected cowpea from infection by *E. polygoni.*

Pythium soft stem rot. Soft stem rot of cowpea, induced by *Pythium aphanidermatum*, is a mature plant disease that is distinct from seedling damping-off induced by the same fungus. The disease appears to be important only in warm, humid tropical conditions such as those of the rainforest, the southern part of the southern Guinea savanna of West and Central Africa (Onuorah 1973), and the humid, subtropical zones of India (Verma and Mishra 1989). We have also observed damaging levels of the disease in the northern Guinea savanna of Nigeria during long periods of very wet weather. Pythium soft stem rot caused crop loss of 11% under rainforest conditions in Ibadan, Nigeria (Onuorah 1973) but the disease is unimportant in Brazil (Lin and Rios 1985).

The characteristic symptom of Pythium soft stem rot is a gray-green, water-soaked rot that completely girdles the stem and kills the plant. The slimy stem base is covered by abundant growth of white, cottony mycelium during periods of high humidity. The pathogen is soilborne, surviving for many years in the soil in the form of perennating oospores; in addition, it has a broad host range of > 100 higher plant species. It has not been established whether the seedling disease and soft stem rot are induced by the same strain(s) of *P. aphanidermatum.*

Control of Pythium soft stem rot is difficult. However, Emechebe and Shoyinka (1985) have suggested that the infection rate can be reduced in moderate plant populations, since

the disease is enhanced by high plant populations. Application of some fungicides, such as benomyl, which are effective against other diseases of cowpea, can increase the severity of Pythium stem rot (Williams and Ayanaba 1975). However, Ogundana (1986) showed that some fungicides (e.g., thiram and fentin acetate) better controlled the disease when used as a seed treatment than as a soil drench.

Septoria leaf spot. Septoria leaf spot of cowpea is induced by three species of *Septoria*, namely *S. vignae*, *S. vignicola,* and *S. kozopolzanskii* (Emechebe and Shoyinka 1985). The most prevalent and most economically important across Africa is *S. vignae*, with reports of *S. vignicola* in East Africa and of *S. kozopolzanskii* in Zimbabwe (Mariga et al. 1985). By contrast, *S. vignicola* has been consistently reported as the pathogen of the disease in India (Rawal and Sohi 1981, 1984, 1986), while *S. vignae* is a minor pathogen in Nicaragua (Lin and Rios 1985).

The disease is characterized by red or reddish-brown leaf spots, which are regular to irregular and 2–4 mm wide, with the lesions on both surfaces of the leaf being essentially identical. The lesions coalesce to give the leaf a freckled appearance. Severe spotting results in generalized chlorosis and premature defoliation.

The pathogen is seed transmitted (Emechebe and McDonald 1979) and survives the dry season on infected seed as well as on infected leaf tissue lying on the soil (Tarfa 1986). We observed that secondary spread is by rain splash, wind-driven moisture, air currents, and contact with man, animals, and farm implements. Severe epidemics of the disease occur in the Guinea savanna zone of West Africa (Emechebe 1988; Konate and Ouedraogo 1988). At Zaria, Nigeria, no consistent relationship was found between disease severity and sowing date, although in 1 of 2 years, the crop sown in mid-July sustained more disease than crops sown in early August (Tarfa 1986).

Tarfa (1986) showed that grain yield losses due to *S. vignae* varied in the Nigerian northern Guinea savanna from 56.5% in 1984 to 42.5% in 1985. In India, Rawal and Sohi (1984) reported that the infection of cowpea at one week of age by *S. vignicola* reduced green pod yield by about 65%.

Although Septoria leaf spot causes high yield losses in susceptible cowpea in both India and Africa, it can be effectively controlled. The most economic and effective method is growing resistant varieties and such varieties are available (Singh 1994). The problem is that some of the most popular varieties grown in the northern Guinea savanna are susceptible to Septoria leaf spot. The incidence of leaf spot in such varieties can be reduced by using pathogen-free seeds, which can be further protected by seed treatment with benomyl or carbendazim (Emechebe et al. 1994). Foliar sprays with the same chemicals are also effective (Tarfa 1986). Similarly, foliar application of benomyl or carbendazim gives effective control of *S. vignicola* in India (Rawal and Sohi 1986).

Sphaceloma scab. Scab, induced by the Sphaceloma (conidial) stage of *Elsinoe phaseoli* Jenkins, produces characteristic lesions that are oblong to elongate, dark brown, buff, or white on stems, peduncles, and petioles. Lesions may coalesce. Heavy stem scabbing of a young plant results in severe stunting. An infected young leaf has a puckered lamina with white spots; the centre of old lesions frequently falls out to produce shot holes. Pod lesions, varying from a few to up to 200 per pod, are ovoid, with dark brown borders which become

black as chlamydospores form; heavily scabbed young pods abort or remain attached to the plant as mummified black masses. Heavy scabbing of the flowering axis either completely prevents flower formation or causes flower and pod abortion (Emechebe 1980).

The longevity of survival is probably mediated by chlamydospores produced on pod and stem tissues. The role of the ascospores in the epidemiology of the disease in the tropics is not known. Infected seed and plant material provide primary inocula (Donli 1983; Lin and Rios 1985; Emechebe 1988), while the subsequent dispersal of secondary conidial inoculum is by rain splash and wind-driven moisture (Emechebe and Shoyinka 1985).

Sphaceloma scab is probably the most important disease of cowpea wherever it occurs in both the northern and the southern Guinea savanna zones of West and Central Africa (Emechebe and Shoyinka 1985). Under conditions conducive for disease development (i.e., moderate temperatures of about 23–28 °C, 3 or more consecutive days of wet weather, and consequent high relative humidity) (Emechebe 1980) in the northern Guinea savanna of Nigeria, we have observed grain yield losses of 70% in Zaria in 1989 and 1990 (Mungo et al. 1995) and complete crop loss in susceptible varieties in Kachia. The disease is also one of the most destructive diseases of cowpea in Central America, Suriname, and Brazil (Lin and Rios 1985). We are not aware of any reports of the occurrence of Sphaceloma scab in India or the USA; in the latter, however, a different scab, induced by *Cladosporium vignae*, occurs (Table 1).

There are several options for the control of Sphaceloma scab. Much success has been achieved through deployment of resistance genes both in Latin America (Lin and Rios 1985) and in Africa (Singh 1994). However, TVx 3236, which is resistant to scab in Nigeria, is susceptible in Burkina Faso (Konate and Ouedraogo 1988), suggesting the existence of at least two races of the pathogen in West Africa. Good control of the disease has been achieved through fungicidal seed treatment (Emechebe et al. 1994) and foliar-applied fungicides (Mungo et al. 1995). Crop rotation and sanitation might be viable options for the control of a highly specialized pathogen like *E. phaseoli.* Preliminary results suggest that rotation does not affect scab incidence although scab symptoms were less severe in fields where cowpea followed another crop in rotation than in those where cowpea followed cowpea (C. Mungo, unpublished data, IAR, Zaria, Nigeria). Further study of the effectiveness of these measures is needed.

Web blight. Cowpea web blight is induced by an aerial type of *Rhizoctonia solani* (teliomorph = *Thanatephorus cucumeris*), the pathogenicity and biology of which are distinct from those of the strains that induce root rots and seedling diseases. Whereas the strains of *R. solani* that induce the latter diseases are strongly soilborne, the web blight strain, as suggested by Onesirosan (1977), has only a transient association with the soil.

Web blight symptoms range from small, circular brown spots to large irregular lesions with zonate banding, surrounded by water-soaked borders (Allen 1983). Under humid conditions, heavy blighting and premature defoliation occur, with affected leaves often bound together by webs of fungal hyphae (Singh and Allen 1979). The affected aerial parts of the plant may be covered with sclerotia, which resemble a dark coarse sand deposit.

The fungus has a broad host range (Lin and Rios 1985) and survives on infected crop debris (mostly as sclerotia), weed hosts, and seed (Onesirosan and Sagay 1975; Emechebe

Table 1. Occurrence of minor fungal shoot and pod diseases of cowpea in the major regions of the world.

Common name	Pathogen	Regions	Reference
Alternaria leaf spot	*Alternaria* sp.	Southern Africa	Maramba (1983); Mariga et al. (1985)
Aristatoma white leaf spot	*Aristatoma guttulosum* Sutton; *A. oeconomicum* (Ellis and Tracy) Tehon	West Africa; USA	Emechebe and Shoyinka (1985); Patel (1985)
Basal stem rust	*Aecidium* sp.	West Africa	Emechebe and Shoyinka (1985)
Chaetoseptoria leaf spot	*Chaetoseptoria wellmanii* Tehon	USA; Central America	Patel (1985); Singh and Allen (1979)
Choanephora pod rot (lamb's tail pod rot)	*Choanephora cucurbitarum* (Berk. and Rav.) Thaxt. *C. infundibulifera* (Currey) Sacc.	West Africa; India; Brazil; USA	Singh and Allen (1979); Bashir et al. (1985); Patel (1985); Toler and Duke (1965)
Cladosporium scab	*Cladosporium vignae*	USA; Southern Africa	Patel (1985); Mariga et al. (1985)
Corynespora target leaf spot	*Corynespora cassiicola* (Berk. and Curt.) Wei	West Africa; Central America	Emechebe and Shoyinka (1985); Lin and Rios (1985)
Dactuliophora zonate leaf spot	*Dactuliophora tarrii* Leakey	West, Central, East and Southern Africa; India	Emechebe and Shoyinka (1985); Chandrashekariah and Hiremath (1982)
Leptosphaerulina leaf spot	*Leptosphaerulina vignae* Tehon and Stout	USA	Patel (1985)
Myrothecium leaf spot	*Myrothecium roridum* Tode ex Fries; *M. graminum*	India	Singh and Shukla (1986); Mahrishi (1986)
Phyllosticta leaf spot	*Phyllosticta* spp.	Southern Africa	Mariga et al. (1985)
Pink rust	*Phakopsora pachyrhizi* Syd.	West Africa	Emechebe and Shoyinka (1985)
Red stem canker	*Phytophthora cactorum* (Lebert and Cohn) Schroet.	USA	Patel (1985)
Diaporthe stem rot	*Diaporthe phaseolorum* (Cook and Ellis) Sacc.	USA	Patel (1985)
Yellow blister (false rust)	*Synchytrium dolichi* (Cooke) Gaum.	East and Southern Africa	Emechebe and Shoyinka (1985); Kannaiyan et al. (1987)

and McDonald 1979). The only data on crop losses caused by web blight are those of Oyekan (1979), who reported losses of 28–40% in southwestern Nigeria. However, the pathogen can cause complete destruction of the leaf canopy during periods of heavy rain with long periods of overcast skies. We have observed further aggravation of the disease in portions of fields that contain stagnant water for 24 hours or more. In Latin America and India, the disease is destructive in hot, humid regions (Lin and Rios 1985; Verma and Mishra 1989).

Very little research effort has been devoted to developing a practicable control strategy against web blight. However, since the disease is favored by dense planting, a moderate plant population could reduce disease severity, as could any practice that ensures good drainage of the field. Latunde-Dada (1991) has demonstrated the potential use of a foliar-applied spore suspension of *Trichoderma koningii* as a biocontrol agent against the web blight pathogen. The level of disease control and the yield increase compared favorably with those obtained with a foliar fungicide spray.

Minor fungal diseases

Table 1 lists the minor fungal diseases of cowpea based on previous reviews (Emechebe and Shoyinka 1985; Lin and Rios 1985; Patel 1985), as well as on some new references. Although yellow blister (false rust) induced by *Synchytrium dolichi* is reported to be severe on rainfed cowpea in Zambia (Kannaiyan et al. 1987) and causes localized epidemics at medium elevations in Uganda, there are no published reports of crop losses caused by the disease. As Zambia and Uganda are, as yet, minor cowpea producers, yellow blister is listed with the minor diseases.

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16

Nematodes and other soilborne pathogens of cowpea

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Abstract
Since the First World Cowpea Conference was held in 1984, over 200 papers have been published on soilborne organisms parasitizing cowpea,*Vigna unguiculata* (L.) Walp. More than a dozen nematode genera and numerous soilborne fungi—including *Rhizoctonia solani*, *Sclerotium rolfsii*, *Phytophthora* spp., *Macrophomina phaseolina*, *Fusarium* spp., and *Pythium* spp.—have been implicated in root rot, seed rot, damping off, and basal stem canker of cowpea. Most of these papers have reported on the control of nematodes and fungal pathogens. A few studies have attempted to elucidate the mechanisms of resistance to these pathogens. Several authors investigated interactions of nematodes with soilborne fungi, mycorrhizae, and *Rhizobium* spp. This paper summarizes pertinent information from many of those published reports.

Nematodes

New species

Caveness and Ogunfowora (1985) listed 51 species in 23 genera of nematodes associated with cowpea. Cowpea has since been cited as a host for nine further species. *Pratylenchus scribneri*, *Criconemella sphaerocephala*, *Paratylenchus* spp. (Gallaher and McSorley 1993), *Hemicycliophora poranga* (Chitambar 1993), and *Tylenchorhynchus germanii* (Baujard and Martiny 1991a) reproduced well on cowpea. *Ditylenchus destructor* (Basson et al. 1990), *Paralongidorus bullatus* (Baujard et al. 1993), *Hoplolaimus galeatus* (Rhoades 1984), and *Xiphinema longicaudatum* (Lamberti et al. 1992) were reported to survive on cowpea but were not considered serious pathogens of the crop. The pathogenicity of *X. ifacolum*, however, was confirmed; the nematode formed a coenocyte in the swollen root tips of cowpea and reduced growth by 37% (Lamberti et al. 1992).

Studies of *T. germanii* explained its impact as a pathogen in West Africa. As few as 250 nematodes per plant damaged root systems of cowpea, millet, sorghum, and groundnut (Baujard and Martiny 1991b). The nematode became anhydrobiotic during the 9-month long dry season (Baujard and Martiny 1991a) and multiplied at high soil temperatures (30–36 °C) and low soil moisture levels (5–11%) (Baujard and Martiny 1991b).

Controlling nematodes using cowpea in cropping systems

In various cropping systems tested, cowpea reduced population densities of several nematodes. Rodríguez-Kábana et al. (1988a,b) concluded that 'Iron,' a cowpea cultivar,

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could be grown in rotation with soybean because it had no root-knot galls and very low root and soil population densities of *Meloidogyne arenaria*, *M. incognita*, *Heterodera glycines* (race 4), *Paratrichodorus christiei*, *Pratylenchus brachyurus*, and *Helicotylenchus dihystera* in greenhouse trials. In field trials at seven locations in Florida, lower soil population densities of *M. incognita* resulted after cowpea cultivar California Blackeye No. 5 than after maize cultivar Pioneer 3098 or sorghum cultivar Asgrow Chaparral (McSorley and Gallaher 1993). Both microplot and field trials showed that a 3-month summer cover crop of the cultivar California Blackeye No. 5 reduced population densities of *Belonolaimus longicaudatus*, *Hoplolaimus galeatus*, and *P. christiei* more than a sorghum-sudangrass cover crop (Rhoades 1984; Rhoades and Forbes 1986), bare fallow, weedy fallow plus nematicide, or a cover crop of *Sesbania exalata* (Rhoades and Forbes 1986). Despite these results, cowpea was not recommended as a summer cover crop because the few *B. longicaudatus* which developed on cowpea were able to build up to damaging levels on later maize crops (Rhoades 1984). Similarly, although cowpea was a poor host for *Paralongidorus bullatus* (Baujard et al. 1993) and *Ditylenchus destructor* (Basson et al. 1990), the few nematodes which survived on cowpea could be potentially damaging to groundnuts in a rotation.

Intercropping maize and cowpea was recommended to provide some control of nematodes on each crop. Maize and cowpea growth was reduced by *Pratylenchus sefaensis* and *M. javanica*, respectively, but intercropping maize and cowpea significantly reduced population densities of *P. sefaensis* compared to maize monoculture, and of *M. javanica* and *R. reniformis* compared to monocropped cowpea (Egunjobi et al. 1986).

Controlling nematodes with organic products

Mulches and soil amendments have often been tested as methods to control soilborne pathogens. Population densities of *M. incognita*, *Helicotylenchus* sp., and *Pratylenchus* sp. were lower on cowpea when 1 t/ha of dried pulverized kolanut (*Cola nitida*) pod was applied to ridges 5 weeks after planting (Oyedunmade et al. 1995). Amending the soil with 6 t/ha of partially decayed, flaked, dry cocoa pod husks reduced root-knot galling by 27% in field trials and increased dry grain yield by 7% (Egunjobi 1985); while larger yield increases were obtained in greenhouse and microplot trials, the amount of husks used was impractical. In field trials, although the lowest population densities of *M. incognita* were found in plots treated with the nematicide carbofuran, the highest net revenue per hectare and the best crop growth were obtained by adding 10 t/ha of cocoa pod husks or cassava peels to soil (Egunjobi and Olaitan 1986). Microplot studies showed that amending soil with a mixture of 4 t/ha soybean meal, 2 t/ha urea, and 2 t/ha Clandosan 601 (a chitinous material from blue crabs) was more effective than the nematicide aldicarb in reducing juvenile *M. incognita* populations in soil at harvest and increasing crop yield (Rodríguez-Kábana et al. 1990). Several mechanisms seemed to explain the effect of this soil amendment: (1) increased soil chitobiase activity which correlated with fewer nematode galls, and (2) increased soil urease activity, indicating that soil microorganisms produced ammonia that is toxic to nematodes. Soil amendments, however, are not always beneficial. Although rice hulls reduced the population densities of *M. incognita* in field trials, many plants died prematurely from *Fusarium semitectum*, *Colletotrichum linde-muthianum*, and *Phoma* spp., which were stimulated by the rice hulls (Egunjobi and Olaitan 1986).

Controlling nematodes with host plant resistance

Many cowpea cultivars have been screened for resistance to nematodes. Characterization of new resistance to root-knot nematodes is reported in this volume (Roberts et al. 1997) and references in that paper will not be repeated here. Criteria used to assess resistance to nematodes include galling, numbers of eggs or juveniles produced, the reproductive ratio (final nematode population densities divided by initial population densities), and plant yield or damage.

A gall index or the actual number of galls per root system are often used to rate resistance of many cultivars. Of eight cowpea cultivars tested, only IT82E-77 had few galls per plant 36 days after inoculation and so was considered resistant to *M. javanica* in pot tests (Onyeigwe and Ogbuji 1991). Cultivar IC 20447 was classified as highly resistant to *M. incognita* and *M. javanica* because it had no galls after 45 days (Patel et al. 1990). Neither the gall index nor the number of galls per root system, however, accounts for differences in the size of a plant's root system. Counting the number of galls per gram of root took longer than using a gall index, but Witcher and Ogle (1987) felt the counts distinguished biologically significant differences among cultivars. Eight of 16 cultivars they tested were resistant to both *M. incognita* race 3 and *M. arenaria* race 1.

Galling does not always predict the reproductive efficiency of root-knot nematodes on a particular host, so many researchers count the numbers of egg masses, eggs, and/or juveniles produced from infected root systems. Based on galling and the number of eggs produced, cultivars IT89KD-288 and IT90K-76 (M.S. Gaya, B.B. Singh, D.A. Florini, IITA, Kano, unpublished) as well as 33 of 76 cowpea cultivars or germplasm accessions (Idowu and Diboh 1987) were rated highly resistant to *M. incognita* in Nigeria. For pot studies, Khan and Husain (1989b) used three criteria to characterize a cultivar's level of resistance: number of galls per root system, nematode reproduction ratio, and reduction in plant growth. Using their index, they rated only cultivar IC-503 to be moderately resistant.

Unfortunately, this rigorous index has no simple terms for cases in which a cultivar is susceptible but tolerant or when a cultivar is resistant but intolerant. A tolerant cultivar should show no growth reduction even when infected. For example, yield losses on cowpea cultivars Tennessee Brown and California Blackeye No. 5 suggested that these resistant cultivars are not tolerant to infection by *M. incognita* race 1 (Gallaher and McSorley 1993).

Pathogenic variability in nematode populations and genetic variability in seedlots of cowpea cultivars have been cited as reasons for susceptible reactions of resistant cultivars. *M. incognita* races differed in their ability to reproduce on 2 of the 12 cultivars tested by Swanson and Van Gundy (1984); California Blackeye No. 3 was resistant to races 3 and 4 but Queen Ann was resistant only to race 2. Although not described as a race, population J7c54 of *M. javanica* was virulent on cultivar Mississippi Silver, which was resistant to most *M. javanica* populations. Interestingly, plants from one seedlot of California Blackeye No. 5 were resistant to races 1, 2, 3, and 4 of *M. incognita*, but plants of the same cultivar from another seed source were susceptible (Swanson and Van Gundy 1984).

Mechanisms of resistance to nematodes

Mechanisms of resistance to root-knot nematodes were studied in several cowpea cultivars. As for many cultivars, resistance to *M. incognita* in cowpea cultivars IC 9642-B and TVu 2430-P is controlled by a single dominant gene (Singh and Reddy 1986). Their

resistance was associated with reduced juvenile penetration, root galling, and fecundity, with delayed development of juveniles to adult females. Resistant cultivars had fewer and smaller giant cells than susceptible lines (Singh et al. 1984). Cells around root-knot nematode larvae died in the roots of the resistant line IC 9642-B before feeding sites could be established (Singh et al. 1984). In resistant cultivars, the cork layer was thicker than in susceptible cultivars and sclereids were present in the cortex. Within 96 h of inoculation with *M. incognita*, plants of the resistant cowpea cultivar C-152 synthesized mRNA six times more rapidly than uninoculated plants (Raja and Dasgupta 1986). In the susceptible cultivar, mRNA was produced more slowly than in the resistant cultivar and a second type of mRNA was produced that blocked the synthesis of some polypeptides which could activate host plant defenses (Raja and Dasgupta 1986).

Soilborne pathogens

New species

So many fungal pathogens had previously been reported from cowpea that new reports of pathogens in the crop have been rare in the past 10 years. Root infection in cowpea was recorded for *Fusarium equiseti* (Ramachandran et al. 1982), *Pythium myriotylum* (Croft 1988), and *Phytophthora dreschleri* (Erwin et al. 1991). In addition to *P. dreschleri*, many other pathogens including *M. phaseolina*, *R. solani*, two *Pythium* spp., *Thielaviopsis basicola,* and *Fusarium solani* f. sp. *phaseoli* were isolated from rotting cowpea roots in California fields, but only the latter two caused early dying of plants in the field and yield loss (30–50%) in pot studies. *Fusarium oxysporum*, was surprisingly not associated with the early dying of cowpea plants, although it was present in 74% of the fields surveyed. Unfortunately, the effect of joint inoculation with the pathogens was not studied.

Macrophomina

Macrophomina phaseolina is not a new pathogen of cowpea, but, since 1984, there have been many studies on its biology and the conditions for infection. *M. phaseolina* was reported to be the most important single fungal pathogen in the Bay region of Somalia (Gray et al. 1990). Temperature studies confirmed why it is one of the major pathogens of cowpea in such hot, arid zones. An Indian isolate grew best and formed most sclerotia on potato dextrose agar (PDA) at 30–35 °C (Ratnoo and Bhatnagar 1991). An isolate from Niger grew best at 35 °C on PDA, with poor growth occurring < 10 °C and > 40 °C (Adam 1990). Soil samples from a survey conducted in Niger had up to 139 sclerotia per gram of soil, while soil collected in France had none (Adam 1990). In India, 42–71% of plants died and there was no grain yield in plots containing 46–148 sclerotia per gram of soil at the time of symptom appearance (Lodha and Singh 1984).

Both the age of the plant at inoculation and drought stress affect the susceptibility of cowpea to *Macrophomina*. Plants younger than 45 days were found to be most susceptible, but only 30% were infected following inoculation at 60 days (Ratnoo and Bhatnagar 1993a). Senescing plants and drought-stressed plants of all ages, however, are commonly colonized by the fungus (Burke et al. 1986). More plants are infected in areas of low annual rainfall and without irrigation in Niger (Adam 1990) and Botswana (de Mooy et al. 1986). Seed is easily infected. *M. phaseolina* was found in 64% of seed samples collected in Niger; some seedlots had as much as 100% of the seeds infected (Adam 1990).

When cowpeas were grown in soil infested with *Macrophomina* sclerotia, infection occurred underground in emerging cotyledons and hypocotyls; roots were colonized but appeared healthy (de Mooy and Burke 1990). When young plants were inoculated, the pathogen spread more rapidly downwards to the roots and upwards in the stems, causing rapid wilting (Ratnoo and Bhatnagar 1993a). In another study, de Mooy and Burke (1990) postulated that ashy stem blight symptoms that appear in the field when plants approach maturity or are under drought stress may be due to activation of dormant hypocotyl lesions; they found no evidence of internal growth of the fungus from the cortical lesions or from the roots, and they did not detect mycelium in microscopic examinations of stem pith, phloem, and xylem. Adam et al. (1991), however, found mycelium around cells of the vascular bundles 96 h after seedlings were inoculated.

Macrophomina phaseolina has a very wide host range, but two studies suggested that isolates may differ in pathogenicity. Burke et al. (1986) found that some cowpea genotypes were infected more often than others, but suggested that the Botswana strain was specific to legumes since sorghum intercropped in the same fields was not susceptible. Byadgi and Hegde (1985) reported variation in virulence, morphology, and pycnidial production. They found that isolates of *M. phaseolina* obtained from *Phaseolus vulgaris*, *Cicer arietinum*, and cowpea grew faster, and were more virulent than those from sorghum, soybean, or *Gliricidia*. In another study, isolates of *M. phaseolina* and *R. solani* from cowpea were more virulent on cowpea than on tomato or *Lagenaria siceraria*; however, modifications in the pathogenic behavior of the isolates were attributed to sucrose as the carbon source and L-asparagine as the nitrogen source in culture media (Naresh et al. 1992).

Sources of resistance to many soilborne pathogens have been identified, but highly resistant cultivars are often not available for generalist pathogens such as *M. phaseolina*. Moderate levels of resistance were reported in 5 of 33 cowpea cultivars (Singh and Lodha 1986) and in 4 of 141 cultivars (Sohi and Rawal 1983) in India. None of the 89 varieties was resistant to *Macrophomina* in Niger, but 30% were regarded as tolerant because 20% or fewer of their plants were infected (Adam 1990). Better cowpea stands were attributed to moderate resistance of one cultivar in Senegal (Gaikwad and Sokhi 1987).

Phytophthora

Races and formae speciales of *Phytophthora vignae* were identified in two reports. Previously reported as a problem on cowpea in Australia and Tanzania, *P. vignae* was detected in Sri Lanka in a greenhouse (Sivakadadcham and Fernando 1991) and was later found in many of the 25 fields surveyed (Fernando and Linderman 1993) even though the symptoms of Phytophthora wilt were seen in only one of the fields. Different races of the fungus were identified using differential cultivars (Fernando and Linderman 1993). Tsuchiya et al. (1986) found that isolates of *P. vignae* from *Vigna radiata* were virulent to *V. radiata* but not to cowpea, while isolates from cowpea were virulent only to cowpea. The isolates could be distinguished by pathogenicity tests and not by soluble protein and isoenzyme patterns. The authors, therefore, proposed two formae speciales: *P. vignae* f. sp. *adzukicola* Tsuchiya, Yanagawa, and Ogoshi for that on *V. radiata*, and *P. vignae* f. sp. *vignae* for that on cowpea (Tsuchiya et al. 1986).

Host plant resistance is the preferred method of controlling *P. vignae*, and several sources of resistance have been identified. Of the 1781 cowpea lines planted in an infested

field and sprayed with a spore suspension of *P. vignae*, only KU235 and TVu 3861 were moderately resistant after the third inoculation (Mligo 1988). In a root inoculation assay using 0.01 g mycelium/kg of potting mix, cultivar CPI 84853 expressed partial resistance to races 1, 2, 3, and 4 of *P. vignae* although this cultivar was highly susceptible to race 4 following hypocotyl inoculation (Davis et al. 1993). Resistance to *P. vignae* race 2 was found to be dominant and controlled by a single gene or gene complex (Bateman et al. 1989). The resistance was mediated by phenylalanine ammonia-lyase (PAL) (Ralton et al. 1988). Only low levels of PAL were produced by the near-isogenic cultivars, Poona and Caloona, in response to inoculation with race 3, which was virulent on both cultivars. Race 2 invaded the susceptible cultivar, Poona, faster than PAL levels could build up except at high temperatures (35 °C), at which Poona seemed resistant. Enzyme activity increased rapidly in the resistant cultivar which became susceptible if compounds which inhibited the production of PAL were applied to the cut bases of hypocotyls (Ralton et al. 1988).

Chemicals that did not control *Phytophthora* spp. in vitro induced defense reactions in cowpea plants which helped in controlling the pathogen (Guest and Bompeix 1990). Phosphite (a breakdown product of Fosetyl-Al) stimulated the production of several phytoalexins in cowpea susceptible to *Phytophthora cryptogea* (Saindrenan and Bompeix 1986). Within 24 hours of inoculation with *P. cryptogea*, enough kievitone accumulated in lesions treated with phosphite to inhibit *P. cryptogea* growth. Phosphite treatment also induced high levels of phaseollidin at inoculation sites, which reached levels inhibitory to fungal growth by 48 h after inoculation (Saindrenan et al. 1988).

Pythium

Koleosho et al. (1987) found that production of oxalic acid and polygalacturonase coincided with decreased pH in hypocotyls of the susceptible cultivars IT 81D-1020 and VITA 5 infected with *Pythium aphanidermatum*, whereas resistant cultivars IT 82E-32 and TVx 3236 had only low levels of oxalic acid and polygalacturonase with little change in pH. They postulated that the oxalic acid chelated calcium and magnesium ions and reduced the pH enough to permit polygalacturonase to degrade the middle lamella of plant cell walls. They suggested that oxalic acid levels in cowpea cultivars 8–10 days after inoculation could be indicative of resistance to *P. aphanidermatum*. Cowpea cultivars with dark seeds were more resistant to *Pythium* spp. than those with cream-colored or beige seeds, perhaps because light-colored seeds imbibed water more rapidly and leaked more solutes which could favor infection (Legesse and Powell 1992).

Fusarium

The severity of Fusarium root and stem rot in cowpea varied with host genotype and plant age, but not with the different levels of inoculum tested. Cultivar CES 42-2 showed less infection than TVx 289-4G or VCS 6-1. The percentage of infected plants was highest in 22-day-old plants, while 5-day-old seedlings were not infected (Sajise 1988). Cowpea cultivars Blackeye, TVu 1330, and TVx 3236-01G were susceptible to Fusarium wilt, while TVu 1560 was resistant (Shihata et al. 1988). Xylem extracts of TVu 1560 were more toxic to *F. oxysporum* than those of Blackeye, the susceptible cultivar, and may explain why the xylem vessels of Blackeye but not TVu 1560 were extensively colonized by the pathogen (Shihata et al. 1989). In the wilt-susceptible cowpea cultivar California Blackeye

No. 5, *F. oxysporum* f. sp. *tracheiphilum* spread quickly upward in plants, colonized most tissues within 6 weeks, and caused severe wilt (Harris and Ferris 1991c). In wilt-resistant cultivar California Blackeye No. 3, however, there was little proliferation of *F. oxysporum* in any tissue whether or not plants were infected by *M. javanica*. Split-root experiments provided no evidence that infection by *M. javanica* results in a translocatable factor that reduces wilt resistance (Harris and Ferris 1991c).

Sclerotium

Field trials conducted from 1982 to 1985 revealed genetic variability in cowpea for resistance to *Sclerotium rolfsii*. The accessions Carolina Cream and CR61N exhibited good levels of resistance (Fery and Dukes 1986). Screening in pots also showed large varietal differences in resistance to *S. rolfsii*. When two sclerotia were set against wounded cowpea stems, symptoms ranged from the enlargement of the initial wound with no further disease development for cultivars IT 82D-699 and IAR-339-1 to wilting and death of plants for cultivar K-59 (Nwakpa and Ikotun 1988).

Control of fungal diseases

Weed mulch (Gupta and Gupta 1986), wheat straw, and neem cake (Ratnoo and Bhatnagar 1993b) controlled *Macrophomina phaseolina*. Neem cake improved growth of plants inoculated with *R. solani* or *M. incognita*, but not with *R. reniformis*; while groundnut cake only improved growth of plants inoculated with *R. solani* (Khan and Husain 1988c). Leaf extracts of *Adhatoda vasica* suppressed mycelial growth of *S. rolfsii*, *R. solani*, *Phytophthora vignae*, and *Pythium* spp., and also suppressed sexual reproduction of the latter two when incorporated into PDA (Sivakadadcham 1988). Cowpea and neem extracts enhanced oospore production in *Pythium butleri* and *Phytophthora vignae*, respectively, while none of the leaf extracts tested controlled *F. solani*. (Sivakadadcham 1988). Green manure, farmyard manure, and biogas sludge all increased seedling rot induced by *R. solani* in growth chamber experiments (Kataria and Grover 1987).

Over the past 10 years, there have been many reports of fungicide efficacy, often based on in vitro or pot tests, but few studies found a correlation between such tests and field results (Adam 1990; Ramadoss and Sivaprakasam 1988; Singh and Lodha 1986). Efficacy of fungicides can be modified by the inoculum ratio and virulence of different pathogens (Gangopadhyay and Grover 1984, 1986); by temperature, moisture, and soil nutrients (Gangopadhyay and Grover 1984; Kataria and Sunder 1985); by soil type (Gangopadhyay and Grover 1984; Kataria and Sunder 1987, 1988); and by soil amendments (Bandyopadhyay et al. 1982; Gangopadhyay and Grover 1984; Kataria and Grover 1987; Kataria and Sunder 1988). Insecticides and herbicides were occasionally reported to have fungicidal or nematicidal activity in vitro (Kataria et al. 1989) but not in vivo (Ramadoss and Sivaprakasam 1989).

Biological control of *M. phaseolina*, *S. rolfsii*, and *R. solani* was demonstrated in pot studies. *T. viride* reduced *M. phaseolina* growth in vitro both alone and in combination with carbendazim, but pelleting seed with the fungicide plus *T. viride* increased germination, reduced postemergence mortality, and increased shoot and root length and dry matter of cowpea (Alagarsamy and Sivaprakasam 1988). *Trichoderma* spp. isolated from sclerotia successfully controlled *S. rolfsii* both in culture and in the greenhouse (Almeida and

Landim 1981). Another biocontrol agent, *Paecilomyces lilacinus*, was antagonistic to *R. solani*, reduced the multiplication of *R. reniformis* and *M. incognita,* and reduced the damage to cowpea. *P. lilacinus* was more effective on single pathogens than on combinations of the pathogens (Khan and Husain 1988b; Khan and Husain 1990).

Pathogen interactions

Many papers have reported interactions among nematodes, pathogenic fungi, mycorrhizal fungi, and *Rhizobium* spp. Examples of five general trends are given below.

Trend 1. A pathogen which first infects a root usually suppresses reproduction of the second pathogen. For both *M. incognita* and *R. reniformis*, inoculation of one nematode before the other reduced the multiplication of the second (Khan and Husain 1988a). Furthermore, the nematode reproduction factor on cowpea was highest when nematodes were inoculated alone, lower when they were inoculated concomitantly with *Rhizoctonia solani,* and lowest when the fungus was inoculated 15 days before the nematodes (Khan and Husain 1988a). Culture filtrates from *R. solani* reduced hatching of *M. javanica* eggs, leading Singh et al. (1986) to postulate that infection of roots by *R. solani* prior to nematode infection permits a buildup of fungal metabolites detrimental to hatching. When *R. solani* or *M. phaseolina* were inoculated on cowpea 7 days prior to *H. cajani,* nematode multiplication was inhibited, resulting in few or no cysts (Walia and Gupta 1986a,b). *M. javanica* inhibited penetration into the roots of cowpea and maize by *Pratylenchus sefaensis* and *R. reniformis* (Egunjobi et al. 1986).

Trend 2. Plant damage is greater and fewer nodules are formed when *Meloidogyne* spp. or *Heterodera* spp. are inoculated before pathogenic fungi or *Rhizobium* spp. When *H. cajani* was inoculated on cowpea 2 weeks before *R. solani,* there was a significant reduction in the top growth of plants (Walia and Gupta 1986a). Vascular discoloration was greatest when *M. javanica* was added 4 weeks before *F. oxysporum* f. sp. *tracheiphilum* (Harris and Ferris 1991c). Fewer nodules were produced on cowpea when *H. cajani* was added before *M. phaseolina* (Walia and Gupta 1986b).

Trend 3. Fungi can suppress multiplication of nematodes when inoculated at the same time as the nematodes. The number of *M. javanica* galls was decreased when *R. solani* was present, especially when the two pathogens were inoculated simultaneously (Kanwar et al. 1988). Concomitant inoculation of *F. solani* and *H. cajani* resulted in lower populations of the nematode and greater shoot weight of cowpea than when the pathogens were inoculated alone (Varaprasad et al. 1987). The low nematode population may have been due to toxic substances produced by *F. solani*. Mani and Sethi (1984) found that high concentrations of culture filtrates of *F. solani* and *F. oxysporum* f. sp. *ciceri* killed eggs and immobilized juveniles of *M. incognita*, and low concentrations inhibited egg hatch.

Trend 4. Vescicular-arbuscular mycorrhizae and/or *Rhizobium* spp. increase plant growth, decrease nematode population densities, and reduce fungal infection when inoculated before or simultaneously with nematodes or fungi. *M. incognita* and *R. reniformis* reproduced least when cowpea was inoculated with *Rhizobium* sp. before inoculation with

nematodes (Khan and Husain 1988a). The best cowpea growth and nodulation were recorded in treatments containing both mycorrhizae and *Rhizobium* sp. without nematodes; however, plant growth was good and nematode multiplication suppressed in *M. incognita*-infested treatments containing the endomycorrhizal fungus *Glomus etunicatum* (Sivaprasad et al. 1990). In roots highly colonized by mycorrhizae, Sivaprasad et al. (1990) postulated that reduced penetration and slower development of *M. incognita* juveniles led to fewer nematodes, fewer roots with galls, and fewer galls per length of root. Sundaresan et al. (1993) found that *F. oxysporum* infection of roots and resulting disease severity were reduced when roots were previously colonized by *Glomus fasciculatum*. The degree of root colonization by mycorrhizae was correlated with the quantity of three phytoalexins, one of which inhibited germination of *Fusarium* conidia (Sundaresan et al. 1993).

Trend 5. Host plant resistance may not be effective when two or more pathogens are inoculated together on cowpea cultivars resistant to one of the pathogens. *M. incognita* and *R. reniformis* reduced the resistance of cultivar IC-244 to *R. solani* when either nematode was inoculated at the same time as *R. solani* (Khan and Husain 1989a). In the same study, cultivars RC-8 and EC-4213A were found to be resistant to *R. solani* and moderately resistant to *R. reniformis* when inoculated with each pathogen individually or simultaneously; however, the resistance was not expressed when either pathogen was inoculated in combination with *M. incognita*. Similarly, cultivar S488 was no longer resistant to *R. reniformis* and cultivar CO-4 was no longer resistant to *R. solani* when *M. incognita* was inoculated with either pathogen. Interestingly, cultivar IC-503, which was moderately resistant to *M. incognita*, did not become more susceptible to this pathogen even when the other pathogens were present (Khan and Husain 1989a). *M. javanica* increased the wilt symptoms caused by three races of *F. oxysporum* f. sp. *tracheiphilum* in the wilt-resistant cultivar California Blackeye No. 3, but did not similarly increase the wilt caused by two isolates of race 3 of the fungus in the wilt-resistant cultivar CB7977 (Harris and Ferris 1991a, 1991b). As for many experiments on pathogen interactions, root-knot nematodes seem more able to disrupt the mechanisms of resistance to other pathogens.

Looking ahead

Soilborne pathogens of cowpea have been studied alone and in various combinations over the past 10 years. Although good information on pathogens, control measures, and mechanisms of resistance has come from work on individual pathogens, experiments in which several pathogens were inoculated together have provided exciting new insights into pathogen-host interactions. More studies of this type will improve the deployment of cowpea cultivars resistant to many pathogens. Inoculation of cowpea with *Rhizobium* spp. and mycorrhizal fungi may become an integral part of integrated pest management practices because these organisms mitigate the effects of pathogenic fungi and nematodes. Studies on the effect of organic amendments on pathogen survival and pesticide efficacy will contribute to the development of control packages that minimize the use of chemical pesticides. Research is still needed on cropping systems and biocontrol agents to explain why certain combinations of crops and microorganisms suppress plant diseases. If undertaken, such complex studies should help formulate strategies for controlling most soilborne pathogens in the next 10 years of cowpea research.

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Characterization of new resistance to root-knot nematodes in cowpea

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Abstract

Valuable new sources of host plant resistance to *Meloidogyne* spp. in cowpea are described according to phenotype and mode of inheritance. Breeding line IT84S-2049 possesses a high level of broad-based resistance that controls *M. incognita* and *M. javanica*, including populations of both species that are virulent to resistance gene *Rk* present in commercial cowpea cultivars. Resistance in IT84S-2049 is conferred by a single dominant nuclear gene, which is allelic to gene *Rk* or very tightly linked to *Rk* within ~ 0.17 map units. Several heat-tolerant blackeye dry bean selections (H-lines) developed at Riverside were also found to contain a higher level of resistance than that conferred by gene *Rk*, but not as high as that in IT84S-2049. Resistance in the H-lines appears to be conferred by gene *Rk*, which is modified to a higher resistance by an independent gene expressed in the homozygous recessive condition.

Introduction

Resistance to root-knot nematodes in cowpea (*Vigna unguiculata* [L.] Walp.) was one of the first examples of nematode resistance to be identified in plants (Webber and Orton 1902). From analysis of F_3 progenies derived from resistant lines, Hare (1959) concluded that the resistance to *Meloidogyne incognita* [Kofoid and White] Chitwood was under the simple genetic control of a single dominant gene, and this was confirmed in a derived cultivar (Amosu and Franckowiak 1974). A more definitive analysis by Fery and Dukes (1980) of the resistance in the cultivars Iron, Colossus, and Mississippi Silver, using F_1, F_2, F_3, and backcross progenies from resistant × susceptible crosses, demonstrated that these lines possessed the same single dominant resistance gene; they designated it *Rk* (for root-knot resistance). Fery and Dukes (1980) further showed that gene *Rk* conferred resistance to *M. incognita*, *M. javanica* (Treub) Chitwood, and *M. hapla* Chitwood. Hare (1959) had reported earlier that the resistance in cv. Iron and line M755 (parental to Mississippi Silver) also controlled *M. arenaria* (Neal) Chitwood. A range of cowpea cultivars with root-knot resistance based on the *Rk* gene have been developed for dry or fresh bean production; for

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example, blackeye dry bean cultivars California Blackeye No. 5 (CB5) and CB46, and CB88 in California. Many other cowpea cultivars possessing gene *Rk* have been developed for other regions of the United States and in other countries. In all these areas, the growing of root-knot resistant cultivars based on resistance gene *Rk* provides the primary nematode management tactic (Thomason et al. 1959; Dukes et al. 1979; Fery and Dukes 1980; Swanson and Van Gundy 1984; Roberts et al. 1995).

Although a broad spectrum of *Meloidogyne* species are controlled by gene *Rk*, studies on California isolates of *M. javanica* revealed differential virulence or aggressiveness of these isolates on cowpeas with *Rk*, such as CB5 and CB7 (Thomason et al. 1959; Thomason and McKinney 1960). Low or no resistance expression to *M. javanica* by gene *Rk* has been further demonstrated in growth pouch, pot, and field plot experiments (Swanson and Van Gundy 1984; Roberts et al. 1992; Roberts et al. forthcoming). Recently, field populations of *M. incognita* caused extensive root-galling and plant injury to cowpea cultivars possessing gene *Rk*, and their virulence to *Rk* has been confirmed in comparative studies with *Rk*-avirulent *M. incognita* populations (Roberts et al. 1995). Fields where significant root-knot infection of resistant cowpeas was observed were generally assumed to be infested with *M. javanica*, which is widely distributed in cowpea production areas (Thomason et al. 1959; Thomason and McKinney 1960; Swanson and Van Gundy 1984; Harris and Ferris 1991a). However, in two cowpea fields ~ 200 km apart, *M. incognita* populations caused extensive root-galling and injury to plants with gene *Rk* (Roberts et al. 1995). A survey of cowpea production sites is required to determine both the extent and pattern of distribution of these *M. incognita* infestations.

These fields also contained the cowpea Fusarium wilt organism, *Fusarium oxysporum* Schlechtend. Fr. f. sp. *tracheiphilum* (E.F. Sm.) W. C. Snyder and H.N. Hansen (Roberts et al. 1995). Differences in Fusarium wilt incidence and severity were observed in both fields, associated with wilt susceptibility in the presence of the nematode. *M. incognita* isolates, whether virulent or avirulent against gene *Rk*, did not predispose wilt-resistant genotypes to disease, but virulent isolates exacerbated the disease in wilt-susceptible genotypes (Roberts et al. 1995). On the other hand, breakdown of Fusarium resistance by predisposition in the presence of *M. javanica* has been reported, based on both greenhouse and field studies (Thomason et al. 1959; Harris and Ferris 1991b).

Three essential aspects of resistance to root-knot nematodes in cowpea have emerged from these considerations. First, there is a strong dependence on a narrow genetic base of gene *Rk* in current cultivars. Second, the resistance expressed by gene *Rk* is effective against some but not all *Meloidogyne* species and populations; thus virulence and aggressiveness to *Rk* have been identified within the two most common species, *M. incognita* and *M. javanica*. Third, resistance to root-knot is important in limiting the damaging effects of the disease complex with Fusarium wilt, because nematode infection can exacerbate wilt disease.

Characterization of *Meloidogyne* spp. and populations

There are > 50 described species of the genus *Meloidogyne*, but only four species (*M. arenaria*, *M. hapla*, *M. incognita*, and *M. javanica*) consistently cause significant economic loss of yield on numerous host crops worldwide (Sasser 1980). All four species parasitize cowpea; *M. incognita* and *M. javanica* are the most important economically,

while *M. arenaria* and particularly *M. hapla* (a cooler climate species among the four) are of more local importance. Most studies have focused on *M. incognita* and *M. javanica*, including variation within each species for ability to parasitize resistant cowpea genotypes (Fery and Dukes 1980; Swanson and Van Gundy 1984; Harris and Ferris 1991a,b; Roberts et al. 1995). The parasitic ability is conveniently categorized into virulence and avirulence. These terms are used to designate nematode populations that are able (virulent) to reproduce significantly on resistant host plants that suppress or prevent reproduction of other populations (avirulent) of the same nematode species (Roberts 1995). Virulent populations are further classified by their aggressiveness. An aggressive population or species has an enhanced parasitic ability, which may involve more than single virulence genes, including aspects of parasitic fitness and competitiveness.

Resistance gene *Rk* has been shown to control all four common *Meloidogyne* spp. Resistance is measured typically by assessing the reproduction level of nematode populations on resistant plants as a proportion (index) of the reproduction on standard susceptible genotypes. Reproduction is usually measured as the number of eggs or egg masses produced on a root system or unit weight (e.g., gram) of root, and/or numbers of nematode eggs and juveniles in soil around roots. Typically, these measurements are made following controlled inoculations of plants in pots or growth pouches, or after planting into replicated plots in infested field soil (Omwega et al. 1988; Roberts et al. 1995).

Characterizing the strength of resistance in a resistant genotype × nematode isolate interaction is important, i.e., is the resistance strong (very low reproduction rate), or at an intermediate or moderate level (reproduction occurs but at a significantly lower level than on susceptible cultivars)? A second characterization of resistance is qualitative, and it determines the breadth of resistance to a range of defined isolates of nematode species and populations. Both aspects are of primary importance, as illustrated here in characterizing the gene *Rk*, and some new cowpea resistance gene factors.

Virulence levels (egg production) of several isolates of *M. incognita* populations are shown for some California blackeye dry bean genotypes carrying the *Rk* gene (Table 1). The *M. incognita* isolates can be grouped into two classes: isolates from sites I and II are virulent on plants with gene *Rk* (CB5 and CB46), whereas those from site III, Pixley, and UCR are avirulent, with fewer eggs produced on roots of *Rk* plants (Roberts et al. 1995). The avirulent populations reproduce to some extent on *Rk* plants; therefore, *Rk* plants are not immune. Thus, both *Rk* gene-virulent and -avirulent populations occur within a *Meloidogyne* species, in this case *M. incognita*. Furthermore, Table 1 shows that the virulent and avirulent *M. incognita* population groups contain isolates belonging to the classic host races 1 and 3 (Sasser 1980; Hartman and Sasser 1985). In other words, *Rk* gene virulence does not conform to the classic host races (Roberts et al. 1995). This result supports the apparent large variation in virulence within *Meloidogyne* spp. to resistance genes in a range of host crop plants (Roberts 1995).

Differences occur in reproduction (egg masses on roots of plants raised in growth pouches) on plants with the *Rk* gene (CB5 and CB46) by virulent and avirulent isolates of *M. incognita* and an aggressive *M. javanica* isolate (Table 2). Data in Tables 1 and 2 indicate the limitations of *Rk* in its ability to control some populations of the important root-knot species. In practice, *Rk*-based resistance is very useful and can control numerous root-knot infestations in cowpea production areas. However, the occurrence of virulent and

Table 1. Egg production by five isolates of *Meloidogyne incognita* on resistant (gene *Rk*) and susceptible cowpea genotypes in a greenhouse experiment†.

	Mean eggs (10^3) per gram fresh root				
	Race 1 isolates		Race 3 isolates		
Genotype	Site I (Avir)§	UCR (Avir)	Site II (Vir)	Site III (Avir)	Pixley (Avir)
8679 (– *Rk*)	5.37a¶	20.89a	12.59a	12.59a	9.55a
CB3 (– *Rk*)	1.23b	16.98a	17.78a	6.46a	22.91a
CB5 (+ *Rk*)	6.17a	0.17b	24.55a	0.43b	1.82b
CB46 (+ *Rk*)	11.22a	0.71b	26.30a	0.19b	0.26b
Isolate means	4.57B‡	1.48C	15.49A	0.85D	2.14C

† ANOVA was performed on log10 (x +1) transformed egg count data.
§ Vir and Avir denote classification of nematode isolates as virulent or avirulent to gene *Rk*, respectively.
¶ Values in columns followed by same lower case letter are not significantly different at $P \leq 0.05$, based on LSD *t* test for genotype x isolate interaction.
‡ Values for overall effects among isolates followed by same upper case letter are not significantly different at $P \leq 0.05$, based on LSD *t* test.
Source: Roberts et al. (1995).

Table 2. Egg mass production in growth pouches on cowpea genotypes possessing different resistance factors to *Meloidogyne incognita* and *M. javanica*†.

	Mean egg masses per root system		
Genotype	*M. incognita* (*Rk*-avirulent)	*M. incognita* (*Rk*-virulent)	*M. javanica* (*Rk*-virulent)
CB3 (– *Rk*)	211a	201a	386a
CB5 (+ *Rk*)	4b	79b	207b
CB46 (+ *Rk*)	2b	64b	191b
H8-8R (+ modified *Rk*)	0b	30c	86c
H8-14R (+ modified *Rk*)	0b	16d	81c
IT84S-2049 (+ Rk^2)	0b	10d	60c

† Means of 12 replicates. Values in columns followed by same letter are not significantly different at $P \leq 0.05$, based on Duncan's multiple range test. Symbol Rk^2 designates the novel resistance gene found in cultivar IT84S-2049.
Source: Roberts et al. (1992).

aggressive nematode infestations makes the deployment of *Rk* resistance very difficult and potentially much less effective, because it is often not known whether an infested field contains virulent populations that will not respond to resistance conferred by *Rk*. Species identification does not provide an answer, in that a virulence identity can only be revealed through bioassay. Bioassays are not practical for short-term grower management decisions, although grower records of previous infection levels or responses of resistant cultivars on an infested field are helpful.

Stability of nematode virulence

In addition to searching for resistance effective against *Rk* gene-virulent populations, we have initiated studies on the genetic stability of the virulence condition in these nematode

isolates (Roberts and Matthews 1995). The site I population was ~ 75% virulent on CB5 and CB46 plants with *Rk* (i.e., a mixture of about three virulent to one avirulent individuals) compared to a typical reaction on susceptible CB3 control plants. During 4 years of continuous culture on susceptible tomato host plants in the greenhouse, this population declined progressively in virulence down to ~ 15%; the virulence percentage on CB46 over time (days) was described by the model $y = 75.695 \times 10^{-3.8675e-4x}$ ($r^2 = 0.68$). Little is known about the genetic control of virulence in *Meloidogyne* (Roberts 1995), and studies are hampered by the parthenogenetic mode of reproduction of these polyploid nematodes that precludes Mendelian-type analysis of inheritance. However, data accumulating on tomato, cowpea, and some other crops suggest that inheritance of virulence is simple (Roberts 1995). In cyst nematodes, simple inheritance (with virulence recessive to avirulence) that conforms to a gene-for-gene interaction with host resistance genes has been demonstrated (Roberts 1995).

In tomato, resistance interaction virulence in *M. incognita* appears to be genetically stable, but our results imply that with gene *Rk* in cowpea, the virulence is not stable and can be selected against fairly readily in the absence of *Rk* gene directional selection pressure (Roberts and Matthews 1995; Roberts 1995). Thus, virulence appears to have, or be linked to, some disadvantage for the nematode in the absence of resistance selection. If so, virulence could be managed in the field by crop rotation that provides multi-year breaks between plantings of cultivars with gene *Rk*. Further detailed analysis of the underlying mechanisms will help in exploiting and managing avirulence in the field.

Characterization of new resistance factors

The spectrum of virulence to gene *Rk* in the *Meloidogyne* spp. provides the framework for identifying and characterizing new resistance gene specificities in cowpea germplasm. Comparisons of phenotype specificity and inheritance between the *Rk* gene and new resistance traits has resulted in the identification of several potentially important resistance factors.

A screening of > 300 entries from the USDA cowpea germplasm collection maintained at UC Riverside revealed only two accessions with high levels of resistance distinct from the *Rk* gene resistance phenotype. This screening specifically targeted resistance to *Rk* gene-virulent *M. incognita* isolates in order to select novel resistance factors. The two accessions (IT84S-2049 and IT84S-2246-4) differ slightly in resistance phenotype, but might contain the same genetic factors for resistance. Accession IT84S-2049 has been studied in detail. In Table 2, the phenotype of the resistance in IT84S-2049 is shown relative to *Rk* gene expression. The resistance is highly effective against *Rk*-avirulent isolates, being nearly immune with little or no nematode reproduction on inoculated plants. The resistance in IT84S-2049 is also effective against *Rk*-virulent isolates; high levels of resistance have been demonstrated by low egg mass and egg production levels on root systems. Furthermore, this resistance is also moderately effective against California isolates of *M. javanica* that are aggressive (or virulent) to gene *Rk*. Resistance to *M. javanica* is not as high as that to *M. incognita* (Table 2), but it is significant in its control of *M. javanica*. This has been confirmed in repeated growth pouch, greenhouse pot, and field experiments (Roberts et al. 1996; forthcoming). Thus a broad-based resistance is expressed to a diverse range of nematode variants.

Table 3. Reaction of cowpea breeding line IT84S-2049 to a *Meloidogyne incognita* isolate avirulent to both *Rk* and *Rk*^{2} resistance genes†.

Population or genotype	Number of plants		Egg masses (mean ± SD)
	Resistant	Susceptible	
IT84S-2049 (+ *Rk*^{2})	64	0	0
CB46 (+ *Rk*)	43	0	0.3 ± 0.5
CB5 (+ *Rk*)	18	0	0.5 ± 0.8
CB3 (– *Rk*)	0	124	117.9 ± 58.4
F_2 (CB46 x IT84S-2049)	654	0	< 0.1 ± 0.4
F_2 (CB5 x IT84S-2049)	552	0	0.4 ± 0.9
TC_1 ([IT84S-2049 x CB46] x CB3)	1114	0	1.6 ± 2.2

† Tests were made in growth pouch experiments in controlled environment chambers. Susceptibility was assessed according to numbers of egg masses produced per root system following inoculation of 1500 ± 150 nematode juveniles per plant.

Source: Roberts et al. (1996).

Genetic analysis of the resistance in IT84S-2049 revealed several important results (Roberts et al. 1996). Egg mass production on inoculated root systems of plants in growth pouches was used to assess resistance of F_1, F_2, F_3, BC_1, BC_1:F_2 and TC_1 populations generated from crosses of IT84S-2049 with CB3 (susceptible), CB5, or CB46 (both possessing *Rk*). Expression and segregation of resistance to *Rk*-virulent *M. incognita* in progenies from IT84S-2049 × CB3 showed that resistance in IT84S-2049 is governed by one dominant nuclear gene (Roberts et al. 1996). No susceptible recombinants were found among totals of 1206 F_2 and 1144 TC_1 progeny from IT84S-2049 × CB5 or CB46 and inoculated with *Rk*-avirulent *M. incognita* (Table 3). The avirulent isolate was used to detect both resistance phenotypes, and the TC_1 progeny were generated by crossing the resistant IT84S-2049 × CB46 hybrid as female parent with susceptible CB3, to ensure that any possible selfed progeny would be resistant and could not be mistaken for susceptible recombinants (Roberts et al. 1996). Therefore, resistance in IT84S-2049 is conferred by an additional dominant allele of the *Rk* locus, or by another gene locus very tightly linked to *Rk* within 0.17 map units.

We proposed the symbol *Rk*^{2} for the single dominant resistance gene in IT84S-2049, in order to indicate (with the superscript ^{2}) that it represents a different resistance phenotype associated genetically with the root-knot resistance gene locus *Rk* which was described by Fery and Dukes (1980), and that it may well be another allele at the *Rk* locus (Roberts et al. 1996). With each nematode, the *Rk*^{2} resistance is higher than the resistance conferred by gene *Rk*, but the relative effectiveness of both *Rk* and *Rk*^{2} to the range of populations that were tested in this study is similar. For example, the highest resistance expressed by both factors is to the *Rk*-avirulent *M. incognita*; the next highest is to virulent *M. incognita*, and this is followed by the resistance expressed to *M. javanica* (Roberts et al. forthcoming). This pattern of expression implies that the novel resistance factor may be a variant of *Rk* (a modified allele or a duplicated and modified adjacent locus). If recombination between *Rk* and *Rk*^{2} is found, then *Rk*^{2} could be redesignated as *Rk*–2 to denote a separate gene locus. Dominant expression and simple inheritance of *Rk*^{2} should expedite incorporation through breeding.

Gene *Rk* as a complex locus

The characterization of Rk^2 suggests that *Rk* may be a complex nematode resistance locus, analogous to those reported for other plant pathogen-host combinations; both multiple allelism and clustering of resistance loci have been identified in some plants for resistance to certain biotrophic fungi and bacteria (Roberts 1995). Some other lines of evidence add support to the apparently complex nature of the *Rk* locus. Previously, Fery and Dukes (1982) reported the occurrence of a recessive resistance gene in the cowpea cv Pinkeye Purple Hull conferring an intermediate level of resistance that they indicated to be another allele (designated rk^i) at the *Rk* locus. In addition, Fery et al. (1994) reported finding new highly expressed *M. incognita* resistance traits in three accessions of cowpea (PI 441917, PI 441920, and PI 468104) which exhibited less root galling, egg mass formation, or egg production than in the case of the cultivar Mississippi Silver (that has gene *Rk*). Their analyses of the F_2 populations of each accession crossed with Mississippi Silver revealed no susceptible recombinants, suggesting that these resistance factors are also allelic or tightly linked to gene *Rk*. Their relationship to Rk^2 and to each other remains to be determined.

One additional important finding is the presence of an independent modifier gene of the *Rk* locus in some advanced breeding lines (H-lines) of blackeye dry beans originally selected at UC Riverside for heat tolerance (J.D. Ehlers and P.A. Roberts, unpublished data). The H-lines were found to possess elevated levels of resistance to *M. incognita* and *M. javanica*, and a broader spectrum of resistance to *Rk* gene-virulent isolates, compared to gene *Rk* resistance (Table 2). This resistance is not quite as high as the resistance conferred by Rk^2 in IT84S-2049, but shows a similar spectrum of effect. Allelism tests showed that the H-lines possess the gene *Rk*. From their crosses with genotypes carrying *Rk* and expressing the typical *Rk* resistance phenotype, preliminary evidence has been obtained for the presence of an independent locus that modifies *Rk* resistance to a higher level. The single modifier locus was found to promote *Rk* resistance when it (the modifier) is present in the homozygous recessive condition. Conversely, when the modifier is in the heterozygous or homozygous dominant condition, *Rk* is expressed at the normal level. The modifier gene can be viewed as a recessive enhancer or helper gene to resistance gene *Rk*, or alternatively, it is a dominant suppressor to *Rk* that only partially suppresses *Rk* gene expression.

Conclusions

Variation in virulence to resistance gene *Rk* occurs in the important species *M. incognita* and *M. javanica*. Virulent populations that render *Rk* ineffective emphasize the need for additional traits that can broaden the genetic base of resistance through breeding. Important new resistance factors have been identified for this purpose. Superior resistance has been identified and characterized in accession IT84S-2049. This dominant trait is another allele of *Rk* or a locus tightly linked to *Rk*, and it confers resistance to nematode populations able to attack plants with *Rk*. Other accessions have been shown to possess additional superior resistance traits that are also linked to or are alleles of gene *Rk*. An independent gene, which in the recessive condition modifies gene *Rk* to a higher level of resistance expression, has also been identified. The *Rk* gene has characteristics of a complex nematode resistance locus.

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Advances in research on cowpea *Striga* and *Alectra*

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Abstract

The parasitic weeds, *Alectra vogelii* Benth., and *Striga gesnerioides* (Wild.) Vatke, have become major yield-reducing factors in cowpea. Currently, *Striga* is more prevalent in the Sudan savanna and Sahelian regions and *Alectra* in the northern Guinea savanna and southern Sudan savanna regions of West Africa, as well as in East and southern Africa. However, both are fast spreading beyond these limits. Therefore, concerted efforts are being made to develop cowpea varieties resistant to *Striga* and *Alectra*, as well as other control measures to minimize yield losses. Systematic research on *Striga* started in Burkina Faso and on *Alectra* in Botswana in the early 1980s, which subsequently evolved into a collaborative research effort involving IITA, the Semi-Arid Food Grain Research and Development (SAFGRAD) project, the Natural Resources Institute (NRI), Long Ashton Station (UK), and various national programs. This has led to the identification of several sources of resistance to *Striga* and *Alectra* and the development of resistant varieties, as well as systematic studies on strain variation and integrated control. Progress is reviewed.

Introduction

Cowpea (*Vigna unguiculata* [L.] Walp.) is attacked by two parasitic plants, *Striga gesnerioides* [Wild.] Vatke and *Alectra vogelii* [Benth.], both belonging to the family Scrophulariaceae (Kuijt 1969). Of the two, *Striga* is more widely distributed, covering sub-Saharan Africa and parts of Asia and USA, whereas *Alectra* is presently restricted to Africa (Musselman et al. 1991; Parker and Riches 1993). Both parasitic plants cause considerable damage to cowpea, with substantial yield reductions, especially in Africa (Emechebe et al. 1991; Lagoke et al. 1994). At present, the Sudano-Sahelian belt of Africa is more affected by *Striga* and the Guinea-Sudan savanna belt by *Alectra*, but both parasites are fast spreading beyond these limits. For example, severe *Striga* attack has been noticed in the coastal savanna of Benin Republic (Lane et al. 1994) and Togo (Agbobli 1991), as well as in the southern Guinea savanna of Nigeria, and *Alectra* is becoming a serious threat in several countries in East and southern African, particularly Botswana, Kenya, Tanzania, Zambia, and Zimbabwe (Riches 1989; Singh et al. 1993). With more monocropping under growing population pressure, the *Striga-Alectra* problem is becoming even more acute, particularly in areas with sandy soils, poor fertility, and low

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rainfall. Both plant parasites are rather difficult to control because they produce large amounts of seeds, and their adaptation/dormancy mechanisms permit the seeds to stay alive in the soil for several years (Saunders 1933; Kust 1963). Therefore, a combination of different control options, including host plant resistance, crop rotation, chemical/biological control, seed treatment, and other phytosanitary practices, needs to be developed to achieve satisfactory and sustainable control. The International Institute of Tropical Agriculture (IITA) is working closely with several national and regional programs, as well as with selected research stations in Europe and the USA, to develop an integrated scheme for controlling these plant parasites, and good progress is being made (Singh 1994; Berner et al. 1995).

Nature of parasitism

The biology of the cowpea parasites, *S. gesnerioides* and *A. vogelii*, and the histology of infected cowpea plants has been extensively studied (Botha 1948, 1950; Kuijt 1969; Visser 1975, 1978; Dörr et al. 1977, 1979; Okonkwo and Nwoke 1978; Reid and Parker 1979; Okonkwo and Raghavan 1982; Ba 1984; Herb et al. 1987; Riches 1989; Igbinnosa and Okonkwo 1991; Lane et al. 1991; Okonkwo 1991; Samb and Chamel 1992; Dörr 1995). *Striga* and *Alectra* seeds germinate when exposed to root exudates from cowpea, other hosts, and a few nonhosts. Radicles elongate, showing a chemotropic response to a concentration gradient of root exudates. Once in contact with host roots, the radicular apex develops numerous hairs, which attach to host roots. The *Striga* radicle penetrates and stimulates cell division in the host root. The new host cells, together with growing parasitic tissues, form a large haustorium, uniting the parasite with tissue in the host's stele which permits transfer of water and nutrients from host to parasite. *Alectra* radicles stimulate profuse root formation by the host and form a larger haustorium than *Striga*. Both *Striga* and *Alectra* shoots emerge from the haustorium about 2 weeks after infection and grow into plants which may be 15–25 cm tall. Our studies (unpublished) in soils jointly infested with *Striga* and *Alectra* and planted with cowpea show that *Striga* attaches and emerges faster than *Alectra*. The plants of both parasites usually branch below ground and emerge as a bunch above the ground. *Striga* leaves are very small, succulent, scale-like, and appressed to the stem; *Alectra* leaves are a bit larger and more open. Flowers are normally borne when the plants emerge above the ground and may be white, pink, yellow, or purple for *Striga* and yellow for *Alectra*. Seeds are produced in capsules, each of which may have 400–500 seeds; over 50,000 seeds may be produced by a single plant, depending upon the branching and growth. The seeds are very small, measuring 0.15–0.25 mm in length (Visser 1978), and these are dispersed over long distances by water, wind, and animals (Parker and Riches 1993), and with crop seeds (Berner et al. 1994b).

Symptoms of infection and yield loss

Symptoms of infection by *Striga* and *Alectra* can be noticed on cowpea plants much before emergence of the parasitic plants above ground, because the parasitism begins about 2–3 weeks before emergence. The common symptoms of *Striga* infection are interveinal chlorosis, general stunting, and smaller leaves. In the case of severe infection, straw-colored necrotic spots develop on the lamina, followed by complete desiccation of the leaves (Emechebe et al. 1991). Plants infected by *Alectra* have symptoms similar to, but

less drastic than, plants infected by *Striga.* Infected plants usually show general stunting and wilting with reduced numbers of flowers and pods, but plants may be completely wilted if there is acute moisture deficit (Mugabe 1983).

The extent of yield reduction depends upon the time and level of infection. Aggarwal and Ouedraogo (1989) reported a mean yield loss of 30% due to *Striga* infection in Burkina Faso, with 50% loss in the most susceptible local variety. Our observations in northern Nigeria indicate even greater yield losses. Total destruction of cowpeas has been reported from Sokoto, Kano, Katsina, Borno, and Bauchi states due to the *Striga/Alectra* complex (Emechebe et al. 1991).

The effect of *Alectra* is equally devastating in heavily infested cowpea fields, as has been observed in Botswana (Riches 1989; Parker and Riches 1993), Kenya (Bagnall-Oakeley et al. 1991), and Nigeria (Singh and Emechebe 1991). We have observed that in fields that are heavily infested with both *Striga* and *Alectra*, the crop is mostly infected by *Striga* if the host cultivar is equally susceptible to both parasites (Singh and Emechebe 1991). However, if the host cultivar is resistant or moderately resistant to *Striga* and susceptible to *Alectra* (as in the case of IT82D-849 and Suvita-2), *Alectra* infection is very severe, causing substantial yield losses. Therefore, in West and Central Africa, where *Striga* as well as *Alectra* are present, control measures to be adopted have to be effective for both.

Effect of cultural practices

Deep cultivation, intercropping, rotation, early planting, use of nitrogenous fertilizers, and seed treatment have been observed to reduce infection of cereals by parasitic weeds. However, only limited studies have been done on cowpea. Emechebe et al. (1991) reported gradual reduction in *Striga* infection with increasing nitrogen concentration in sandy soil in pots. Using 0, 60, and 120 ppm nitrogen in the watering solution, they observed 88%, 25%, and 12% *Striga* infection, respectively. There was no *Striga* infection at all at concentrations greater than 120 ppm. However, nitrogen fertilization studies under field conditions did not confirm these results. In any case, adding fertilizer N may not be a practical control option because most traditional farmers seldom use nitrogenous fertilizers on cowpea.

Preliminary studies at IITA Kano Station have shown that certain varieties of millet and sorghum stimulate germination of *Striga gesnerioides* seed, thereby reducing the concentration of seed in the soil when these crops are planted as intercrops with cowpea or as sole crops in rotation with cowpea. Late-planted cowpea (10 Aug) had a significantly higher number of *Striga* plants/plot than early-planted cowpea (21 Jul), which was reflected in lower yields (Lagoke et al. 1994). Berner et al. (1994a) reported that soaking cowpea seeds in an aqueous solution of imazaquin at a concentration of 3.6 mg a.i./ml for 5 min before planting in pots greatly reduced *Striga* and *Alectra* infection. However, the chemical was somewhat toxic to cowpea plants and further studies are needed to establish an appropriate dose. We have observed moderate to high incidence of *Smicronyx* spp. (an insect) on *S. gesnerioides* at Kano, indicating a possibility for reducing *Striga* by biological control. Our preliminary studies also indicate that growing *Striga*-resistant cowpea varieties will not only protect the current crop from *Striga* infection, but will also reduce the *Striga* seed bank, thus minimizing infection level in the succeeding crop.

Host plant resistance

Excellent progress has been made in controlling *Striga* and *Alectra* on cowpea by host plant resistance. This has involved collaborative work among a number of national, regional, and international programs over the past 14 years. Initial work on resistance to *Striga* in cowpea was done by IITA scientists based at Kamboinse, Burkina Faso, working on a joint project with the International Development Research Centre (IDRC), Canada, and the Semi-Arid Food Grain and Development (SAFGRAD) project of the Organization of African Unity. Resistant varieties, identified in field screening of 54 cowpea varieties at Kamboinse in 1981 (IITA 1982; 1983), were evaluated by the IITA/SAFGRAD project at many locations in Burkina Faso, Cameroon, Mali, Republic of Niger, and Nigeria from 1983 to 1986 to ascertain the stability of *Striga* resistance across the West African savanna. 'Gorom local' and '58-57' had shown a high level of resistance to *Striga* in Burkina Faso, but their susceptibility in other countries indicated the presence of different strains (Aggarwal 1985). Therefore, the search for sources of resistance continued and two new resistant sources, B 301 (a landrace from Botswana) and IT82D-849 (an improved breeding line from IITA), were identified in 1987 through collaborative work of the IITA/SAFGRAD project with Long Ashton Station, UK, and various national programs. These new sources showed stable resistance to *Striga* across Burkina Faso, Mali, Republic of Niger, and Nigeria (Parker and Polniaszek 1990; Aggarwal 1991; Emechebe et al. 1991). In addition, a number of other lines were identified which are less susceptible to *Striga,* as shown by a lower number of *Striga* plants as well as delayed emergence of *Striga* (Singh and Emechebe 1991). These lines display varying degrees of resistance to the *Striga* strain found in Kano, Nigeria. IT82D-849 is resistant to *Striga* but susceptible to *Alectra*, whereas IT86D-534, IT86D-371 and IT84D-666 are moderately resistant to *Striga* and highly resistant to *Alectra.* B 301 is completely resistant to both. Suvita-2 is highly resistant to the *Striga* strain from Burkina Faso, moderately resistant to *Striga* from Nigeria, but highly susceptible to *Alectra.* IT82D-957 is highly susceptible to both, and yield loss due to both parasitic plants can be significant. Resistance to both *Striga* and *Alectra* must be incorporated into cowpea varieties intended for production in the region.

Screening methods

Reliable and fast screening methods have been developed for genetic studies and evaluation of segregating materials.

Field screening. Most of the experimental fields at IITA Kano Station are infested with *Striga* and *Alectra.* One of these fields (0.5 ha) was developed as a *Striga* sick plot by evenly spreading 20 bags of matured *Striga* plants and 10 bags of matured *Alectra* plants on it, and incorporating them into the soil by repeated harrowing about 3 weeks before planting. More inoculum is added each year. Sick plots have also been developed/ identified in Benin Republic, Burkina Faso, Mali, Niger Republic, and Nigeria, in collaboration with the national programs. Test lines are planted in these plots along with known susceptible varieties and data on number of emerged *Striga/Alectra* are taken, beginning 5–6 weeks after planting. The days taken to first emergence of *Striga* and/or *Alectra* in each line is recorded, and then weekly counts are made to study the pattern of *Striga* and/or *Alectra* emergence. Lines free from the parasitic weeds and those showing delayed or less

emergence of the parasites in the field are further tested in a screenhouse, using the pot culture technique described next.

Pot screening. Plastic pots (13 cm diameter and 13 cm depth) are used for screening (Singh and Emechebe 1990). Each pot contains about 1 liter of a mixture of unsterilized sieved sand and sandy loam top soil (1:1 v/v) previously inoculated uniformly with about 800 seeds of *Striga* or *Alectra*. The pots are kept on benches in a screenhouse and planted with test cowpea lines (two plants per pot). The pots are watered daily and weeds other than *Striga* and *Alectra* are removed. Emergence of *Striga* and *Alectra* plants in pots containing susceptible plants normally begins from 6 weeks after planting. The experiments are terminated 10 weeks after planting, when the differences between resistant and susceptible plants become quite marked. The levels of *Striga* and *Alectra* infection are determined by counting both emerged and attached parasites. To count attached *Striga* and *Alectra*, the soil is washed off the plant roots after submerging each pot in a 20-liter bucket of water for about 5 min. The roots of each plant are gently separated from the others, and the number of *Striga* and/or *Alectra* attached to each plant are counted. Plants permitting attachment and healthy development of these parasitic weeds are classified as susceptible, and those free of infection or showing only minute *Striga/Alectra* plants are grouped as resistant. Plants with a few, small *Striga/Alectra* plants are rated as moderately resistant.

Level and manifestation of resistance in different cowpea varieties

Major differences in expression of resistance have been observed in different varieties. Lack of emergence or delayed and reduced emergence are observed in resistant and moderately resistant lines, as compared with severe infection of susceptible lines. Pot and in vitro culture tests revealed that B 301 roots stimulate germination of *Striga* seeds and permit attachment, but haustorial formation and/or further growth are inhibited (Lane et al. 1991).

Genetics of resistance to Striga *and* Alectra

Good progress has been made in elucidating the genetics of resistance to *Striga* and *Alectra* in cowpea, as reported earlier in this book (Fery and Singh 1997).

Development of varieties resistant to different strains of Striga

A systematic breeding program for resistance to *Striga,* using B 301 as a resistance source, was undertaken in 1987. This line was crossed to a susceptible variety, IT84S-2246-4, which is otherwise a high-yielding variety with resistance to aphid, bruchid, thrips, and several diseases. The F_1 was backcrossed to IT84S-2246-4. From the resistant BC_1 F_1 plants, F_2, F_3, F_4, F_5, and F_6 progenies were developed and selected under suitable disease, insect, and *Striga/Alectra* pressures. This led to the selection of a number of F_6 breeding lines, which are very similar to IT84S-2246-4 and have combined resistance to aphids, bruchids, thrips, *Striga,* and several diseases. These were evaluated for yield and other characters in a replicated trial in 1991 (Table 1), and promising lines have been distributed since to various national programs in Africa. IT90K-76-6 has already been released for general cultivation in Nigeria, and IT90K-59-5 in South Africa. Further breeding continues, using these lines as parents.

Table 1. Grain yield (kg/ha) of *Striga*-resistant cowpea varieties at indicated locations in Nigeria, 1991†.

Variety	Kano	Gumel	Maiduguri	Mean	*Striga* reaction§
IT90K-59-5	1289	1653	1763	1568	1
IT90K-59-3	1055	1544	1171	1257	1
IT90K-101-1	1164	1081	1117	1121	2
IT90K-102-6	1089	1657	1027	1258	2
IT90K-82-2	1104	1320	778	1067	1
IT90K-76-6	1114	1106	976	1065	1
IT84S-2246-4	1028	583	733	781	4
LSD (5%)	337	474	475		

† *Striga* incidence was severe at Gumel and Maiduguri.
§ 1 = completely resistant, 5 = highly susceptible.
Source: Adapted from Singh (1994).

Table 2. Number of cowpea plants† infected with *Striga* at Zakpota, Republic of Benin.

Variety	1990	1991	1992
IT82D-849	0.5	15.5	33
B 301	2.8	6.0	28
IT86D-371	32.5	25.8	nt§
TVx 3236	22.8	22.3	41
IT86D-534	22.5	20.0	nt
IT86D-472	19.3	26.8	nt
IT84D-666	14.3	13.8	nt
IT81D-985	7.8	1.5	8
IT81D-994	0	0	0
Suvita-2	0.8	0	1
IT90K-76¶	nt	nt	28
IT90K-59¶	nt	nt	21
IT90K-77¶	nt	nt	2

† Average number per $6m^2$ plot.
§ nt = not tested.
¶ These lines have a gene for *Striga* resistance derived from B 301.

In 1990, a few plants of IT82D-849 and B 301 were found to be susceptible to *Striga* at Zakpota, Republic of Benin. Systematic studies were then undertaken to elucidate whether this was due to seed mixtures or the existence of a new strain (Lane et al. 1994). Lines known to be susceptible and resistant to *S. gesnerioides* were evaluated in 1991 and 1992 at and around Zakpota (Table 2). The level of susceptibility in both B 301 and IT82D-849 indicated the presence of a new strain at Zakpota. It is interesting that IT81D-994 and Suvita-2, which are resistant to the Burkina Faso strain and moderately resistant to *Striga* in Nigeria, appeared to be completely resistant to the new strain at Zakpota.

Using *Striga* isolates from different countries and suitable host differentials, five different strains of *Striga* have been identified in West Africa (Lane et al. 1997). B 301 and

IT82D-849 are completely resistant to four strains, while IT81D-994 and 58-57 are resistant to three, including the Zakpota strain. Therefore, a cross involving B 301 or IT90K-76 (derived from B 301) with 58-57, IT81D-994, or other lines listed in Table 2 should give recombinants with complete resistance to all five strains. A large number of crosses has been made and segregating populations are being evaluated. Advanced progenies from the cross "58-57 × IT90K-76" were tested in the field at Kano in 1995 and 1996. Advanced cowpea breeding lines with resistance to all five strains of *Striga* will be made available to various national programs.

New sources of resistance

In view of the fact that strain diversity exists in *Striga*, it is desirable to have genetically diverse sources of resistance, so that stable resistance can be bred into new improved cowpea varieties. Therefore, 1600 cowpea germplasm lines were screened in 1992 in a "sick" field infested with *Striga* and *Alectra* at IITA Kano Station. Each test line was planted in a single 3-m long row, with rows spaced 1.5 m apart. Two plants per hill were maintained within the rows, with a hill-to-hill distance of 20 cm. The days taken to first *Striga* emergence and the number of emerged *Striga* plants per plot were recorded each week from 5 weeks after planting. At maturity, 104 lines remained free from *Striga*. These lines were then further tested in the screenhouse, using the pot culture technique (Singh and Emechebe 1990), and 17 lines were found to have high levels of resistance to *Striga:* TVu 1271, TVu 1272, TVu 1330, TVu 1331, TVu 1332, TVu 4642, TVu 8337, TVu 8453, TVu 9238, TVu 11788, TVu 12415, TVu 12430, TVu 12431, TVu 12432, TVu 12449, TVu 12470, TVu 13035. Of these, TVu 9238, TVu 11788, TVu 12415, TVu 12432, and TVu 12470 were also resistant to *Alectra*. As with B 301 and IT82D-849, all the new resistant lines were from East and southern Africa, where *Striga* is not a problem but *Alectra* is a major problem.

Future prospects

The past and current work on host plant resistance and other aspects of *Striga* and *Alectra* in cowpea suggest that yield losses due to these parasitic plants can be minimized. In addition, the further spread of these pathogens can be contained by reducing their seed bank in the soil. Strain variation in *Striga* has been documented, but at the same time resistant sources have been identified for each strain. These sources are being combined in order to provide protection from a broad range of strains. The *Striga* strains of Burkina Faso, Mali, Cameroon, and Benin Republic (Zakpota) are presently restricted to small areas, and only the Nigerian strain is widely spread. *Striga*-resistant varieties that contain B 301 or IT82D-849 genes—resistant to both the Nigerian and Burkina Faso strains and moderately resistant to the Zakpota strain—already give some broad range protection. When they are crossed with IT81D-994, 58-57, and Suvita-2—all resistant to the Burkina Faso and the Zakpota strains—the resulting varieties will provide even greater protection. In addition, several other lines that are resistant to the Zakpota strain have now been identified, and they are being crossed with B 301. All identified resistant lines are being tested in a range of environments, to monitor the emergence of new strains and to identify sources of resistance to these strains. This will enable the development of host plant resistance to a broad range of strains, even in the wake of new strains. Ongoing studies on

false hosts as intercrops or rotation crops and on chemical/biological control will complement resistance breeding. Thus, the prospects for controlling *Striga* and *Alectra* on cowpea appear bright.

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Variation in virulence of *Striga gesnerioides* on cowpea: new sources of crop resistance

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Abstract

Variation in virulence of *Striga gesnerioides* was mapped using a differential series of cowpea varieties grown using an in vitro technique. The geographic distribution of the three known parasite races was established. Two new races were also identified. One race came from Cameroon and northeastern Nigeria, while the other was found only in southern Benin. The latter race was unique in that it was virulent on cowpea variety B301, previously thought to be resistant across West Africa. Two new sources of resistance to *S. gesnerioides* have been identified in cowpea landraces (varieties 87-2 and APL-1); they are resistant to all races of *S. gesnerioides*, except the one found in Niger and northern Nigeria. The mechanisms of resistance of these cowpeas have been characterized. Individual resistant plants selected using in vitro methods were clonally propagated to multiply seed.

Introduction

The parasitic angiosperm, *Striga*, is an obligate root pathogen which infests cereal and legume crops in sub-Saharan Africa causing yield losses in excess of 50% (Parker 1991). Each *Striga* plant can produce up to 90,000 seeds, which may remain viable for 15–20 years (Parker 1991). Control of *Striga* is difficult for subsistence farmers, and crop resistance offers an appropriate and sustainable control method. Resistance has been identified in several varieties of cowpea (*Vigna unguiculata* [L.] Walp.), including B301, which is resistant to *S. gesnerioides* [Willd.] Vatke across West Africa (Parker and Polniaszek 1990). This germplasm is being widely used in breeding programs in West and Central Africa, and cowpeas with resistance to *S. gesnerioides* are available to farmers (Singh and Emechebe 1991; Lane and Bailey 1992; B.B. Singh 1995, IITA, Ibadan, personal communication).

An in vitro system was developed which allowed the expression of resistance of cowpea to *S. gesnerioides* to be studied. Two different mechanisms of resistance were characterized in cowpeas 58-57 and B301 (Lane et al. 1991; Lane et al. 1993). Each mechanism was expressed after penetration of cowpea roots by *S. gesnerioides*; one killed the parasite, while in the other, parasite infections failed to develop normally. Most cowpea varieties, e.g., 58-57 and IT81D-994, were only resistant to *S. gesnerioides* in one or two countries, due to variation in parasite virulence. Originally, three parasite races were identified, each with a discrete geographic distribution (Parker and Polniaszek 1990).

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Recently, a differential series of cowpea varieties was used to identify a new race of *S. gesnerioides* (Lane et al. 1994).

In 1990, a survey in seven West African countries assessed the incidence of the parasite on cowpea and collected samples of *S. gesnerioides* (Cardwell and Lane 1995). The aim of this study was to determine the virulence of *S. gesnerioides* across West Africa, and to screen cowpea germplasm for resistance to *S. gesnerioides*.

Materials and methods

The sources of the cowpea seeds used for the series were Blackeye from the USA, 58-57 from Burkina Faso, and IT81D-994 and B301 from IITA in Nigeria. Cowpea seeds were grown in moist Vermiculite for 6–12 days in a Fisons F600H growth cabinet at 30/25 °C (light/dark temperature), 67% RH, with a 16 h daylength. Seeds of *S. gesnerioides* collected from 1984 to 1993 from parasitized cowpea plants in farmers' fields in Benin (3 sites), Burkina Faso (12), Cameroon (3), Mali (10), Niger (7), Nigeria (11), and Togo (2) were surface sterilized and then soaked in water for 17 days in petri dishes in the growth cabinet (Lane et al. 1996). Soaked *Striga* seeds were pipetted on to 6-mm discs of glass fiber filter paper, which were then placed on host roots growing on glass fiber filter paper and tissue paper in plastic trays (Lane et al. 1991). The trays were enclosed in a polyethylene bag and wrapped with aluminium foil. Nutrient solution was added to the filter paper at daily intervals as described by Lane et al. (1991).

From 10% to 60% of parasite seeds had germinated after 72 h. *Striga* seedlings were viewed with a stereo-microscope and transferred from the filter paper onto host roots, using a fine paint brush. Up to 50 parasite seedlings were placed on each host root system, and 2–5 plants were used for each cowpea variety. The plants were maintained in the growth cabinet as already described. Using a stereo-microscope, parasite development and the responses of infected roots were assessed at 6, 13, and 20 days after inoculation of parasite seedlings. The parasite tubercle diameter was measured at 20 days and used to determine resistance (a tubercle diameter of < 1.5 mm was classified as resistant). The hypersensitive response following the penetration of host roots with associated parasite death was also used to classify resistance.

Individual plants of variety 87-2 grown using the in vitro system, which were found to be resistant to *S. gesnerioides* from Cinzana in Mali, were propagated by taking nodal cuttings. Cuttings were grown in compost in a propagator in a glasshouse (minimum temperature 25 °C, 12 h daylength) for 14–21 days. Rooted cuttings were transferred to soil in pots, and grown to produce seed. Resistance to *S. gesnerioides* was also assessed by growing cowpea plants in pots containing soil mixed with *S. gesnerioides* seeds from Cinzana in Mali (about 1000 seeds per pot). The number of parasite stems above the soil surface was counted after 10 weeks. This experiment allowed resistance to be assessed over a longer period than for the in vitro tests.

Results

Virulence of *S. gesnerioides* and distribution. The virulence of *S. gesnerioides* from 48 sites in seven West African countries was characterized using the series of four cowpea varieties (Fig. 1). Of the 14 parasite samples that were only virulent on cv. Blackeye, 10 were from Burkina Faso, 2 from southern Togo, 1 from Badeggi in central Nigeria, and 1

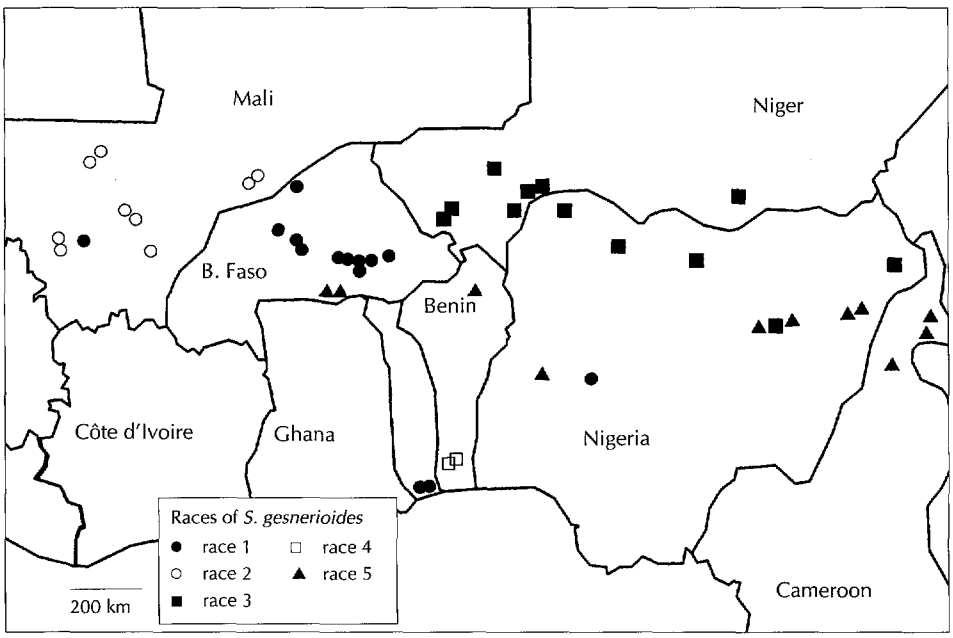

Figure 1. Distribution of *Striga gesnerioides* virulence in West Africa.

from Saintiguila in central Mali. The 9 other *S. gesnerioides* samples from Mali were virulent on varieties Blackeye and 58-57. The 12 parasite samples from Niger and northern Nigeria were virulent on all cowpea varieties, except B301. Two samples of *S. gesnerioides* from Zakpota in southern Benin were virulent on cultivars Blackeye and B301. Virulence on variety B301 was unique to *S. gesnerioides* from Zakpota. All samples from Cameroon were virulent on cowpea varieties Blackeye and IT81D-994, as was *S. gesnerioides* from central and eastern Nigeria, Zabre in southern Burkina Faso, and Kandi in northern Benin.

Resistance of cowpea to *S. gesnerioides*. Parasite tubercles of 2–4 mm diameter formed within 13 days on the susceptible cv. Blackeye. *S. gesnerioides* seedlings placed on the roots of varieties APL-1 and 87-2 died, with an associated necrosis of host tissue around sites of parasite penetration. No successful parasite development occurred on the roots of varieties 87-2 and APL-1, except for two tubercles on one 87-2 plant (Table 1). There were numerous parasite tubercles on the roots of the other eight cowpea varieties.

Variety APL-1 and progeny of cuttings of 87-2 plants that were resistant to *S. gesnerioides* were grown in pots in soil mixed with *S. gesnerioides* seeds. All four plants of variety APL-1 and four of the six 87-2 plants tested were resistant to *S. gesnerioides*; no parasite stems emerged. There were seven *S. gesnerioides* stems on two 87-2 plants, compared to 111 stems on six Blackeye plants.

Discussion

There are at least five distinct races of *S. gesnerioides* in West Africa. In addition to the three existing races, a fourth race was identified with virulence on variety B301 and a fifth

Table 1. Responses of cowpea germplasm to *Striga gesnerioides* from Cinzana in Mali using the in vitro system.

Cowpea variety†	Origin	Cowpea plants	*Striga* inoculated	*Striga* penetrated	*Striga* tubercles	Diameter of tubercles (mm)
APL-1	IITA/Nigeria	3	150	24	0	0
87-2	Nigeria	6	300	42	2	nd§
KVx-65-114	IITA/B. Faso	2	83	24	7	1.1
183-1	Mali	2	74	46	7	1.1
TVu 7614	IITA	2	77	16	3	1.2
KVx-30-166-3G	IITA/B. Faso	3	63	22	6	1.6
KVx-30-305G	IITA/B. Faso	4	166	30	4	1.9
Cipea	Mali	2	70	13	5	2.7
90-168	Nigeria	3	67	8	3	3.3
90-164	Nigeria	3	70	8	5	3.4
Blackeye	USA	2	100	9	1	2.3
Blackeye	USA	2	91	44	43	3.6

† Variety APL-1 was selected from a field where *S. gesnerioides* was present, but APL-1 was not infected (B.B. Singh, personal communication, 1990, IITA, Ibadan). Two plants of cowpea cv. Blackeye were used in each experiment, so the range of values for cv. Blackeye are presented.
§ nd = no data.

Table 2. Responses of *Striga gesnerioides* on a differential series of cowpea varieties, including two new resistant varieties, APL-1 and 87-2.

Differential cowpea varieties	Races of *S. gesnerioides*†				
	1	2	3	4	5
Blackeye	S	S	S	S	S
58-57	R	S	S	R	R
IT81D-994	R	R	S	R	S
B301	R	R	R	S	R
APL-1	R	R	S	R	R
87-2	R	R	S	R	R

† S = susceptible, R = resistant to *S. gesnerioides*.

race was characterized in samples from Cameroon and three other regions. On the basis of the complex distribution of virulence revealed by this survey, a numerical system of classification is proposed (Table 2). In contrast to earlier findings, race 1 was not exclusive to Burkina Faso. However, race 2 was restricted to Mali, which confirmed earlier studies (Parker and Polniaszek 1990). Race 3 occurred in Niger and northern Nigeria. Race 4 was restricted to Zakpota in Benin. Field trials at Zakpota confirmed the susceptibility of variety B301 to *S. gesnerioides* (Berner et al. 1995). None of the other samples tested in this study was virulent on B301. Reports of the susceptibility of B301 to *S. gesnerioides* at various sites across West Africa (Parker 1991) therefore appear to have been due to the use of impure B301 seed (Lane and Bailey 1992).

The overlapping distribution of the five parasite races has important consequences for breeding resistant cowpeas. Firstly, multilocational field trials will be essential in Benin,

Burkina Faso, and Nigeria to encompass the full extent of parasite variation. Alternatively, cowpea genotypes could be assessed rapidly against the full range of virulences using in vitro tests. Information on parasite virulence is essential for determining the optimum deployment of resistance across West Africa. The virulence data can also facilitate monitoring the changing distribution of *S. gesnerioides*. This will become increasingly important with the greater deployment of *Striga*-resistant germplasm in West Africa.

The identification of a new race (race four) of *S. gesnerioides* with virulence on B301 has several implications for cowpea breeding programs. Resistance genes from cowpeas other than B301 will be needed to control *S. gesnerioides* in southern Benin. Resistance genes from varieties 58-57 or IT81D-994 should be effective. In addition, two new sources of resistance to *S. gesnerioides* were identified in cowpea landraces. In field trials in Mali, resistance to *S. gesnerioides* was confirmed (Moore et al. 1995). Varieties 87-2 and APL-1 are resistant to most parasite races (Table 2). They have good grain characteristics and could probably be used immediately in those areas where variety B301 is susceptible (Moore et al. 1995). The resistance responses of these two landraces to *S. gesnerioides* was the same as observed in other resistant cowpea varieties (Lane et al. 1993). The in vitro techniques allow individual cowpea plants to be screened and resistant material to be propagated. Such methods are essential for utilizing heterogenous landrace material, such as variety 87-2. It is recommended that when screening for new sources of resistance, screening should focus on landrace material from West Africa. This appears to be a promising gene pool for resistance to *S. gesnerioides*. In addition, when resistant germplasm has been identified, it could be quickly deployed, without extensive adaptation, using the virulence distribution map for *S. gesnerioides*.

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Further characterization of cowpea aphid-borne mosaic and blackeye cowpea mosaic potyviruses

C. Huguenot, M.T. Furneaux, and R.I. Hamilton[1]

Abstract

The molecular weights of the capsid protein of cowpea aphid-borne mosaic virus (CABMV) and blackeye cowpea mosaic virus (formerly designated BlCMV, now bean common mosaic virus, blackeye cowpea strain BCMV-BlC) were estimated to be 32 kDa and 35 kDa, respectively, following immunoblot analysis of crude sap from infected plants by specific monoclonal antibodies. The 35 kDa proteins of BCMV-BlC, peanut stripe mosaic virus (BCMV-PSt), bean common mosaic virus (BCMV), and adzuki bean mosaic virus (BCMV-Az) showed equal reactivity. Unexpectedly, those of CABMV and the bean common mosaic necrosis virus (BCMNV-NL3) were serologically related, suggesting a possible similarity between these viruses. Analysis of capsid protein tryptic peptide profiles by high performance liquid chromatography showed that those of BCMV-BlC, BCMV-PSt, BCMV-Az, and BCMV were almost identical, confirming that this group comprises strains of one virus. In contrast, those of BCMNV-NL3 and several CABMV serotypes were distinct from the first group, and exhibited limited similarities.

Introduction

Potyviruses are generally differentiated as individual viruses or strains according to their biological and physicochemical properties, such as virion morphology, types of cytoplasmic inclusions, host range, serology, amino-acid and nucleotide sequences, and genomic organization (Moghal and Francki 1976; Fauquet et al. 1986a,b; Milne 1988; Shukla and Ward 1989; Jordan and Hammond 1991; Ward and Shukla 1991).

Cowpea aphid-borne mosaic virus (CABMV) and bean common mosaic virus, blackeye cowpea strain (BCMV-BlC), have very similar properties. Because they occur worldwide (Lovisolo and Conti 1966; Bos 1970; Iwaki et al. 1975; Behncken and Maleevsky 1977; Lima et al. 1979; Mali and Kulthe 1980; Pio-Ribeiro and Kuhn 1980) and are seed-transmissible (Zettler and Evans 1972), they are economically important pathogens. The identification of the two viruses has been ambiguous for many years, since neither virus has been fully characterized. The genome of CABMV is one of the potyvirus genomes that has not been sequenced. The only individual characteristics reported are the existence of serological differences, differential cowpea hosts (Taiwo and Gonsalves 1982; Huguenot et al. 1993, 1995), and different cowpea resistance genes (Provvidenti et al. 1983).

In order to further characterize CABMV and BCMV-BlC, electrophoresis and immunoblotting were used to distinguish their capsid proteins, and high-performance liquid

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chromatography (HPLC) was used to analyze tryptic digests of these proteins. Capsid proteins of four other legume-infecting potyviruses (bean common mosaic virus, BCMV; BCMV strain peanut stripe mosaic, BCMV-PSt; BCMV strain adzuki bean mosaic, BCMV-Az; and bean common mosaic necrosis virus, BCMNV-NL3) were also analyzed by these techniques. Each analysis confirmed the classification of the viruses into at least two groups that may be of taxonomical importance.

Methods

Viruses. The potyvirus isolates used in this study and their origins are described in Table 1. All isolates were mechanically propagated in *Vigna unguiculata* ssp. *sesquipedalis* or *Phaseolus vulgaris* (cv. Red Kidney). For immunoblot experiments, virus isolates were used directly as extracts from infected plants. For HPLC experiments, isolates were purified according to Huguenot et al. (1993).

Antibody production. Polyclonal antibodies were raised in rabbits against seven Nigerian isolates of BCMV-BlC and CABMV (Huguenot et al. 1993). Monoclonal antibodies that

Table 1. Identification, origin, and source of potyvirus isolates.

Virus isolate	Origin	Source
Bean common mosaic virus, blackeye cowpea strain		
BCMV-BlC-81.11	IITA, Nigeria	H.W. Rossel, G. Thottappilly
BCMV-BlC-IT.16	IITA, Nigeria	H.W. Rossel, G. Thottappilly
BCMV-BlC-Onne	IITA, Nigeria	H.W. Rossel, G. Thottappilly
BCMV-BlC-AYB	Nigeria	E.C.K. Igwegbe
BCMV-BlC-Fl-V346	Florida, USA	D.E. Purcifull
Cowpea aphid-borne mosaic virus		
CABMV-Monguno	Nigeria	H.W. Rossel, G. Thottappilly
CABMV-Baga	Nigeria	H.W. Rossel, G. Thottappilly
CABMV-Fekam	Cameroon	H.W. Rossel, G. Thottappilly
CABMV-Nkechi's	Cameroon	H.W. Rossel, G. Thottappilly
CABMV-70.12	IITA, Nigeria	H.W. Rossel, G. Thottappilly
CABMV-Maputo	Mozambique	H.W. Rossel, G. Thottappilly
CABMV-Mo	Morocco	R.O. Hampton
Bean common mosaic virus, peanut Stripe strain		
BCMV-PSt-P12	USA	G.I. Mink
Bean common mosaic virus, adzuki bean mosaic strain		
BCMV-Az-MJS	Washington, USA	G.I. Mink, M.J. Silbernagel
Bean common mosaic virus		
BCMV-NY15Z	New York, USA	G.I. Mink
Bean common mosaic necrosis virus		
BCMNV-NL3	Netherlands	G.I. Mink

were produced against BCMV-BlC-81.11 (mAb 10G5), CABMV-Fekan (mAb 5H5), or CABMV-70.12 (mAb 6C10) (Huguenot et al. 1993) were used as crude ascitic fluids in immunoblot experiments. In addition, antisera raised against CABMV-Mo (Ab^R-Mo) and BCMV-BlC-Georgia (Ab^R-Ge) were supplied by Dr D. Gonsalves (Cornell University, Ithaca, New York, USA) and Dr C.W. Kuhn (University of Georgia, Athens, Georgia, USA), respectively.

Immunoblotting. Crude sap extracts from infected plants, mixed with a standard denaturation buffer containing SDS (Laemmli 1970), were separated in a 12% polyacrylamide gel by electrophoresis in the presence of low molecular weight protein standards (Bio-Rad, Hercules, California, USA). The protein bands were electrically transferred onto an Immobilon membrane (Millipore, Bedford, Massachusetts, USA) according to Towbin et al. (1979). Molecular marker strips were cut out and stained with a 0.1% amido-black solution in 40% methanol, 10% acetic acid. The remaining portion of the membrane was soaked for 2 h in blocking solution of 3% bovine serum albumin in phosphate-buffered saline (PBS-BSA 3%). Subsequently, the membrane was soaked in PBS-BSA 0.3% containing polyclonal or monoclonal antibodies for 1 h at ambient temperature. After three rinses with PBS-BSA 0.3%, the membrane was soaked in goat anti-rabbit (GAR) or goat anti-mouse (GAM) alkaline phosphatase conjugate for 1 h. Finally, the membrane was rinsed in 0.1 M Tris, 0.1 M NaCl, 50 mM $MgCl_2$, pH 9.5 buffer (Alkaline Phosphatase [AP] buffer) and a freshly prepared substrate solution of nitroblue tetrazolium chloride (NBT, 0.33 mg/ml) and 5-bromo-4-chloro-3-indolylphosphate p-toluidine salt (BCIP, 0.16 mg/ml) (from Gibco, Gaithersburg, Madison, USA) in AP buffer was added. Virus-specific bands appeared within a maximum of 10 min and the reaction was stopped by immersion of the membrane in 20 mM Tris, 5 mM EDTA, pH 7.5.

Peptide preparation and HPLC profiling. Peptides of the viral coat protein were prepared from freeze-dried virus, as described by McKern et al. (1992), using modified, tosyl lysyl chloromethyl ketone (TPCK)-treated, trypsin (Promega, Madison, Wisconsin, USA). The HPLC profiles were obtained by separating the soluble peptides on a C18 reverse-phase column (5 µm C_{18} - 300 Å, 3.9 × 150 mm Deltapak, Waters) connected to a Waters chromatography system (Millipore Waters, Milford, Massachusetts, USA) in a buffer of 0.115% trifluoroacetic acid (TFA). The peptides were eluted from the column using a 0–49% acetonitrile (in 0.1% TFA) linear gradient over a period of 60 min at a flow rate of 1 ml/min at 45 °C. The eluted peaks were monitored at 214 nm. Each sample was run in duplicate. Before comparison, the baseline was subtracted from each profile.

Results

Molecular weight comparison of coat proteins. Crude extracts of cowpea infected with African isolates of BCMV-BlC and CABMV were separated by electrophoresis on the same gel as reference isolates (BCMV-BlC-Fl and CABMV-Mo), and another related potyvirus, BCMV-PSt. The immunoblot obtained from this gel, after incubation with a mixture of monoclonal antibodies (mAb 10G5 [10^{-4}], BCMV-BlC- and BCMV-PSt-specific; mAbs 5H5 [10^{-3}] and 6C10 [10^{-4}], CABMV-specific), is shown (Fig. 1). Antibodies detected a coat protein of 32 kDa for the CABMV isolates and 35 kDa for the

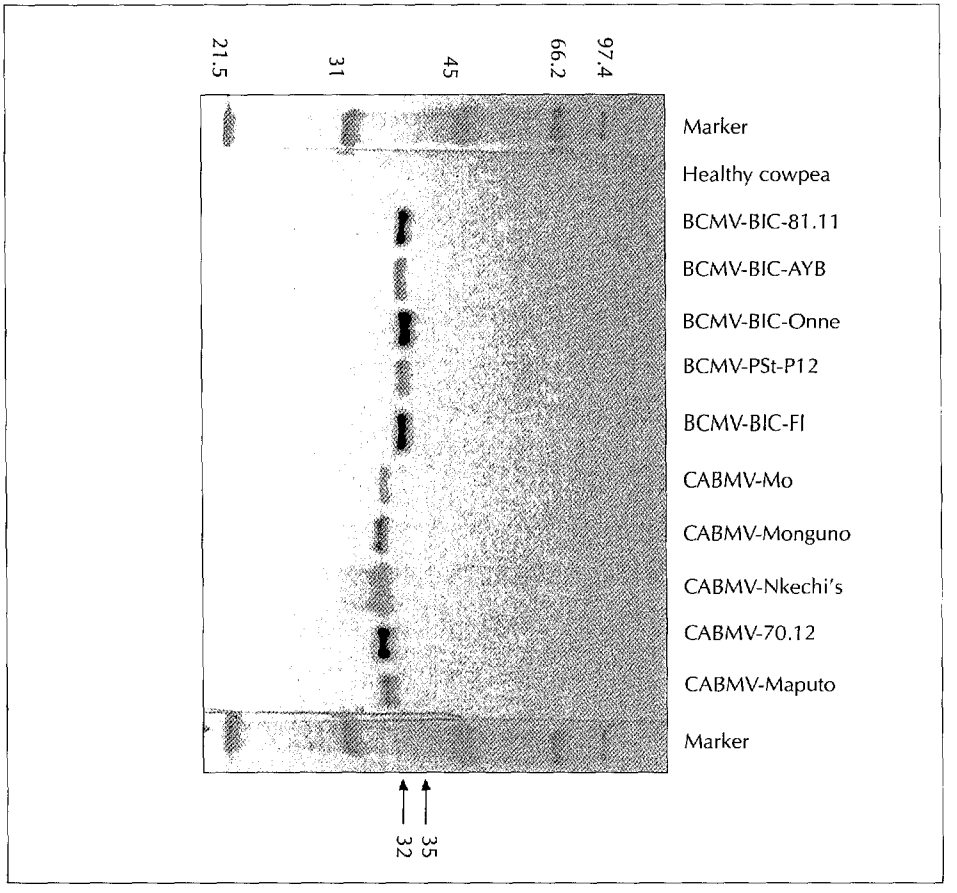

Figure 1. Immunoblot of crude cowpea extracts of African BCMV-BIC and CABMV isolates, after 12% SDS-PAGE, using a mixture of monoclonal antibodies 10G5, 5H5, and 6C10. Numbers to the left are molecular weights ($\times 10^3$) of marker proteins. Origin, source, and identification of virus isolates are shown in Table 1. Letters between brackets indicate serotypes.

BCMV-BIC isolates and BCMV-PSt. This difference in the molecular weights of the coat proteins of CABMV and BCMV-BIC corroborates the previous serological differentiation of the two viruses by ELISA (Huguenot et al. 1993). This structural difference between CABMV and BCMV-BIC could be used as a criterion in virus identification and, therefore, in diagnosis and in taxonomic studies.

When the same experiment was run with a panel of different legume-infecting potyviruses consisting of BCMV, BCMV-PSt, and BCMV-Az, which are serologically related (Mink and Silbernagel 1992), as well as CABMV and BCMNV-NL3, the same difference in molecular weight was observed (Fig. 2). Polyclonal antibodies to BCMV-BIC (Ab^R Ge) and CABMV (Ab^R Mo) were used at 1 µg/ml and 10 µg/ml, respectively. The molecular weights of the coat proteins of BCMV, BCMV-BIC, BCMV-PSt, and BCMV-Az isolates were 35 kDa, while those of CABMV and BCMNV-NL3 were 32 kDa.

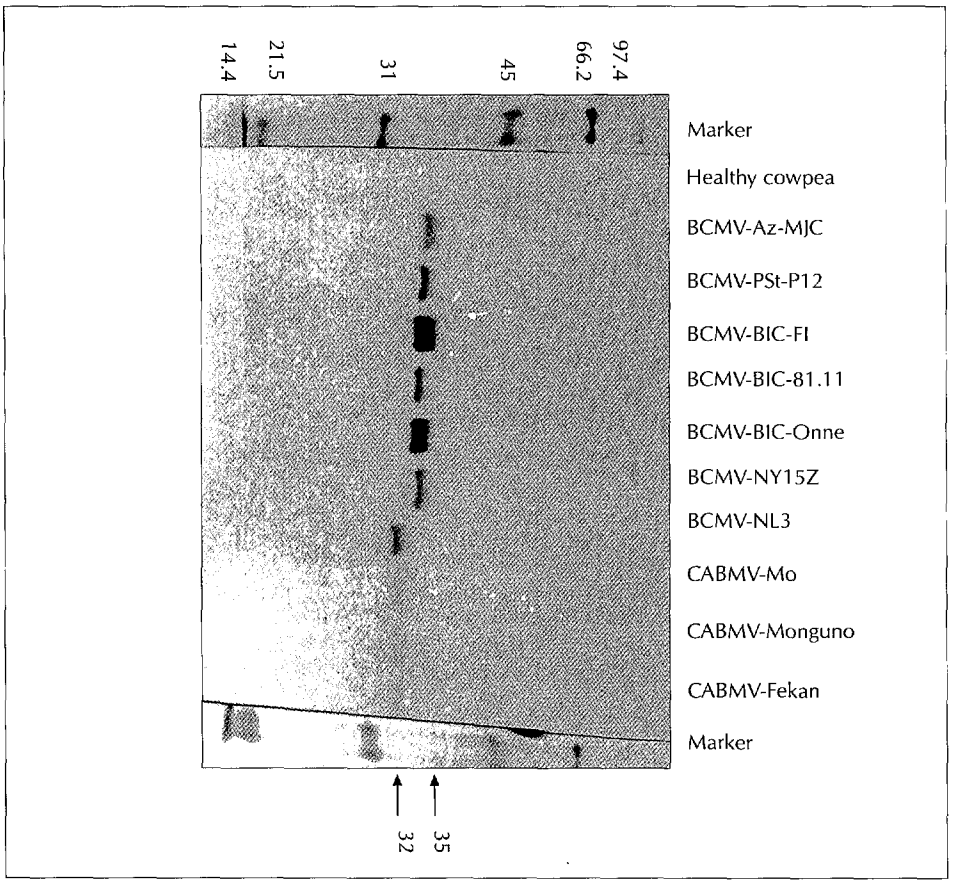

Figure 2. Immunoblot of crude cowpea extracts of different potyviruses, after 12% SDS-PAGE, using polyclonal antibody Ab^R-Ge. Numbers to the left are molecular weights ($\times 10^3$)) of marker proteins. Origin, source, and identification of virus isolates are shown in Table 1. Letters between brackets indicate serotypes.

Comparison of coat protein tryptic peptides by HPLC. Coat proteins from BCMV-BIC-IT.16 and BCMV-BIC-Fl, both representing serotype I (Huguenot et al. 1993, 1995), BCMV-BIC-Onne (serotype II), BCMV-PSt-P12, BCMV-Az-MJS, and BCMV-NY15Z were analyzed by HPLC after trypsin digestion. Their peptide profiles were generally very similar (Fig. 3a) and among the 6 isolates, peak heights were comparable. In these 6 profiles, retention times were the same for at least 65% (peaks 1–18) of the 28 major peaks. Seven peaks (a–g) were common to 4 or 5 of the 6 isolates and 2 other peaks (α–ß) were common to 2 virus isolates (BCMV-Az-MJS and BCMV-NY15Z). In addition, some peaks were specific to a particular isolate such as peak I, which was only observed with BCMV-BIC-Onne.

When isolates of different serotypes of CABMV (Mo, Baga, Fekan, 70.12, and Maputo), as well as BCMNV-NL3, were analyzed in the same conditions (Fig. 3b), HPLC

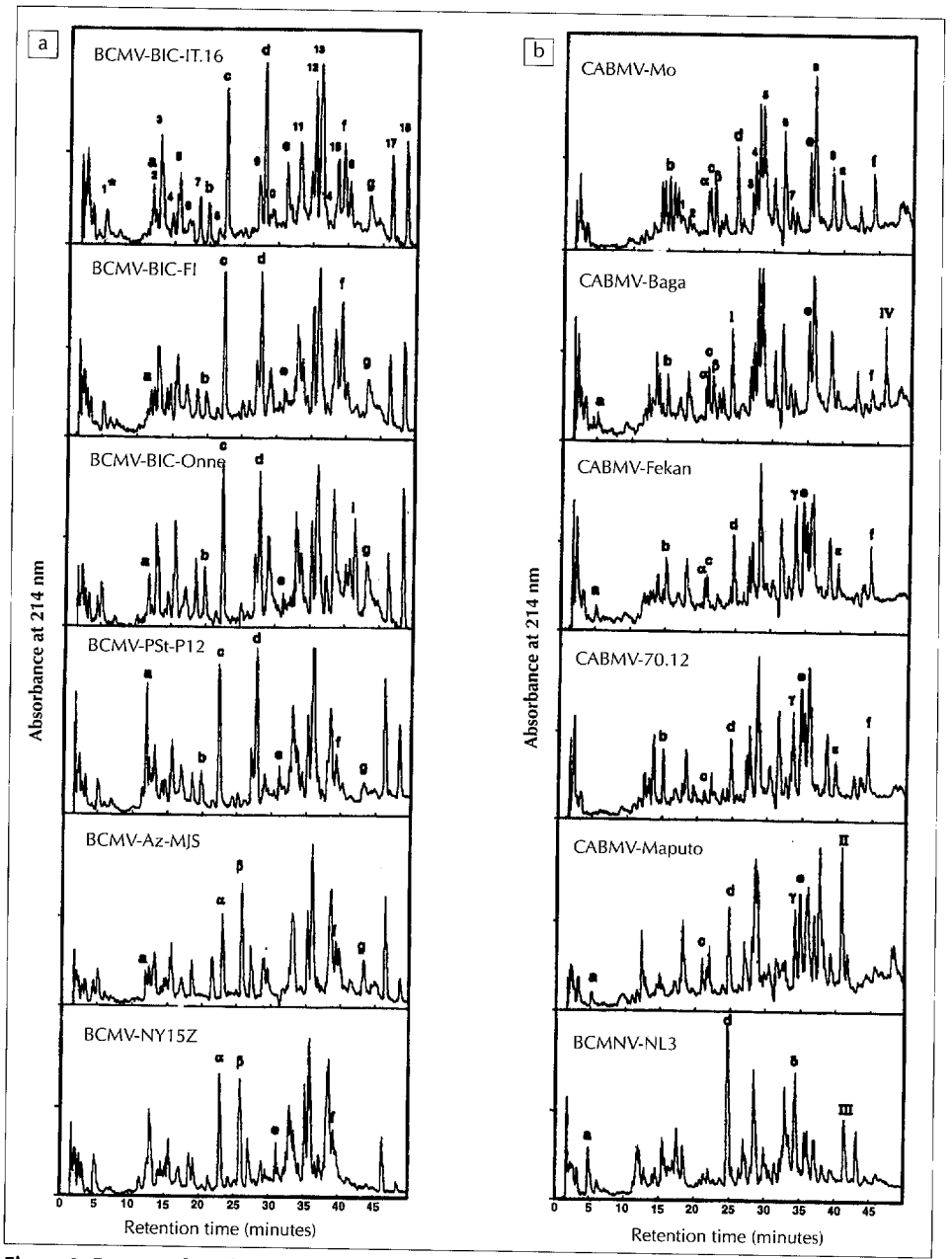

Figure 3. Reverse phase high-performance liquid chromatography of tryptic digests of coat protein from BCMV-BIC isolates, BCMV-PSt, BCMV-Az, BCMV, CABMV isolates, and BCMNV-NL3. Table 1 gives origin, source and identification of virus isolates. For each of the 12 isolates, peptides bound to the column were eluted with a linear gradient of 0–49% acetonitrile in 0.1% trifluoroacetic acid over 60 min (flow rate 1 ml/min; temperature 45 °C). Numbered peaks (1–18) in profiles of (a) or (b) are present at the same retention time in the six profiles. Peaks identified by a letter (a–g) are common to 4 or 5 isolates; by Greek characters (α–δ) are common to 2 or 3 profiles; and by roman letters (I–IV) are specific for one isolate. Peaks in (a) do not correspond to those in (b).

profiles were very different from those of the first group (Fig. 3a). None of the 18 common peaks identified among the isolates represented in Figure 3a could be found in this second group of 6 isolates (Fig. 3b). Moreover, profile similarities among CABMV isolates were limited. Of the 24 major peaks, only 37% (1–9) were common to the 6 isolates, 6 peaks (a–f) were common to 4 or 5 isolates, and 5 peaks (α–δ) were common to 2 or 3 isolates. Two peaks (I and IV) were observed only in tryptic digests of CABMV-Baga, whereas peaks II and III were observed only in CABMV-Maputo and BCMNV-NL3, respectively.

These observations confirm the existence of two distinct groups based on properties of the capsid proteins; the first one, consisting of all BCMV strains (BCMV, BCMV-BlC, BCMV-PSt, and BCMV-Az) studied by us, is very homogeneous; the second one is more heterogeneous and includes various serotypes of CABMV and possibly BCMNV-NL3.

Discussion

The distinction between CABMV and BCMV-BlC has already been established by serology and by their symptoms on differential hosts (Huguenot et al. 1993). Coat protein properties, such as molecular weight and HPLC peptide profiles, are additional criteria to discriminate between these two potyviruses (Huguenot et al. 1994; McKern et al. 1994). A 35 kDa capsid protein was consistently observed for several isolates of BCMV-BlC, including the type isolate BCMV-BlC-Fl, while one of 32 kDa was characteristic of the isolates of CABMV, including the type isolate CABMV-Mo. HPLC analysis of coat protein tryptic peptides also showed a clear distinction between the two viruses. The very homogeneous group revealed by the serological study on BCMV-BlC isolates (Huguenot et al. 1993) is confirmed by HPLC analysis, which shows 89% of profile similarities, i.e., 25 out of 28 peaks are identical (Fig. 3a). On the other hand, CABMV isolates, which were very heterogeneous serologically (Huguenot et al. 1996), exhibited extensive variations with a consequent low similarity (37%) in the HPLC profiles of their capsid tryptic peptides (Fig. 3b).

Among other potyviruses, BCMNV-NL3, which has been characterized serologically as serotype A of BCMV and symptomatically as the necrotic strain of BCMV (Vetten et al. 1992; Drijfhout and Bos 1977), has also been analyzed by HPLC peptide profiling (McKern et al. 1992, 1994). Using this technique, BCMNV-NL3 was already shown to have only 30–50 % similarity with BCMV isolates (B serotypes) and characterized as a separate virus. In the present study, a 32 kDa coat protein was identified for this isolate, similar in size to that of CABMV, whereas BCMV-NY15Z (B serotype) exhibited a 35 kDa coat protein. The molecular weight difference was significant in the immunoblot experiments. Since fresh crude sap extracts were used, protein degradation, which is known to occur during purification or storage, was never observed and experimental results were reproducible. However, partial sequence comparison between CABMV-Mo and BCMNV-NL3 (McKern et al. 1994) established that the two viruses are distinct. Despite their 32 kDa protein molecular weight and some common epitope (Fig. 2), the two viruses are very distantly related.

The use of immunoblotting and HPLC in the analysis of capsid proteins of several legume-infecting potyviruses allows us to clearly distinguish at least two groups among them. The first one, with a 35 kDa coat protein and 65% peptide profile similarity, includes all BCMV strains (BCMV, BCMV-BlC, BCMV-PSt, and BCMV-Az). These viruses are so

closely related that they are now considered strains of the same virus (Mink et al. 1994; Shukla et al. 1994; Fauquet and Martelli 1995). The second group, exhibiting a 32 kDa coat protein and consisting of CABMV serotypes, appears to be very heterogeneous in HPLC analysis (37% similarity). The nucleotide sequence of CABMV is only partially known but this HPLC study as well as the previous serological study (Huguenot et al. 1993) suggest that CABMV isolates include those so distantly related that they could be considered different viruses.

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21

Pest management practices in cowpea: a review

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Abstract

Cowpea yields are known to be low in most parts of the tropics because of heavy insect pest problems. In Africa, cowpea growers do not generally use synthetic insecticides; however, in most parts of Asia, dependence on the use of insecticides is common, often with serious environmental consequences. Such misuse of insecticides on cowpea, coupled with low yields, has led to an intensive search for pest control options that will increase yields with little or no input from insecticides, or biointensive integrated pest management (IPM). The major elements of this strategy include host plant resistance, use of beneficial organisms, agronomic practices, and (where adequate results are not obtained) some insecticide input, preferably from plant-based insecticides. This paper reviews the status of each of these interventions in cowpea production and discusses new initiatives in cowpea pest management. We also identify gaps in research and discuss options for developing IPM on cowpea.

Introduction

Much has happened in the art and science of insect control since a paper with a similar title (Jackai et al. 1985) was presented at the 1st World Cowpea Research Conference. People have become wiser, and grown more sensitive to environmental problems; research on nonchemical control has been intensified, and the clamor for system sustainability has reached unprecedented levels. Along with these events, and the new visions for pest management, cowpea production has undergone changes, but these are inadequate to address the more difficult pest problems.

Pest problems on cowpea persist, at least in part because of a lack of diversity in research interests in the control of pests. Much effort is devoted to the easier problems (aphids, bruchids, leafhoppers, etc.), while the major problems (e.g., thrips, pod borer, and pod bugs) remain unsolved. The pest problem on cowpea is complex, and requires diversified efforts. Without a major breakthrough in the control of the more recalcitrant postflowering field pests of this crop, bridging the gap between present and potential production of cowpea will be a slow and frustrating process. This notwithstanding, the future of cowpea production looks brighter today than ever before for two main reasons:

1. New advances in biotechnology have provided enormous impetus to host plant resistance research. Recombinant DNA and other molecular techniques are being used to seek answers to pest problems that do not lend themselves to conventional solutions (see later in this volume).

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2. The renewed interest in basic ecological research as the foundation for sustainable pest management, and the drive to understand and sustain the system as a whole rather than an individual crop is bound to provide greater insight into pest bionomics.

Cowpea is a popular—and nutritionally important—legume crop in many parts of the tropical world. Despite this, it is considered too risky an investment by many growers, because of the numerous pest problems associated with it. Insect pests damage cowpea from seedling emergence to storage. The pest complex (Table 1) ranges from two to four key pests, often including as many as four minor or sporadic pest species. Different pest guilds specialize on every cowpea plant part, and in the worst cases these pests overlap in their incidence and damage (Fig. 1). It is not unusual to find four or more pests on the crop at the same time.

The most damaging of all pests are those that occur during flowering and podding (i.e., the postflowering pests or PFPs). They include flower thrips (Fth), dominated by *Megalurothrips sjostedti* Tryb. (Thysanoptera: Thripidae); the legume pod borer, *Maruca vitrata* Fabricius (syn. *M. testulalis*) (Lepidoptera: Pyralidae), known more commonly as maruca pod borer (MPB); and a complex of pod and seed suckers, of which *Clavigralla tomentosicollis* Stal (Hemiptera: Coreidae) is the dominant species in tropical Africa, where 70–80% of the world crop is grown (Jackai and Daoust 1986; Singh et al. 1990). These pod sucking bugs (PSBs), as they are called, cause similar damage to cowpea and can be controlled using the same methods. To this list can be added the cowpea curculio, *Chalcodermus* spp., and leafhoppers found in South America (Daoust et al. 1985) and parts of southern US (Chambliss and Hunter 1997), and the beanfly, *Ophiomya* spp., which occurs in Asia and parts of Africa. These are the most important pests associated with cowpea in much of its geographical distribution. However, it is not uncommon to encounter specialized, location-specific, pest species such as *Amsacta moorei* (Butler) (Ndoye 1978), *Apion* species (Nonveiller 1984), and *Alcidodes leucocephalus* (Erichson).

Storage pest species of cowpea are more cosmopolitan (Southgate 1978), and they are discussed in greater detail later in this book (Murdock et al. 1997).

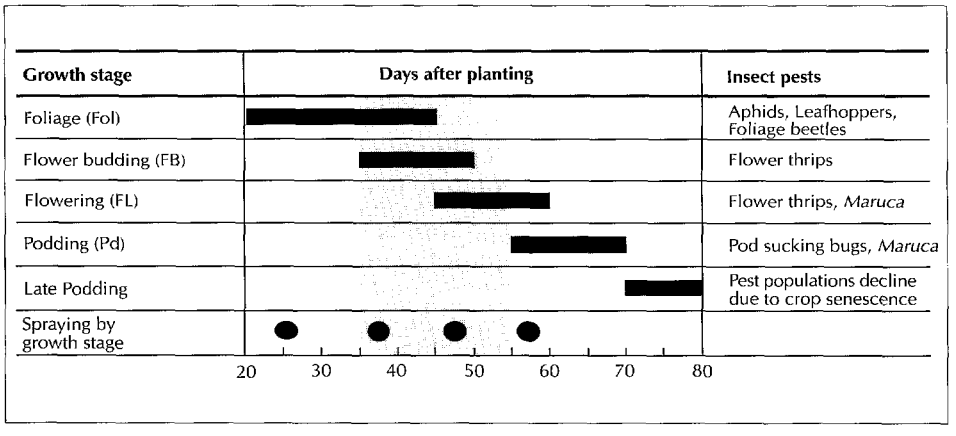

Figure 1. Cowpea growth stages and pest incidence.

Table 1. Major insect pest species found on cowpea worldwide.

Pest species (Order: family)	Geographical distribution	Plant part attacked	Importance
Aphis craccivora Koch (Homoptera:Aphididae)	Cosmopolitan	Foliage, flowers, pods	Key
Empoasca kraemeri, Ross & Moore (Homoptera: Cicadelidae)	S. America	Leaves	Key
Empoasca dolichi Paoli (Homoptera: Cicadelidae)	Africa	Leaves	Sporadic
Empoasca biguttula (Shiraki) (Homoptera: Cicadelidae)	Asia	Leaves	Unknown
Ophiomyia phaseoli (Trybon) (Diptera: Agromizidae	Asia Africa	Stem Stem	Key Sporadic
*Amsacta moore*i (Butler) (Lepidoptera: Arctiidae)	Africa (Senegal)	Leaves	Sporadic
Megalurothrips sjostedti (Trybon) (Thysanoptera: Thripidae)	Africa Asia Americas	Floral structures Floral structures Floral structures	Key Not important Unknown
Thrips palmi (Thysanoptera: Thripidae)	Asia	Floral structures	Sporadic
Thrips tabaci Lindeman (Thysanoptera: Thripidae)	Asia, S. America	Floral structures	Sporadic
Maruca vitrata (Fab.) (Lepidoptera: Pyralidae)	Cosmopolitan (rare in S. America)	Stem, flowers, pods	Key
Elasmopalpus lignosellus (Zeller) (Lepidoptera: Pyralidae)	S. America	Stem	Key
Etiella zinckenella (Treitschke) (Lepidoptera: Pyralidae)	Asia	Pods, flowers	Sporadic
Clavigralla tomentosicollis Stal (Hemiptera: Coreidae)	Africa Asia S. America	Pods Pods Pods	Key Minor Minor
Leptoglossus sp. (Hemiptera: Coreidae)	USA	Pods	Sporadic
Crinocerus sanctus (Fab.) (Hemiptera: Coreidae)	S. America	Pods	Key
Riptortus dentipes (Fab.) (Hemiptera: Alydidae)	Africa	Pods	Sporadic

Table 1. continued.

Pest species (Order: family)	Geographical distribution	Plant part attacked	Importance
Lygus hysperus (Hemiptera: Miridae)	USA	Pods, leaves	Key
Nezara viridula Linnaeus (Hemiptera: Pentatomidae)	USA	Pods	Key
	Africa	Pods	Sporadic
	Asia	Pods	Sporadic
	S. America	Pods	Sporadic
Chalcodermus spp. (Coleoptera: Curculionidae)	USA, S. America	Pods	Key
Callosobruchus spp. (Coleoptera: Bruchidae)	Cosmopolitan	Seeds (storage)	Key

The pest problem on cowpea is clearly more severe in Africa than elsewhere, probably because many of the pests are considered indigenous to the continent and/or have had ample time to co-evolve with the crop in its center of origin and domestication (Ng and Maréchal 1985). Other views on the origin of cowpea pests have recently been expressed (Tamo et al. [in press]; see also Tamo et al. 1997).

Pest management philosophy

Insects are considered pests because of the socioeconomic and medical threat they pose to man and his property. Biologically, an insect is a pest because its population density and/or damage level exceeds a preestablished or conceptualized threshold (the economic injury level, EIL) below which the insect does not constitute an economic threat (Horn 1986). This is defined as the lowest population or damage level capable of causing economic impact (Poston et al. 1983). If the population of an organism exceeds the EIL, the organism becomes a pest. When an insect is introduced into a favorable environment, its population density tends to increase to the carrying capacity, K, of the resource. This is not usually exceeded because of the balance in environmental stress factors (e.g., predation, competition, and other natural mortality factors), constituting the environmental resistance. The EIL is usually below the carrying capacity of the resource. In order to maintain a pest population below this level may require some manipulation using one or more of the interventions at the disposal of growers (e.g., resistant cultivars, beneficial organisms, insecticides, etc.). Usually, we do not let the damage or population density of the pest reach levels that would result in economic loss before action is taken. This resource damage level, or pest population density prior to the EIL, is the economic or action threshold (ET or AT) (Stern et al. 1959), or damage boundary (Pedigo et al. 1986). This is when control measures must be introduced, augmented, or applied to the system. (Horn 1986; Metcalf and Luckmann 1994). Alterations in crop-pest dynamics, for instance by many of man's agricultural activities, dictate how pest management proceeds and the tools that can be used.

Identifying control interventions for cowpea pests

The ecology of most tropical insect pests has been inadequately studied. As a result, many control programs are *ad hoc* activities driven by crises resulting from perceived insect pest outbreaks. In cowpea the situation is somewhat different, though far from perfect, thanks to research at the International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria, and many national research programs (Singh et al. 1978).

Cowpea pest incidence and diversity dictate that a single control strategy is unlikely to produce satisfactory results. Even if this were chemical control, the pests respond differently to different insecticides. As a result, the "best mix" approach is currently advocated. This involves the most logical combination of different compatible tactics for the control of pests on cowpea, as for other crops, in what might be termed, very simply but appropriately, intelligent pest management.

Chemical control using synthetic insecticides

Insecticide use on cowpea has a long history (Booker 1965; Jackai 1983; Jackai et al. 1985; Singh et al. 1990). It is the most widely known form of pest control on this crop. Traditional cowpea growers in Africa do not habitually use insecticides, as reflected in the poor yields they obtain. In many countries in Asia, pest control is mainly insecticide based, and for many commercial growers it is the only way. It is not surprising that insecticide resistance is already evident in certain areas (M. Tamo, personal communication). However, insecticides are the fire-fighting analog in cowpea pest control, a function for which they remain unrivaled (National Academy of Sciences 1969).

The landscape of insecticide use has changed over the years from dependence on the highly toxic and/or persistent insecticides (e.g., DDT, endosulfan, monocrotophos, etc.) of the 1960s and 1970s to an era of great skepticism and reduced usage in the 1980s, typified by a shift towards less toxic and more environmentally friendly and narrow spectrum, target-specific technology (e.g., Electrodyn sprayers). Currently, economic necessity and sensitivity to environmental destruction have rendered insecticide use socially unacceptable, although somewhat unrealistically so. There is also increased advocacy for monitored rather than calendar-based insecticide application, if insecticides must indeed be used (Afun et al. 1991).

The insecticides used on cowpea can be grouped into seed dressings, foliar sprays, storage sprays, and dusts.

Seed treatments. Getting a good crop stand is paramount to getting good yield. Damage from beetles, leafhoppers, beanfly, and birds can cause poor stands. One way this can be avoided is by treating seeds with an insecticide dust or slurry before they are planted (Breniere 1967). Even though poor stands are a persistent problem in a great many locations, it is surprising how little use is made of seed dressings. Detailed studies conducted with carbosulfan (Marshal® 25 ST, FMC, Pa, USA) (Jackai et al. 1988) show that as little as 10 g/kg of seed is required to protect cowpea seedlings from aphids, foliage beetles, and tunneling herbivores such as beanfly for up to 3 weeks in the screenhouse, and for longer periods under field conditions. More recently, another seed dressing, Apron Plus®, was also evaluated using two cowpea cultivars, one susceptible (Vita 7) and the other resistant (IT84S-2246) to *Aphis craccivora* (Koch) (Fig. 2). The results show that

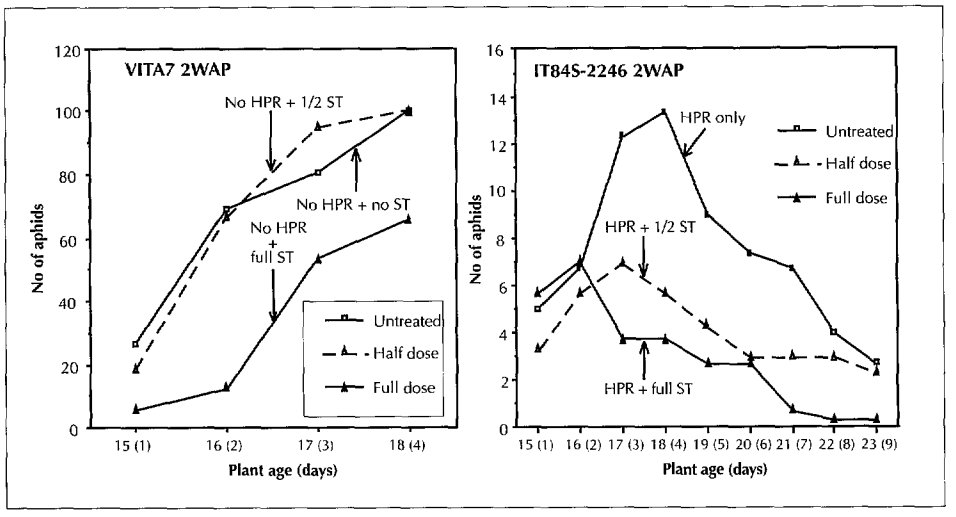

Figure 2. Evaluation of Apron Plus as a seed treatment for the control of seedling pests in cowpea. Vita 7 is susceptible to aphids. Half dose (2.5 g/kg seed) did not reduce aphid infestation after 2 weeks. On the aphid-resistant cultivar, IT845S-2246, there was a marked reduction in the number of aphids even by half dose. This demonstrates how plant resistance and insecticides can be used additively, and safely.

when varietal resistance is combined with seed treatment, the effects are additive and extend over considerable periods.

Other seed dressings that were popular in the past, such as Fernasan-D® (25% Thiram + 20% Lindane) and Aldrex-T®, are no longer recommended because of their organochloride content. They are nonetheless found on sale in the open market in many developing countries. Liquid seed dressing formulations are usually more toxic than dusts, and require special devices for mixing. Dust formulations such as carbosulfan can be applied to seed in small amounts (less than 1 kg), using paper bags or in covered cans. These are suitable for use by small farmers. Medium- to large-scale growers also use seed dressing, the only difference being one of scale. An additional advantage of seed dressing is that it has minimal impact on parasitoids and predators, and it can, therefore, be used in conjunction with biological control. Proper use of seed dressing ensures good initial plant stands, which are critical to successful farming, and many farmers would adopt this technology without too much difficulty. Its major drawback is the potential danger posed to people who consume cowpea leaves. Seed dressing should, therefore, not be recommended in areas where leaves are consumed.

Foliar sprays. Many insecticides used on cowpea are foliar sprays, either of emulsifiable concentrates (EC) or wettable powders (WP). Several of these chemicals are effective against most cowpea pests, although there is greater specificity in some cases against specific groups, a distinction related to the feeding behavior of the different pests.

The most commonly used insecticides include endosulfan, Lambda cyhalothrin, cypermethrin, permethrin, and dimethoate (Table 2). A more complete list is given by Jackai (in press). Despite their differential efficacy, most of these chemicals will increase

Table 2. Most commonly used insecticides for pest control on cowpea in the tropics.

Common name (chemical group)	Trade name†	Target pest
Lambda-cyhalothrin (Synthetic pyrethroid)	Karate	*Maruca vitrata*, foliage beetles, flower thrips, pod bugs
Cypermethrin (Synthetic pyrethroid)	Cymbush Sherpa	*M. vitrata*, flower thrips, pod bugs
Deltamethrin (Synthetic pyrethroid)	Decis	*M. vitrata*, flower thrips, pod bugs
Cypermethrin + Dimethoate (Synthetic pyrethroid + organophosphate)	Sherpa Plus Cymbush Super	All cowpea pests
Monocrotophos (Organophosphate)	Azodrin Nuvacron	Beanfly, leafhopper, aphid (only in Asia), flower thrips, pod bugs
Endosulphan (Organochloride)	Thiodan Perfekthion	*M. vitrata*, pod bugs, beetles, leafhoppers
Carbofuran (Carbamate)	Furadan	Flower thrips, leafhopper, aphid, beetles, beanfly
Carbosulphan (Carbamate)	Marshal	Flower thrips, leafhopper, aphid, beetles, beanfly
Carbaryl	Sevin	*M. vitrata*, other lepidoptera
Aluminum phosphide (Carbamate)	Phostoxin Detia, Gastoxin	Storage pests
Permethrin (Synthetic pyrethroid)	Coopex	Storage pests
Pirimiphos methyl + Permethrin (Organophosphate)	Actellic Actellic Super	Storage pests
Deltamethrin (Synthetic pyrethroid)	K-othrin	Storage pests

† The list is not exhaustive. Use of a trade name is not necessarily an endorsement of the product.

cowpea yields by at least tenfold with 2–4 applications (Franks et al. 1987; Afun et al. 1991).

The introduction of the more target oriented electrostatic (Electrodyn®) sprayer was a significant innovation in the control of cowpea pests in the early 1980s, capturing the interest of many cowpea growers in northern Nigeria (Coffee 1979; Gowman and Durand 1986). However, the popularity of this spray technology was relatively shortlived because of its high cost and the limited number of insecticides that could be used with it.

Consequently, the more versatile, and less expensive, low volume knapsack sprayer has remained the dominant sprayer although it is clearly less suitable (because of the water needed) for use in the drier savannas, where most cowpea is grown.

Insecticide use on cowpea will always have an element of controversy, but its use on cowpea may never be completely eliminated without a substitute that gives comparable results; right now, there is none. In the end, it is perhaps those farmers (the medium- and large-scale growers) that can afford the cost of chemical control who will influence the future of insecticide use on cowpea. Their influence creates a market for chemicals, thereby making insecticide use by others inevitable. To counter this, scientists must make the use of chemicals less attractive by providing viable and realistic alternatives. Our observations in Asia and several African countries indicate that the number of growers who use chemical control is on the increase (Bernardo and Adalla 1992), despite the escalating costs of spraying and nonavailability of appropriate products.

Insecticide use in storage. Use of insecticides to protect cowpea grain in storage is probably more commonplace and controversial than their use on the field crop, because chemical residues are erroneously feared to persist in the bean after cooking. This is a common misconception. If the right insecticides are used in the appropriate manner, there should be little or no concern about residues in cooked food.

The most commonly used insecticides for the protection of cowpea in storage are pirimiphos methyl (Actellic® and the formulation synergized with permethrin, Actellic Super®), aluminum phosphide (Phostoxin®, Gastoxin®, Detia®), malathion, permethrin (e.g., Coopex®, Kaothrin®), deltamethrin, etc. (Table 2). Those available in dust formulations or as liquid-based sprays usually do not pose the same degree of danger as do fumigants (gastoxin, phostoxin, etc.). However, despite the greater risks posed by fumigation, especially if used close to living quarters, fumigants are among the most effective products for disinfesting stored cowpea. Furthermore, the relative ease with which they can be dispensed (as tablet or pellet formulations) has greatly expanded the use of fumigants. In general, most clean and uninfested cowpea sold in the marketplace 3 months or more after the end of the growing season is generally treated with insecticides.

Use of insecticides invariably raises questions about resistant pest strains. Fortunately, this has really not been an issue in the case of cowpea, probably because compared to other crops such as cotton, the use of insecticides on cowpea is small. Reducing insecticide use on cowpea as currently advocated will make the development of pest resistance to insecticides less likely. Unfortunately, many research institutions which conducted research on insecticides have either shifted emphasis or completely abandoned work on this subject in favor of topics that attract more funding. As a result, we may never know if, or when, resistance to insecticides ever develops.

Plant-derived insecticides

Jackai (1993) reviewed the current status of the use of neem (*Azadirachta indica* A. Juss) on cowpea. Research in this area has intensified, possibly because of the high cost and/or the unavailability of conventional insecticides. Neem is only one of the many plants in the African landscape that are being investigated as a source of pest control on food crops (Olaifa et al. 1987; Saxena 1989; Schmutterer 1990). Although most research work on this

aspect of plant protection has dwelled on storage protection of cowpea (Ivbijaro 1983; Sowumni and Akinnusi 1983) and maize (Kossou 1989), there has been increasing interest in the application of plant-based insecticides (PBIs) against field pests (Schmutterer 1990). For instance, extensive use has been made of neem extracts to control field pests of rice in Asia (see Saxena 1989, for review), cassava in West Africa (Olaifa and Adenuga 1988) and a few cases on cowpea (Cobbinah and Osei-Owusu 1988; Tanzubil 1992). The main groups of insects that show sensitivity to PBIs, especially neem, include Lepidoptera, Coleoptera, Agromyzidae, and Orthoptera (Schmutterer 1985).

Current work on the use of PBIs on cowpea is dominated by neem; different extracts from neem are under investigation on field pests in Nigeria. The impetus for this work came from the results of laboratory research at IITA and elsewhere, which showed high activity against two of the major pests of the crop, *M. vitrata* and *C. tomentosicollis* (Jackai and Oyediran 1991; Jackai et al. 1992). In Ghana, Cobbinah and Osei-Owusu (1988) and Tanzubil (1992) have also shown that neem has great potential as a field insecticide for use on cowpea. Whereas the emphasis in the past was on using the kernel and seed, ongoing work at IITA has included leaf extracts, to utilize the abundance of leaves. The active principles are, however, known to be more concentrated in the seed and bark of the tree (Saxena 1989; Schmutterer 1990). There is evidence of growth disruption, feeding inhibition, deterrence, and outright mortality associated with neem-based insecticidal products (Table 3) (G. Forjoe and L.E.N. Jackai, unpublished). A number of neem-based commercial insecticides are now available in many countries, especially in India, USA, and Germany. In the Philippines, other plants including *Vitex negundo, Derris* sp., and *Tinospora rumphi* have shown varying levels of toxicity against a wide range of field pests.

The interest in neem is driven mostly by need and economics. There is a gap created by the inaccessibility of conventional insecticides. An additional incentive to explore this and protectants such as vegetable oils (e.g., groundnut oil and dinnetia oil, etc.) (Osisiogu and Agbakwuru 1978; Singh et al. 1979) is their perceived compatibility with the environment and other pest management interventions (Schmutterer 1985, 1990). PBIs are generally not as effective as their synthetic counterparts, but their use can be augmented with other controls, such as natural enemies and entomopathogens, to provide acceptable levels of protection. These plants are grown or grow locally; therefore, educating farmers and the

Table 3. Effect of neem extracts on the hatchability of *Maruca vitrata* eggs†.

Concentration (%) of neem extracts	Egghatch (% ± SE)			
	Leaf extract		Seed extract	
	24 h fermentation	48 h fermentation	24 h fermentation	48 h fermentation
0	81.8(±6.08)a	81.8(±6.08)a	81.8(±6.08)a	81.8(±6.08)a
5	57.4(±13.14)b	50.6(±5.35)bc	35.6(±58.26)b	34.3(±4.36)b
10	40.1(±1.25)bcd	40.7(±10.7)bcd	25.9(±1.61)bc	26.1(±3.15)bc)
20	30.6(±6.72)d	34.8(±3.13)cd	19.8(±0.91)cd	16.0(±4.96)d

† Analysis of observed differences was based on transformed data (Arcsine transformation). Means within a column followed by the same letter are not significantly different at $P < 0.01$ (Student-Newman-Keul test).

general public on their use in plant protection should lead to an increase their use. Farmers in many parts of the tropics use botanicals for grain protection in storage as well as against field pests (Schmutterer 1990). Neem is also useful as a fertilizer and nematicide (Radwanski and Wickens 1981; Cobbinah and Osei-Owusu 1988; Colin and Pussimier 1992; Krishnamurthy 1993).

Plant resistance

A decade ago (Jackai et al. 1985), the emphasis was on chemicals and habitat modification. Mention was made of plant resistance as the focus for future sustained control. The story is now different, for two main reasons: (1) chemical control cannot be sustained by the fragile economies of most African states; and (2) despite the recalcitrance of certain pests, there is greater readiness to exploit the benefits of low or partial resistance to cowpea pests, given the knowledge that these can be used in conjunction with botanicals for a greater payoff. In addition, the traditional cowpea grower appears somewhat better informed of the existence of resistant cultivars, particularly to aphids and bruchids, with the result that there is a marked increase in demand for these cultivars (B.B. Singh, personal communication).

Preflowering pests. Resistance to seedling pests was first reported after evaluating a few hundred germplasm accessions from the gene bank at IITA. Resistance to aphids was identified in TVu nos. 36, 408, 801, 3000, to mention only the most prominent (Singh 1980). According to Ansari (1984), resistance in these accessions is due to antibiosis, but we believe other modalities are involved. Most of the aphid-resistant cowpea cultivars (e.g., IT83S-728-5, IT84S-2246-4, IT85D-3577, IT87S-1394, and KVx 426-1, among others) were developed from crosses involving either TVu 3000 or TVu 36. This narrow resistance base is a potential weakness in these cultivars, particularly since it is controlled by a single dominant gene (Singh and Ntare 1985). Fortunately, as far as we know, only US strains of *A. craccivora* have been reported to survive on some of these resistant lines. Clearly, the potential exists for more of this to happen.

The resistance to aphids identified at IITA was assessed only at the seedling stage (Singh and Jackai 1985). In a recent study to determine the reaction of these resistant cultivars to aphid challenge at different growth stages of the plant, it became clear that some cultivars were susceptible to infestation at the postflowering stage, thus suggesting stage-specific rather than a generalized form of resistance. This finding confirms reports from several colleagues in national programs (e.g., Burkina Faso) that a number of aphid-resistant cowpea cultivars developed at IITA were susceptible to this insect at the reproductive phase. Because of this, a study has been initiated to determine the mechanisms of resistance in known resistant germplasm accessions. This exercise could lead to a wider genetic base for resistance to aphids.

Cowpea growers no longer need to spray their crop against aphids if they plant the right cultivars. Among the best of these is IT84S-2246, a brown-seeded cultivar recommended for release in Nigeria and other countries. This recommendation notwithstanding, many farmers do not grow this cultivar, for reasons unknown to us which deserve investigation.

Leafhoppers present a similar success story (Rahman 1975). In addition, cowpea seedling resistance to the beanfly has been studied in the Philippines (Adalla 1994) and in Taiwan (IITA 1986).

Postflowering pests. While plant resistance can presently provide adequate protection for cowpea against seedling pests, the same cannot be said of the postflowering pests. The gravity of this situation is better understood when one realizes that except where the beanfly is a problem, the fate of cowpea production in many parts of the tropics hinges on what happens during the reproductive phase of the crop. Many insects that attack the crop at this stage are either oligophagous or sternophagous in their host range, with a few being (narrowly) polyphagous. The development of resistant varieties against these pests has eluded efforts over for several years. The most recalcitrant pests are the flower thrips, the maruca pod borer, the pod and seed sucker, *C. tomentosicollis,* and the cowpea curculio. Against this background, the progress achieved in the development of resistance to this group of insects, and the increase in our understanding of the phenomena that are involved in cowpea resistance to this group, becomes quite significant. After screening over 10,000 germplasm accessions of cultivated cowpea, a few were found to possess low to moderate levels of resistance. This has not solved the problem, but clearly represents significant progress in the long-term objective of developing cowpeas resistant to PFPs.

In multilocational trials conducted from 1990 to 1993 on a north-south axis in Nigeria, to evaluate a range of cultivars for their performance under varying intensities of the pod borer, *M. vitrata,* at different sites, it was evident that the pest pressure became less from south (Ibadan) to north (Kano). Under no-spray conditions, most genotypes performed better in the drier northern locations than in the more humid southern sites, as measured by the pod evaluation index (Ipe) (Table 4; see also Jackai 1995). MPB develops and reproduces better under high relative humidity and low to moderate temperatures (Jackai et al. 1990; Oghiakhe et al. 1992). Therefore, its population density tends to be lower in drier weather. Low levels of resistance would be most useful at such locations. Similar information is needed for all other important pests associated with cowpea. Resistance should be tailored to suit different locations (and needs) where possible, rather than seeking to develop varieties that can be planted everywhere.

Table 4. Performance of selected cowpea cultivars for *Maruca vitrata* resistance across three locations on a north–south axis in Nigeria†.

	Location mean (±SE)		
Variable	Ibadan (n = 37)	Mokwa (n = 36)	Kano (n = 30)
Pod evaluation index (Ipe)	28.59 (±0.14)	31.76 (±1.72)	44.3 (±1.88)
Plant resistance index (Ipr)	23.76 (±0.68)	22.76 (±1.76)	37.9 (±2.03)
Relative performance ratio			
Ipe	1.0	1.1	1.6
Ipr	1.1	1.0	1.7

† Ipe = pod load x (9–Pod damage) (see Jackai 1995 for more details)
Ipr = $(FP_{w1} + MF_{w2} + N_{w3} + Z_{w4})/\Sigma_{wi}$), where FP = full protection; MF = spray at midflowering; N = Nuvacron spray; Z = no spray; wl–w4 = different weights in monocrotophos spray; Z = no insecticide protection.

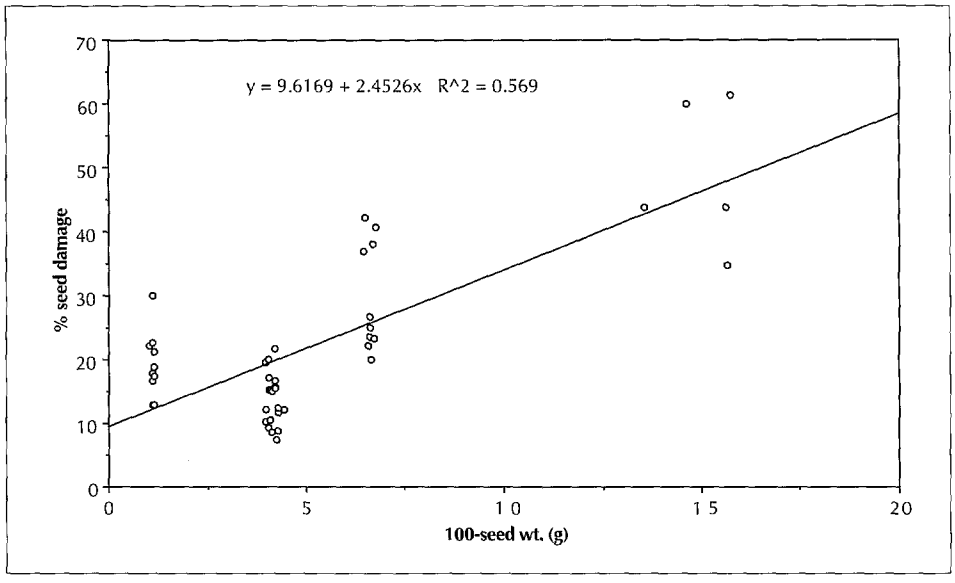

Figure 3. Regression of percentage seed damage by *Clavigralla tomentosicollis* per 100-seed weight of cowpea.

Several of the newly identified resistant germplasm have small seeds, or seed colors that are unacceptable to many consumers. This is important, because seed size is directly related to damage by the pod borer and pod bugs (Fig. 3). Although we need to keep consumer requirements in focus, researchers must recognize that consumers in different regions of the world have different preferences.

With the recent emphasis on the wild relatives of cowpea, intensive and systematic screening has resulted in the identification of good levels of resistance among the wild *Vigna* species. Those that can be easily crossed to cultivated cowpea have already been used in hybridization programs that seek to pyramid the genes for partial resistance, both in the cultivated group, on the one hand, and the uncultivated group in the *V. unguiculata* ssp. *dekindtiana,* on the other.

The resistance mechanisms seem to be quite variable, and they include the disruption of physiological growth processes of the insects, resulting in less crop damage than in the commonly grown cultivars (Jackai et al. in press). Unfortunately, some of these attributes appear to be associated with undesirable features, such as small seed size.

Use of biological control

Natural stress factors have obviously played an important part in ensuring that cowpea pests are contained as much as possible. Unfortunately, until recently, not much was studied about these agents and their impact on cowpea pests. Two papers in this volume (Tamo et al. 1997; Bottenberg et al. 1997) confirm that more attention is being directed towards this important subject. Given the status quo, we know of no case where biological control agents, either arthropods or pathogens, have been deliberately introduced for the control of cowpea pests. However, according to Tamo and his colleagues (Tamo et al. in

press; 1997), the future landscape of pest control on cowpea will include the introduction, conservation, and augmentation of natural enemies. By implication, the overall equation of pest control on this important crop will also change, and thus promote biodiversity and sustain environmental quality.

Environmental management practices

Pest control tactics on cowpea that involve manipulating the insect's environment are well-known among traditional cowpea growers. They have practiced these tactics for ages, usually for different reasons than those proposed by scientists (Richards 1985). Several of these agronomic or cultural interventions are used in different parts of the tropics, but the greatest diversity is in the African tropics (Okigbo and Greenland 1976). One of the most common is intercropping, which will be discussed in some detail. Others include date of sowing, tillage, mulching, crop residue management (e.g., rice stubble management in rice fields in the Philippines [Litsinger and Ruhendi 1984]), and trap cropping.

The scientific basis for intercropping as a tactic in the management of insect pests was brought into focus by the work of Tahvanainen and Root (1972) and Root (1973) on *Brassica* sp. In a nutshell, their work indicated that with an increase in vegetational diversity in the agroecosystem, there is usually a corresponding decrease in pest species density, which generally leads to stability of the system.

Intercropping does not necessarily reduce the pest load in any given situation, as is often assumed; it depends on the crop(s) and pest(s) in question. Unfortunately, assessment of the impact of intercropping on pest populations is usually conducted on only one of the associated crops (Ezueh and Taylor 1983). Insects not present on that crop may indeed be found on the associated crop. This leads to an underestimation of the pest density in the whole system, and of the amount of damage caused by a given pest in a mixture, compared to that in the monocrop. Another misconception is that some cowpea pests can be controlled simply by intercropping. This view persists, despite insufficient experimental evidence to support it. Intercropping can reduce damage (or the rate of damage accumulation) on a crop; it can certainly contribute to the control of a pest in an integrated control context. However, in the final analysis, and with a few incidental exceptions, damage to intercropped cowpea is generally no less than that of the monocrop at the time of harvest.

Cowpea is generally intercropped with cereals, root crops, coffee, plantains, and cotton. Millet/sorghum-cowpea mixtures are perhaps the most prevalent form of intercropping involving cowpea in West Africa. Other forms are found in other parts of the tropics. Different patterns of intercropping are used in different locations. One common feature of most studies on intercropping of cowpea, irrespective of the associated crop or intercropping pattern, is the lack of response by the pod borer, *M. vitrata* (Matteson 1982; Lawson and Jackai 1987; Agbo-Noameshie et al. unpublished). Notable exceptions to this assertion were reported by Seshu Reddy and Masyanga (1987) who claimed to have got a 46% reduction of *M. vitrata* in a 1:3 sorghum/cowpea intercrop. Karel et al. (1980) working in Tanzania also reported less damage by flower thrips and the maruca pod borer on cowpea intercropped with maize. In fact, simultaneous sowing of cowpea and maize appears to increase infestation by the borer (Ezueh and Taylor 1983). This is perhaps because higher humidity and relatively lower temperatures, typical of intercropped cowpea, are generally favorable to the borer (Oghiakhe et al. 1992).

Several studies have shown that the population density of flower thrips is consistently lower in cowpea intercropped with maize, or sorghum (Matteson 1982), cassava (Lawson and Jackai 1987), and beans (Kyamanywa and Ampofo 1988), for exactly the same reasons that foster increase in the borer population. Kyamanywa and Ampofo (1988) have shown convincingly that shade, high humidity, and lower temperatures keep the population of thrips down in intercropped cowpea and field beans. Interestingly, in the same ecosystem, we find opposing requirements for two major pests of cowpea. Although this is not a genuine case of intercropping, leafhoppers and the beanfly are also effectively controlled by proper management of rice stubble in the Philippines (Litsinger and Ruhendi 1984).

Despite such evidence, we know of no case where the farmer intercrops for the sole purpose of pest control. We, therefore, consider this benefit as "incidental pest control." Further, the merits and demerits of intercropping are not necessarily dependent on numerical changes of the pests (Helenius 1991). Spatial and temporal changes of pest distribution may result in significant changes in crop damage, even if pest population densities remain unchanged.

Even though plant species diversity (crop-crop and weed-crop diversity) results in a reduction of pest populations (Ballidawa 1985), not all intercropping with cowpea confers entomological advantage. For example, blister beetles (Meloidae) and pod and seed sucking bugs (Coreidae) increased in population when cereals and cowpea were intercropped in Nigeria (Ochieng 1977; Matteson 1982). It is worth noting, however, that other agronomic tactics have been adopted because they help reduce damage by pests, sometimes because of increase in natural enemy activity (Letourneau 1990). Risch (1983) provides a commendable review on intercropping.

Other pest control interventions, which could appropriately be referred to as "cultural controls," vary from one ethnic group to another, and are truly culture-dependent. They include use of wood ash, fine sand, orange peels, various spices, and vegetable oils for the preservation of cowpea grain. Generally, these interventions have no adverse effects on the environment or their user. Their efficacy is quite variable, but they should work well in combination with resistant cultivars.

Future directions of pest control in cowpea

Pest control on cowpea is still primarily centered around the use of insecticides and resistant cultivars. And worse, the decision to spray is not based on pest threshold levels, despite the increasing body of knowledge on this subject. In parts of Asia, the effect of such misuse of insecticides is already being felt as more cases of resistance are reported yearly (Adalla 1994). Unless this trend is stopped, we can expect the same problems of the insecticide treadmill that characterized agricultural systems in the developed world (Edwards 1985). Bio-intensive pest management should be advocated for cowpea, regardless of its subsidiary status in the farming system. A good first step is to strive to reduce the number of insecticide sprays to the barest minimum (usually 2). This has been accomplished in some countries, and is an initial target in many projects on pest control on cowpea. However, the success of this reduction will depend largely on the existence of alternative control options for the farmer. Landmark studies on the cost-effectiveness of monitored insecticide applications show a 50% reduction in costs and clearly unquantifiable benefit to the environment (Afun et al. 1991). In the end, the reduction in synthetic

insecticides should be balanced by an increased use of plant-based insecticides, where this input is essential.There is already a reduction in the use of agrochemicals as a result of the poor economic health of these countries.

Ecological studies are necessary for all control interventions to be meaningful. Several gaps exist in our knowledge of the interactions between insect pests and their environment, especially with respect to farmers' fields in the varying ecological zones where cowpea is grown. As more information is obtained, new ideas should be developed and control interventions modified accordingly.

Conclusions

Clearly, several sustainable pest control interventions are available for use in cowpea production. Except for the use of chemicals (synthetic and botanical), there is insufficient evidence to show, or suggest, that growers apply these measures deliberately for the control of cowpea pests. Research workers should be interested in determining why this is so, and try to change the status quo.

The socioecological tenets of pest control require that we apply those control measures we can influence or manipulate only if, or when, pest densities exceed a tolerable threshold and threaten to destabilize natural equilibria or threaten man's welfare more directly. So far, this philosophy does not seem to have been internalized by those involved in tropical agriculture, perhaps on account of ignorance. The use of "incidental controls" and biologically driven, self-sustaining tactics, such as host plant resistance and the use of natural mortality factors, needs to be encouraged in efforts to inculcate the norms of "alternative pest management" (APM), as this approach can be aptly described.

With the exception of chemical control, the different methods of intervention discussed in this paper comprise the basic components of "bio-intensive pest management". Pest control must be sustainable if long-term impact is expected or desired. Every crop has peculiarities, which should be addressed in designing control measures. For cowpea, control strategies need to be neutral in both access and scale, particularly because the target end-user is not expected to remain a small peasant farmer for life, and also because certain areas are better suited than others for larger scale farming. This scenario imposes an enormous challenge on research workers to develop technology that is focused on the small-scale farmer, yet sufficiently flexible to be adapted to other scales of farming, farming systems, and income levels. Tactics such as host plant resistance and the use of beneficial organisms clearly meet these criteria.

Finally, it must be pointed out that insecticides are not necessarily bad, and they can be intelligently integrated in cowpea pest management without the destruction of the environment that has characterized their use on other crops. Their use should be considered only if, or when, other controls fail to provide the desired protection. In the end, only IPM strategies with a sound economic foundation (Mumford and Norton 1984) will succeed. No one wants to grow a crop at a loss!

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The feasibility of classical biological control of two major cowpea insect pests

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Abstract

Biological control, as a key component of biointensive IPM in cowpea, is evaluated for its practical feasibility. The case study of one of the major insect pests, the bean flower thrips *Megalurothrips sjostedti* (Trybom) (Thysanoptera, Thripidae), is used to indicate the most important criteria for this evaluation: the nature of the pest and the release habitat, the availability and effectiveness of biological control agents, and the current status of biocontrol against the target pest. Although taxonomic aspects need further consideration, present knowledge suggests a southeast Asian origin for *M. sjostedti*. In the savannas of West Africa, cultivated and wild host plants are always available to sustain the feeding and reproduction of the pest throughout the year, while the locally present natural enemies are unable to control its population. A first exploration in search of *M. sjostedti* and efficient natural enemies, undertaken in Malaysia in November-December 1994, yielded an endophagous larval parasitoid, tentatively identified as *Ceranisus menes* Walker (Hymenoptera, Eulophidae). This parasitoid was able to parasitize up to 70% of the larvae of the closely related species, *M. usitatus,* found in flowers of *Pueraria phaseoloides*, a commonly grown cover crop. In a second, less detailed case study, the biocontrol feasibility for another key pest, the legume pod borer, *Maruca vitrata* Fabricius (previously *M. testulalis* Geyer) (Lepidoptera, Pyralidae), is assessed, using the same criteria.

These feasibility studies indicate that (1) both pests might be of foreign origin; (2) the alternative host plant habitat is conducive to the perennial presence of the pests; (3) the indigenous antagonists are not effective in controlling the pests; and (4) potential natural enemies of both pests have been identified in southeast Asia. Additional foreign explorations are needed to substantiate the results of these studies.

Introduction

Severe yield losses of cowpea, *Vigna unguiculata* (L.) Walp., are caused in tropical Africa by the interplay of abiotic (e.g., drought, and poor soil fertility) and biotic (e.g., arthropod pests, diseases, birds, and rodents) constraints. Ranked first among the latter group, a wide array of insect pests can cause total yield failure in cases of severe attack (Jackai and Daoust 1986). Two of them are among the most noxious and least amenable to available control measures (with the exception of chemical control): the bean flower thrips, *Megalurothrips sjostedti* Trybom (Thysanoptera, Thripidae), and the legume pod borer, *Maruca vitrata* Fabricius (formerly *M. testulalis* Geyer) (Lepidoptera, Pyralidae).

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At present, the only method for the effective control of these insect pests (and thus for maintaining reasonable yield levels) is the use of synthetic insecticides. This, however, is not a sustainable practice and should be used only in emergency situations (Jackai and Adalla 1997, this volume). The approach adopted by IITA to sustainable pest control in cowpea is to develop a biointensive IPM strategy (Jackai and Adalla 1997), in which the key components are host plant resistance, cultural control, and biological control. Reviews on the use of cultural practices to control insect pests in cowpea have been provided by Ezueh (1991) and Jackai (in press). Biological control is discussed in the remainder of this paper.

Present status of biological control in cowpea

First, we would like to clarify the term "biological control", to avoid misinterpretation. As outlined in Huffaker and Smith (1980), biological control is defined as both the undisturbed activity of antagonists naturally present in a given ecosystem ("naturally occurring biological control"), and the manipulation of natural enemies in order to achieve better control levels ("applied biological control"). Generally, when we talk about biological control as an intervention tactic, we refer to the latter form and, more specifically, to "classical biological control" as the introduction of exotic antagonists against exotic pests. One of the best documented examples of classical biological control is the successful introduction of the solitary endoparasitoid *Epidinocarsis lopezi* (De Santis) (Hymenoptera, Encyrtidae) to control the cassava mealybug *Phenacoccus manihoti* Mat.-Ferr. (Homoptera, Pseudococcidae) in Africa (reviewed by Herren and Neuenschwander 1991).

In the literature concerning pest control in cowpea, the term "biological control" has usually been used to indicate the naturally occurring interactions between pests and their antagonists (Daoust et al. 1985; Jackai and Daoust 1986; Singh et al. 1990; Ezueh 1991). Therefore, recommendations for biological control were merely aimed at preserving the available natural enemies (Ezueh 1991). Up to now, classical biocontrol has never been adequately evaluated for cowpea, although there have been attempts at introducing and establishing natural enemies for *M. testulalis*, a pest of other legumes in areas where cowpea is not cultivated or of minor importance (Waterhouse and Norris 1987).

In this paper, the feasibility for classical biological control in cowpea is assessed, using three criteria discussed in Barbosa and Segarra-Carmona (1994):

1. the nature of the pest and release site, which includes information concerning both the origin of the pest and the host plant habitat;
2. the availability of biocontrol agents, i.e., the inventory and impact of natural enemies;
3. the current status of biocontrol against the target pest.

This approach is illustrated in more detail with *M. sjostedti*, which has been the object of in-depth ecological investigations more recently.

The feasibility study presented in this paper focuses only on the use of introduced arthropod beneficials. Although entomopathogens could in principle be of great potential value against cowpea pests from a biological perspective (Jackai, in press), there are predictable technical, economic, and institutional constraints regarding their production,

formulation, and application (Moore and Prior 1993). In fact, biocontrol by means of predators and parasitoids is easier to implement in the field than the application of myco-insecticides; as opposed to entomopathogens, arthropods are able to spread actively from the original release site and effectively colonize other areas. Also, once the natural enemies have become established, no further introductions are required, particularly if parasitoids are released. Nevertheless, the use of entomopathogens for the control of cowpea pests might gain importance in the future, if the difficulties concerning their production and application are overcome.

The bean flower thrips, *M. sjostedti*

Nature of pest and release site

As with many other successful examples of classical biological control, including that of the cassava mealybug, the discovery of the appropriate natural enemy was only possible after the pest's native home had been correctly identified. Hence, investigation of the origin of the pest is probably the most crucial step in assessing the feasibility of a biocontrol project (Bellows and Legner 1994). This information is relatively easy to gather in the case of recent pest introductions, due mainly to the existence of worldwide databases on agricultural pests. However, for organisms such as *M. sjostedti*, for which there is no historical record of an earlier introduction, and which are, therefore, considered indigenous pests, it is difficult to ascertain their origin from the available literature on taxonomy and distribution.

Since its first description in East Africa in 1905 (Trybom 1908), *M. sjostedti* has never been found outside the African continent (Palmer 1987). However, the fact that six other distinct species of *Megalurothrips* are uniquely found in tropical Asia, while *M. sjostedti* is the only species of this genus present in Africa (Palmer 1987), suggests that, in all probability, the center of origin of the genus *Megalurothrips* is tropical Asia. In addition, *M. sjostedti* is the only species of this genus that is considered an important crop pest, whereas none of the Asian species is considered an agricultural pest (Kalshoven and van der Vecht 1950; Litsinger et al. 1978; Singh et al. 1990). In fact, ecological studies in Southeast Asia indicate that *Megalurothrips* spp. are pollinators (Velayudhan et al. 1985), and only seldom cause feeding damage on flowering structures.

On the other hand, the strongest argument against an Asian origin of *M. sjostedti* is that it has never been found there. However, taking again the example of the cassava mealybug, that insect was not known to science in its native habitat until it was accidentally introduced into Africa without its natural enemies and became a pest. A comparable example is given by the *Megalurothrips* species described as most similar to *M. sjostedti*, *M. typicus* Bagnall, which is a rarely collected insect (Palmer 1987) and is not present on commonly sampled legume flowers. Similarly, if present in Asia, *M. sjostedti* might be confined, e.g., by interspecific competition, to host plants of no agricultural importance and might, therefore, have never been observed. Evidently, the present knowledge on the taxonomy and distribution of *M. sjostedti* does not allow us to draw a definitive conclusion about its origin but, together with the apparent lack of co-evolution in its environment as presented in the following sections (i.e., the inefficacy of available natural enemies and substantial damage on wild host plants), it leads us to hypothesize a southeast Asian origin for this insect (Tamò et al. 1993b; in press).

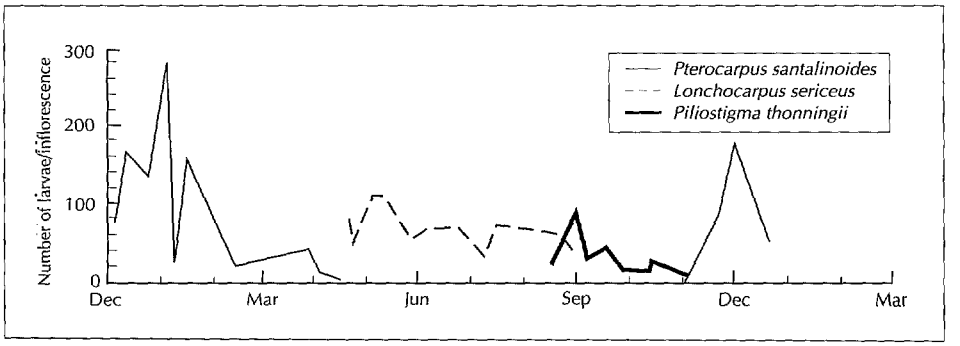

Figure 1. Seasonal abundance of larvae of *Megalurothrips sjostedti* on three alternative host plants in southern Benin.

Once a suitable biological control candidate has been identified in the pest's area of origin, and released in the new environment, its successful establishment depends largely on the availability of perennial habitats where it can find hosts during periods when the crop plant (i.e., cowpea) is not cultivated.

For *M. sjostedti,* there is already a long list of alternative host plants (Tamò et al. 1993b) where this insect can be found during their respective flowering periods. This list, though far from exhaustive, shows that, from the coast in the south to the dry savanna areas in the north of West Africa, there are a number of host plants which play an important role in the population dynamics of the pest. Using as examples the three most common wild host plants in the moist savanna, we observed (Fig. 1) that there is always a plant at the flowering stage to ensure survival and reproduction of the thrips, even in the complete absence of cowpea. This observation will have implications for the establishment and survival of the hypothetical natural enemy to be released against *M. sjostedti*: populations of *M. sjostedti* with a suitable demographic profile will be available throughout the year, but the biocontrol candidate should be able to recognize most of these plants as being hosts for *M. sjostedti.*

Availability of biological control agents

The mortality inflicted by natural enemies is an important factor regulating pest populations in tropical climates, and we would expect to find well-adapted antagonists if *M. sjostedti* was of African origin. Although the search for natural enemies associated with *M. sjostedti* yielded a number of antagonists (Table 1) comparable to the numbers for other flower thrips (e.g., Lewis 1973), the only quantitative data available for both parasitoids and pathogens indicate a minor influence on the population dynamics of *M. sjostedti* (Tamò et al. 1993b).

Mortality rates due to the activity of egg parasitoids of the genus *Megaphragma* (Hymenoptera, Trichogrammatidae), although reaching peaks > 30% on cowpea and 53% on one alternative host plant, *Pueraria phaseoloides* (Leguminosae, Fabaceae), are inconsistent throughout the season and differ greatly from region to region. A reason for this inefficacy can be sought in the ephemeral character of *Megaphragma* sp., the smallest insects known, whose movements and survival on the host plant are largely influenced by

Table 1. Parasitoids and predators of *Megalurothrips sjostedti* from West Africa.

Organism	Stage affected†	Country	Source
Parasitoids			
Hymenoptera			
Eulophidae			
Ceranisus menes	L	Benin	Tamò et al. (1993b)
Trichogrammatidae			
Megaphragma sp.	E	Benin	Tamò et al. (1993b)
Oligosita sp.	E	Benin	Tamò et al. (1993b)
Predators			
Acari			
Phytoseiidae			
Iphyseius sp.	E	Benin	Tamò et al. (1993b)
Coleoptera			
Coccinellidae			
Cheilomenes sulphrea	L	Benin	Tamò et al. (1993a)
Staphilinidae	L	Benin	Tamò et al. (1993b)
Paederus sabeus			
Hemiptera			
Anthocoridae			
Orius sp.	E, L, A	Nigeria	Matteson (1982); Rösingh (1980)
		Benin	Tamò et al. (1993b)

† E = egg; L = larva; A = adult.

the microclimate. Also, *Megaphragma* spp. are known to be rather nonspecific. They parasitize eggs of several thrips species in the Terebrantia (Lewis 1973) and *M. sjostedti* may not be the primary host.

In 1992, an indigenous larval parasitoid of *M. sjostedti* was recorded for the first time in Benin Republic in the flowers of an exotic shrub, *Tephrosia candida* (Leguminosae, Fabaceae). The solitary endoparasitoid was tentatively identified as *Ceranisus menes* (Hymenoptera, Eulophidae) (Tamò et al. 1993b), although the authority stated clearly that, for this genus, there were no keys for species outside Europe (J. LaSalle, British Museum, personal communication). Further studies on *M. sjostedti* larvae collected from cowpea revealed very low parasitism rates. After having reared over 12,000 thrips larvae, sampled from different ecological zones in Benin Republic, with a total parasitism rate of < 1%, we are now convinced that *C. menes* cannot effectively recognize cowpea as a host plant for *M. sjostedti*. Also, the low parasitism rates observed on naturally occurring alternative host plants (Table 2), which represent a much more stable ecosystem than the cowpea field, indicate that *C. menes* is not an efficient natural enemy of *M. sjostedti*.

Our laboratory experiments, conducted in small rearing jars under no escape conditions, revealed a parasitization rate of only about 15%, indicating low host acceptance. Often the parasitoid inside the attacked larva could not complete its life cycle (Tamò, unpublished data). All these facts suggest that the parasitoid is more likely to be associated with other thrips than with *M. sjostedti*, and that it is probably more attracted to *T. candida* than to cowpea or indigenous alternative host plants.

Table 2. Parasitism rates of larvae of *Megalurothrips sjostedti* and *Maruca vitrata* collected on major alternative host plants in Benin (Tamò and Arodokoun, unpublished data).

Host plant	% larval parasitism	
	M. sjostedti	*M. vitrata*
Piliostigma thonningii	0.30	–
Lonchocarpus sericeus	1.71	1.87
Pterocarpus santalinoides	3.82	20.97
Tephrosia platycarpa	–	2.38

The interactions between a hypothetical parasitoid, the larval population of *M. sjostedti,* and the yield of cowpea have been evaluated using simulation models (Tamò et al. 1993a), and the results indicate that a parasitoid that can kill 35% of the larval population should have a beneficial effect in the cowpea field.

Current status of classical biocontrol

As mentioned earlier, there has never been any attempt to implement classical biological control against cowpea pests in the past. Very recently, to test the foreign origin hypothesis for *M. sjostedti* and its implications for biological control, an exploration in search of both *M. sjostedti* and efficient natural enemies was undertaken by the first author in Peninsular Malaysia in November–December 1994.

The search did not yield a specimen of *M. sjostedti*, but some rare female specimens of *M. usitatus* were collected which had asymmetrical positioning of the median posteromarginal setae on sternite VII, one seta being on the posterior margin (which is typical of *M. sjostedti*), and the other being anterior to it (typical of *M. usitatus*).

During the same exploration, an endophagous larval parasitoid, also tentatively identified as *C. menes*, was found in flowers of different cultivated and cover crops, together with populations of *Megalurothrips* spp., mainly *M. usitatus*. Up to 70% parasitism was observed from *Megalurothrips* spp. larvae collected from flowers of *Pueraria phaseoloides*, a commonly grown cover crop (Table 3). This parasitoid did recognize long beans (*V. sesquipedalis*), whose flowers are quite similar to those of cowpea, as a host plant for *Megalurothrips* spp. However, preliminary observations (Table 3) indicate that the application of insecticides affected the presence of this parasitoid on cultivated legumes.

The suitability of using the collected strain of *C. menes* as a biological control agent for *M. sjostedti* is now being studied under quarantine.

Table 3. Parasitism of larvae of *Megalurothrips* spp. by *Ceranisus menes* in peninsular Malaysia.

Total collected	Larvae of *Megalurothrips* spp.			
	Parasitized	Pathogens	other	% parasitism
Sprayed beans 215	1	19	31	0.5
Unsprayed beans 326	146	33	35	44.8
Unsprayed cover crop 188	129	14	11	68.6

The legume pod borer, *M. vitrata*

Nature of pest and release site

The origin of the pod borer, *M. vitrata* (syn. *M. testulalis)*, a cosmopolitan pest in the tropics, is uncertain (Waterhouse and Norris 1987). In addition, the taxonomic classification of this genus is still unclear and needs to be revised. In the past, several species of the genus were considered as a complex, but later all were synonymized under *M. testulalis* (Taylor 1967). Apart from the widespread species *M. vitrata,* the genus *Maruca* includes only two other species: *M. amboinalis* (Feld and Rog), and *M. nigroapicalis* (De Joannis). These two other species have been exclusively observed in the Indo-Malaysian and Tonkin area, and the latter has never been found again after the first description (Ghesquière 1942). Nevertheless, the Indo-Malaysian region was given as the most probable area of origin of the genus *Maruca,* including *M. vitrata* (Prof. Munroe, Ottawa, Canada, personal communication).

Detailed studies on the importance of alternative host plants for the population dynamics of *M. vitrata* have revealed that this insect is oligophagous, feeding and reproducing on a number of cultivated and wild host plants, all of which belong to the Fabaceae (Leumann 1994; Arodokoun 1996). Further, the alternation of the flowering pattern of these plants on a south-north gradient has been found to influence the migration of *M. vitrata* from the coast to the dry savannas of West Africa (Bottenberg et al.1997, in this volume). During this migration, the population of *M. vitrata* finds favorable conditions for multiplying on the different host plants, thereby increasing the size of each new generation. When this huge population reaches the main cowpea growing areas in the northern regions, it is too late to intervene unless highly resistant varieties are available, or intensive pesticide use is envisaged. To prevent the buildup of such large populations, a suitable biocontrol agent should be able to arrest their migration from the south to the north. Therefore, any efficient biocontrol candidate should be able to recognize the most important host plants for *M. vitrata,* in order to follow the pest migration through host switching.

Availability of biocontrol agents

Although *M. vitrata* is attacked by several different natural enemies (Table 4 lists the parasitoids and predators that have been recorded in Africa), the available quantitative data indicate that the overall parasitism rates on cowpea are low, mostly between 5 and 15% (Taylor 1967; Okeyo-Owuor et al. 1991). On the most common alternative host plants for *M. vitrata* in the moist savanna, recent investigations (D.Y. Arodokoun, unpublished data) indicate significantly higher parasitism rates in these more stable ecosystems, often averaging over 20% (Table 2). However, the same study suggests that, despite the higher biotic mortality, these wild host plants suffer considerable feeding damage through *M. vitrata* larvae.

A life tables study by Okeyo-Owuor and Oloo (1991) indicates very high mortality rates from egg to adult in western Kenya. Although disappearance accounts for > 50% of the compounded mortality, the impact of pathogens is considered an important mortality factor, as confirmed by the data of Otieno et al. (1983) and Odindo et al. (1989). Still, field data on pest infestation strongly indicate that the available biotic mortality is not sufficient to keep *M. vitrata* populations under the damaging level.

Table 4. Parasitoids and predators of *Maruca vitrata* in Africa.

Organism	Stage affected†	Country	Source
PARASITOIDS			
Diptera			
Muscidae			
Musca domestica f. *callara*	L	Nigeria	Taylor 1967
Tachinidae			
Pseudoperichaeta laevis	L	Nigeria	Usua 1975; Usua and Singh 1978; Ezueh 1991
Thelaitrodoms palposum	L	Nigeria	Usua 1975; Usua and Singh 1978; Ezueh 1991
undetermined	P	Kenya	Okeyo-Owuor et al. 1991
undetermined	P	Benin	Arodokoun (unpub. data)
Hymenoptera			
Braconidae			
Apanteles sp.	L	Kenya	Okeyo-Owuor et al. 1991
Bracon sp.	L, P	Kenya	Okeyo-Owuor et al. 1991
Braunsia sp.	L	Nigeria	Taylor 1967; Usua 1975; Usua and Singh 1978; Ezueh 1991
	P	Kenya	Okeyo-Owuor et al. 1991
B. kriegeria	L	Benin	Arodokoun (unpub. data)
Chelonus sp.	L	Kenya	Okeyo-Owuor et al. 1991
Phanerotoma sp.	L	Nigeria	Taylor 1967; Usua 1975; Usua and Singh 1978
	E, L	Benin	Arodokoun (unpub. data)
Pristomerus sp.	L	Benin	Arodokoun (unpub. data)
Chalcididae			
Antrocephalus sp.	P	Kenya	Okeyo-Owuor et al. 1991
Brachymeria sp.	P	Benin	Adango 1994
Eulophidae			
Tetrastichus sp.	L	Nigeria	Usua 1975; Usua and Singh 1978; Ezueh 1991
T. sesamiae	P	Kenya	Okeyo-Owuor et al. 1991
Trichogrammatidae			
Trichogrammatoidaea sp.	E	Benin	Tamò (unpub. data)
PREDATORS			
Aranea			
Selenopidae			
Selenops radiatus	L, A	Nigeria	Usua 1975; Usua and Singh 1978; Ezueh 1991
Dermoptera			
Diaperasticus erythrocephala	L, P	Kenya	Okeyo-Owuor et al. 1991
Dictyoptera			
Mantidae			
Polyspilota aeruginosa	A	Nigeria	Usua 1975; Usua and Singh 1978
Spodromantis lineola	A	Nigeria	Usua 1975
Hymenoptera			
Formicidae			
Campanotus sericeus	L	Nigeria	Usua 1975; Usua and Singh 1978; Ezueh 1991
C. rufoglaucus	L	Kenya	Okeyo-Owuor et al. 1991

† E = egg; L = larva; P = pupa; A = adult.

Current status of classical biocontrol

Based on the taxonomist's assumption that *M. vitrata* is native to southeast Asia, we would expect to find in that region more efficient natural enemies for this pest. In fact, in the Indo-Malaysian region, *M. vitrata* has been reported in the past only as a minor pest (Kalshoven and van der Vecht 1950). The same authors indicate a larval parasitoid, *Phanerotoma philippiniesnsis*, and a pupal parasitoid, *Bassus* sp. (Hymenoptera, Braconidae), as the natural enemies responsible for this low occurrence. More recently, however, *M. vitrata* has become one of the key pests on legumes in southeast Asia. Personal observations by the first author in Malaysia suggest that *M. vitrata* is probably an induced problem, caused by the misuse of pesticides in vegetable legumes. In fact, in regions where vegetable farmers apply an average of two sprays a week with combinations of up to three pesticides, it is easy to imagine the buildup of pesticide resistance and loss of natural enemies.

The next logical step for the assessment of the feasibility of biocontrol would be to test both the efficacy and specificity of *M. vitrata* parasitoids from southeast Asia, and introduce the most promising ones into Africa. Though the long list in Table 4 indicates that there are already many parasitoids in Africa, careful ecological studies would be needed before any releases are made.

It is important that there are no known parasitoids recorded from South America, where *M. vitrata* was first discovered. One implication of this could be that *M. vitrata* is economically more important in Africa than in other geographical regions, and it is consequently better investigated there. Another could be that *M. vitrata* is not a big problem in South America because it is kept under control by antagonists, whose identity is not yet known. Evidently, the actual knowledge about the regional distribution and importance of natural enemies of *M. vitrata* presents some gaps, which need to be filled before further assumptions concerning the origin and the chances of biocontrol can be made.

At the same time, one should keep in mind the unsuccessful attempts at biological control of *M. vitrata* cited in Waterhouse and Norris (1987), and learn from the possible causes of these failures.

Conclusions

There is, thus, a potential for biocontrol against the two major cowpea pests discussed. The investigation of pest origin, one of the most important assumptions for classical biological control, has revealed that both *M. sjostedti* and *M. vitrata* might have originated in Southeast Asia. This hypothesis is further strengthened by the apparent lack of co-evolution in their natural environment, as indicated by the frequent severe damage on the flowering structures of alternative host plants, as well as the overall low biotic mortality inflicted by natural enemies. Also, given the case that exotic natural enemies are to be released, the alternative host plants available throughout the year could be critical for their survival when cowpea is not available.

The discovery and collection of a first larval parasitoid from a related thrips species in Malaysia is encouraging. However, further exploration elsewhere in tropical Asia is needed to ascertain the presence of different strains of *C. menes*, or of other parasitoid species.

Concerning the search for biocontrol candidates for *M. vitrata*, it is recommended that explorations be conducted on wild alternative host plants in its area of origin, to avoid interferences caused by excessive pesticide applications.

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Population dynamics and migration of cowpea pests in northern Nigeria: implications for integrated pest management

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Abstract

The population dynamics of major cowpea pests was studied in northern Nigeria during 1992–95 in both the wet and dry seasons. Rainfed cowpea is grown as a subsistence crop, mixed at low densities with cereals. Dry-season cowpea is irrigated or grown on residual moisture in monocultures as a cash crop. Light-trap monitoring and sampling of cowpea fields, throughout the wet and dry seasons, showed that *Maruca vitrata* (previously *M. testulalis)* does not occur during the dry season in northern Nigeria, even if cowpea is present. Corroborative data from more southern locations within the study region showed that *Maruca* is a migratory pest, which survives the dry season on alternate hosts in the more humid south and migrates to the north following the pattern of rainfall and cowpea cultivation. Similarly, populations of *Megalurothrips sjostedti* and *Clavigralla tomentosicollis* are very low on cowpea during the dry season but develop rapidly during the rainy season. Aphids and Lycaenids are present year round but predominate during the dry season. The importance of seasonal changes in the pest complex and cropping system for integrated pest management (IPM) of cowpea is discussed.

Introduction

Cowpea, an important food crop and source of protein in West Africa, is grown mainly in the savanna regions in the wet season (WS) (Taylor 1967). The dry season (DS) in Kano, northern Nigeria, lasts from October through May (Griffiths 1972; FAO 1984). No crop is left in the field and most herbaceous vegetation dies off, except in irrigated crop land and swampy areas (Hill 1972). Yet, every year, cowpea is attacked by a wide variety of pests, particularly aphids *Aphis craccivora* Koch (Homoptera: Aphididae), pod borers *Maruca vitrata* (Fabricius) (previously *M. testulalis* [Geyer]) (Lepidoptera: Pyralidae), flower thrips *Megalurothrips sjostedti* Trybom (Thysanoptera: Thripidae), and pod-sucking bugs, especially *Clavigralla tomentosicollis* Stål (Hemiptera: Coreidae) (Singh et al. 1990).

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It is not clear if these pests survive the DS in northern Nigeria or if they migrate and fly in from more southern areas at the onset of the WS. The desert locust (*Schistocerca gregaria* [Forskål]) and the African armyworm (*Spodoptera exempta* [Walk.]) are two examples of migratory pests in Africa that follow wind-convergence systems and seasonal rains to areas with abundant vegetative growth (Betts 1975). In the Middle East, windborne migration of 17 species of moths from breeding areas in the Nile Delta has recently been demonstrated (Pedgley and Yathom 1993). Nonmigratory pests may survive locally on alternate hosts, or on cowpea planted in soils with residual moisture or in irrigated land. Pests may also retreat in a dormancy stage in a protected site (Denlinger 1986). Hammond (1983) found inactive, quiescent *C. tomentosicollis* adults in cowpea leaf litter during the DS in Mokwa, Nigeria. The maize stem borer, *Busseola fusca* Fuller, and the millet stem borer, *Acigona ignefusalis* Hampson, pass the DS in northern Nigeria as diapausing larvae in stems of millet and sorghum, stored as fodder for farm animals (Harris 1962; Usua 1973). Potential estivation sites for cowpea insects may include soil and cowpea fodder.

DS cowpea production is popular on soils that retain residual moisture after recession of flood waters in alluvial flood plains (fadamas), such as the Hadejia Wetlands (Thomas 1992; Hanssens 1993) and Bida area (Alghali 1991a) in northern Nigeria, and the Lake Chad flood basin covering parts of Niger, Nigeria, Cameroon, and Chad. Cowpea is also becoming an economically viable DS cash crop on irrigated land. Because of the increasing potential of DS cowpea production in northern Nigeria, there is an urgent need to document its pest profile and develop appropriate control methods.

During 1992–95, a number of surveys and studies were carried out in northern Nigeria, to determine which of the cowpea pests persist during the DS and those which occur only on rainfed cowpea. This paper synthesizes the results of those studies and discusses strategies to control cowpea pests during the DS and WS.

Methods

Potential diapausing sites

The following sites were investigated for the presence of diapausing cowpea pests in the 1992–93 DS in the Minjibir Local Government Area, Kano, Nigeria.

Cowpea fodder. Cowpea fodder bundles from 15 farmers' fields (3 bundles per field) around Minjibir village were stored on trees near the field of origin between 7 and 28 Oct 1992. The bundles were weighed and checked for insects between 20 and 28 Jan 1993.

Cowpea leaf litter and topsoil. Two cowpea fields, planted on 21–23 Jul 1992 with cv. IT86D-715 (extra-early maturity) and cv. Dan 'Ila (a local cultivar, spreading type), were selected. The fields, never treated with insecticides, were attacked heavily by *M. vitrata* (3.2 larvae/flower, mean of two sampling dates) and less intensely by pod-sucking bugs (1.4/m-row, mean of two sampling dates, nymphs + adults, all species combined). After pod harvest on 13–14 Nov, the leaf litter and topsoil were sampled for arthropods during 19–30 Nov. The leaf litter was collected by placing a 100 × 50 cm wireframe lengthwise on top of the cowpea row, thus enclosing four (cv. IT86D-715) or two (cv. Dan 'Ila) stands, and removing all leaf litter within. This was followed by removing the topsoil within the wireframe down to the level of the furrow between the rows (~ 30 cm deep). This

procedure was replicated 40 times in the field with cv. IT86D-715 and 48 times in the field with cv. Dan 'Ila, resulting in a total of 88 samples. The leaf litter of each sample was weighed, and checked in the laboratory for arthropods; the soil was sieved, weighed, and checked for arthropods in the field. Preserved arthropod specimens were identified and deposited at the IITA Insect Museum, Cotonou, Republic of Benin.

Alternate hosts in the DS

A wide variety of leguminous trees and herbaceous weeds in Kano state during the 1992–93 and 1993–94 DS was examined for the presence of cowpea pests.

Pests of DS cowpea

The following surveys and experiments were conducted to monitor DS cowpea pests in 1993–95 and were extended through the WS.

Farmers' fields planted on residual moisture. Farmers' fields were visited with a local extension agent about every two weeks from mid-Feb to mid-Oct 1994, starting with cowpea at the flowering to early podding stage. The fields were situated within a 20 km radius of Nguru, Yobe state, northern Nigeria. DS cowpea production on residual moisture after WS rice cropping is a traditional practice in this area. During the WS, rainfed cowpea is planted mixed with millet and sorghum on higher, drier, and more sandy soils surrounding the marshy flood plains. Each month, 5–19 fields were visited. In each field, 150 flowers were collected: 25 flowers in a vial with 50% ethanol and 125 flowers in a paper bag. The flowers in the vials were checked in the laboratory for insects, particularly thrips, with a stereomicroscope. The flowers in paper bags were checked carefully for live Lepidopteran larvae, which were placed in petri dishes with fresh cowpea pod and reared out to adults to determine the species (in the case of Lycaenids) and parasitization rates. Specimens were sent for identification to taxonomists at the Smithsonian Institution, USA and deposited at the IITA Insect Museum, Cotonou, Benin. Farmers were interviewed for information on cowpea variety, planting date, expected yield, pest control practices, and observed pests.

Irrigated planting date trials at the IITA Minjibir farm. In 1993, plantings of cowpea Dan 'Ila were made on 18 Mar, 24 Apr, 15 May, 28 May, and 9 Jun. The plots measured 25 × 25 m and were located on previously flooded land along the irrigation reservoir, adjacent to the IITA research farm at Minjibir, near Kano, Nigeria. Residual moisture was supplemented with overhead sprinkler irrigation when necessary. Cowpea flowers (up to 100 per day) were collected, stored in 50% ethanol in vials, and checked in the laboratory for insects with a stereomicroscope. Starting on 1 Jan 1994, cowpea variety TVx 3236 was planted every 15 days at 25 × 75 cm in plots measuring 10 × 10 m. The last plot was planted on 15 Dec 1994. During the DS, water was provided when necessary by sprinkler irrigation. Planting dates were not replicated due to constraints imposed by the irrigation system.

Vegetative tips, racemes, and flowers were collected daily during the DS and every other day during the WS at the rate of 50 per plot, stored in 50% ethanol in vials, and checked in the laboratory for insects with a stereomicroscope. In addition, 100 flowers per

plot were collected daily or every other day in paper bags; each flower was checked carefully in the laboratory. Live Lepidopteran larvae were removed, placed in petri dishes with fresh cowpea pods, and reared out to determine parasitization rates. Populations of pod-sucking bugs were assessed visually every other day between 8–10 AM, from the onset of podding to pod maturity. Counts were made by walking along each row in the plot and recording the species and number observed. All pods from the central row were hand picked at maturity. Before threshing, the percentage of pods that were damaged by Lycaenids, *M. vitrata,* and pod-sucking bugs was determined.

Flight activity of cowpea pests during dry and wet seasons

Sticky traps for thrips and aphids monitoring in Kano. We used 30 × 50 cm plastic transparent sheets coated with Tanglefoot® on one side and mounted in wooden frames at different heights on a 330 m tall TV transmission tower (Kano Broadcasting Corporation) near Kano. The frames were attached with strong steel wire to the tower at 35 m, 110 m, and 190 m, with the sticky sides facing E, SW, and NW directions (as per the orientation of the tower). The sheets were changed weekly and taken to the laboratory, where all arthropods were removed with forceps. Arthropods were stored in vials with kerosene to dissolve Tanglefoot®, and counted and categorized into 42 groups, using a stereo-microscope.

Light trap for *M. vitrata* monitoring. These were set up in Kano, Nigeria; Cotonou, Republic of Benin; and Niamey, Niger. An insect light trap was developed, based on a trap designed by O. Youm at the ICRISAT Sahelian Center, Niger. The trap consisted of a mercury vapor pressure (MVP) lamp with baffles, a funnel, and a cage. Insects, attracted to the light at night, hit the baffles, and dropped through the funnel into the cage. The cage, made of wood or metal, measured about 1.5 × 1.5 × 2 m, with sides made of 2 × 2 mm screen mesh. The cage had a door for a person to enter; two opposite sides of the cage could be opened to facilitate cleaning. Holes were drilled in the floor to drain the rainwater. *M. vitrata* adults were collected in the morning by picking the moths individually from the inner sides of the cage. Trapping was done from sunset to sunrise. *M. vitrata* trapping started in July 1993 at all three sites.

Results

Potential diapausing sites

No diapausing *M. vitrata* or pod-sucking bugs were found in cowpea fodder, leaf litter, or soil (Bottenberg, unpublished). Low numbers of bean leaf beetles (2 *Ootheca* sp.) and adult pod-sucking bugs (2 *C. tomentosicollis*, 1 *Mirperus* sp., and 1 *Aspavia* sp.) were found in 2445 kg of topsoil sampled from a cowpea field. These insects appeared active and may have flown in from adjacent surroundings. The only cowpea insects recovered from 46 kg of fodder were 16 bruchid larvae (*Callosobruchus maculatus* [Fabricius]), feeding on cowpea grain contained in the fodder bundles.

Alternate hosts in the DS

Lycaenid larvae were collected from flowers of *Crotalaria* sp. during the early DS (Nov–Dec), and also on pigeonpea (*Cajanus cajan* [L.] Millsp.) and lablab (*Lablab*

purpureus L.) planted on residual moisture in the Hadejia Wetlands. *Maruca vitrata* larvae were found in flowers of *Sesbania* sp. and *Crotalaria* sp., but their infestation was highly variable and noticeable only during the WS when cowpea was also present. *Pterocarpus* sp. and *Lonchocarpus* sp., important off-season hosts for *M. vitrata* in the humid forest zone (M. Tamò, unpublished data), were not found in Kano. *Cassia obtusifolia* L. supported populations of *A. craccivora* at the onset of the WS before cowpea planting commenced in July. During the later part of the DS, various hemipterans, including *Anoplocnemis curvipes* (Fabricius) but excluding *C. tomentosicollis,* were seen feeding on pods of *Cassia occidentalis* L. Flower thrips were found in flowers of *Crotalaria* sp., groundnut (*Arachis hypogea* L.), pigeonpea, and lablab.

Pests of DS cowpea planted on residual moisture in Hadejia Wetlands

Farmers reported heavy damage by aphids (*Aphis craccivora* Koch) on DS cowpea. No larvae of *M. vitrata* feeding on cowpea flowers or pods were found during the DS. Flower infestation by *M. sjostedti* was relatively low (< 1 thrips/flower) during the DS, but after the rains started, it increased rapidly to 7 thrips/flower in mid-July; infestation then dropped to < 1/flower in October after cessation of rains. *Frankliniella* sp. and *Sericothrips* sp. were present during the DS, reaching levels of 4 thrips/flower. Lycaenids comprised the major flower-feeding, lepidopteran pest during the DS, but densities did not exceed 0.09 larvae/flower. Of the 224 Lycaenids that were reared to adulthood, 71% were *Lampides boeticus* (L.), 20% *Lepidochrysops* sp., and 9% *Virachola antalus* Hopffer (determined by R. Robbins, Smithsonian Institution, USA). Flower infestation by *M. vitrata* larvae did not start until mid-July, after the onset of rains, reached a level of 0.12 larvae/flower in mid-August, and declined to zero in October. Lycaenid numbers dropped rapidly as *M. vitrata* increased in the WS.

Pests of irrigated DS cowpea in Minjibir

In 1993, when collection of flowers started on 2 Jun due to delayed flowering in cowpea cultivar Dan 'Ila, the first two *M. vitrata* larvae were collected on 22 Jun from 200 flowers. No *M. vitrata* larvae were collected prior to this date from flowers nor from vegetative tips that were inspected weekly at the rate of 100 per plot in May.

In 1994–95, aphids, not observed during the DS, appeared in July, but populations did not persist beyond August and are not reported here. Flower thrips were scarce during the early DS but increased rapidly (up to 12 thrips/flower) in the last week of April (Fig. 1). The first peak comprised mainly adults. Flower thrips were the major thrips species from May through September, after which the numbers dropped (it must be noted that cowpea TVx 3236 is known to be somewhat resistant to flower thrips; their incidence could have been higher otherwise). *Frankliniella* and *Sericothrips* were more common in the DS than in the WS. Although *Sericothrips* sp. feeds more on foliage than on flowers, we assume that the number of thrips present within the flowers is a spillover from the populations feeding on the foliage and is, therefore, indicative of its population size. *Sericothrips* leaf damage was observed in mid-May, but disappeared soon thereafter. Although the first plot was planted on 1 Jan 1994, *M. vitrata* larvae did not begin to infest cowpea flowers until the last week of June in a plot planted on 15 May (Fig. 1). *M. vitrata* flower infestation peaked in mid-August (1.2 larvae/flower) and declined to < 0.1 larvae/flower in October;

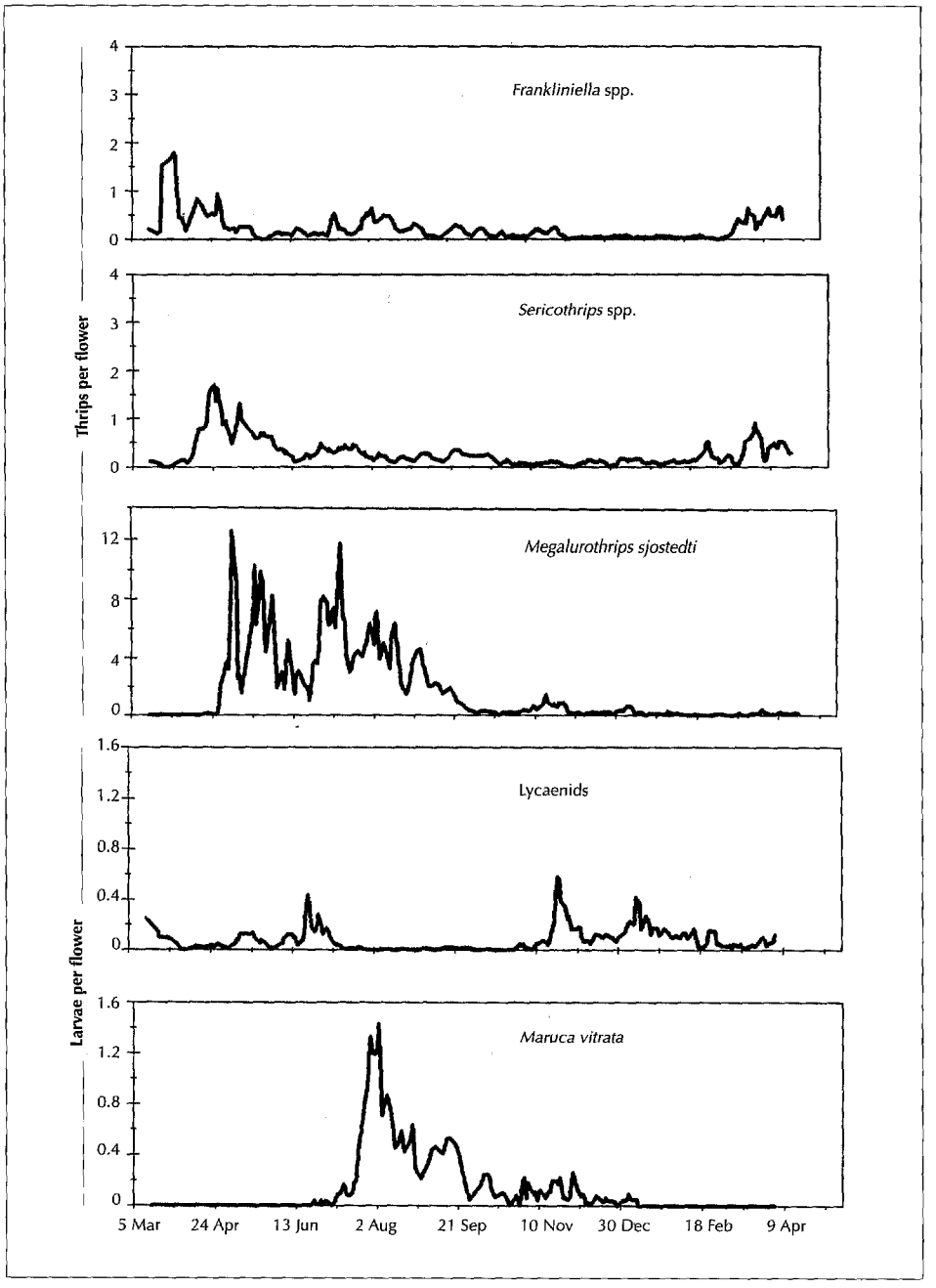

Figure 1. Cowpea (TVx 3236) flower infestation (3-day moving averages) by *Frankliniella* sp., *Sericothrips* sp., and *Megalurothrips sjostedti* (larvae and adults combined) and larvae of Lycaenids and *Maruca vitrata* in plots planted every 15 days from 1 Jan to 15 Dec 1994, at the IITA research farm in Minjibir, Kano state, Nigeria.

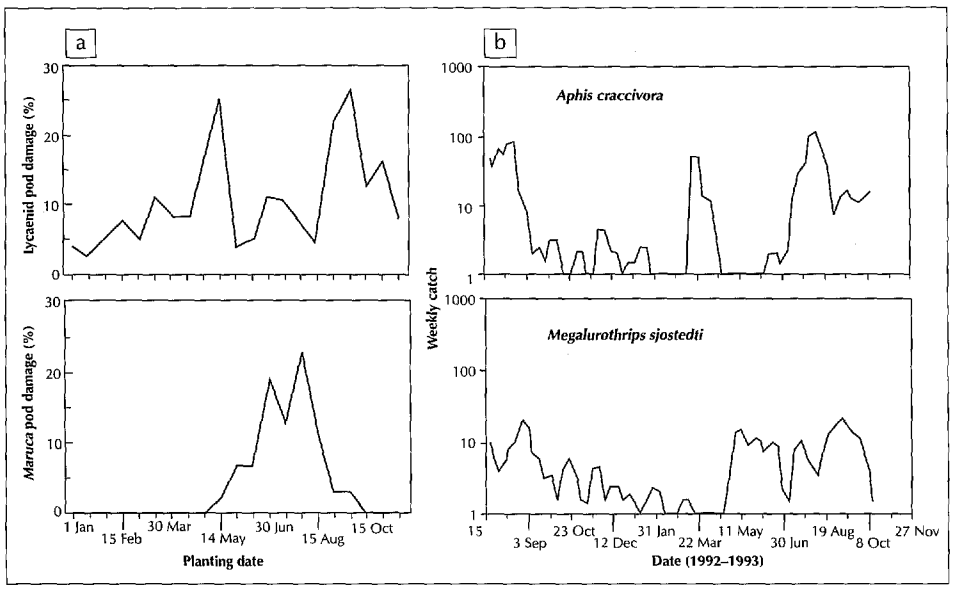

Figure 2. (a) Cowpea (TVx 3236) pod damage by larvae of Lycaenids and *Maruca vitrata* in plots planted every 15 days from 1 Jan to 15 Dec 1994, at the IITA research farm in Minjibir, Kano state, Nigeria. (b) Weekly total catch of *Aphis craccivora* and *Megalurothrips sjostedti* on sticky traps mounted on the 300-m tall Kano Broadcasting Corporation TV transmission tower, Kano city, Nigeria, from July 1992 to October 1993. Numbers shown are the sum of catches made with traps at 30, 100, and 190 m, facing E, SW, and NW directions.

low levels of infestation persisted throughout November and December. The last *M. vitrata* larvae were collected on 9 Jan. *M. vitrata* pod damage (Fig. 2a) was first recorded from plots planted on 14 May (2.4%), reached a peak in plots planted on 31 Jul (23.2%), and dropped to zero in plots planted after 15 Oct. Lycaenids were always present during the DS and WS. However, they declined during the WS as *M. vitrata* populations increased. Pod damage caused by Lycaenid feeding followed the same pattern. It was relatively high during the DS but did not have a significant impact on yield. Damage by *M. vitrata* is easily distinguished from Lycaenid damage by the presence of frass around the entry hole.

The pod-sucking bugs complex changed rapidly from month to month. During the 1994 DS, the pentatomid *Agonoscelis ?haroldi* Bergroth (1/m-row), the lygaeids *Spilostethus rivularis* Germar (0.3/m-row) and *S.* sp. *nr? elegans* Wolff (0.8/m-row), and the pyrrhocorid *Dysdercus voelkeri* Schmidt (1.2/m-row) were tmost common (determined by G. Georgen, IITA, Cotonou, Republic of Benin). Only *A. ?haroldi* was recorded again in the 1995 DS. *Clavigralla tomentosicollis* counts were very low during the DS; they started to increase rapidly in the first week of June, peaked in early August (0.8/m-row) and declined in September, probably due to migration to the larger fields surrounding the test site that were podding in September. Other, less common PSB species were *Aspavia armigera* Fabricius, *Mirperus jaculus* Thunberg, *Clavigralla shadabi* Dolling, *Cletus notatus* Thunberg, *Nezara viridula* L., *Riptortus dentipes*, and *Anoplocnemis curvipes*.

Grain yields were highest in plots planted on 16 Mar (681 kg/ha), and lowest in plots planted on 31 Jul (150 kg/ha). Yield was negatively and significantly (df = 15, $P < 0.05$)

correlated to pod damage caused by *M. vitrata* (r = –0.50), but not with pod damage caused by PSB (r = –0.42) or Lycaenids (r = 0.03).

Flight activity of cowpea pests during DS and WS: sticky traps

Cowpea aphids and flower thrips were collected from sticky traps throughout the year (Fig. 2b). Adult *M. vitrata* and pod-sucking bugs were never collected. *Megalurothrips sjostedti* numbers suddenly increased in May when winds were shifting from the NE to the SW. Cowpea planting had not yet commenced around Kano at this time. A second peak of flight activity developed in September as cowpea in northern Nigeria was in the flowering to podding stage. *Aphis craccivora* flight activity was high in the early part of the WS and decreased in September. However, a distinct peak developed in March.

Populations of cowpea pests during DS and WS: M. vitrata *light trap monitoring*

Results of the first two years of trapping showed that *M. vitrata* did not occur in the Kano and Niamey locations during the DS from about November to July (Fig. 3). It occurred only during the WS from July to October, when rainfed cowpea is cultivated. In Kano, three peaks developed, each about one month (or one generation) apart. The first peak occurred in mid-August, the second (the highest) in mid-September, and the third in mid-October. In Niamey, the most northern site, only one peak developed around mid-September. In the southern location (Cotonou), there were two periods of flight activity. The first lasted from about mid-May to mid-August, and the second from mid-October to mid-February during periods of cowpea cultivation. Small numbers of the pest were trapped in between these periods. However, most *M. vitrata* moths were trapped in the second wave when *Pterocarpus* sp., an important alternate host common in forests and along streams (Tamò, unpublished data), was flowering after cowpea harvest.

Discussion

Pests of DS cowpea

Lycaenids, foliage thrips, and aphids were the only pests that prevailed during the DS. Afun et al. (1991) and Alghali (1991a) also reported low insect pressure on cowpea grown during the second half of the dry season in a fadama area in the Bida region, 450 km southwest of Kano. Cowpea aphids and foliage thrips occurred throughout the year because groundnut, cowpea, and other leguminous hosts were always available. Aphids and thrips can be carried year round by prevailing winds over long distances, as demonstrated by our sticky trap catches. In the DS, northeasterly harmattan winds carried aphids from DS cowpea production areas in the Hadejia Wetlands, about 200 km NE of Kano. In the WS, aphids were carried from areas south of Kano. Aphid infestation on DS cowpea can be severe. In March 1993, we noticed widespread aphid infestations on cowpea planted on soils with residual moisture in the Lake Chad basin (H. Bottenberg, unpublished data). Lycaenids are the prominent lepidopteran pests during the DS. The amount of economic damage attributable to lycaenids is not known. *Sericothrips* may cause leaf chlorosis and distortion, but cowpea plants are normally able to outgrow this damage. However, *Sericothrips* and *C. impurus* outbreaks have been reported on DS cowpea in Bida rice fallows (Afun et al. 1991), and the former more recently in the

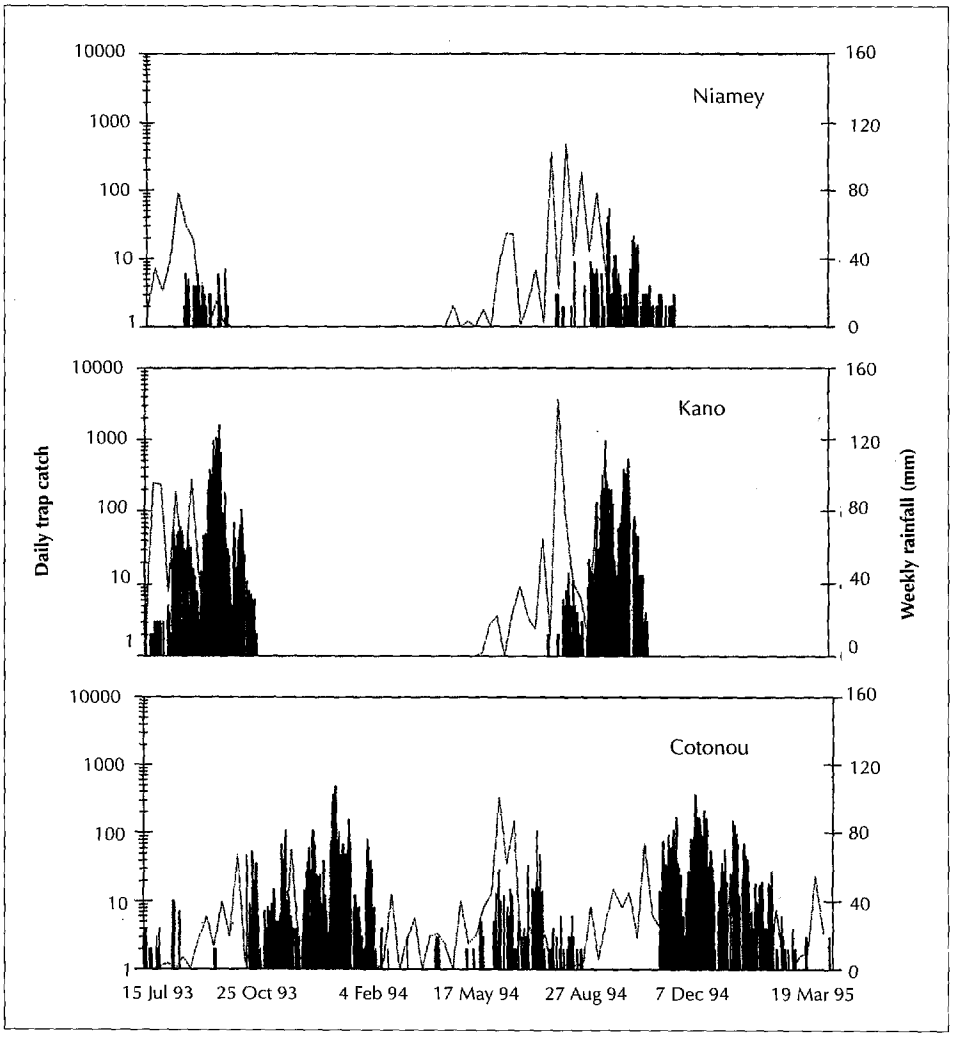

Figure 3. Daily light trap catch (S–N) of *Maruca vitrata* moths (vertical bars) and daily rainfall (mm) (dotted line) in Cotonou (Republic of Benin), Kano (Nigeria), and Niamey (Niger) from July 1993 to March 1995.

Hadejia wetlands (Hanssens 1993). Late-planted DS cowpea risks heavy infestation by flower thrips that are carried by southwesterly winds in early May, as shown by our sticky traps and field sampling. A varied complex of pentatomids, pyrrhocorids, and lygaeids may invade the crop temporarily during flowering and early podding, but because they also feed on extra-floral nectaries, the extent of pod damage is not clear.

Pests of WS cowpea

The major WS pests of cowpea are *M. vitrata*, flower thrips, and *C. tomentosicollis*. These are also the pests that are most destructive to cowpea grain production because they feed on

flowers and pods. *M. vitrata* is probably a migratory pest because: (1) it does not diapause during the DS (Taylor 1967); (2) we did not find it associated with any alternate hosts or cowpea during the DS in northern Nigeria; and (3) adult moths fly in at the onset of the rainy season, when cowpea is still in the vegetative stage. Tamò (unpublished data) found *M. vitrata* feeding on flowers of leguminous trees (*Pterocarpus* sp. and *Lonchocarpus* sp.) during the DS along streams and rivers as far north as Parakou in the Republic of Benin and Abuja in Nigeria. It is also present in southeastern Nigeria throughout the dry season (L.E.N. Jackai, unpublished). Atachi and Djihou (1994) found 22 host plants distributed in eight families, of which 77% are leguminous. Although these authors considered *M. vitrata* a polyphagous feeder, it is more likely an oligophagous insect. However, the apparent scarcity of these hosts during the DS in northern Nigeria and/or unfavorable climatic conditions limit the DS survival of *M. vitrata* to more southern regions.

Rainfall in Nigeria below latitude 9 °N (Bida) follows a bimodal pattern: the first season is from April to July, and the second is from September to November (Griffiths 1972). Earlier results from light trapping in Ibadan (Taylor 1967) showed that in populations of *M. vitrata*, flight activity follows a bimodal pattern, corresponding to the early and late planting season, with peaks in July and December. Akingbohungbe (1982) also reported two periods of peak activity of cowpea pests, including *M. vitrata*, at Ile-Ife in southern Nigeria: from April to July and October to December. This bimodal population pattern was confirmed by Alghali (1993b), who also found that larval counts are significantly related to cumulative rainfall and number of rainy days but stressed that the even distribution of rainfall over time is more crucial.

Very low infestation levels of *M. vitrata* (< 1 larvae/100 flowers) were found on DS cowpea planted in the fadamas in Bida (Alghali 1991a), suggesting that the northern limit of DS survival coincides with the northern limit of bimodal rainfall in Nigeria. Bimodal rainfall may not have a direct impact on *M. vitrata* populations, but it probably dictates the distribution of important host plants (such as *Pterocarpus* sp. and *Lonchocarpus* sp.) in the off-season. *Maruca vitrata* moths were never collected on our high-altitude sticky traps. Therefore, the moths probably fly at lower altitudes within the surface boundary layer and can, therefore, only make short flights. In contrast, *Heliothis* moths fly above the surface boundary layer and are considered long-distance migrants (Farrow and Daly 1987).

Maruca vitrata populations probably move from south to north over a period of several months or generations, following the northward progression of rainfall, cowpea planting, and possibly the flowering pattern of leguminous trees. The farther north, the later the moths arrive; also, the fewer the generations that can be completed, the lower the population buildup. Our light trap records from Niamey and Kano and field surveys from Nguru and Minjibir give some credence to this hypothesis. *M. vitrata* does not survive the DS in the north, even if cowpea is available in the fadamas, possibly because of some unfavorable climatic conditions other than the absence of rain, such as temperature or relative humidity. The upper and lower temperature thresholds for *M. vitrata* are 15.6 and 34 °C, respectively (Jackai and Inang 1992). In Kano, the minimum temperature during December and January averages 13.0 °C; in May the maximum temperature is 38.0 °C (FAO 1984). Relative humidity is lower during the DS and may also play a role.

Flower thrips are known to survive the DS in the southern Benin Republic on a wide range of alternate hosts (Tamò et al. 1993). However, unfavorable temperature extremes

in northern Nigeria may suppress populations of flower thrips during the DS. Temperatures < 15 °C and > 35 °C severely reduce survival of all developmental stages of flower thrips (Tamò 1991). Alghali (1991b) attributed crashes in thrips populations to mean daily temperatures of > 30 °C and scotophases of less than 18 h. Our sticky trap records show that flower thrips can potentially cover large distances on prevailing winds and invade the north around the end of May, when suddenly large populations of adult thrips show up in DS cowpea fields. When the intertropical convergence zone (ITCZ) passes through northern Nigeria from south to north, which normally occurs around April–May, wind direction shifts from NE to SW. Wind direction reverses to NE again around November when the ITCZ moves southward (Griffiths 1972; Udo 1982; Grove 1989). The northward movement of the ITCZ may explain the sudden influx of flower thrips in May.

Populations of *Clavigralla tomentosicollis* were also very low on cowpea during the DS, but they increased rapidly during the WS. Their pest status may also be related to migratory movement from southern refugia and sensitivity to unfavorable climatic conditions during the DS. Jackai and Inang (1992) reported temperature thresholds of 18.5 and 37 °C for *C. tomentosicollis*. Our data on PSB are from a single location only and should, therefore, be interpreted with caution.

Implications for pest management

Different pest management strategies are required for DS and WS cowpea, because DS cowpea is exposed to a drastically reduced pest complex than WS cowpea. Also, DS cowpea is grown in monocultures, while WS cowpea is grown in much lower densities in mixtures with millet and/or sorghum. For DS cropping, varieties with aphid resistance (Ansari et al. 1992; Ofuya 1993) and favorable agronomic traits will be important. Insecticides may not be required because *M. vitrata* is absent and flower thrips are of minor importance. Alghali (1991a) found that insecticides did not increase grain yield of cowpea grown on fadamas during the DS in Bida.

Pest management is more complex in WS cowpea. To harvest an acceptable grain yield, chemical insecticides are necessary to control flowering pests (Amatobi 1994, 1995). However, chemical control for cowpea may not be feasible in traditional mixed row systems because of (1) its low yield potential in such systems, and (2) difficulty in applying insecticides to the lower-growing cowpea plants (Norman et al. 1982; Fischer et al. 1987; Ampong-Nyarko et al. 1994). Mixed cropping may reduce cowpea aphids (H. Bottenberg, unpublished data), thrips (Ezueh and Taylor 1984; Kyamanywa and Ampofo 1988; Alghali 1993a; Kyamanywa et al. 1993), and pod-sucking bugs (Alghali 1993a). It does not, in general, reduce damage by *M. vitrata* (Ezueh and Taylor 1984; Gheti and Khaemba 1985; H. Bottenberg, unpublished data), though there are contradictory reports from East Africa for some crop combinations (Amoako-Atta et al. 1983; Karel 1993).

Cowpea fodder provides nutrition for farm animals during the DS, which in turn provide milk and meat for human consumption (Norman et al. 1982). Fodder production is not reduced by flower thrips and *M. vitrata* damage (Suh and Simbi 1983); it may, in fact, be stimulated because photosynthates that would have been invested in flowers and pods are used for foliage. Alghali (1991a) found that fodder production was enhanced by not applying insecticides. In traditional farming systems, grain and fodder varieties are planted in relay with millet or sorghum. When pest attack is heavy and grain yield is minimized,

fodder production for animal nutrition guarantees the supply of animal protein for the human diet. Sustainable pest management for WS cowpea should, therefore, include traditional varieties and farming practices. Variation in planting time in such cropping systems could be further explored, as it may have some scope in avoiding *M. vitrata* attack (Taylor 1967; Akingbohungbe 1982; Alghali 1993b). In northern Nigeria, planting early-maturing cowpea in September (at the end of the rainy season, after harvest of the cereal crop) could be a feasible option (Blade and Singh 1994). However, risk of late-season drought may limit the adoption of this practice.

DS cowpea has a high grain production potential because flowering pests are minor and the crop can be planted in much higher densities than WS cowpea. In Nguru, the average yield of cowpea in pure stand in the 1990–91 DS was 855 kg/ha (Hanssens 1993). In a farmer-participatory trial in Minjibir during the 1993 DS, cowpea var. 89KD-941-1, planted on residual moisture along an irrigation reservoir, averaged 691 kg/ha. These yields compare very favorably with those obtained from mixed crops (25–60 kg/ha) in farmers' fields or sole crops (150–250 kg/ha) on station (Blade and Singh 1994). The major pest of DS cowpea is aphids, which can be easily controlled with resistant cultivars. WS cowpea pests, particularly those that attack flowers and pods, are more difficult to manage because resistant cultivars are still scarce, biological control is ineffective, chemical control is often not economically viable, and cultural control is of limited value. DS production for cowpea grain on residual moisture and on irrigated land should be further studied and promoted.

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Vigna unguiculata germplasm evaluated for resistance to insects

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Abstract
Germplasm accessions and advanced lines were evaluated for resistance to cowpea curculio, *Chalcodermus aeneus*, southern green stink bug, *Nezara viridula*, and leaffooted bug, *Leptoglossus phyllopus*, under natural infestations in southeastern Alabama, USA. No insecticide was used. Based on prior knowledge of performance, we screened 300 entries from the following sources: 79 from the IITA advanced breeding lines; 100 accessions being evaluated in the cowpea breeding program at University of California (UC) Riverside, USA; 101 plant introductions previously reported to have resistance to cowpea curculio or other insects or diseases; and 20 check lines with known resistance or susceptibility to cowpea curculio, pod bugs, or cowpea storage bruchid, *Callosobruchus maculatus*.

Introduction

Cowpea curculio, *Chalcodermus aeneus* Boheman, is the most serious insect pest of cowpea in southern USA, where most of the US crop is grown and marketed as a fresh, frozen, or canned product (Fery 1990). Adequate control is necessary because the presence of immature larvae in the frozen or canned product constitutes a contaminant. Economically feasible control is difficult to attain and may become impossible if restrictions are placed on the insecticides now in use. The *Vigna* Crop Advisory Committee to the USDA National Plant Germplasm System has identified cowpea curculio resistance as a characteristic important enough to assign it top priority in germplasm evaluation projects. Higher levels of resistance to cowpea curculio than currently available are needed to enhance control and decrease the overuse of insecticides. There is evidence of three bases for the resistance of cowpea to the curculio: (a) antixenosis (nonpreference), (b) antibiosis, and (c) pod wall/factor deterrence, as yet not classified as either (a) or (b). (Cuthbert and Davis 1972; Cuthbert et al. 1974). Pod strength has been suggested as one characteristic contributing to pod factor resistance (Ennis and Chambliss 1976; Rymal and Chambliss 1979; Hossain 1983). In recent years, the southern green stink bug, *Nezara viridula* Linnaeus, and the leaffooted bug, *Leptoglossus phyllopus* (L.), have caused very heavy damage to our research plots. In some cases of high infestation, total loss occurred due to flower abscission and pod dehiscence or ovule abortion in young pods. At lower infestation levels, seeds were misshapen and blemished to the extent of being unmarketable.

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Materials and methods

Germplasm screened

Germplasm accessions known to be of value for insect resistance, disease resistance, or other attributes were screened for resistance to insects in field plots in southeastern Alabama, having high natural populations of cowpea curculios and pod bugs. Based on past performance, we screened 300 accessions from the following sources: 79 breeding lines from the IITA advanced trials; 100 accessions being evaluated at the University of California (UC) Riverside, USA; 101 plant introductions with known resistance to cowpea curculio, or other insects or diseases; and 20 check lines. The list of these entries is available upon request. Cowpea curculio resistant checks were AU85-CCR-20, Freeze-green, CR-17-1-13, CR-18-13-1, CR-22-2-21, and PI 255815; susceptible checks were PI 343449, California Blackeye No. 5, and TARS 36. Cowpea storage bruchid resistant checks were IT-81D-1137, IT-84D-449, IT-84D-460, IT-86-472, IT-86D-534, and IT-86D-641. Lines resistant to southern green stink bug were PI 293476, PI 293557, and PI 293570. TVu-1890 was the resistant check to the pod sucking bug (*Clavigralla tomentosicollis* Stal.), and VYA the susceptible check. Entries were grown in 0.9 × 4.5 m plots, in a randomized block design with 3 replications. Plots were planted in mid-June to synchronize pod development with the natural cowpea curculio population (Arant 1938). During pod maturation, adult insect densities were estimated by sampling plots in an adjacent field of susceptible California Blackeye No. 5. There were ~ 17600 adult curculios and 30,000 pod stink bugs/ha (Sudbrink 1992, unpublished data). No insecticide was applied.

Insect data collected

An assessment of pod bug damage to immature pods was made, by counting the shriveled and sound pods in a 0.6 m section of the plot. We did not distinguish between damage caused by stink bug and leaffooted bug, because both species were present and caused similar damage to pods and seeds. Seed damage by pod bugs was assessed on dry seed samples after harvest, when most of the pods in a plot were dry. A sample of ~ 100 dry pods per plot was taken to provide insect damage data. Curculio larvae were collected as they emerged from these samples, oven dried to a constant weight, and average larval weights determined.

Cowpea curculio damage data were collected from two subsamples. One sample had 25 pods taken randomly from the plot sample of dry pods, and the other sample had a selection of 10 pods with pod punctures caused by adult curculios. The selected sample was to determine the genotype effect on overall survival in pods known to have been punctured by adult curculios. These data would give some general indication of antibiosis and tend to discount preference, since only damaged pods were included in this sample. Data collected from the 25 pod sample included a number of larval exit holes in the pod walls, as well as average pod length and pod weight. Data collected from the 10-pod sample included pod punctures due to adult curculio feeding/oviposition, seed damaged by curculios and pod bugs, sound seed (undamaged by insects), exit holes made by curculio larvae leaving the pod to pupate, and pod strength required (in kg) to puncture a pod on an Instron testing machine (Model 1122 Food Testing System) with a 1-mm diameter probe.

The above data were used to calculate pod insect resistance scores, which identify germplasm having potential for insect resistance breeding programs. Components used to arrive at this score and their weighted values (in parentheses) were as follows: exit holes per 25-pod sample (3), exit holes per 10-pod sample (3), percentage of sound seed (3), percentage of curculio-damaged seed (3), pod punctures per exit hole (1), curculio-damaged seed per pod puncture (1), exit holes per pod puncture (1), percentage of sound pods in field plots (1), pod strength (1), and average larval weight (1). The top-ranking 25 lines for each component were assigned weighted values for each component. The sum of the weighted values received by each of these top lines across all components resulted in the pod insect resistance scores by which top lines were ranked.

Results and discussion

Cowpea curculio resistance

Results were obtained from 288 of the 300 entries in the evaluation. Larval exit hole data is a measure of the overall curculio resistance of an entry, regardless of the nature of the resistance. A few exit holes per pod indicates that either a few eggs were deposited in the seed or a few curculios survived, for whatever reason, to exit from the pod to pupate. About 60% of the germplasm entries were distributed in almost equal frequencies in class intervals from 6 to 12 exit holes/25 pods with 48 of the 288 entries in the most frequent class, 8 (Fig. 1). Known curculio resistant lines had 7.7 or less exit holes/25 pods. Among six check lines with known curculio resistance (Table 1), AU 85-CCR-20 ranked in the top 1%, Freezegreen ranked in the top 8%, and CR-18-13-1 and CR-22-2-21 ranked in the top 20% of the entries, with 2.0, 4.3, 6.7, and 7.0 larval exit holes/25 pods, respectively. Only

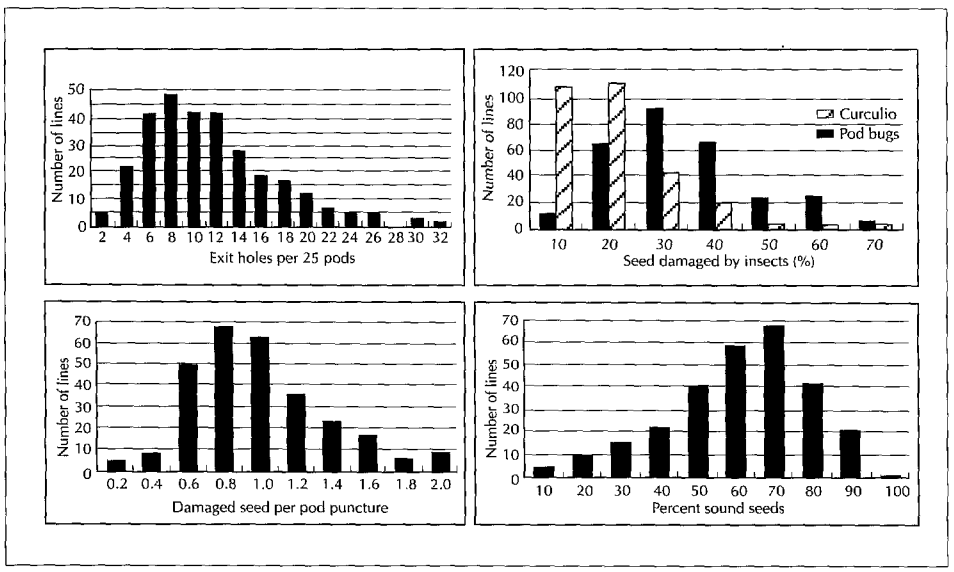

Figure 1. Frequency distributions for variables evaluated in screening *Vigna unguiculata* germplasm for resistance to cowpea curculio and stink bugs, Headland, Alabama, USA, 1992.

Table 1. Highest ranking entries in the evaluation of *Vigna unguiculata* germplasm for resistance to cowpea curculio and pod bugs, Headland, Alabama, USA, 1995.

Rank	Description	100-seeds (wtt. g)	Seed description	Pod length (cm)
1	Bambey-5	12.2	Large, speckled + hilar ring, kidney	17.5
2	IT82D-713	9.6	Medium, browneye + bicolor, rhomboid	13.0
3	UCR194=BBR-42	8.7	Small, black/white bicolor, kidney	17.8
4	PI 218122	4.5	Small, brown, kidney	10.9
5	TVu-3046	6.3	Small, brown, kidney	11.9
6	UCR200=24-1A	5.3	Small, cream, kidney	13.5
7	PI 115679	7.3	Small, black, kidney	17.8
8	PI 145198	6.2	Small, brown, marbled, ovoid	15.0
9	AU85-CCR-20	5.7	Small, cream, globose	10.7
10	UCR-202	7.5	Small, brown, kidney	14.5
11	IT83S-911	16.5	Large, browneye + bicolor, kidney	21.6
12	VITA-5	8.8	Medium, brown hilar ring, rhomboid	15.2
13	Charodi	5.5	Small, brown, rhomboid	11.2
14	N'Diambour	14.2	Large, brown hilar ring, kidney	16.0
15	UCR347=MN-150	12.6	Medium, brown and white bicolor, kidney	16.3
16	IT86F-2062-5	10.1	Medium, brown + bicolor, kidney	23.6
17	PI 175959	8.6	Small, brown, ovoid	15.5
18	PI 293467	8.9	Small, brown, marbled, ovoid	13.7
19	CPI-30783	9.6	Medium, blue, marbled, ovoid	16.8
20	PI 142779	8.2	Medium, brown, marbled, ovoid	14.2
21	PI 255815	7.3	Small, black hilar ring, kidney	14.7
22	PI 148674	8.7	Medium, brown, ovoid-rhomboid	16.0
23	PI 214354	4.3	Small, brown, kidney	10.7
24	PI 353074	8.8	Medium, brown and white bicolor, kidney	26.7
25	PI 189374	6.9	Small, brown, kidney	12.7
26	UCR189=BBR-23	6.3	Small, brown, kidney	18.5
27	IT82D-380-5	9.1	Small, black hilar ring, kidney	17.8
28	UCR-240	10.5	Small, black hilar ring, kidney	17.8

one entry, PI 218122, had less exit holes than AU 85-CCR-20, with 1.4/25 pods. The most susceptible entry, IT82E-41, had 46 holes/25 pod sample, almost 2 exit holes/pod. About 72% of the entries sustained more curculio damage (as expressed by larval exit holes/25 pods) than the best of the resistant check lines (those with 7.7 or less exit holes/25 pod sample). Of the eight lines reported to have pod resistance (Kitch et al. 1991) to cowpea storage weevil (92.3–99.9% larval mortality) evaluated for curculio resistance, four ranked in the top 27%, with 7.7 or less exit holes/25 pods.

Similar data were obtained for larval exit holes on the 10-pod samples having pod punctures selected for comparison with the 25-pod sample taken at random, without predetermined pod damage (Fig. 1). A higher number of exit holes per pod was apparent in the data from the selected 10-pod sample, as compared to the 25-pod sample (0.6 compared to 0.3 for the most frequent class in the distribution). Since all pods in the 10-pod sample had been punctured by adult curculios, there was a higher probability of larvae being in the pod and surviving to exit the pod than in the 25-pod sample in which some pods were not damaged by adults, by chance. The distributions are similar for both sets of data. Among

the entries ranked in the top 10 for larval exit holes, five entries were in the top 10 in both the 25-pod sample and the 10-pod sample. These were Charodi, PI 218122, Vita-5, Bambey-5, and TVu-3046. Our most resistant check, AU85CCR-20, ranked second in the 25-pod sample and 14th in the 10-pod sample. Among the entries ranked in the bottom 10 (those with the highest number of exit holes), IT86D-792 was the only one ranking this low in both the 25-pod sample and the 10-pod sample. It ranked second from the bottom with 40 exit holes in the 25-pod sample, and third from the bottom with 25 exit holes in the 10-pod sample.

The average number of punctures per pod gives an indication of the extent of anti-xenosis or nonpreference. Entries with very few pod punctures are not attractive to curculio for feeding or oviposition. Slightly > 75% of the entries had 3–7 pod punctures/pod. Five entries, the most resistant based on antixenosis, had only 1 puncture/pod. The 12 entries which had 11 or more punctures/pod were most preferred by insects, and thus the most susceptible.

A reduction in the number of damaged seeds per pod puncture is an indication of pod-factor resistance (Fig. 1). These data show that some factor(s) in, or on, the pod interfere(s) with the attempts of the adult curculio to feed and/or oviposit in the seed. Our past observations of known resistant lines are that only ~ 25% or less of the punctures made in the pod wall by the adult result in seed damage. Less than 2% of the entries in this evaluation would be classified as pod-factor resistant by that criterion. We observed in past evaluations that susceptible lines usually sustain seed damage from ~ 75% or more of pod punctures. The most frequent class in the distribution is 0.8 (80%) seed damage per pod puncture.

Few entries expressed an antibiosis effect, based on average weights of larvae recovered as they exited pods to pupate. Average larval weight for > 85% of the entries was above 7 mg, the most frequent class being 7.5 mg. When the rankings for larval weight and larval mortality are compared, they seem unrelated, with one exception. PI 189378 had a low larval weight and a higher mortality (29.2%) than other lines. This level of mortality should not be considered high as it relates to an antibiosis effect. Only 11 lines (3.8% of the entries) had > 25% mortality. The highest mortality was 35% in two lines, PI 165493 and IT 82E-32. Among the ranking lines in the curculio resistant group, which had very low numbers of exit holes per pod puncture, were PI 175959, Bambey-5, N'Diambour, 83S-899, PI 255815, UCR 202, AU85-CCR-20, UCR 200, UCR 191, and Freezegreen. In most cases, lines with few exit holes per pod puncture indicated repeated attempts by adults to feed and/or oviposit, resulting in large numbers of pod punctures, but with limited success in oviposition or larval survival.

Those in the resistant group with few damaged seed per pod puncture were IT82D-713 and 83S-899. Other lines with < 0.30 seed damage per pod puncture were 86D-371, 83S-911, UCR 194, PI 293573, PI 170869, and IT90K-59. We have observed the relationship of increasing pod strength and decreased success of the adult curculio to damage and oviposit in seed. Pod strength contributes to pod-factor resistance (Rymal and Chambliss 1979; Hossain 1983). Pod strength of entries in this evaluation ranged from a high of 0.47 kg to a low of 0.07 kg. Our resistant check, Freezegreen, was in the upper 33% of the entries, with pod strength of 0.28 kg. The known susceptible, California Blackeye No. 5, had a pod strength of 0.19 kg. The three entries which had the highest pod strength were PI 293467,

PI 293468, and PI 293514, with 0.47, 0.4 and 0.46 kg, respectively. Two of these lines were derived from Brabham, an old USA ety known for its curculio resistance, and the other line is the old USA variety, Groit, known to be resistant to curculio (Cuthbert and Chambliss 1972).

About 77% of the entries sustained 20–40% seed damage from curculio (Fig. 1). IT82-D 713, BBR 42 (UCR 194), and MN 150 had the lowest percent damaged seed: 3%, 4%, and 6%, respectively. Other entries which had losses of < 10% (ranked in increasing order) were: PI 189374, TVu 3046, BBR 23 (UCR 189), 24-1A (UCR 200), CPI 30783, PI 218122, PI 353074, UCR 240 (754), and AU 85 CCR-20. The entries sustaining the most seed damage (64–70%) from curculio were Bambey-21, IT 88DM-361, IT 82E-56, 87D784-1, and IT 82E-41.

Pod bug resistance

Resistance to pod bugs was evaluated, in part, from percent sound pods. Resistant lines (checks) produced ~ 90% or more sound pods in field plot counts. The best southern green stink bug resistant check, PI 293476, produced ~ 96% sound pods, and the pod sucking bug resistant check, TVu 1890, produced 94% sound pods. Three of the entries, PI 194207, CPI-30783, and TVu-354, ranked higher than the best check, producing 96%, 97%, and 99% sound pods, respectively. Among the curculio resistant lines, PI 142779, PI 175959, PI 293468, and N'Diambour had 10% or less of their pods destroyed by pod bugs. The resistant check lines TVu 1890, PI 293476, PI 293570, and PI 293557 had pod strengths of 0.36, 0.27, 0.27, and 0.26 kg, respectively. In addition to contributing to pod factor resistance for curculio, higher pod strength also appears to be a contributing factor to pod bug resistance.

Some 91% of the entries had 30% or less seed damage from pod bugs (Fig. 1). Among the curculio resistant lines, GUJ-1 (UCR 168), Bambey-5, PI 218122, TVu 3046, PI 353074, and UCR 202 produced the highest percent sound seed (> 80%).

Percentage sound seed is a measure of the overall performance under both biotic and abiotic stress in the field. It is noteworthy that ~ 74% of the entries produced 50–80% sound seed (Fig. 1). Lines which showed > 80% sound seed could provide new germplasm having higher resistance to insect pests (and perhaps abiotic stress) than the resistant check lines used for this evaluation.

Using the pod insect resistance scores calculated from the characteristics measured in this evaluation, entries were ranked from most to least resistant. The 28 lines with the highest scores (i.e., ~ 10% selection intensity) can be considered to have potential use in breeding programs for improving the level of insect resistance (Table 1).

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Indigenous knowledge and cowpea pest management in sub-Saharan Africa

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Abstract

We review studies on indigenous knowledge of cowpea pest management in sub-Saharan Africa, considering both field and postharvest insect pests. Farmers appear to be well aware of the insect and non-insect pests inflicting losses, and to have accurate perceptions of the degree of damage caused by insect pests. While nearly all farmers employ control measures to reduce storage losses, few farmers report using control measures against field insects. Postharvest pests were controlled primarily with traditional methods, including ash, ash/herb mixtures, storage in pods, and smoking or heating. Control measures for field insects included agronomic/cultural practices (plot location, crop rotation, burning of cleared land, timing of weeding, and mixed cropping strategies), and direct control measures, either traditional (hand picking/killing, removal of infested plants, use of local herbs) or modern (insecticides).

Our review indicates that (1) knowledge of many traditional production and pest control practices is in danger of being lost; (2) non-insect pest constraints (rodents, animals, diseases, weeds, drought, etc.) are perceived by many farmers to be more important than insect pests; and (3) considerations of gender are essential in working with indigenous knowledge systems in Africa. Why did so few farmers report taking control measures against field insect pests? Our studies suggest that farmers place a high value on the use of cowpea for fodder, leafy vegetable consumption, and soil conservation. From the more holistic viewpoint of the farmer, concentration on fodder and leafy vegetable production may in fact be viewed as a means of controlling (or avoiding) losses to field insects, by emphasizing utilization of plant parts which are relatively undisturbed by insects. We also outline needs for future research, to be carried out by teams of biological and ethnoscientists.

Introduction

In agricultural development, it is important to understand indigenous knowledge systems (IKS) because they govern the local-level decision making processes of a culture or society. A creative synthesis of indigenous agricultural knowledge with science is coming to be regarded as a cornerstone for sustainable development.

An essential starting point for development efforts in the low-resource agricultural areas of Africa is research to better understand indigenous or local-level knowledge. This recognition is a reflection of (1) the comparatively weak impact of agricultural research on

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African agriculture to date, and (2) the increasing concern of the world scientific community that research efforts foster diversity and sustainability of the agricultural resource base.

A general revitalization of traditional agriculture is viewed as perhaps the most effective means of coupling higher productivity with ecological considerations (OTA 1988). That revitalization will require two efforts: (1) a clearer understanding of the indigenous knowledge systems, which shape the agricultural decision making processes of local farmers; and (2) the input of additional technology from the scientific community. The Office of Technology Assessment (OTA) of the US Congress (1988) states that "technological innovation to enhance low-resource agricultural systems will be a major factor in determining Africa's ability to meet the challenges ahead," and that "the technological framework with the most promise for promoting food security in Africa calls for an evolution of existing agricultural systems." A key task for researchers and development workers is to find ways to bring indigenous agricultural knowledge into creative synthesis with science.

Low-resource agriculture is the predominant form of agriculture in sub-Saharan Africa. It is the major source of food production, employment, and rural income. Farmers in low-resource areas of sub-Saharan Africa strive to minimize risk rather than maximize yields; they depend on local knowledge and renewable biological resources. Their farming systems involve low cash costs but often require relatively high amounts of labor, they are adapted to local cultures and environments, and they are highly diversified, producing a wide array of crops and several types of livestock. This diversification helps provide food throughout the year, reduce the risk of total crop failure, and modulate peak labor demands (OTA 1988).

In low-resource production systems, cowpeas are grown in mixtures with cereals, root and tuber crops, or other legumes. Due to severe losses to field insect pests, cowpea grain yields in these systems seldom exceed 100 to 150 kg/ha (Singh and van Emden 1979; Jackai and Daoust 1986). Postharvest insect pests are responsible for yet additional losses, which can exceed 87% by weight (Caswell 1968). Given the clear importance of insect damage in cowpea production, it is logical to suppose that local farmers have developed indigenous technologies or practices to cope with this problem.

There have been a number of studies conducted to better understand indigenous knowledge (IK) pertaining to cowpea pests and their control. In this review, we focus on studies carried out in Nigeria, Cameroon, and Kenya, concerned with both field and postharvest pests. We consider IK as reflected in production practices, perceptions, and knowledge of insect and non-insect pest constraints, and the control methods utilized. Our objective in this review is to (1) provide an overview of the concept of IKS; (2) to review work that has been done to better understand IK as it pertains to cowpea pest management in sub-Saharan Africa; and (3) to discuss ways in which this IK can be used in developing new technologies.

Indigenous knowledge systems

"Indigenous knowledge systems are learned ways of knowing and looking at the world. They have evolved from years of experience and trial-and-error problem solving by groups of people working to meet the challenges they face in their local environments, drawing

upon the resources they have at hand" (McClure 1989). Indigenous knowledge is dynamic and is shaped by indigenous creativity, innovativeness, and contact with other knowledge systems (Warren 1989).

Embedded in the concept of IK is the assumption that things are the way they are for good reasons, and that we need to understand the dynamics of the indigenous knowledge systems before recommending changes that may or may not improve local conditions (McClure 1989). In agricultural development projects, it is important to understand indigenous knowledge systems because (1) they govern the local-level decision making processes of a culture or society, thereby forming the framework within which productive farmer participation can be fostered; (2) they often offer the best solutions to technical problems; and (3) solutions developed without regard to IKS may be inappropriate (Warren 1989).

Cowpea production and utilization in sub-Saharan Africa

Cowpea production in sub-Saharan Africa is dominated by smallholder low-resource farmers. The majority of farmers grow cowpea in traditional mixed cropping production systems. Sixty-seven percent of the farmers interviewed by Wolfson et al. (1990) in northern Cameroon intercropped cowpea with sorghum, millet, cotton, peanut, bambara groundnut, and sesame or in a mixture of these crops. The same authors also found that the median interplant distance was 1 m, but ranged from 0.5 m to 3 m. Eighty-four percent of the farmers they interviewed rotated their fields, and those who could not rotate fields rotated crops within rows. The average household in northern Cameroon devoted about 1 ha to cowpea production (Wolfson et al. 1990). Alghali (1991) reported that the average farm size in Minjibir, Kano state, northern Nigeria (Sudan savanna), was 3.47 ha, with cowpea occupying 30–50% of the land area. Production area in villages further south in Nigeria (in the Guinea savanna and forest zones) averaged about 1/3 ha (Alghali 1991). In the three villages surveyed by Alghali (1991), the majority of farmers grew cowpea in mixed cropping systems. Similarly, in Kenya, Saxena et al. (1989) reported that 95% of the farmers interviewed grew cowpea as an intercrop.

Cowpeas are utilized for a variety of purposes, as described in studies cited here (Saxena et al. 1989; Wolfson et al. 1989; N'tare 1990; Alghali 1991) and elsewhere in this volume (Nielsen et al. 1997; Singh et al. 1997; Tarawali et al. 1997).

Farmer knowledge and perceptions of pests (constraints)

Field insect pests

Farmers are generally quite knowledgeable of the pests that damage their crops. Several studies (Saxena et al. 1989; Alghali 1991; Chitere and Omolo 1993; Bottenberg 1995) have reported that farmers are able to identify (by local names) specific insect pests, the crop species they damage, and the type of damage. Atteh (1984) interviewed 120 farmers from 4 villages in Kwara state of Nigeria, and reported that all farmers were able to name each of 25 different insect species and also identify which ones damaged crops. Bottenberg (1995) in northern Nigeria reported that nearly one-half of the farmers he interviewed correctly identified cowpea aphids, and different species of pod sucking bugs. Similarly, Alghali (1991) reported correct identification of pod bugs by 64% of interviewed farmers in the area of Bida, northern Nigeria. Saxena et al. (1989) reported that in a survey of 150

farmers in Kenya, as many as 48% were aware that some insects kill insect pests (beneficial insect predators).

There is also evidence that farmers have accurate perceptions of the degree of damage caused by field insect pests. Saxena et al. (1989) found that farmer estimates of cowpea losses due to insects closely approximated on-farm scientist observations of actual losses.

Non-insect pests (constraints)

The importance of non-insect pest constraints was stressed in several studies. In Kenya, nearly 50% of the farmers surveyed by Chitere and Omolo (1993) considered damage due to non-insect pests to be greater than that from insect pests. Similarly, Alghali (1991) found that as many as 33% of the farmers in the Bida area considered non-insect pests more important than insects. Non-insect pest constraints are undeniably very important in these traditional production systems because they can affect yields of several crops in the system. Non-insect constraints listed by farmers are numerous and include birds, rodents (rats, mice, moles), other animals (gazelles/antelopes, porcupines, squirrels, monkeys, mongooses, rabbits), plant diseases, weeds (including *Striga*), drought, poor germination, bad climate (rainfall—too much, too little), fire, sickness, enemies, lack of labor to weed, etc. It is important for entomologists to be aware of these additional farm-level pressures or constraints (as perceived by farmers) when considering cowpea pest management technologies that farmers would find acceptable.

Postharvest insect pests

Farmers are almost universally able to identify the primary storage insect pest of cowpea, *C. maculatus*, and many can differentiate between *C. maculatus* and *Bruchidius atrolineatus*, a pest of secondary importance (Wolfson et al. 1989, 1990). Farmers are well aware of the losses they suffer in storage and, in many cases estimate that everything will be eventually lost if no control measures are taken (Saxena et al. 1989; Wolfson et al. 1990; Goldman 1991).

Pest control practices

Field insect pests

In the earliest survey paper that we reviewed (Atteh 1984), all of the 120 farmers interviewed in Kwara state, Nigeria, reported using either traditional methods or pesticides to control their field insect pest problems (Table 1). Those farmers employed a number of integrated pest control/farming practice methods (covert measures, meaning those employed for reasons other than just insect control) and although pest control was not the sole reason for these farming practices, farmers were clearly able to articulate the linkage between pest control and these farming practices. Covert control measures employed by farmers included (1) farm plots grouped together to avoid isolated plots, and to spread pest risk among many farmers; (2) plots and crops rotated to avoid insect pest buildup; (3) newly cleared land burned to reduce pest populations; (4) field weeding timed to interfere with the egg-laying and breeding times of the major pests; and (5) multicropping and mixed cropping strategies based on the fact that different crops are attacked by different pests, and believed to prevent buildup of a single pest to an unacceptable level. Direct control measures employed included use of herbal concoctions as seed dressings, herbal

Table 1. Principal insect field pest control practices cited by farmers in various studies.

Covert measures (agronomic or cultural practices)	Direct measures
Alghali (1991)†	
Weeding around surrounding plots	Manual removal
	Chemical insecticides
Atteh (1984)§	
Farm plots grouped together (avoid isolation)	Use of herbal concoctions
Plot and crop rotations	Burning dried herbs
Burning newly cleared land	Insecticides
Weeding to interfere with egg laying	
Mixed cropping strategies	
Bottenberg (1995)†	
	Sprinkling ash on plants
	Beating with branches
	Manual removal
	Apply manure + petrol or kerosine
	Chemical insecticides
	Praying
Saxena et al. (1989)†	
Planting early and simultaneously	Manual removal
Removal and destruction of crop residues	Use of resistant varieties
Mixed cropping strategies	Removal of infested plants
Crop rotation	Insecticides of plant origin
	Chemical insecticides

† The average percentage of farmers who reported using no control measures for field insect pests were as follows: Bottenberg 1995, 80%; Saxena et al. 1989, 73.5%; Alghali 1991, 64%.
§ Control methods were employed by all farmers interviewed.

smoke, and pesticides. At the time of the Atteh surveys (1981), pesticides were much more widely available and affordable in Nigeria, and nearly 80% of the interviewed farmers reported using them.

In contrast to the 1981 findings of Atteh, surveys conducted in the 1990s in Nigeria found that the majority of farmers were taking no active measures to control field pests. In a study by Alghali (1991), roughly 64% of the farmers interviewed reported taking no control measures. Of the remaining farmers, about 28% used insecticides, and in one village, 21% of the farmers practiced weeding around the surrounding plots for control (Table 1). Farmers in the Alghali survey did not report use of any agronomic or cultural practices to control field pests. On average, 80% of the farmers interviewed by Bottenberg (1995) reported taking no control measures. Pest control practices cited by farmers in the Bottenberg survey (1995) included insecticides (reported by roughly 10% of farmers), beating with branches, praying, manual removal, and applications of ash and/or manure + petrol (Table 1). No agronomic or cultural practices for controlling pest populations were reported. Similarly, an average of 73.5% of Kenyan farmers surveyed by Saxena et al. (1989) reported taking no control measures for field pests. Control measures, when employed (Saxena et al. 1989), included both agronomic/cultural practices (covert measures) and direct measures (Table 1).

Bottenberg (1995) discusses possible reasons for the low percentage of farmers who report using field pest control in Nigeria. One hypothesis is that partial subsidies of pesticides during the 1970s and 1980s led to their widespread use (80% of the farmers inteviewed by Atteh [1984] reported using pesticides) and subsequent loss of knowledge of traditional methods. This hypothesis is supported by the findings of Alghali (1991). A second factor, according to Bottenberg (1995), is the underreporting of the use of traditional methods because they may be perceived by farmers as backward or primitive.

An additional explanation for the limited use of field pest control measures relates to how the cowpea crop is utilized. Several studies report that farmers place a high priority on cowpea fodder production (Saxena et al. 1989; N'tare 1990; Wolfson et al. 1990; Alghali 1991). In certain areas, farmers even plant separate varieties for fodder production and grain production, with grain types planted earlier than fodder types (IITA 1993). Another often reported use of cowpea is as a source of leafy vegetables. This was reported by 85% of the farmers interviewed by Wolfson et al. (1990), and 56–72% of the farmers surveyed by Saxena et al. (1989). From the more holistic viewpoint of the farmer, covering the entire production system, concentration on fodder and leafy vegetable production may in fact be viewed as a means of controlling (or avoiding) losses to field insects, by emphasizing utilization of plant parts that are relatively undisturbed by insects.

The studies reviewed here suggest that farmers have not been very successful in developing specific control methods for field insect pests, but they have been successful in developing agronomic/cultural practices and production systems which stabilize their overall production, and thus minimize losses from a myriad of production constraints (field insect pests, vertebrate pests, plant diseases, weeds, drought, poor germination, labor constraints, etc.). From the farmer's viewpoint, these various agronomic/cultural practices are not employed specifically for field insect control (Saxena et al. 1989; Alghali 1991; Bottenberg 1995), but rather are viewed as a means of minimizing the risk of total crop failure due to the combined effects of all their various production constraints (Saxena et al. 1989; Alghali 1991; Bottenberg 1995).

Postharvest pests

With postharvest insects, farmers seem to have been more active in developing specific control measures, at least for small quantities of grain. In contrast to the low percentage of farmers using specific control measures against field insects, nearly all farmers take some action to limit postharvest losses (Saxena et al. 1989; Wolfson et al. 1990; Goldman 1991; Sagnia and Schuette 1991). The unbounded nature of the losses that can occur in storage, and the somewhat less complicated nature of the storage environment compared to the field environment, appears to have provided a strong incentive for farmers to develop and use control measures.

Traditional methods are widely used to limit the storage losses. Only 12% of the farmers interviewed by Wolfson et al. (1990) in northern Cameroon reported using insecticides, but in areas where availability and cost are not too limiting, insecticide use is much higher (Goldman 1991; Sagnia and Schuette 1991). Numerous traditional methods of storage are reported by farmers: (1) storage in wood ash, or ash/herb mixtures; (2) storage in pods; (3) storage in sand; (4) smoke fumigation of granaries; (5) roasting grain; (6) sealed hermetic storage; and (7) smoking grain.

Wolfson et al. (1990) reported important gender differences with respect to cowpea storage practices. They found that women were much more likely to take active measures to preserve their cowpeas than men farmers. They also found a strong association between the gender of the person storing the cowpeas and the method used (e.g., women used ash proportionally more than men).

Implications for technology development

Generally, our findings indicate that there is a substantial amount of IK about cowpea pests and their control, but it is probably not adequate by itself to foster the production increases needed to sustain current population growth rates. We need to ask what these findings imply with respect to technology development, and what can be done to bring this IK into synthesis with scientific knowledge.

Postharvest insects

Wolfson et al. (1991) have shown that storage in wood ash is very effective if (1) the ratio of ash to cowpea is at least 3 parts ash to 4 parts cowpea; (2) the ash and cowpea are thoroughly mixed and stored in a container (such as a clay granary); and (3) 2–3 cm of ash is used to cover the mixture. In northern Cameroon, Wolfson et al. (1991) found that in certain localities this indigenous method is used correctly and is effective, whereas in other areas, presumably as a result of poor communication, the method is improperly used, with less efficacy. Scientists have been involved in validating this indigenous knowledge through developing effective and simple technical bulletins for distribution to extension groups and helping facilitate farmer-to-farmer communication.

Another example of technology development based upon indigenous practices is the concept of combined seed and pod resistance to *C. maculatus*. Storage of cowpea in pod form on elevated pole shed-type structures is widely practiced in northern Cameroon (Wolfson et al. 1991). Kitch et al. (1991) conducted studies to determine whether storage in pod form was effective in limiting *C. maculatus* damage. Their findings indicated that pods which resist breakage and are non-dehiscent, form a physical barrier to the developing larvae, and can reduce *C. maculatus* emergence by as much as 50%. In addition, some varieties also possess pod-wall resistance factors that are believed to account for an additional 20–30% mortality above that, due to the physical barrier effect alone. Since bruchid larvae have to penetrate both the pod wall as well as the testa of the underlying seed, pod-seed interactions which involve specific seed characteristics (such as seed coat texture) and pod characteristics (such as pod strength or thickness) also play an important role in resistance. Based upon this scientific understanding, researchers of the Bean/Cowpea CRSP Cameroon/Purdue University cowpea storage project are currently conducting a breeding program to combine pod resistance characters with seed resistance. Advanced lines have already been developed which possess very high levels of resistance to *C. maculatus* when stored in pod form. This resistance should be more durable than either seed or pod resistance alone.

Insect field pests

The surveys of IK about cowpea field pests just discussed represent a small but significant step towards understanding cowpea production practices in sub-Saharan Africa. These

studies suggest several avenues for bringing indigenous agricultural knowledge into creative synthesis with science:

1. Training and exchange activities between scientists and farmers would increase farmers' knowledge of pest biology, increase motivation for local pest control innovations, and enhance researcher knowledge of local practices.

2. Farmers already use a vast number of mixed cropping system arrangements, and they could potentially adopt systems which research shows to be most effective in limiting insect population buildup (e.g., reduced populations of flower thrips, *Megalurothrips sjostedti*, in cowpeas intercropped with maize or sorghum [Matteson, 1982]).

3. Use of effective agronomic/cultural practices should be encouraged through farmer to farmer transfer (e.g., ash storage in northern Cameroon).

4. Ethnoscientists need to be involved as team members with biological scientists. The studies reported here were nearly all conducted by entomologists and would be strengthened by methodological improvements. In general, the studies have been reasonably effective in describing what farmers do, but an issue with greater potential payoff for improved pest management is why farmers do what they do. This question can be most effectively addressed by teams of ethnoscientists/biological scientists. With respect to methodology, most studies we reviewed made no specific reference to gender issues. The Bottenberg survey (1995) contained all male farmers, and some of the other studies made no mention whether the farmers interviewed were male or female (Atteh 1984; Alghali 1991). In the surveys conducted by Wolfson et al. (1989, 1990) however, there were clear gender differences within the households interviewed with respect to cowpea production methods, cowpea utilization, and storage techniques. This demonstrates the need to consider gender issues in studies of IK. Norem et al. (1989) have enumerated the following gender differences in knowledge systems: "(a) women and men may have different knowledge about similar things; (b) women and men may have knowledge about different things; (c) women and men may have different ways of organizing knowledge; and (d) women and men may have different ways of preserving and transferring knowledge." Increased attention must be given to gender issues in studies of IK and cowpea insect pest management.

5. Farmers in most areas of sub-Saharan Africa place a high value on cowpea fodder and leaves for vegetables (Wolfson et al. 1989; Alghali 1991). This aspect is very important with respect to the sustainability of the traditional production systems. We suggest that a creative synthesis of IK and science can be brought about by the development of dual-purpose cowpea types through classical breeding efforts. Efforts in this direction are currently under way by breeding programs at IITA, as well as at the Bean/Cowpea CRSP. These efforts appear well founded and should be strengthened.

6. IK concerning cowpea pests is intertwined with farmers' perceptions or knowledge of their entire production system. IK-based decisions always encompass the system as a

whole, and rarely reflect concerns for production of one crop (cowpea) or of one pest (e.g., field insects) in isolation. In interpreting results of IK studies, therefore, it is important to maintain a holistic production viewpoint.

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Postharvest storage of cowpea in sub-Saharan Africa

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Abstract

Technologies are now available to limit postharvest losses to insect pests of cowpea grain. Chief among these pests is the cowpea bruchid, *Callosobruchus maculatus*. Solar disinfestation, combined with subsequent storage under conditions that prevent reinfestation, enables long-term preservation of threshed grain from *C. maculatus* attack. Cowpea lines with seed resistance, pod-wall resistance, and combined seed and pod-wall resistance have been bred through the joint efforts of breeders and entomologists. Cowpea grain storage in air-tight containers, such as metal drums or triple plastic bags, arrests the development of storage insect populations. Mixing the grain with wood ash from cooking fires or other fine-grained inorganic material, such as sand, also stops damage to grain. Treatment of cowpea grain with numerous plant-derived oils, such as that from groundnut, is also effective. None of these technologies involves the use of synthetic chemicals. Additional research, including the use of biotechnology to improve postharvest storage of cowpea, is currently under way to add to this set of management alternatives. Continued improvement of storage technologies is necessary to meet the needs of an extremely diverse spectrum of cowpea producers, traders, processors, and consumers in sub-Saharan Africa.

The storage pest problem

The principal storage pest of cowpea grain in sub-Saharan Africa is the cowpea beetle, *Callosobruchus maculatus* Walp., also known erroneously as the "cowpea weevil" (Taylor 1981). In low-resource farms, *C. maculatus* infestations start in the field and continue in storage. In the field, gravid females deposit eggs on the surfaces of pods still hanging on the plant. The females prefer mature green pods, but will oviposit on dry, mature pods as well (Messina 1984). Females oviposit more readily on exposed grain, as in cowpea lines whose pods dehisce easily.

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Larvae hatching from eggs on either seeds or pods use their mouthparts to bore through the bottom of the chorion. They tunnel onwards, penetrating the pod wall or the seed testa. Larvae hatching from eggs laid on pod walls must not only pass through the pod wall itself but must also gain entry into one of the seeds enclosed by the pod wall. Difficulty in surmounting these physical barriers partly accounts for the higher mortality of cowpea bruchids whose eggs are laid on pod walls compared to those laid on cowpea grain (Kitch et al. 1991).

Within the seed, the larva undergoes four instars, the longest of which is the fourth (Shade et al. 1990). The development from egg to adult at 26 °C and 55% RH takes about 35 days in susceptible seeds. Emerging females mate and lay viable eggs on the same day they emerge. Since each female can produce about 21 female offspring that survive to adulthood in susceptible grain (R.E. Shade, Purdue University, W. Lafayette, Indiana, unpublished observations), the population of bruchids in a cowpea store can grow exponentially in a few months.

Little is known about the applied ecology of *C. maculatus*. Alternative host plants are known, and include numerous wild species. The range of host species anywhere is also poorly understood. Similarly, we know little about the distances adult cowpea weevils actually cover, although it is established that there are two phases, a sedentary and an active dispersing morph (Messina 1987); the dispersing morph accumulates high levels of lipid reserves, which supply it energy for dispersal (Nwanze et al. 1976). Incomplete understanding of many aspects of the life history and bionomics of cowpea bruchid hampers the ordering of applied research priorities, thus rendering the technological products of some kinds of research ineffective.

Besides *C. maculatus,* there are numerous insect pests of cowpea in storage (cf. Singh et al. 1990). Infestations of an important one, *Bruchidius atrolineatus* Pic., begin in the field, like those of *C. maculatus*. Unlike *C. maculatus*, however, the *B. atrolineatus* populations do not increase in storage; instead, adults which emerge in grain stores enter a reproductive diapause until cowpea begins to flower during the subsequent rainy season (Huignard et al. 1984). Another bruchid pest of cowpea, ranked by Taylor (1981) as next in importance to *C. maculatus*, is *Callusobruchus chinensis* L.

Cowpea on sale in markets in sub-Saharan Africa often has bruchid emergence holes. In most cases, such holes would be due to either *C. maculatus* or *B. atrolineatus.* Unfortunately, the term "bruchid-resistant" is often used for cowpea lines with resistance to *C. maculatus* only, but not to all bruchids. When, for example, such varieties are planted in areas with *B. atrolineatus* or another bruchid, cowpea grain may develop as many holes as bruchid-susceptible varieties. Were this to happen, farmers could easily (and erroneously) lose confidence in the value of the resistant lines.

The financial and nutritional losses of cowpea to storage pests in sub-Saharan Africa are not well documented, but are clearly high. Low-resource farmers often sell their cowpea at harvest, when prices are lowest in the year, partly because they anticipate storage losses. Being aware of the storage problem, they are interested in better techniques for preserving their grain after harvest. Caswell (1984) has documented the loss of cowpea grain during traditional postharvest storage in Nigeria. Pods stored for 8 months had 50% of the grain damaged by bruchids, but when stored as grain, 82% of the grain had one or more holes. Since emergence holes represent insects that have developed and left the seed,

mated, and laid additional eggs, counting emergence holes to assess damage undoubtedly represents only a part of the problem. The next generation of larvae, more numerous yet, will generally still be developing within the grain. Visits to virtually any village market in sub-Saharan Africa reveal that the cowpeas for sale are typically damaged by bruchids. When the damage exceeds one emergence hole per seed, the price is usually discounted. We presently have no systematic methodology for assessing the economics of storage losses of cowpea grain. Though important, we still do not have good techniques for the cost-benefit analysis of the technology to reduce losses. A start in this direction has been made by Schulz (1993). A careful documentation of storage losses and of the benefits of addressing them through research could help persuade donors of the need for such research.

This paper reviews major features of cowpea grain storage in sub-Saharan Africa, and describes available technologies for reducing losses to storage pests, particularly the cowpea bruchid.

Use of oils and botanicals

An effective way to protect dry beans such as cowpea from bruchids is to mix the grain thoroughly with small amounts of vegetable oil (Schoonhoven 1978; Singh et al. 1979). The oil coats the testae, acting as an ovicide, by plugging the egg micropyle, thus hindering the oxygen supply to the embryo. Other factors may be involved as well; in some cases, the oil may deter oviposition or cause mortality of the adult bruchids. Only small amounts of oil are needed to preserve the grain for months (1–5 mL of oil per kg of threshed grain) (Singh et al. 1979). A variety of oils can be used, including oil from palm kernel, cottonseed, groundnut, neem, soybean, citrus peels, shea nut (karite), and others (Pereira 1983).

Though effective, oil treatment has some negative attributes. It is essential that the oil be thoroughly and evenly applied to the surfaces of the seeds, so that any eggs already present or subsequently placed on the seeds come into contact with it. Treating much grain (e.g., > 10 kg) thoroughly is tedious. Some oils become rancid with time, or have inherently negative properties; neem oil, for example, stains the hands and has an unpleasant "garlic" odor. Also, oil treatment is often messy and it is easy to pick up dust and debris while applying it. Surveys of hundreds of households in rural northern Cameroon revealed only one in which cowpea grain was being preserved with oil treatment (Wolfson et al. 1989). Thus, while oil treatment is effective, its degree of acceptance appears to be limited. The reasons for this are not clearly understood.

The use of other plant materials for the preservation of cowpea is common on low-resource farms in sub-Saharan Africa. This is often as whole plants or leaves of various mints, aromatic or pungent plant materials that are mixed with the stored cowpea grain. Ofuya (1986) noted that onion scales and dried chili pepper fruits confer some degree of protection against *C. maculatus*. There is a need to study and document the plant species of value as botanicals for plant protection.

Sealed container storage

Anthropologists have surmised that clay-lined pits in the earth, dug thousands of years ago by the Celts of northern Europe, served as grain stores. Experiments indicated that enough

moisture was initially present in the pits to allow limited germination of some of the grain and that the associated respiration eliminated O_2 in the enclosed space; this O_2 elimination contributed to the preservation of the grain (James 1993). A consequence of O_2 removal from a grain store is the suppression of insect infestations that could develop in the grain. In modern times, it has been proven that air-tight storage suppresses insect infestations in stored grain. Sealed containers may be huge, elaborate underground silos, or simple metal drums (Bailey 1954). For a long time, it was thought that the accumulation of CO_2 released from insect respiration would reduce insect populations. Certainly insects have high metabolic rates, and with severe infestation of a grain store, much CO_2 is produced; metabolic rates of 1 mL/g body weight/hr are typical. Thus, if 1 kg of grain in a closed space is infested by 100 insects weighing 10 mg each, 12 mL of CO_2 will be produced during the first day alone, raising the CO_2 level to > 1.2%. With time, the CO_2 levels increase. High levels of CO_2 are toxic to insects, suppressing them in grain stores, but high CO_2 levels, ca. 40% v/v, are required for 100% mortality of the granary weevil, *Sitophilus granaria* (Bailey 1954).

Actually, depletion of O_2 by insect respiration in a closed space may more directly suppress infestations in stored grain; *S. granaria* may die when ambient O_2 levels fall from the normal 20% to about 2% v/v (Bailey 1954). Over the years, researchers have sought practical and low-cost techniques to take advantage of insect sensitivity to low O_2 tensions, such as a variety of containers sealed with butyl rubber and plastic sheeting (O'Dowd 1971).

Metal drums. In some developing nations, 50 gallon (200 L) metal drums are used for the shipment of petroleum products and other high value liquids. Once they have served their primary purpose, used drums in good condition can be obtained in markets at relatively low cost (e.g., CFA 3000 = $5–7 in Senegal in 1994). After being thoroughly washed with detergent, such drums serve as sealable containers for long-term storage of cowpea grain. With support from the Bean/Cowpea Collaborative Research Support Program (CRSP), Dogo Seck of ISRA, Senegal, in collaboration with A.E. Hall of the University of California, Riverside, USA, has developed a practical drum storage technology and tested it extensively with cooperating farmers in Senegal (A.E. Hall, University of California, Riverside, California, personal communication). The procedure involves drying the threshed cowpea grain in the sun, then filling the drum, which will hold about 150 kg of grain. The Senegal-CRSP project recommends that the drum be sealed for a minimum of 2 months prior to opening. It is vital that the drum has no openings such as cracked seams that might admit O_2 and that the cap be tightly sealed. To ensure that it is air-tight, the cap should be greased before tightening. Filled drums are kept in the shade or storehouses so that the cowpea grain does not get too hot due to absorption of solar radiation by the drum. In addition to their relatively low initial cost, these drums can be used repeatedly. One disadvantage of the technique is that a drum filled with cowpea is heavy, but wooden racks can be devised to store and move the drums. Smaller 60 L drums are available, but they are more expensive per kg of grain stored than the 200 L drums. Another disadvantage of bulk drum storage is the long interval during which they must remain sealed for the treatment to be effective. Even a brief opening can admit enough O_2 to allow insects to resume activity and further damage grains.

Triple plastic bagging. In the Bean/Cowpea CRSP cowpea storage project, involving the Institut de la Recherche Agronomique of Cameroon and Purdue University, West Lafayette, Indiana, USA, researchers have devised a simple and inexpensive bagging technique for long-term storage of cowpea. Their work built on an observation by the late Moffi Ta'Ama, who was seeking a low-cost technique for storing cowpea grain after harvest and was conducting experiments with fumigants added to plastic bags. The storage system under test involved double plastic bags—one inside the other—filled with cowpea. The test fumigant was added, and the bag was sealed and stored for months. As a control, cowpea was sealed in double bags without fumigant. When the bags were eventually opened, Ta'Ama noted, perhaps as expected, that there was little bruchid damage to cowpea grain stored with fumigant. The surprise was that grain stored in sealed double bags without fumigant was also practically undamaged. This led to systematic experiments carried out by L.W. Kitch, G. Ntoukam, and others that resulted in a recommended technique for preservation of cowpeas after harvest (Kitch and Ntoukam 1991b). The technique makes use of widely available, low-cost, clear plastic bags, which will hold about 50 kg of threshed grain. Cowpea grain (40–50 kg) is put in a bag and tightly sealed by tying it shut with strong twine. The first bag is then completely enclosed in a second bag, which is likewise tied shut around the first. The third bag is then similarly used to enclose the first two. Tests in many villages in northern Cameroon have established that the technique is effective and readily accepted by low-resource farmers. Because the bags are transparent, it is easy for the owner of the grain to visually monitor grain storage. A disadvantage of the technique is that the bags are somewhat fragile and can burst with rough handling. They may also be vulnerable to rodents.

Co-storage with ash and other abiotic materials

Ash. A survey of postharvest storage methods in northern Cameroon revealed that the most common traditional method was co-storage of cowpea with ash (Wolfson et al. 1989). Numerous authors have noted ash storage in use at various sites in sub-Saharan Africa (Golob and Webley 1980; Ofuya 1986). The method commonly used in northern Cameroon consists of mixing cowpea grain with ash from the cooking fire. The mixture is placed in a mudpot granary, clay jar, or any other vessel, and sometimes tamped down to compress it. Results varied with variations in the mode of application, and to the proportion of ash to cowpea grain used.

Systematic studies at Purdue University (Wolfson et al. 1991) revealed that the method can work extremely well under certain conditions. The most important of these is the ash:grain ratio. Ash storage does not provide complete protection against a buildup of cowpea bruchids unless the ratio of ash to grain is 3 or more parts ash to 4 parts grain. If immature *C. maculatus* are already present in grain at the time the grain is mixed with ash, they will complete their development within the seed and may even emerge from the seed even if covered with ash. Consequently, farmers sometimes put infested grain—but apparently undamaged—into ash and discover when they remove it later that it now has emergence holes, thus evoking doubts about its usefulness. When properly used, ash storage arrests cowpea bruchid population development within the store, but it does not kill the generation already within the seeds (Kitch and Ntoukam 1991a). For this reason, it is important to mix the grain well with ash soon after threshing. The method recommended is

to mix sieved ash from cooking fires with cowpea grain in equal volumes, then put this mixture in a closed vessel, tamp it down firmly, and cover with a 3 cm layer of loose ash (Kitch and Ntoukam 1991a).

The mode of action of ash in protecting against cowpea bruchid population buildup is twofold. First, adults developing from larvae already in the grain at storage with ash are immobilized when emerging from seeds; they die before they find mates. Second, gravid females approaching the store from outside will not burrow into the ash and so will not oviposit on the cowpea grain.

Use of resistant cultivars

Bruchid-resistant seeds. Moderate seed resistance to *C. maculatus* has been bred into cowpea lines at IITA (Singh and Singh 1990). This seed resistance in three landrace varieties (TVu 2027, TVu 11952, and TVu 11953), is controlled by two recessive genes, designated *rcm1* and *rcm2* (Adjadi et al. 1985). Studies indicated that each of these three landraces have the same genes for bruchid resistance (Kitch 1987). Resistance conferred by *rcm1* and *rcm2* leads to delayed developmental time and reduced adult emergence (Singh and Singh 1990).

Dick and Credland (1986) and R.E. Shade (Purdue University, W. Lafayette, Indiana, personal communication), have shown that *C. maculatus* populations can overcome the resistance genes in TVu 2027 when they are reared on its seeds for several generations. An alternative genetic source of seed resistance to *C. maculatus* is yet to be found in the entire cowpea germplasm collection at IITA of approximately 12,000 accessions.

Seed resistance is a valuable tool against *C. maculatus* but must be carefully deployed to avoid the rapid development of a TVu 2027-virulent cowpea bruchid biotype. The durability of TVu 2027 resistance genes could be increased by introducing them into a pod resistant line. Although all pods provide a mechanical barrier, which increases bruchid mortality compared to development in seeds alone, certain varieties can reduce bruchid survival on infested pods to 1% (Kitch et al. 1991).

Cowpea bruchid-resistant pods. Varieties differ with respect to both ovipositional non-preference (Fitzner et al. 1985) and adult emergence from infested pods (Akingbohungbe 1976; IITA 1980; Fatunla and Badaru 1983; Fitzner et al. 1985; Owusu-Akyaw 1987; Kitch et al. 1991). Akingbohungbe (1976) suggested that pod-wall thickness may be responsible for reduced adult emergence from resistant pods; whereas Owusu-Akyaw (1987) reported that pod resistance was significantly correlated with pod-wall toughness measured by a penetrometer.

Combined seed- and pod-resistant lines. The IRA-Cameroon/Purdue University CRSP cowpea storage project is conducting a cowpea breeding program focused on developing germplasm and/or varieties with combined seed and pod resistance to *C. maculatus*. Farmer surveys and observations in northern Cameroon showed that > 85% of smallholder farmers store cowpea in pod form on pole platforms, locally known as "dankis," for at least 2 months (M. Kamuanga, NCRE Project, IRA, Maroua, Cameroon, personal communication; Wolfson 1989). This practice suggests that developing varieties combining both seed and pod resistance could result in an effective approach to achieving a durable and

high level of bruchid resistance and that this technology is likely to be adopted because it suits the current handling of cowpea after harvest.

To understand better the basis of pod resistance in cowpea and to develop the basis for seed and pod resistance breeding, researchers at IRA/Purdue screened 30 cowpea varieties to identify lines possessing high levels of pod resistance. Twenty of these 30 selected varieties were developed at IITA from the seed-resistant local variety TVu 2027. Attempts were made to determine whether reduced adult emergence from pods was the result of seed resistance, pod-wall resistance, or interactions between the pod and seeds. Pod wall and seed characteristics associated with resistance were identified. Results of this research (Kitch et al. 1991; Kitch 1992) guide the current breeding efforts of the CRSP project:

1. For effective pod resistance, the pod must first be resistant to breakage during and after harvest and it must also be nondehiscent. Genotypes vary widely in their ability to resist breakage. In general, local landrace cultivars are very resistant to breakage whereas most improved, exotic varieties have lost this trait, presumably after being bred for easier threshing. For cowpea storage as pod, nondehiscent pods that resist breakage are sought during selection.

2. Resistance in intact pods of cowpea is manifested by delayed bruchid development, preestablishment larval mortality (PreM), and/or postestablishment within-seed mortality (PostM).
   - PreM represents larvae dying after egg hatch but before penetrating seeds, and it is expressed as a percentage of the total number of hatched eggs on the pod.
   - PostM represents larvae dying after establishment in the seeds, and it is expressed as a percentage of the total number of entry holes in the seeds.
     - Among the 30 varieties studied, PreM ranged from 57.9 to 99.4% and PostM ranged from 6.7 to 82.6%.
     - The total percentage intact pod mortality (TM), which results from the combined effects of PreM and PostM, ranged from 65.9 to 99.9%.
     - Ten of the varieties in this study exhibited TM > 95%, and they are being used as parents in the breeding program.

3. Seed coat texture and thickness are significantly correlated with both PreM ($r = 0.843$, $P < 0.001$). Varieties with smooth and glossy seed coats were consistently more resistant than rough-seeded varieties. However, PreM was variable (59.9 to 98.9%) among resistant smooth-seeded varieties, suggesting that other factors besides seed coat thickness affect resistance. This is important as rough-seeded varieties are preferred in many areas.

Better understanding of the basis of combined seed and pod resistance has helped the Cameroon/CRSP breeding program to develop cowpea germplasm with high resistance to bruchids. Crosses of pod-resistant smooth-seeded lines with Cameroon-adapted rough seeded lines possess nondehiscent, nonbreakable pods, showing that breeders can combine high levels of pod resistance with rough seed coat types. Studies are presently under way to determine the mode of inheritance of PreM, PostM, and TM.

Solar and other heat disinfestation techniques

Susceptibility of insects to thermal death. Insects die when exposed to high temperatures because of their limited physiological capacity to thermoregulate. Cowpea bruchid eggs, larvae, and pupae do not thermoregulate and, being immobile, are unable to escape from a hot environment. Therefore, bruchids living within grain are excellent targets for management using elevated temperatures. This was discovered long ago by farmers in sub-Saharan Africa, who disinfest cowpea by heating them on iron plates over wood fires. For example, in markets of some villages and towns in northern Cameroon today, cowpea that has obviously been scorched can occasionally be found on sale. This fire-heating technique undoubtedly works, but it is clearly troublesome to use and hard to keep the cowpea from overheating and burning.

Inception of the CRSP plastic solar heater. Scientists of the IRA Cameroon/Purdue CRSP project seek to exploit the thermal susceptibility of storage insect pests of cowpea for pest management in sub-Saharan Africa with abundant sunshine during postharvest storage. This sunlight can easily be translated into heat, as anyone knows who places his hand on a hot tin roof on a bright day. Besides, villagers are accustomed to spreading their grain to dry in the sunlight. Thus, a disinfestation procedure mimicking this traditional activity ought to be easy to adopt. The problem was to devise a practical method for translating sunlight into heat that would be low-cost, use easily available materials, and raise cowpea seed temperatures above the thermal death points of the cowpea bruchid.

Entomological studies at Purdue established that all stages of the cowpea weevil are killed by exposure for one hour to temperatures > 57°C (Murdock and Shade 1991). Exposure to temperatures > 60°C were lethal after shorter periods. A simple solar heater was devised, consisting of a sheet of black polyethylene laid on the ground. Cowpea grain was spread on it, and then covered with a similar size of translucent plastic sheeting. The edges of the two plastic sheets were folded under and secured with stones, making a plastic envelope containing cowpea. This simple heater, when exposed to the sun for 2 hours, killed all stages of cowpea weevil in the seeds. Exposure to temperatures attainable in the solar heater does not change cooking time, rate of germination, or vigor of seedlings. With regard to the latter, exposures of several hours to 80°C did not significantly reduce germination of the exposed seeds—reflecting the high temperature hardiness of cowpea.

Subsequently, the CRSP solar heater was adapted and improved for use by low-resource farmers in Cameroon (Kitch et al. 1992). First, the heater was expanded in size to 3×3 m to allow 50 kg of cowpea to be disinfested in one treatment. Second, an insulating layer was added that increased the temperatures attainable in the heater. Straw or dried grass is first spread on the ground, and the plastic heater laid upon this. Thanks to collaboration with the USAID-funded and IITA-managed Cameroon National Cereals Research and Extension project, and with MINAGRI, the national agricultural extension agency, and SODECOTON, the cotton parastatal, the solar heater has been field-tested and introduced in northern Cameroon.

The CRSP plastic solar heater is only one way of using heat to disinfest cowpea. Another is to use corrugated galvanized tin or aluminum sheeting that is widely available at fairly low cost in many areas. A metal heater may prove to be more durable and more economical in the long run than plastic heaters. It is not known which form of the solar

heater technology will be most adopted. Although individual low-resource farmers were the targeted users, entrepreneurs who provide a disinfestation service for profit could be more effective and efficient in bringing the benefits of the technology to villagers. Alternatively, groups of farmers may cooperate to purchase and use the heaters.

Summing up

Each of the technologies described above for the postharvest storage of cowpea has limitations, as well as merits. It is important to recognize that none of the methods described involves the use of synthetic insecticides. Were any of these alternative technologies to be adopted widely, the use of insecticides on stored cowpea grain in sub-Saharan Africa could practically be eliminated. This would have economic and health benefits for the applicators, consumers, and the environment.

Cowpea producers and traders with grain to store would have to choose among the methods. The options available make it more likely they will find a technology suited to their individual needs. Clearly, many factors affect the choice of method suitable for an individual wishing to store cowpea grain. These include the availability of inputs (e.g., metal drums, or plastic for solar heaters), the costs of inputs, the labor involved, the time frame for application of the technology, the level of know-how for using the technology, the economic status of the person, the amount of grain involved, cultural biases, etc.

While progress has been made, the problem of cowpea storage free from insect pests is far from solved. New technologies that improve upon or add to the above range of technologies are needed. Indeed, some are being developed. For example, although we have useful genetic resistance to cowpea bruchid, thanks to the efforts of breeders and entomologists at IITA and elsewhere, only a single set of resistance genes is involved. Biotypes or ecotypes of the cowpea bruchid may appear that can overcome this resistance. Indeed, this is likely as the use of this source of resistance becomes more widespread. For that reason, tools of biotechnology are now being used by CRSP scientists in the USA, in Italy, at IITA, and elsewhere to find novel genes for cowpea weevil resistance and introduce them into cowpea. By introducing a variety of resistance genes through biotechnology and deploying them wisely, we may be able to combine resistances in such a way that overall resistance is enhanced.

Improved solar heaters that make use of inexpensive and widely available aluminum or galvanized metal roofing are being developed, as are single-unit plastic bags that will simplify the procedure of bag storage.

In the end, the cowpea supply in sub-Saharan Africa can be increased through reduction of postharvest losses, and the quality of available grain chanced as well. This will require the efforts of production researchers, socioeconomists, extension experts, nongovernmental organizations, and government leaders.

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Cowpea haulms as fodder

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Abstract
The use of cowpea haulms as fodder is attractive in mixed crop/livestock systems where both grain and fodder can be obtained from the same crop. Traditional farming systems which use the haulms as fodder in Asia, Australia, Africa, and other regions are reviewed. ILRI and IITA have undertaken joint research to identify accessions with improved grain and fodder yields. Trials were conducted in northern Nigeria to monitor yields in (1) cropping systems trials planted in the rainy season at Kano (Sudan savanna) and (2) dry season evaluation (including fodder quality) at Kano and Kurmin Biri (northern Guinea savanna), where the fodder potential of cowpea was compared with selected forages. Dry-matter yields from selected cowpea lines at Kano were good, some exceeding 10 t/ha. The role of cowpea as a fodder source is discussed in relation to the major cropping systems in the West African savannas.

Introduction

Cowpea is grown in over two-thirds of the developing world, usually as a companion or relay crop with major cereals. Its major importance is as a staple in the diet of many millions of people. Development of new varieties that are resistant to insects and pests or have shorter life cycles have contributed to increased cultivation of the crop (Rachie 1985). In addition, throughout the developing world, there is increasing emphasis on integrating crop and livestock production to promote more sustainable agricultural systems. Cowpeas can make a valuable contribution towards livestock fodder and supply nitrogen to the soil (Lal et al. 1978); their use as dual-purpose crops, providing both grain and fodder is attractive where land is becoming increasingly scarce. This paper focuses on the use of cowpeas as fodder; its development in Asia, Australia, and Africa (especially dry areas of West Africa); and ways to improve fodder production.

The use of cowpea fodder

Asia/India. The use of cowpea as fodder is most advanced in Asia, especially India, where green material is used for grazing, or, more commonly, cut and mixed with dry cereals for stall feeding. Dry cowpea haulms are not stored. Relwani (1970) recommended the use of cowpea in combination with cereals and other crops in an intensive scheme for lactating cows, to maintain milk yields of > 5 L/cow/day. Although green cowpea pods are eaten by

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humans in India, if the crop is being grown for fodder, inclusion of the pods in the fodder is considered important to raise its nutritive value (Mital et al. 1960).

Trials on fodder varieties of cowpea in India gave dry-matter yields of > 4 t/ha, with crude protein contents of up to 26% (Relwani et al. 1970). Bhatti et al. (1983) recommended forage cowpeas for use in Pakistan, recording dry-matter yields of 5.7 t/ha for the best variety. Cutting trials have indicated that harvesting at 60 days after planting (DAP) gave the best dry-matter yields of highest quality (Kandaswamy et al. 1976), although Sandhu et al. (1976) reported best yields and qualities from early planting and cutting at 70 DAP. In the same study, applying 25 kg P_2O_5/ha significantly increased fodder quantity and quality. Variety GFC-3 was recommended in 1983 as a superior fodder type, growing quickly to cover the ground 30 DAP, with dry-matter yields > 20 t/ha, crude protein 17.5–19.5%, in vitro dry-matter digestibility (IVDMD) 67%, and aphid resistance (Sanghi and Raj 1983). Earlier, Verma and Mishra (1981) reported yields of 27.5–30 t/ha, with crude protein of 15–20%, for a range of cultivars. Variety UPL 5286 yielded up to 38 t/ha and was released as a fodder variety (Mishra and Verma 1984). By 1984, nine varieties of cowpea had been released as fodder/green manure types in Asia (Mishra et al. 1985). Dry-matter yields can be positively associated with days to flower. The longer the vegetative period, the more forage was produced (Tyagi et al. 1978). In addition, the numbers of leaves and branches were positively correlated with green fodder yield, and the authors suggested that these traits could be selected for in breeding programs for forage cowpea. Insect pests significantly reduced the quality of cowpea fodder (Ram et al. 1990).

Australia. In Australia, as early as 1958, cowpea was regarded primarily as a fodder crop, with grain harvesting being the exception (Kavanagh 1958). Cowpea was recommended for use in mixtures for grazing (Douglas 1959), as part of crop rotations (Philips and Norman 1962), and was referred to as "annual forage legumes" (Davies 1960). Generally, cowpea fodder was higher in crude protein, digestibility, and intake than lablab (Milford and Minson 1968). The intake of lablab was related to the amount of leaves present because the stems are tough (Hendricksen and Minson 1980). This is not a problem with cowpea where both leaves and stems are readily consumed. Cowpea fodder tends to deteriorate more rapidly than lablab, implying its main feed value was in summer/autumn, whereas lablab could be maintained later in the year. Imrie and Butler (1983) found that for determinate cowpea accessions, seed yield was positively related to forage yield.

Africa. Africa is the largest producer of cowpea in the developing world, with Nigeria and Niger producing the most. In eastern and southern Africa, the crop is grown for human consumption of its leaves and beans, whereas in West Africa cowpea fodder plays a major role in the drier areas.

In the humid areas of West Africa, cowpea is grown as an early crop, often intercropped or sequentially cropped with cereals (maize), cassava, or yams, and used exclusively for the production of dry beans for human consumption. In a sequential cropping system, any herbage remaining after the cowpea seed harvest may be incorporated into the soil prior to planting the cereal. In the northern Guinea savanna, intercropping cowpea with groundnut is common. In the more northern Sudan and Sahelian areas (semiarid and arid zones), cowpea fodder is an important resource for livestock. Farmers there plant cowpea varieties

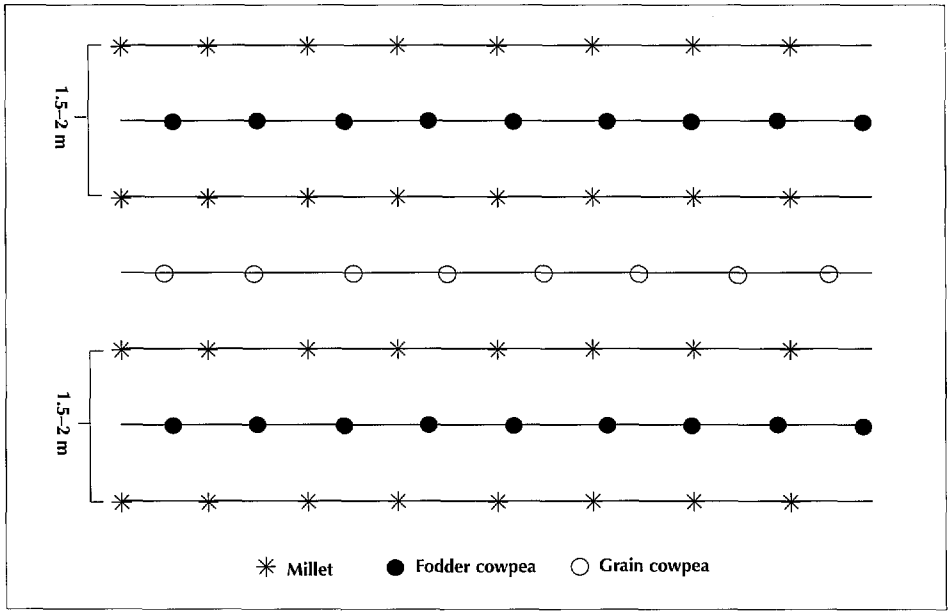

Figure 1. Schematic representation of the common cropping pattern for millet/cowpea in northern Nigeria (inter- and intrarow spacings are not drawn to scale).

or use intercropping arrangements which favor forage production. Cowpea/millet or cowpea/sorghum intercropping is the dominant pattern (Steiner 1982). In detailed studies of farmers' fields in northern Nigeria (Singh 1993), the predominant pattern was to intercrop millet and cowpea, with the cowpea rows consisting alternately of grain and fodder types (Fig. 1). Both grain and fodder cowpea varieties are spreading and photosensitive, but the latter type is later maturing. Grain cowpea is planted between millet rows 2–3 weeks after planting millet, followed by the fodder cowpea 3–4 weeks later. Following the millet harvest, the grain cowpeas are harvested and the fodder cowpea is left to continue growth. At the first sign of drought at the end of the rainy season, the fodder is cut and rolled, with any grain produced considered a bonus. Typical yields from farmers' fields are 400–500 kg/ha dry cowpea fodder. Bundles of harvested fodder are stored on rooftops or on trees for use, and for sale as "harawa" (feed supplement) in the dry season. In semiarid and arid areas of West Africa, cowpea hay constitutes 25–50% of crop sales (ICRISAT 1991). The millet/cowpea intercropping system is complex, with many factors influencing the farmers' decisions about planting and the resultant fodder and grain yields (Ntare 1989; Ntare and Williams 1992).

The nutritive value of cowpea haulms

Estimates of the nutritive value of dry cowpea haulms from various parts of the world (Table 1) indicate that cowpea haulms compare very well with other forage legumes, mostly with higher crude protein, digestibility, and mineral contents, but lower fiber. It should be noted, however, that these values for cowpeas cover a wide range of varieties, plant ages, growth conditions, etc. The forage legume data in Table 1 are dry-season

Table 1. Nutritive value of cowpea haulms; values are the range presented in the various papers, some for different ages and/or varieties†. At the bottom of the table, a summary of data for forage legumes, and native northern Guinea savanna vegetation, is presented for comparison. All values are in % of dry matter.

Crude protein	Ether extract	Crude fiber	N-free extract	Total ash	Ca	P	IVD§	NDF¶	Reference
Cowpea haulms									
16.5–26.4	2.25–3.25	18.73–28.01	32.06–50.77	10.07–12.99	1.55–2.23	0.26–0.39	–	–	Relwani et al. 1970
10.0–25.9	–	–	–	–	–	0.15–0.34	–	–	Kandaswamy et al. 1976
17.5–19.5	–	–	–	–	–	–	66.9	–	Sanghi and Raj 1983
9.5–21.8	1.67–3.51	24.6–47.0	27.5–42.7	–	1.95–3.16	0.23–0.51	–	–	Sandhu et al. 1976
12.91	2.20	36.53	39.42	8.93	1.27	0.22	–	–	Shah et al. 1977
24.5–29.2	–	–	–	–	–	–	56.7–64.4	30.8–43.4	Ram et al. 1990
12.5–20.6	–	–	–	–	–	–	56.6–63.8	–	Milford and Minsen 1968
22.6–31.0	1.2–2.0	22.0–38.2	3.4–24.5	3.6–4.1	0.96	0.72	32.0–68.6	–	Buamah 1971
20.3	1.0–5.0	18.5	52.4	7.3	0.55	0.40	–	–	Ranjhan 1970
17.4	2.5	33.1	–	–	2.0	0.79	–	–	Relwani and Kumar 1969
17.9–21.4	4.84–5.01	18.29–29.05	–	7.43–11.97	–	–	–	–	Piper 1914
Forage legumes									
5.6–35.8	–	12.4–43.4	–	–	0.27–2.24	0.06–1.00	36–69.3	–	Minson 1977
5.0–11.0	–	–	–	0.31–14.30	0.69–1.11	0.02–0.09	40.0–55.0	49.5–69.7	Peters 1992
Native vegetation (dry season)									
<3.0	–	–	–	2.9–3.8	0.26–0.39	0.02–0.05	40.0–45.0	60.1–77.8	Peters 1992; Crowder and Chheda 1982

† – = no data.
§ in vitro digestibility.
¶ Neutral detergent fiber.

values, and quality would be higher in the wet season. In the dry season, both cowpea and forage legumes contain sufficient protein and minerals to meet the needs of ruminants for relatively high levels of production (Minson 1977). Thus, in this sense they are better than the native vegetation, which are deficient in crude protein for the maintenance of livestock (Humphreys 1991).

Dual-purpose cowpea varieties

Improved varieties of cowpea, developed at IITA, yield adequate leaf fodder and grain (Akundabweni et al. 1990). Recently, the IITA station at Kano initiated a breeding program to develop dual-purpose cowpea varieties that produce both grain and fodder, to suit diverse needs of farmers in the savanna of West Africa. Singh et al. (1994) reported the grain and fodder yield of cowpea varieties in pure crop and intercrop, with and without insecticide protection (Table 2). Early- and medium-maturing varieties yielded higher

Table 2. Performance (yield kg/ha) of promising early-, medium-, and late-maturing cowpea varieties in different cropping systems at Kano, 1993 (after Singh et al. 1994).

	Pure crop (2 sprays)		Pure crop (no spray)		Intercrop (no spray)	
Cowpea variety	Seed	Fodder	Seed	Fodder	Seed	Fodder
Early-maturing varieties						
IT90-K284-2	2453	–	815	2166	252	595
IT90K-56	1946	–	715	1166	525	838
IT88D-6431	1780	–	626	1666	241	353
IT89KD-389	1750	–	848	1166	328	443
IT91K-93-10	1628	–	541	1916	408	550
IT90K-594	1872	–	883	1250	495	431
IT84S-2246-4	1159	–	594	1000	284	483
LSD 5%	511	–	275	1377	201	ns†
Medium-maturing varieties						
IT90K-277-2	2371	–	1082	3250	452	656
IT90K-372-1-2	1871	–	600	1416	211	152
IT88DM-363	1824	–	366	2500	569	542
IT89KD-374-8	2045	–	539	1583	279	308
IT89KD-374-57	1592	–	461	1083	196	148
IAR 48	1575	–	383	2833	494	681
Dan 'Ila	353	–	68	1666	507	1484
LSD 5%	664	–	526	1498	201	ns
Late-maturing varieties						
IT81D-985	1258	4900	470	3300	118	1042
IT89KD-252	630	5800	201	6900	102	833
IT260-1	617	5100	106	3100	74	646
IT89KD-288	302	5700	129	3700	19	1250
Kanannado	205	4700	0	3900	55	1083
Borno Local	204	4800	0	4300	0	2292
LSD 5%	309	ns	ns	ns	ns	1319

† ns = not significant.

grain but lower fodder than the fodder-type cowpea varieties which yielded > 5 t/ha of fodder and less grain. This supports farmers' practice of growing different cowpea varieties for grain and fodder production.

Grain and fodder yields of cowpea varieties in different cropping systems

In a 1993 trial, five improved cowpea lines and a traditional variety (Dan 'Ila) were evaluated for grain and fodder yield in three cropping systems at IITA Kano Station. The systems were: cowpea sole crop, strip cropping (four rows of cowpea bordered by single rows of millet), and intercropping (alternate rows of cowpea and millet). The improved cowpea lines outyielded Dan 'Ila when grain yields were combined across all cropping systems (Table 3). In addition, IT89KD-391 also gave high fodder yields.

Considering the land covered by the cowpea crop, the strip should produce 80% of the sole crop yield, and the intercrop 50%. The mean yields of grain under strip cropping as well as under intercropping were near the expected values, 687 kg/ha (vs 679 kg/ha) and 432 kg/ha (vs 425 kg/ha), respectively. In contrast, the fodder yield in strip cropping was as expected (1608 kg/ha vs 1654 kg/ha) but the fodder yield of the intercrop was below expectation (607 kg/ha vs 1034 kg/ha). Therefore, strip cropping seems beneficial, since its expected grain yield was similar to that of the sole crop, but fodder yields were higher than the intercrop.

Higher yield of cereals is the chief aim of savanna farming systems in West Africa. Farmers ensure they produce enough cereal to maintain their families, since they are the main staples. Table 3 shows that without millet production, the sole crop cowpea system would not be adopted by subsistence farmers. Considering the proportion of the land that

Table 3. The grain and fodder yield of millet and cowpea lines in pure crop, strip crop, and intercropping systems, 1993.

Cowpea line	Pure crop cowpea	Strip crop cowpea	Millet	Intercrop cowpea	Millet
Grain yield (kg/ha)					
86D-715	871	936	609	577	1094
89KD-284-2	623	569	600	300	1259
89KD-391	1028	810	574	506	1250
89KD-277-2	1014	861	579	387	1109
89KD-374-57	858	387	470	347	1258
Dan 'Ila	698	555	438	421	1052
Mean	849	687	545	423	1171
Fodder yield (kg/ha)					
86D-715	2239	1559	1717	732	2886
89KD-284-2	1401	896	1525	616	3125
89KD-391	2787	2595	1725	880	3282
89KD-277-2	2368	1948	1500	781	2938
89KD-374-57	1145	551	1646	297	2656
Dan 'Ila	2462	2103	1538	458	3573
Mean	2067	1608	1609	627	3077

Table 4. Cowpea grain and fodder yields (kg/ha) from a dry-season irrigated trial at Kadawa, Kano state, Nigeria.

Cowpea variety	Grain	Haulms
89KD-374-57	1887	2570
Dan 'Ila	1495	1890
87D-941-1	1773	4145
84S-2246-4	1293	6536
LSD (5%)	ns	1450
CV (%)	33	30

was covered by millet in the strip crop (20%) and in the intercrop, the strip crop yield of 545 kg/ha of millet grain vs the expected yield of 468 kg/ha, and 1608 kg/ha of fodder vs the expected 1231 kg/ha, indicates an advantage of the strip crop over traditional intercrop as regards fodder yield.

Recent studies have shown that some improved cowpea lines produce good yields of grain and fodder during the dry season (January–April) in irrigated areas (Table 4), where wheat could not be planted due to late harvest of the previous rice crop. In 1993, IT84S-2246-4 yielded 1.3 t/ha of grain and 6 t/ha of fodder under gravity-fed irrigation in northern Nigeria. Such high production, during a period of high fodder demand, seems attractive and is being adopted by the regional agricultural development programs. There is also some spontaneous adoption by farmers of such cowpea lines.

Performance of fodder type cowpea versus forage legumes

Joint ILRI/IITA trials at Minjibir (8° 36' E; 12° 03' N) in the semiarid zone, and at Kurmin Biri (7° 55' E, 10° 10' N) in the subhumid zone compared the forage yield of cowpea varieties with other forage legumes. In 1991, 1992, and 1993 total rainfall was 1308, 1511, and 1564 mm at Kurmin Biri and 905, 642, and 675 mm at Minjibir, respectively. The soil at Kurmin Biri is very poor and shallow with a ferrallitic hardpan (Adeoye 1988) 15–25 cm below the soil surface; pH is 5.2, N 0.03–0.05%, and available P (Bray 1) 2.3 ppm.

In 1991, four IITA cowpea breeding lines, and the local, late-maturing fodder-type "Kanannado" were tested with seven forage legumes. At the end of the wet season, the fodder was cut and dry-matter yield determined. Two more cuts of fodder were made at 6 and 12 weeks into the dry season. The cowpea lines yielded no grain at Kurmin Biri due to a severe attack of scab (*Elsinoe phascoli*); and only Kanannado produced < 100 kg/ha of fodder. *Centrosema pascuorum* (ILCA 9857) and *Stylosanthes guianensis* (ILCA 164) were the best forage legumes. At Minjibir, the highest fodder yield at the end of the wet season was from cowpea IT89KD-260. *Lablab purpureus* (ILCA 147) also had a good yield of fodder.

In 1992, the performance of cowpea varieties at Kurmin Biri was again poor, with no grain produced; but at Minjibir, yields for all lines were higher, with *L. purpureus* (ILCA 147) and the cowpea lines yielding > 10 t/ha of dry forage and 60–500 kg/ha of grain.

The experiments show that forage legumes, especially *C. pascuorum* and *S. guianensis*, yield better than cowpea at the wetter site at Kurmin Biri, but that cowpea lines yield as

good as or better at the drier site at Minjibir. *L. purpureus* showed its potential for a dual-purpose crop, as its grains are also consumed in northern Nigeria. A more detailed trial in 1993 included selected cowpea lines Kanannado and IT89KD-288, *C. pascuorum* (ILCA 9857), *S. guianensis* (ILCA 164), and *L. purpureus* (ILCA 147). This was to investigate the potential quality and quantity of fodder and grain at each site. The trial had a split plot design with harvest dates as main plots (H1 = end of the wet season, H2 = 8 weeks later, H3 = 16 weeks later) and the five legume accessions as subplots. Subplots were 5 × 2 m planted with four rows spaced 0.5 m apart, and with borders of 0.5 m at each end. The two central rows were sampled for yield. The two forage legumes were sown at 6 kg/ha, distributed evenly along the rows; the grain legumes were sown at a hill spacing of 20 cm within rows. Fertilizer as NPK (15:15:15) was applied at 100 kg/ha and mixed with the soil before planting. Plots were kept weed-free throughout the trial. At H1 and H2, two 1 m^2 quadrats were sampled from the central rows, and the fresh matter from each quadrat was weighed immediately. Material from one quadrat was tied in a bundle and stored on a rooftop. Material from the other quadrat was dried, reweighed, and ground. At H3, samples were taken for dry-matter determination as described, and seeds were harvested from the central rows but no material was stored. At this time, the material stored from the two earlier harvests was reweighed and ground. All ground samples were analyzed for crude protein and *in sacco* digestibility (Osuji et al. 1993).

Dry-matter yields at Minjibir were higher than at Kurmin Biri (Table 5), with the exception of *S. guianensis* which was the best yielder at Kurmin Biri but germinated poorly at Minjibir. The two sites had opposite trends in terms of yield variation with harvest date; at Minjibir, highest yields were at H3, but these were at H1 for Kurmin Biri. *L. purpureus* had the highest grain yields at both sites. The two cowpea accessions gave moderate fodder yields at Minjibir, but very low yields at Kurmin Biri. In general, losses from the earlier harvest were much lower than from the later harvest; and those at Kurmin Biri were more than at Minjibir. At H2, stored cowpea accessions and *C. pascuorum* had the highest losses. Crude protein and digestibility are shown in Table 6.

Forage yields at both sites were comparable with previous results for forage legumes (Tarawali 1991, 1994a, 1994b) in these regions. Cowpea forage yields were low at Kurmin Biri, but those at Minjibir were similar to yields of dual-purpose cowpea recorded at the more humid Ibadan site (Akundabweni et al. 1990). The low grain yields of cowpea could be related to the poor growth at Kurmin Biri and to the absence of protective spraying, even though these were monocultures. From this preliminary study, it appears that dual-purpose legumes, such as cowpea and *L. purpureus,* are suited to the semiarid areas. It would be appropriate to develop improved breeding lines of both species and better management practices to obtain higher yields of grain and fodder. Early cutting for fodder to be stored before it regenerates to produce grain could be useful. The potential of the grain legumes for fodder in the subhumid area may be limited, whereas forage legumes tend to give better yields. In this area, the local farmers, unlike those further north, are not accustomed to keeping fodder from such crops for livestock and to them, *in situ* grazing of crop residues or range is the common practice.

The study showed that late harvest of fodder caused substantial losses, mainly because the material was drier and shattered easily. The farmers were obviously aware of this, since they cut and roll the cowpea fodder as soon as there is any sign of drought at the end of the

Table 5. Forage dry-matter and grain yields (kg/ha) for the three harvest dates in the dry season (H1 = beginning of dry season; H2 = 8 weeks later; H3 = 16 weeks later) for selected forage and grain legumes at Minjibir and Kurmin Biri (Kano state, northern Nigeria). The yields in kg/ha are those at the time of harvest, the % loss figures refer to the loss of dry matter during storage until the time of H3.

Species	Accession	H1 DM†	H1 % loss	H2 DM	H2 % loss	H3 DM	H3 Grain
Kurmin Biri							
Centrosema pascuorum	I 9857	3875	8	2657	74	1783	256
Stylosanthes guianensis	I 164	4663	10	6540	40	3964	0
Lablab purpureus	I 147	3663	10	2432	42	1813	383
Cowpea	Kanannado	886	0	128	46	180	0
Cowpea	IT89KD-288	537	10	167	37	229	18
LSD 5% (grain) = 89 kg/ha							
For dry-matter yield:							
LSD (5%) between accessions = 724 kg/ha							
LSD (5%) between harvests = 561 kg/ha							
For loss:							
LSD (5%) between accessions = 10.08%							
LSD (5%) between harvests = 8.00%							
Minjibir							
Centrosema pascuorum	I 9857	4465	2	4605	37	4975	0
Stylosanthes guianensis	I 164	1489	12	476	0	3191	0
Lablab purpureus	I 147	5295	8	4340	7	819	869
Cowpea	Kanannado	2572	8	1599	46	3480	12
Cowpea	IT89-KD-288	1923	5	1336	28	3201	84
LSD (5%) (grain) = 319 kg/ha							
For dry-matter yield:							
LSD (5%) between accessions = 755 kg/ha							
LSD (5%) between harvests = 584 kg/ha							
For loss:							
LSD (5%) between accessions = 7.42%							
LSD (5%) between harvests = 5.75%							

† DM = dry matter.

rainy season. Quality was best when harvested early, and this was maintained during storage, thus confirming the importance of time of harvest, rather than the storage.

It is noteworthy that Minjibir with lower rainfall and a shorter growing season had higher dry-matter yields than Kurmin Biri. This could be related to the soil, which was very shallow Kurmin Biri, implying that plants had less access to residual moisture and dried up very quickly. The lighter and deeper soil at Minjibir meant that plant roots could

Table 6. Crude protein and digestibility of forage legumes for the three harvest dates, for not stored (NS) and stored (S) samples, which refers to those kept on rooftops until the time of H3. Replications were bulked before grinding material for analysis.

Location/ species	Accession	H1 NS	H1 S	H2 NS	H2 S	H3 NS	H1 NS	H1 S	H2 NS	H2 S	H3 NS
		Crude protein (%)					Digestibility (%)				
Kurmin Biri											
Centrosema pascuorum	I 9857	10.26	10.38	5.50	5.75	6.06	nd†	61.5	41.2	44.7	42.0
Stylosanthes guianensis	I 164	11.51	12.69	5.75	7.31	5.19	nd	55.7	43.3	54.1	49.1
Lablab purpureus	I 147	9.84	7.56	5.94	7.06	4.56	nd	59.9	59.9	49.4	47.5
Cowpea	Kanannado	12.87	12.94	11.44	nd	9.00	nd	67.0	57.0	nd	51.8
Cowpea	IT89KD-288	8.57	8.94	8.69	8.75	9.06	nd	52.7	50.7	47.3	45.4
Minjibir											
Centrosema pascuorum	I 9857	12.98	13.25	8.34	7.56	8.50	nd	68.2	54.5	54.1	55.6
Stylosanthes guianensis	I 164	10.18	12.75	11.25	11.25	8.56	nd	66.0	64.7	60.6	60.6
Lablab purpureus	I 147	15.42	12.13	12.25	10.69	8.63	nd	74.7	64.0	67.6	57.3
Cowpea	Kanannado	17.16	17.13	13.56	14.06	14.50	nd	71.8	59.4	60.7	66.3
Cowpea	IT89KD-288	11.94	13.56	nd	10.19	8.06	nd	72.0	62.2	59.1	nd

† nd = not done.

reach residual moisture and thus grew during part of the dry season, resulting in higher yields at H3. The drier plants at Kurmin Biri were also indicated by the higher losses from their stored samples, drier material (as at both sites for H2) shattered more and had more losses during collection and storage.

The future of cowpea haulms as fodder

Cowpea can provide a valuable fodder resource, under appropriate management, good quality fodder for *in situ* grazing, silage (in combination with cereals) or hay to be stored can be produced. The management and cultivars selected will depend on the farming system requirements and the mode of use. In India and Australia, cowpea can be grown

exclusively for fodder, with no need to harvest beans from the same crop. Cultivars for such systems would be late maturing with a long vegetative period, to ensure maximum herbage growth, and cutting should be timed to obtain the optimum fodder yield. Protective spraying may not be necessary or economically viable in such situations, although insect damage may affect forage quality (Ram et al. 1990). These are all areas that can be further investigated to maximize output.

In East Africa, cowpea is not used as fodder although the need for a forage resource for livestock is apparent and early studies in the region noted the potential of cowpea (French 1935; Elliot and Croft 1958). Currently, research stations in the region are evaluating forage legumes for livestock (Dzowela 1990). Perhaps, dual-purpose cowpea could be included in forage evaluation schemes as an initial step towards determining their utility, since farmers now grow the crop.

In West Africa, the current practices by farmers indicate the areas where cowpea serves as fodder. In subhumid areas, cowpea fodder is not a viable option, due to the planting of late-maturing varieties, which are exposed to many insect and disease problems and generally yield very little forage. Forage legumes are a better option in such areas. In semiarid/arid areas, however, where cowpea fodder is already at a premium, there is scope for improving the output of the system by developing dual-purpose cultivars and management practices that will yield both fodder and grain, thereby maximizing the output from land and labor.

Investigating areas such as the effects of various intercropping situations on cowpea fodder production (Kamara and Haque 1989), as well as the effect of cowpeas on subsequent crops, is important if cowpea fodder production is to be developed to benefit the whole farming system. The preliminary results of work at IITA Kano Station indicate that strip cropping produces more cowpea fodder and millet yields than the traditional cereal/cowpea intercrop.

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Cowpea leaves for human consumption: production, utilization, and nutrient composition

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Abstract

Although cowpeas (*Vigna unguiculata* [L.] Walp.) are grown most commonly for their edible seeds, their young leaves are also consumed by humans in numerous African and Asian countries. The leaves are often served boiled, but are also consumed fried or fresh in relish. Drying is a common way of preserving cowpea leaves, but canning techniques and polyethylene bag packaging of fresh leaves have also been studied. Planting density and leaf harvest time have been studied in relation to cowpea leaf productivity. Other studies have examined the effect of cowpea leaf harvest on seed yield. Cowpea leaves are a good source of some vitamins and minerals. Their protein content (based on total nitrogen) ranges from 29% to 43% on a dry weight basis, with the higher nitrogen content in younger leaves. However, a portion of the nitrogen contained in cowpea leaves is nonprotein nitrogen. The total dietary fiber content of cowpea leaves increases with leaf age, but fat and ash contents are less affected. The nutrient content of cowpea leaves is affected by cowpea plant growth in controlled environments, where hydroponic nutrient solution, light, and CO_2 levels are regulated to achieve rapid growth rates.

Geographical utilization pattern

Cowpea (*Vigna unguiculata* [L.] Walp.) is grown primarily for its edible seeds in the United States and Africa (Wien and Summerfield 1984). However, young cowpea leaves are also harvested and consumed in at least 18 countries in Africa, and 7 countries in Asia and the Pacific (Duke 1981; Barrett 1987, 1990). Cowpea is among the top three or four leaf vegetables used in many parts of Africa (Barrett 1990). Fresh and dried cowpea leaves are sold in many African markets. Direct consumption of leaf from home gardens and field production is even more widespread (Bittenbender et al. 1984; Bittenbender 1992).

Researchers in the United States have recently begun to study cowpea as a leafy vegetable and grain source for use in space colonization as part of the National Aeronautical and Space Administration's (NASA) Controlled Ecological Life-Support System (CELSS) program (Bubenheim and Mitchell 1987, 1988; Bubenheim et al. 1990; Nielsen et al. 1994; Ohler 1994; Ohler and Mitchell 1995; Ohler et al. 1996). Early inhabitants of a CELSS will subsist on a largely vegetarian diet because of weight and storage restrictions of deploying animals or animal meats in space. Because CELSS would have a limited number of crop species, those that can provide dietary variety are

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advantageous for psychological satisfaction (Ohler 1994). Cowpea may provide nutritional and harvest versatility not available with many other CELSS candidate crops (Bubenheim and Mitchell 1987, 1988; Bubenheim et al. 1990). Cowpea leaves could also provide a more nutrient-dense food source than other proposed leafy vegetables (Ohler et al. 1996).

Production and harvest

While a variety of management practices affect cowpea leaf productivity, research has focused on planting density and harvesting stategies. Cowpea leaf harvest has a cropping period of 21–42 days (Bittenbender et al. 1984), as compared to 70–120 days for cowpea seed harvest (Duke 1981). Cowpea is generally densely sown when grown exclusively as a leaf vegetable, and it is harvested 3–6 weeks after planting (Bittenbender et al. 1984). Cowpea seeds may be broadcast, alone or into a field with a grain crop, then thinned at intervals with the thinnings cooked and consumed (Barrett 1990). If seeds are desired, leaf harvest should stop before pods enlarge (Barrett 1990). Ohler et al. (1995) showed that manipulating planting density and harvest time affects cowpea leaf yield. Increased edible leaf yields were obtained at very high plant densities (85–99 plants/m^2) and late leaf harvest (40–50 days). However, early leaf harvest (20 days) produced the highest shoot harvest indices and yield efficiency rates. For a vegetative harvest only, planting density did not affect the shoot harvest index and yield efficiency rate.

Within limits, cowpea leaves could be harvested without adversely affecting seed yield (Oomen and Grubben 1977; Imungi and Potter 1983; Akundabweni et al. 1990). Even a limited harvest of leaves has a detrimental effect on seed yield of cowpea harvested at maturity (Bubenheim et al. 1990; Nielsen et al. 1994). Some of the conflicting results about the effect of leaf harvest on seed yield may be attributed to cultivar differences: seed yields of some cultivars are adversely affected by leaf harvest, whereas those of others are not (Bittenbender et al. 1984). Seed yield of leafy, determinate types suffers more reduction than that of indeterminate types following defoliation (Wien and Tays 1978). Barrett (1987) showed that the timing of leaf removal greatly affects the ability of the plant to recover from defoliation. Removing too many young cowpea leaves at once impaired seed yield, whereas removing the oldest leaves increased it (Barrett 1987). It has been suggested that, in a CELSS, a given cowpea crop should be grown for either leaves or seeds but not both (Bubenheim et al. 1990). However, a mixed harvest case, in which fully expanded leaves are removed until flowering and then left for harvesting seed, was more productive than a harvest for only seed (Ohler and Mitchell 1995). Decisions on any plan for early leaf harvest must be based on when food is needed by the consumer and in what form the food is desired (Nielsen et al. 1994).

Utilization

Cowpea leaves are most commonly served boiled to accompany a starchy porridge, but are also consumed fried or fresh in relish (Bittenbender et al. 1984; Bittenbender 1992). Cooking before drying of cowpea leaves is a widespread method of preservation (Bittenbender et al. 1984; Bittenbender 1992). For example, in parts of Africa, boiled cowpea leaves are kneaded to a pulp and then squeezed into golf-ball size pellets that are dried and stored (Bittenbender et al. 1984). Dried cowpea leaves are sometimes ground into a powder, and stored for use in the dry season when fresh leaves are not available

(Bittenbender et al. 1984). Canning techniques have been developed for cowpea leaves (Imungi and Potter 1985). Cowpea leaves, dried or fresh, are sold commonly in local and urban markets whenever available (Bittenbender et al. 1984). Bittenbender (1992) studied the effects of temperature and package ventilation on the storage life of fresh cowpea leaves, so as to reduce postharvest losses in households and markets. Optimal packaging was achieved with fresh leaves in a closed 2-mil polyethylene bag at 15–30°C, but chilling injury occurred when stored for 2–5 days at < 15°C.

Nutrient composition

The nutritional value of legume leaves, such as cowpea, has been largely discounted due to their high water content and the difficulty of documenting their production and consumption (Bittenbender et al. 1984). Nutritionally, cowpea leaves compared well with other tropical leaf vegetables (Table 1) and with cowpea seeds (Table 2). Compared to cooked cowpea seeds, cooked cowpea leaves contain seven times more calcium and three times more iron (Bittenbender et al. 1984). The phosphorus content of cooked cowpea leaves is 50% that of cooked seeds. The phosphorus in leaves is not present as phytic acid, a storage form of phosphorus that accumulates in legume seeds (Maga 1982; Carnovale et al. 1990). The minerals of cowpea leaves are more bioavailable than those in seeds, because phytic acid reduces the bioavailability of minerals such as calcium and iron (Maga 1982; Carnovale et al. 1990).

Compared to raw cowpea seeds, raw cowpea leaves (not dried) have about 20% the thiamine, twice the riboflavin, and equal amounts of niacin. Cowpea leaves are significant sources of ß-carotene and ascorbic acid, whereas cowpea seeds have negligible amounts. Like cowpea seeds (Rockland et al. 1977; Augustin and Klein 1989), cowpea leaves are an excellent source of folacin (334 µg and 2012 µg of free and total folacin per 100 g of solids

Table 1. Nutritional composition of some tropical leaf vegetables and lettuce (100 g edible portion)†.

Species	H_2O (%)	Energy (Cal)	Protein (g)	Ca (mg)	Fe (mg)	Carotene (mg)	Ascorbic acid (mg)
Cowpea (*Vigna unguiculata*)	88.4	34	4.2	110	4.7	2.4	35
Amaranth (*Amaranthus* sp.)	84.8	43	5.2	340	4.1	7.7	120
Cassava (*Manihot esculenta*)	81.0	60	6.9	145	2.8	8.3	80
Chinese cabbage, pak-choi (*Brassica chinensis*)	94.2	17	1.7	100	2.6	2.3	55
New Zealand spinach (*Tetragonia expansa*)	91.5	22	2.8	180	3.8	3.5	25
Nightshade (*Solanum nigrum*)	85.0	–	4.6	215	4.2	1.7	30
Pumpkin (*Cucurbita moschata*)	92.6	21	3.0	40	2.1	1.9	10
Sweet potato (*Ipomoea batatas*)	86.7	42	3.2	85	4.5	2.7	20
Taro (*Colocasia esculenta*)	81.4	61	4.1	160	1.0	5.5	65
Lettuce, looseleaf (*Lactuca sativa*)	94.0	18	1.3	68	1.4	–	18

† References for data: Watt and Merrill 1975 (lettuce); Oomen and Grubben 1978 (all other species).

Table 2. Nutritional composition of cowpea leaves and mature seeds (100 g edible portion)†.

Part	Leaf			Seed	
	Raw	Dried	Cooked	Raw	Cooked
H_2O (%)	85.0	10.6	8.9	10.5	80.0
Energy (Cal)	44	277	na§	343	138
Protein (g)	4.7	22.6	3.2	22.8	5.1
Fat (g)	0.3	3.2	0.3	1.5	0.3
CHO (g)	8.3	54.6	na	61.7	13.8
Ca (mg)	256	1556	132	74	17
P (mg)	63	348	41	426	95
Fe (mg)	5.7	12.0	4.7	5.8	1.3
ß-carotene (mg)	2.4	27.0	6.5	0.02	0.01
Thiamin (mg)	0.20	na	na	1.05	0.36
Riboflavin (mg)	0.37	na	na	0.21	0.04
Niacin (mg)	2.1	na	na	2.2	0.4
Ascorbic acid (mg)	56	86	6	na	na

† References for data: Leung 1968 (raw and dried leaves); Imungi and Potter 1983 (cooked leaves); Watt and Merrill 1975 (raw and cooked seeds).
§ na = data not available.

in raw leaves) (Imungi and Potter 1983). However, when freshly harvested cowpea leaves were cooked by a traditional Kenyan method, ß-carotene was well retained, but losses of vitamin C and free and total folacin were 87%, 49%, and 66%, respectively (Imungi and Potter 1983). Recoveries in the cooking water were 5.6%, 20%, and 12%, respectively. Other reports indicate that ß-carotene and ascorbic acid are well retained in fresh or dried (by either traditional or improved solar dehydration methods) cooked leaves (Gomez 1981, 1982). Cowpea leaves commercially canned as "spinach" are a good source of the minerals phosphorus, zinc, iron, and vitamins—ascorbic acid, ß-carotene, and folic acid (Imungi and Potter 1985).

Protein output from cowpea leaves is about 15 times that from cowpea seeds, because the leaves are produced earlier and in much greater quantity than the seeds (Bittenbender et al. 1984; Barrett 1990). Cowpea leaf protein contents range from 29% to 43% protein on a dry weight basis (dwb), but they seem to vary with leaf age (Berry 1981; Imbamba 1973). Leaf protein content is much higher than cowpea seed protein content 21–33%, dwb (Evans and Boulter 1974; Bressani 1985; Akinyele et al. 1986; Baker et al. 1989; Nielsen et al. 1993). However, these seed and leaf protein values are calculated from total N, as determined by the standard microKjeldahl procedure. Part of the N in cowpea leaves is not protein N, because vegetative plant material is known to accumulate nitrate and other nonprotein N (Aldrich 1980). Using procedures to differentiate total, protein, and NO_3 nitrogen, testing of cowpeas grown in a controlled environment chamber indicated that 50–67% of the N contained in cowpea leaves is protein N. Of the nonprotein N, 25–50% was nitrate N (S. S. Nielsen 1995, Purdue University, West Lafayette, Indiana, unpublished data). Such tests of field- and greenhouse-grown cowpea leaves is needed to determine what portion of the leaf is protein N.

The amino acid contents of cowpea seeds and leaves were compared to the amounts required by humans (Table 3). Amino acid analyses indicate that cowpea leaf protein is superior to that of the seed protein (Leung et al. 1972; Hall et al. 1975). Drying (solar) of cowpea leaves has been shown to increase the content of aspartic acid, glutamic acid, and valine, but it decreases the content of histidine and lysine (Maeda 1985).

The proximate composition of cowpea leaves changes as they expand (Bubenheim et al. 1990; Nielsen et al. 1994; Ohler et al. 1996). Protein contents of the greenhouse-grown cowpea (calculated from N) was 43% (dwb) for 7- to 10-day-old expanding leaves and 30.5% (dwb) for 22- to 25-day-old expanded leaves (Bubenheim et al. 1990). Nielsen et al. (1994) found that the protein content (measured as described above) of greenhouse-grown cowpea leaves harvested at 5 or 7 weeks did not differ significantly among five cultivars tested, but the mean protein content of leaves harvested at 5 weeks (40.2%, dwb) was significantly higher than that of leaves harvested at 7 weeks (37.9%, dwb).

Fat (4–5%, dwb) and ash (14–17%, dwb) content of greenhouse-grown cowpea leaves remained constant as they aged (Bubenheim et al. 1990; Ohler et al. 1996). The fat content of cowpea leaves is similar (2–5%, dwb) under field, greenhouse, and controlled environment growth conditions. However, the ash content of cowpea leaves from

Table 3. Essential and nonessential amino acid content (g/16 g N) of cowpea seeds and leaves, compared to required levels.

Amino acid	Seeds†	Fresh leaves	Solar-dried leaves§	Requirements of 2–5 year child¶	Requirements of adult¶
Essential					
Ile	4.2–4.8	6.6	6.6	2.8	1.3
Leu	7.6–8.5	13.4	11.8	6.6	1.9
Lys	6.6–8.1	9.5	5.6	5.8	1.6
Met	1.5–2.3	5.0	2.6	2.5 (Met+Cys)	1.7 (Met+Cys)
1/2 Cys	na‡	–	1.6		
Phe	5.5–6.2	6.1	7.8	6.3 (Phe+Tyr)	1.9 (Phe+Tyr)
Tyr	2.2–3.6	4.5	4.9		
Thr	3.6–4.5	6.6	6.6	3.4	0.9
Val	4.9–5.7	6.1	9.5	3.5	1.3
Trp	na	na	na	1.1	0.5
His	2.9–4.7	4.1	1.8	2.8	1.6
Nonessential					
Ala	4.1–4.8	7.5	9.2		
Arg	5.4–8.0	4.7	4.9		
Asp	10.7–12.9	11.6	17.1		
Glu	16.2–18.7	15.9	19.7		
Gly	3.8–4.6	8.0	7.5		
Pro	3.2–4.9	na	na		
Ser	4.1–5.6	9.5	6.6		

† Range for 24 cowpea varieties, corrected to 100% N recovery, reported by Kochhar et al. (1988).
§ From Maeda (1985), corrected to 100% N recovery.
¶ From FAO/WHO/UNU 1985, converted from mg/g protein.
‡ na = data not available.

controlled environment chambers (18–25%, dwb) is considerably higher than for greenhouse (14–17%, dwb) or field (12–14%, dwb) conditions (Imungi and Potter 1983; Bubeheim et al. 1990; Ohler et al. 1996; S.S. Nielsen 1995, Purdue University, West Lafayette, Indiana, unpublished data). The total dietary fiber content of greenhouse-grown cowpea leaves has been shown to increase with time to harvest, from 19% (dwb) at 20 days after planting to 26% (dwb) at 50 days after planting (Ohler et al. 1996).

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Impact of a cowpea research project in Nigeria, using the rapid rural appraisal technique

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Abstract

An impact assessment study was conducted in 1991. A team of human nutritionists, food scientists and social scientists conducted a 1-month rapid rural appraisal (RRA) survey to evaluate the impact of available cowpea technology on levels and patterns of utilization since a baseline study conducted in 1981–82. The 1991 survey in four communities in Anambra and Imo states used secondary data review, direct observation, interviews, validation, and rapid reporting. It revealed that cowpea consumption has increased in frequency and quantity by 150%. Cowpea marketing channels have not changed. Dried cowpea is sold wholesale in 80–100 kg sacks in large urban and semiurban markets by distributors who buy from producing areas in northern Nigeria and sell through middlemen to retailers. Mark-up through this channel is high, as are storage losses (> 25%). Severe malnutrition in children was reduced by 70–100%. Local production of cowpea has not increased, and increases are unlikely due to unfavorable edaphic and other factors. The high and rising market price relates to these production constraints. High-income urban respondents store significant quantities for up to 6 months in sealed containers, but poor respondents in nonproducing areas do not store cowpea. Improved milling and storage have reduced weevil damage and the long duration and energy requirements for cowpea preparation. The image of cowpea has improved and it is being introduced into children's diets at earlier ages. Use of cowpea in ritual continues to occur now (as in the baseline study) for religious sacrifices and festivities in the villages. Commercial milling services at engine-driven plate mills have increased in number. Because of population demand, there are more mills in urban than rural areas.

Introduction

Nigeria is the largest consumer of cowpea *(Vigna unguiculata)* in the world (Nnanyelugo et al. 1985; McWatters et al. 1990). Cowpeas are an important source of B-vitamins and protein in Nigerian diets. This paper describes the impact of 10 years of a collaborative research project between the University of Georgia Agricultural Experiment Station and the University of Nigeria, Nsukka, on milling processing technology in Nigeria (Chinnan et al. 1987). This project, with a grant from USAID through Bean/Cowpea Collaborative Research Support Program (CRSP), was initiated in 1981 and was the recipient of the 1991 International Award of the Institute of Food Technologists (Phillips and McWatters 1991).

The study used the methods of rapid rural appraisal (McCraken et al. 1988) to evaluate the success and impact of cowpea flour and its technology on the consumption and patterns

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of utilization of cowpea in 1991, as contrasted with the baseline situation in 1981–82 when the project was started.

Methodology

Survey team

A multidisciplinary team—of four human nutritionists, two food scientists/technologists, and two social scientists—surveyed four communities in Anambra and Imo states of Nigeria for 1 month, using RRA core tools, such as secondary data review, direct observation, interview, validation, and rapid reporting (McCraken et al. 1988).

The survey determined quantities of cowpea produced, consumed, and stored at household level; marketing channels; processing practice; health impact; image of cowpea; and constraints in 1991, as contrasted with the 1981 baseline data.

Survey area and selection of subjects

Four communities were selected: two rural and two urban areas. The rural communities were Ogbodu-Aba in Anambra state and Isiala-Ngwa in Imo state. The urban communities were Nsukka and Enugu both in Anambra state. Fifty households in each community (i.e., 200 households) were randomly picked for visit and interview. Also, 24 vendors of akara/moin-moin and 22 mill operators were randomly selected, visited, and interviewed.

Results and discussion

Cowpea consumption and usage patterns

The 1981–82 data showed that 59% of the respondents consumed cowpea three or more times a week (King et al. 1985); this rose to > 90% in 1991. Average weekly consumption per household in 1991 was 8–15 cups (Table 1), as against 54% of households which consumed ≥ 8 cups in 1981–82. Cowpea was used in similar forms, basically eaten in combination with the primary staples in the diet, which include cassava, maize, yam, rice, plantain, cocoyam, and sweet potatoes, and as akara and moin-moin. Ritual usage occurred in 1991 as in 1981, but only in cowpea-producing areas, for religious sacrifices and festivities (Onah 1987). Household food intake measured in 1981 showed that cowpea contributed much to protein (30%), iron (24%), thiamin (35%), and niacin (21%) for preschool children (Nnanyelugo et al. 1985). We conclude that increases in frequency and quantity of cowpea consumed will greatly contribute to the overall nutrient intakes of people in the surveyed communities.

Cowpea production

The 1981–82 data showed that in rural areas around Nsukka, <1% of respondents grew all their cowpea, 72% grew some and purchased some, while 27% purchased all their cowpea (King et al. 1985). In contrast, the 1991 data showed that cowpea was produced ("akidi" variety) only at Ogbodu-Aba, with an average output of 50 kg of unthreshed, fresh pods per household per year. Little of this output is sold and/or stored. Thus, in Ogbodu-Aba and most of the four communities surveyed, cowpea is mainly purchased from local and distant markets. Indeed, it appears from the 1991 survey that local output of cowpeas in rural Anambra and Imo states was less than 1981–82 levels due to unfavorable factors, which

Table 1. Household consumption and storage of cowpea in four survey locations (results from 50 households x 4 locations = 200 subjects).

	Locations			
	Ogbodu-Aba (rural)	Nsukka (urban)	Isiala-Ngwa (rural)	Enugu (urban)
Consumption				
Quantity consumed (cups/hsd†/wk)	11	10–15	8	10 cups/wk/ low-income hsd 15 cups/wk/ high-income hsd
Forms of consumption	In combination with primary staples	Same as in Ogbodu-Aba	Same as in Ogbodu-Aba	Same as in Ogbodu-Aba
Storage				
Quantity stored annually	50 kg/hsd by 60% of respondents	50–100 kg/hsd by 30% of respondents	None	75 kg/hsd by 50% of higher-income hsd's
	Remaining 40% cannot afford bulk purchase	70% cannot afford bulk purchase		50% kg/hsd by 10% of lower-income hsd's
Method of storage	In sealed bottles with pepper and camphor over fire. Dried pods tied in jute bags or tied and stuffed into earthenware pots and held over fire. Dry seeds stacked or held in pots or metal containers.	Dry seeds for consumption stored in plastic can, mixed with pepper and capped and gasketed for airtightness	None	Dry seeds capped in jerry can, stored with pepper and lime peels added
Problems of storage	40% stated weevil attack	None	None	None

† hsd = household.

include declining productivity of soils and high costs of labor and farm inputs. We concluded, therefore, that increasing consumption of cowpea in these communities would not lead to higher production in these communities.

Household storage of cowpea

In 1981–82, 63% of respondents from the rural and urban communities surveyed stored negligible quantities of cowpeas for periods of 4–12 months per year (King et al. 1985). In 1991 (Table 1), more cowpea (average of 50–100 kg per household) was stored for 4–8 months per year, with variations in the community patterns of respondents. It was observed that:

- among poor households (urban and rural) in areas that do not produce cowpea, storage is much less a practice, due to high cost of the commodity;
- among higher income urban households, and in the poor rural households in cowpea producing areas, e.g., Ogbodu-Aba, more cowpea was stored for longer periods (6 months per year).

In 1981–82, the common technique of storage was to keep cowpea in improvised vessels and crude utensils (King et al. 1985), including kerosine tins (30%), jute bags (29%), plastic bags (45%), clay pots (13%), bottles (27.3%), calabash (7.3%), racks-atop-fireplaces (0.9%), and metal drums (1.4%). Cowpea was stored in these vessels with pepper (28%), ash (3%), or sometimes with periodic drying and airdrying in sunshine, but more often without such aids (56%).

Cowpea losses due to weevil, rodent, and mold damage in such storage was high, as reported by > 55% of all respondents.

By contrast, the 1991 study showed that 100% of urban respondents with adequate resources to store cowpea do so effectively, with only negligible losses, in large plastic jerry cans screw-capped against polythene or rubber film gaskets to secure a reasonable degree of airtightness and, most often (60%), mixed with pepper, peels of lime, fruits or ash. At Ogbodu-Aba, the only cowpea producing community surveyed in which poor rural households stored large quantities of cowpea, the mode of storing cowpea for food was crude and ineffective (dried fiber-tied pods in sacks or pots over the fireplace, or as threshed seeds in jute bags mixed with pepper). At Ogbodu-Aba, cowpea seeds intended for planting are carefully dried and effectively stored in capped bottles and held over the fireplace until planting time.

These findings suggest that:

- effective storage of cowpea under almost dry, airtight conditions in gasketed jerry cans is being used by urban respondents who can afford the cost of such storage;
- the cost factor and ignorance militate against the adoption of such effective techniques among the rural poor in cowpea-producing areas.

Cowpea marketing

The forms and channels of cowpea marketing have not changed since the initial study was conducted in 1981–82. In cowpea-producing areas, cowpea is sold as fresh pods and as threshed dry seeds. Fresh pods are retailed directly in village markets by the producers. Dried cowpea seeds are sold wholesale in large sacks (80–100 kg) from market depots in large urban and semiurban markets such as Ogbete in Enugu (urban) and Oye-Orba in Nsukka (rural). From these market depots, large-scale distributors who bring cowpea

stocks from the main producing areas in northern Nigerian sell the stocks through a chain of middlemen to retailers. The retailers are small-scale entrepreneurs, who sell to household consumers. Mark-up through this extensive channel was high, as were the storage losses (over 25%) because of ignorance of bulk-storage techniques. Consumers who can afford a sack of cowpea at a time for storage buy it directly from the wholesale depots at discounts.

Cowpea processing

There were considerable changes in the practice and patterns of cowpea processing in the communities studied between 1981 and 1991:

- In 1991, most households (> 99% of respondents) and all akara/moin-moin vendors processed cowpea (wet and/or dry), using custom-operated engine-driven plate mills in both rural and urban areas. Less than 1% of households were still using the manual grindstone and/or pestle and mortar for this purpose. In 1981–82, only 2% of rural respondents residing within a 20 km radius of Nsukka township used engine-powered plate mills for any form of cowpea milling. Thus, the rural communities experienced a transformation in the cowpea milling technology, shifting from manual to machine milling (Chinnan et al. 1987).

- While this change in the mode of milling implies a rapid increase in the number of engine-powered mills, their cost restricts the majority of commercial mills to urban areas. In urban areas, these mills were being increasingly used by households and vendors. In the rural areas, mills are few and with low client patronage.

  Households and vendors appeared to take advantage of the convenience and flexibility of using cowpea flour. Flour is milled in a local village mill or a nearby township, and this flour is used to prepare akara/moin-moin as required. The flour can be stocked in a village home for a reasonable period without spoilage. The evidence indicates that the greatest impact of cowpea flour technology will be in the rural areas. Because rural dwellers have limited access to milling services, they tend to use cowpea flour more judiciously. Although flour may be inferior to wet paste as regards the quality of akara/moin-moin produced, it is nevertheless more convenient, more flexible, less wasteful (of time, energy, material) and more cost effective (Okeke et al. 1995). The patterns had changed adequately between 1981 and 1991 to support this conclusion.

Added to greater diffusion of new cowpea flour technology, the survey observed changes in old practices due to the cowpea CRSP mill at Ogbodu-Aba and Isiala-Ngwa. Prior to the project, cowpea flour was milled whole at Ogbodu-Aba and its surrounding villages. The flour from whole-milling was speckled and of poor quality. However, the 1991 survey revealed that all akara vendors at Ogbodu-Aba now crack their beans and winnow to remove the hulls and black eyes before milling into flour. Flour prepared in this way has an improved appearance and usage. Also, in large-scale production of traditional wet pastes, vendors in Enugu, Nsukka and other urban areas now crack their beans before soaking and wet-dehulling, so as to ease seed coat removal.

Cowpea as infant food

About 64% of the respondents in the 1981–82 survey introduced cowpea to their infants at ages 7–12 months, with 33% of them introducing it earlier. In the 1991 survey, most of the respondents (91%) introduced cowpea to their infants at an earlier age (5–7 months). In both surveys it was noted that cowpea is fed to infants soft-boiled and mashed, as adult food combined with the primary staples in the diet, or as akara/moin-moin (Uwaegbute and Nnanyelugo 1987).

This earlier introduction of cowpea to infants is attributable to rising awareness, generated partly by health education programs of the Ministry of Health, which extol the nutritional virtues of cowpea and other legumes, and also partly by programs such as the CRSP cowpea project.

In both surveys, health problems were associated with feeding cowpea to infants: these problems included diarrhoea (soft stools), indigestion, and other forms of abdominal discomfort (Ndubaku et al. 1989). Among the higher income group, 10% (at Nsukka) and 20% (at Enugu) reported these complaints. Among the lower income group, 50% (at Enugu) complained of such problems. In 100% of the cases in village households, no abdominal problems were reported. An explanation for these differences in the incidence of health problems may be the extent to which cowpea is cooked (Nnanyelugo et al. 1987). Insufficient cooking may result from ignorance or inadequate cooking fuel. Lower income urban families are more prone, because of their life style and circumstances, to either form of discomfort.

Health impact of cowpea consumption

The 1991 survey noted that increased consumption of cowpea relative to other foods had a positive impact on health in the communities studied. In 1984, severe malnutrition in children at Isiala-Ngwa was estimated to be as high as 74%. In Nsukka and its environs, in 1982, 13% of children studied were mildly wasted, 32% were mildly stunted, and 8% were moderately stunted (Nnanyelugo et al. 1985). The 1991 survey showed a marked improvement in these indices of malnutrition, with the incidence of severe malnutrition among all children studied varying between 0–20%. Children of the higher income households in Enugu recorded 0% incidence of severe malnutrition, while rural households at Isiala-Ngwa recorded 20%.

The benefits of eating cowpea were well known by respondents, who described it variously as giving protein, giving blood, body building, giving strength, and being an adequate substitute for meat.

Cowpea image

In 1981, cowpea was regarded as "poor man's meat". Its consumption implied poverty and was associated with the low income groups. The 1991 survey showed that this perception has changed. Cowpea is considered by all respondents interviewed to be food for the rich, the informed, the salaried worker and those who can afford it. All the households interviewed ate cowpea. Higher income urban households eat more cowpea (15 cups/household/week) than lower income urban households (10 cups), who in turn, eat more cowpea than rural households (8–11 cups). Cowpea consumption was thus directly correlated with income.

Constraints to increased cowpea consumption

The major factors which militated against increased consumption of cowpea in 1981 were:

- consumer aversion to infested cowpeas;
- high energy and time required for the culinary preparation of cowpea-based foods;
- high cost of cowpea relative to other foods.

In 1991, the major constraint identified by all respondents was the escalating market price of cowpea. This was related to an expanding gap between rising demand and insufficient supply of cowpea, compounded by the declining purchasing power of household income. It is evident that new and effective technological measures have been applied to address two of the earlier constraints:

- insect infestation has been addressed by improved storage technique;
- cowpea's high demand of time and energy have been addressed by availability of contrasting levels of cowpea milling technology.

The CRSP cowpea project has contributed to these positive changes, among other factors.

Conclusions

- Cowpea consumption in all areas surveyed has increased.
- The increased consumption of cowpea relative to other foods has reduced the incidence of severe malnutrition in children by 70–100% of the 1981–82 levels across all communities surveyed.
- The image of cowpea, between 1981 and 1991, shifted from that of "food for the poor" to that of a generally cherished health food, which the rich consume more than the poor because they can afford it.
- Consumption of more cowpea in the survey areas has not raised local production of the crop, and would not do so in future because of unfavorable edaphic and other factors. People still purchase the cowpea they can afford from the market.
- Innovation and diffusion of improved cowpea processing technologies, including contrasting levels of milling, have enhanced cowpea consumption.
- Commercial milling services at engine-driven plate mills are now in common use for cowpea. However, because of the cost of installing mills, they are more common in urban areas, where they have more clients. But patronage for cowpea flour and its technology are making more rapid impact in the villages than in the towns. Cowpea is now usually cracked to enhance dry dehulling by winnowing or to speed up the soaking step in wet dehulling. Thus, several aspects of the dry-milling process introduced through the CRSP cowpea project have been adapted by millers and akara/moin-moin vendors to improve the efficiency of earlier practices for both dry and wet milling.

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Opportunities for biotechnology in cowpea*

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Abstract

Several applications of biotechnology have been successfully used recently in cowpea. A molecular map of cowpea has been constructed using RFLP markers, and this has already facilitated the localization of certain quantitative trait loci; gene chromosome localization using in situ hybridization is in progress. Appropriate bioassays have been developed that have facilitated the identification of candidate genes for insect pest resistance in cowpea, including *Bacillus thuringensis* protoxin genes, and genes coding α-amylase inhibitor, protease inhibitor, and lectins. Since cowpeas are "recalcitrant" to regenerate "in vitro", several attempts have been made to develop a reliable protocol for differentiating shoots from calli obtained through in vitro tissue cultures. Thus far, only regeneration from already meristem-rich tissues has been obtained. The best results were obtained using the herbicide, thidiazuron, as a growth regulator to induce multiple bud proliferation. *Agrobacterium*-mediated plant genetic transformation remains an approach that requires considerable further work to be efficient. Direct plasmid DNA transfer into meristematic cells has also been attempted using microprojectile bombardment; rates of genetic transformation are too low to be useful. Recently, two new transformation methods were set up on in vivo plants: the first is based on electroinjection of plasmid DNA directly into meristematic cells, and the second involves the inoculation of buds with *Agrobacterium;* these two methods do not need in vitro regeneration and are giving promising results.

Introduction

The role of cowpea in the nutrition and farming systems of Africa is well known; also well known are the reasons for the poor yields of this crop, prominent among which is its susceptibility to several insect pests and diseases. Though improved varieties have been obtained with modifications of the plant habit and the introduction of genetic resistance to some diseases, the crop still suffers from several insect pests: the cowpea pod borer, flower bud thrips, and the pod-sucking bug complex cause very large losses. No good source of resistance against these pests has been found in cowpea, which is why an international network of institutes was promoted by the International Institute of Tropical Agriculture (IITA), with the purpose of exploring the use of innovative technologies to solve such

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intractable problems. This international effort was considered necessary, because cowpea, compared with cash crops where biotechnologies have been successfully applied in industrialized countries, had received no attention from biotechnologists, being considered a "minor" crop.

This paper reports the main results obtained by the use of molecular techniques to improve our knowledge of the cowpea genome, and to transfer genes from other species into it. As most of the efforts are directed toward obtaining cowpea transformed for insect resistance, studies reported here focus on genes that are, at present, the best candidates for this purpose.

Genome analysis

As with many other seed legume species, there is little knowledge of the cowpea genome. Only recently has each of the 11 chromosomes of *V. unguiculata* been characterized (Saccardo et al. 1992), and cytological differences among some *Vigna* species described.

Improved chromosome characterization of cowpea has been obtained using C-banding techniques. The combined use of C-banding and of fluorochromes (CMA and DAPI) led to the identification of two classes of heterochromatin (Galasso et al. 1993). Further improvement came from the application of molecular cytogenetic techniques. Two rDNA probes were co-localized on metaphase chromosomes of cowpea and wild allies, demonstrating a constant association of one of these probes to the CMA-bright heterochromatin type. Additionally, a repetitive sequence of ~ 500 bp was cloned from cowpea and hybridized in situ on metaphase chromosomes and on membrane to genomic DNA digests from several species of the *Phaseoleae*. The results demonstrate that this sequence, named pVuKB1, is species-specific and localized in the centromeric heterochromatin blocks of cowpea (Galasso et al. 1995). Finally, the extension of the analyses of other accessions of cowpea allowed the recognition of a polymorphism for the number of rDNA sites.

Because of limited conventional genetic studies, only a few morphological and physiological marker genes are known in cowpea. The development of restriction fragment length polymorphism (RFLP) and random amplified polymorphic NDA (RAPD) marker technologies has contributed in the past two years to the construction of a cowpea linkage map. Using clones from common bean, mung bean, soybean, and cowpea (Fatokun et al. 1993a), ~ 100 loci were identified as being distributed into 10 linkage groups. The current map has already facilitated the localization in two genomic regions of some major quantitative trait loci controlling seed weight (Fatokun et al. 1992). The average distance between adjacent markers is ~ 7 cM and an increase in marker density is expected, since other segregating populations are being investigated (Menancio-Hautea et al. 1993). Once a more complete DNA marker-based map is obtained, marker-assisted selection for agronomically important traits will be facilitated. An RFLP map of moderate density in the *Vigna* genus will also improve our knowledge of the origin of cowpea, its evolution, and its phylogenetic relationships with closely related species (Fatokun et al. 1993b).

Genetic engineering

Several attempts are in progress to overcome interspecific barriers to gene flow, in order to transfer pest resistance traits that are present in wild species into cowpea. Histological

studies have shown that after crossing *V. unguiculata* with *V. vexillata*, F_1 embryos start to develop but collapse while still in the globular stage (Barone and Ng 1990; Ng 1992). Successful crossing of *V. luteola* and *V. oblongifolia* resulted in hybrid plants that can be used as bridges for crosses to cowpea (Schnapp et al. 1990).

Molecular technologies have opened up new opportunities for crop breeders by enabling them to use isolated single genes derived from other organisms, and these techniques can now be applied also to cowpea, where recently successful regeneration and genetic transformation experiments were carried out.

In transferring selected genes from one species to another using recombinant DNA techniques, priority has been given to insect resistance genes. This kind of research requires (1) the setting up of effective bioassays for discovering resistance genes for specific pests; (2) the use of those bioassays to search through the plant, fungal, animal, and microbial kingdoms for suitable genes; and (3) the understanding of insects' physiological and biochemical systems that are vulnerable to resistance genes.

Cowpea has many insect pests, but we are still at a rudimentary stage in the process of using biotechnology for practical improvement of cowpea for insect resistance. At present, effective bioassays are available only for a storage pest, the cowpea weevil, and a field pest, the cowpea pod borer. For flower thrips, bioassays are yet to be developed that would allow the identification of candidate resistance genes. For some of the other pests, such as the pod-sucking bugs, preliminary collaborative studies between IITA and Purdue University indicate that the artificial seed system developed for the cowpea weevil may be useful for evaluating candidate genes for control of these pests. Further, it is important to remember that cowpea is grown over a wide geographic area, not only in Africa and the Americas, but in Asia as well. Some of the pests are cosmopolitan, and it is possible that they will exhibit a wide range of adaptations and variabilities, such that one population may be invulnerable to a gene that controls another population. For these reasons, and for the reason that virulent biotypes may emerge against single, highly active genes, it is apt to continue the search for additional genes that can be pyramided or deployed over time to ensure that biotechnological management tools are both effective and durable.

Candidate genes for pest resistance

Following the bioassay methods above mentioned, the active substances coded by known genes were tested on cowpea weevil and on *Maruca vitrata* (formerly *M. testulalis).*

"B.t". Despite some concerns about the practical implementation and sustainability of genes from *Bacillus thuringiensis* "B.t." used in transgenic crop cultivars, research has made it clear that B.t. genes have potential for controlling a number of the insect pests of cowpea. Bioassays carried out at Purdue University in collaboration with Auburn University have demonstrated that the cowpea pod borer, *M. vitrata,* is susceptible to several different forms of the B.t. crystal toxin when these are fed in its diet. Concentrations that caused 50% mortality (LC_{50}'s) ranged from 0.03 µg/g of diet for CryIA(b) crystal toxin to 1.0 µg/g for Cry IA(a) crystal toxin; whereas CryIA(c), CryIC, and CryIIA have an activity intermediate between the two. Genes encoding several of these proteins are available for cowpea transformation and could be used to impart resistance to *M. vitrata.*

Joint research efforts involving Purdue and Auburn universities have also provided evidence that B.t. crystal toxins that are effective against the cowpea weevil may be found through systematic screening. Several well known forms of B.t. crystal toxin (e.g., CryIA(b), a lepidopteran-active form) proved totally inactive in feeding bioassays against *Callosobruchus maculatus*, as did a beetle-active B.t., *Bacillus thuringiensis tenebrionis*. The most active B.t. found to date is CryIA/CryIB, which caused a significant mortality of *C. maculatus* when incorporated into the diet at a level of 16 µg/g, and > 90% mortality at a level of 128 µg/g. (W. Moar and R.E. Shade, Purdue and Auburn Universities, USA, personal communication). These are levels of proteins that could easily be attained in the protein-rich seeds of cowpea.

Protease inhibitors. Limited studies have been carried out to evaluate the impact of proteinase inhibitors on insect pests of cowpea. Lima bean, Bowman-Birk, and Kunitz trypsin inhibitors had no effect on developmental rates or mortality of *M. vitrata* larvae when present in the diet at levels of 1% (w/w). Protease inhibitors I and II (PTI-I, -II) from potato, by contrast, exhibited measurable activity, causing slight developmental delays and increased mortalities at dietary levels of 1.0%. When the diet contained 1% (w/w) of each potato inhibitor, all insects died. In view of the high dose necessary to have a substantial effect on *M. vitrata* it is doubtful if the transfer of the PTI-I or -II genes into cowpea would be worthwhile. However, in view of the fact that low levels of trypsin inhibitors may markedly enhance the activity of B.t. crystal toxins (MacIntosh et al. 1990), knowledge of effective trypsin inhibitors against *M. vitrata* may prove useful.

Resistance of cowpea variety TVu 2027 was, for many years, widely held to result from an elevated level of a trypsin inhibitor (Gatehouse et al. 1979). Seeds of TVu 2027 were reported to contain levels of trypsin inhibitor almost twice as high as those in susceptible seeds. This interesting hypothesis has not been upheld, for numerous reasons. First, several laboratories (e.g., Xavier-Filho et al. 1989) have been unable to verify that this variety has higher levels of trypsin inhibitor than do other, susceptible, varieties. Second, when an appropriate bioassay is utilized (Zhu et al. 1994), the cowpea weevil is not affected by dietary levels of cowpea trypsin inhibitor twice as high as those originally reported by Gatehouse et al. (1979). Third, cowpea weevil larvae do not use a serine protease to digest their dietary protein (Gatehouse et al. 1985; Kitch and Murdock 1986), making it unlikely that a serine proteinase inhibitor could disrupt protein digestion.

Cowpea weevil larvae are susceptible to dietary cysteine proteinase inhibitors. When artificial seeds are made up containing E-64, a specific cysteine proteinase inhibitor, doses as low as 0.02% (w/w) significantly reduce growth rates, mortality, and fecundity (Murdock et al. 1988). The effects of E-64 were reversed by adding free amino acids to the diet (R.E. Shade and L.L. Murdock, unpublished data), indicating that the negative effect of E-64 was to restrict the supply of free amino acids. E-64, from *Aspergillus japonicus*, is an unusual tripeptide, which contains agmatine and trans-epoxysuccinic acid. Multiple genes are involved in its biosynthesis; thus it is not practical to think of using E-64 to protect cowpeas through gene transfer. There are, however, a few proteinaceous inhibitors of cysteine proteinases whose genes might be used to confer protection against cowpea weevil. Such an inhibitor from soybean seed is effective in vitro against the digestive protease of the cowpea weevil (Hines et al. 1991). Further studies are needed with this and

other proteinaceous cysteine protease inhibitors before their potential can be fully assessed.

Lectins. The most thorough studies on lectins, thus far, have focused on the cowpea weevil. Purified *Phaseolus vulgaris* lectin, which occurs in leukocyte agglutinating form (PHA-L), in erythrocyte agglutinating form (PHA-E), and as a mixture (PHA-P), has no effect on cowpea weevil when fed in the diet at concentrations of 1% (w/w) and above (Murdock et al. 1990). This result contradicted that of Gatehouse et al. (1986) who had observed that a preparation of PHA was toxic when present at relatively low levels in the diet of the cowpea weevil. Curiously, however, the authors noted that a purified preparation of PHA was less toxic than the impure preparation.

Furthermore, tests of PHA-E or PHA-L separately revealed that they were "largely ineffective" against *C. maculatus* (Boulter 1986). The disagreement of results using the individual purified isolectins with the earlier published results using the impure lectin preparation was explained away with the assumption that there is a synergistic effect of E and L lectin subunits. Unfortunately, this assumption was not tested, but tests with PHA-P at Purdue revealed no biological activity (Huesing et al. 1991). Since an impure preparation of PHA from Sigma Chemical Co. was active when fed to cowpea weevil and since this preparation was found to contain a substantial part (15–20%) of α-amylase inhibitor as impurity—enough to account for its biological activity against the cowpea weevil—there seems no reason to expect that purified PHA, in any of its forms, has any substantial activity against the cowpea weevil.

Several other lectins have been shown to affect the cowpea weevil when present in its diet. The most interesting of these are specific for N-acetyl-glucosamine (GlcNAc) residues (Murdock et al. 1990). The best GlcNAc-specific lectin was wheat germ agglutinin (WGA), which had significant effects on the insect when fed at levels as low as 0.2% (w/w). The vulnerability of cowpea weevil to GlcNAc-specific lectins may be related to the presence of chitin—a polymer of GlcNAc—in the insect gut.

α-amylase inhibitors. Seeds of common bean, *Phaseolus vulgaris*, do not support growth and development of the cowpea bruchid, *Callosobruchus maculatus*, although the females readily oviposit on the beans and the hatchling larvae bore into them. Much of the resistance is due to the presence of a proteinaceous inhibitor of the digestive α-amylase of the bruchid (Ishimoto and Kitamura 1989; Huesing et al. 1991). The kidney bean α-amylase inhibitor, which occurs in common bean seed at levels of ~ 1% (w/w) (Shade et al. 1994), is active against the cowpea weevil digestive amylase and prevents the insect from digesting the complex carbohydrate of the seeds. Microscopic examination of the midgut contents of insects that have fed on diets containing bean α-amylase inhibitor reveals a massive accumulation of undigested starch granules. It is the deprivation of this major nutrient source that presumably accounts for the effectiveness of α-amylase inhibitor in preventing cowpea weevil growth, development, and survival.

While experiments with artificial seeds clearly show the promise of the bean α-amylase inhibitor gene for controlling this important storage pest, transfer of the gene into cowpea to prove its effectiveness awaits the development of an efficient cowpea transformation procedure. In the interim, powerful new evidence has accumulated that such a transfer will,

indeed, generate a new source of cowpea weevil resistance. A multidisciplinary effort by scientists at Purdue University, the University of California at San Diego, and the CSIRO, Canberra, Australia, successfully transferred the bean gene into garden pea, *Pisum sativum*, and expressed it in the pea seeds at levels comparable to those naturally occurring in common bean (Shade et al. 1994). Pea seeds expressing the bean α-amylase inhibitor gene were immune or highly resistant to the adzuki bean weevil. Seeds from the same plants were either immune, highly resistant, or moderately resistant to cowpea weevil, depending on the level of α-amylase inhibitor expression. Differing responses of the two bruchid species to the transgenic seeds reflect the markedly higher sensitivity of the adzuki bean weevil to α-amylase inhibitor compared to the cowpea weevil.

In vitro regeneration

In general, gene transfer methodologies are based on the regeneration in vitro of a complete plant, or at least of new buds, from a transformed single cell or tissue. In grain legumes, except for soybean, regeneration protocols are not so reliable as for other crops. Despite the existence of a few published reports, these protocols seem to be laboratory-dependent, rather than genotype-dependent. To fulfil all the requirements for a good transformation system, a reliable protocol must be: (a) widely applicable, i.e., genotype/laboratory independent; (b) efficient, i.e., generating as many regenerants as possible per cultured explant; (c) reproducible, i.e., without any constraints such as particular chemicals or manipulations; and (d) fast. In this respect, grain legumes gained the negative label of "recalcitrant crops" to in vitro manipulation.

In order to develop a method suitable for cowpea genetic transformation, considerable efforts are under way to differentiate new buds and hence new shoots from differentiated cowpea tissues, or to induce multiple bud proliferation from already present highly morphogenic tissues, by testing different explant sources and several combinations of natural or synthetic plant growth factors.

Shoot differentiation. Scientists at IITA have tested the differentiation ability of young cowpea tissues cultured in vitro on a medium containing coconut water from fresh local coconuts and a high cytokinin concentration. The rationale is that coconut water extracted from fresh coconuts already contains a high level of natural cytokinins (De Wald et al. 1989). After the explants passed through 3 different media and 3 months of in vitro culture, ~ 33% of explants (primary leaves and hypocotyls isolated from germinating seeds) differentiated some shoots (S.Y.C. Ng and G.Thottappilly 1993, IITA, unpublished data). The histology of these explants carried out in Italy showed that a strong cellular proliferation occurred on the explant surface, at the epidermis level, where callus was formed. Some other experiments were carried out in Italy, to study the morphogenic response of the local cowpea cultivar "Cornetto" when cultured in vitro in the presence of natural Nigerian coconut water, compared to its commercial counterpart (Sigma C5915, deproteinized) and versus coconut water from coconuts available on the Italian market. The overall frequency of regeneration was, under the conditions used and with the above mentioned genotype, lower than that obtained at IITA with other genotypes. Nigerian coconut water seemed more effective in inducing the production of healthy shoots. However, experiments carried out in Italy have shown that only the basal part of young leaflets are able to produce shoots,

perhaps due to the presence of already formed meristems. The histology of some explants is now being studied, to elucidate the shoot origin (whether regeneration or true differentation).

Multiple bud regeneration. Several experiments have been carried out to induce multiple bud proliferation from highly morphogenic cowpea tissues. The rationale of these experiments was to find a different approach to plant differentiation, in order to obtain transformants by regeneration of transformed tissues. Scientists at Purdue University have tested the effect of media containing a high concentration of Benzyl Amino Purine (BAP) (3–6 mg/L) and a low concentration of auxin on cotyledon segments and embryonic axes from different-age embryos of various cowpea genotypes (e.g., CB5, TARS 36, SUV-2, 283, 1137, 275, TN88-63, B301, 849, and 58-57). When the callus produced on explants grown in a medium with high cytokinin concentration and cultured in darkness was transferred into media with reduced cytokinins and cultured under light conditions, proliferation of shoots occurred from regenerated buds. Since mature seed explants gave, on average, the same regeneration frequency as those of immature seeds, the former were chosen for genetic transformation experiments. Cotyledon explants developed shoots at a frequency of 50% at best after 3 weeks of in vitro culture.

Recently, the herbicide thidiazuron has been used in some grain legumes (Malik and Saxena 1992) as a growth regulator to induce multiple bud proliferation from cotyledonary and apex nodes. At the University of Naples, the effect of three different concentrations of thidiazuron (5, 10, and 20 mM) on seed germination, and on apical and lateral bud proliferation, has been studied on the local cultivar "Cornetto" and on three lines selected at IITA (TVu 9062, VITA3, and VITA4). The cultivar Cornetto and the line TVu 9062 gave, on average, the best results in terms of frequency of multiple bud proliferation from apices, with an average of 87% and 85%, respectively. Shoots from these buds produced roots only when transferred into a basal medium without the presence of thidiazuron. The results confirmed that this in vitro regeneration protocol is still genotype-dependent.

On the basis of these experiments, differentiation of new shoots in the presence of coconut water is, at present, a cumbersome protocol: it requires tissues in a particular stage of growth, it is strictly genotype-dependent, and it is slow. Finally, the efficiency of this protocol in producing new buds is very low in comparison with those applied to other crops, which is the major drawback to applying it in genetic transformation experiments. Instead, multiple bud proliferation is a less demanding task, which can be accomplished by using some well-defined cytokinins. The protocol involving the herbicide thidiazuron showed the best performance on more than one genotype: it is fast, highly reproducible, and explant handling is relatively easy for subsequent manipulation for gene transfer experiments, with either physical or *Agrobacterium*-mediated protocols.

Genetic transformation

In the absence of a reliable regeneration protocol for cowpea, scientists working with this species have been forced to seek a different approach for plant genetic transformation. All the methodologies developed in the past few years are based on the rationale that highly morphogenic tissues, i.e., meristems, can be transformed in the same way as other tissues. Therefore, the main goal has been to rescue shoots developed from a bud regenerated from

a previously transformed tissue. Hence, studies were, at first, focused on the ability of meristematic cells to be genetically transformed. Two explant sources were tested: apical vegetative meristems and lateral (cotyledonary) meristems. Moreover, two different gene transfer methodologies were tested: *Agrobacterium*-mediated DNA transfer and direct plasmid DNA transfer into meristematic cells.

***Agrobacterium*-mediated gene transfer.** *Agrobacterium*-mediated plant genetic transformation involves interaction between plant and bacterial genotypes (Lurquin 1987). This requirement is more stringent when meristems are involved in the gene transfer. Scientists at Purdue University and the University of Naples have tested several *Agrobacterium* strains, having various degrees of virulence: the highest was the A281 strain (Hood et al. 1986), a hypervirulent oncogenic strain, followed by the EHA101 strain, a hypervirulent cured strain (Hood et al. 1986), while the lowest was the LBA4404 strain (Hood et al. 1986), a cured strain. Binary vectors carrying multiple *vir*-gene copies, kindly supplied by S. Gelvin of Purdue University, have also been tested.

Frequencies of explants espressing *gus* reporter gene varied between 2% and 84%, depending on the kind of transformed explant (apical or lateral meristems performed poorly in comparison with other tested plant tissues) and the presence of *vir*-gene enhancer (bacteria conditioned with acetosyringone were more efficient in gene transfer than those not conditioned). However, no transformed shoots were obtained from these cultures, even though some evidence of chimeric shoot generation was produced at Purdue University. In all, no experiments in these laboratories ruled out the possibility of obtaining transformed meristems and, thus, transformed shoots. All experiments showed, however, that considerable work and a higher number of explants are needed to pursue our goal of genetic transformation in cowpea.

Other nontissue-culture approaches involving *Agrobacterium*-mediated genetic transformation have been successfully set up on *Arabidopsis thaliana*. These methods are based on seed co-cultivation with bacteria (Feldmann and Marks 1987), in planta *Agrobacterium* infiltration (Chang et al. 1994), and in planta *Agrobacterium* inoculation. All of these techniques produced stably transformed progenies, as verified by Southern analysis. Frequencies of transformation ranged from 0.3% using the seed imbibition technique to ~ 30% using in planta *Agrobacterium* inoculation in buds. This latter method was applied to cowpea in Portici, using a binary vector, harboring NPT II, GUS and the α-amylase inhibitor genes, kindly supplied by Prof. M. Chrispeels (UC San Diego, USA), as part of the joint project with IITA. In the project, more than 2700 T_1 seeds were collected; after a preliminary screening based on NPT II and GUS assays, the material is at present under evaluation at IITA for *C. maculatus* resistance.

Direct plasmid DNA transfer into meristematic cells. Physically mediated plasmid DNA transfer into cells can be accomplished using different methods: microprojectile bombardment, electroporation, DNA adsorption by dry tissues, etc. (Potrykus 1990). All these methods have been tested by scientists involved in the Cowpea Biotechnology Project. It has been shown that using these methods, plasmid DNA can be expressed in the cells. However, results indicate that > 90% of this expression is transient, and that there is a decline in activity a few days after the experiment; only a small percentage of cells

transformed in this way have been found to be stable subsequent to transformation after at least 2 weeks of culture. In all experiments, the *gus* (β-glucuronidase) gene from *Escherichia coli* was used as the reporter gene, in order to verify all transformation events.

A helium-driven gene-gun apparatus, used to shoot cells using tungsten or gold microprojectiles covered by a DNA solution, was tested by scientists at Purdue University and at the University of Naples. The shooting vector was also supplied by M. Chrispeels and contained the same genes mentioned earlier. Several shooting parameters were evaluated: helium pressure, distance of carrier disk from the stopping plate, distance of the stopping plate from tissues, developmental stage of seeds, and different genotypes. Transient expression was first studied by testing GUS activity in explants 3 days after microprojectile bombardment. Even transient expression was found to be genotype-dependent. Scientists at Purdue University showed that among 7 genotypes, the mean number of single transient transformed cell of cotyledonary nodes ranged from 5.4±1.3 for the cowpea line 1137 to 45.5±6.9 for the CB5 genotype. At the University of Naples, some parameters were tested to improve the frequency of transformed explants and the mean number of blue-stained cells per explant. Apical and lateral thidiazuron-induced multiple meristems gave the same results when helium pressure was set to 1100 p.s.i. and the microcarrier was placed 9 cm away from the stopping plate. However, the number of transformed leaf cells steadily decreased from the maximum, an average of 150 per explant 24 h after shooting, to 5 per explant after 14 days, due to DNA transient expression. This value is still too low to be useful for genetic transformation experiments.

Other experiments were performed combining direct with indirect genetic transformation systems: plain microprojectiles bombardment followed by co-culture with *Agrobacterium tumefaciens*. In some pilot tests, ~ 100% of explants showed stable DNA integration in cells very close to the meristematic ones, as revealed by GUS-istochemical assays.

Recently, a novel method for plant transformation, useful for recalcitrant species like cowpea, has been set up by Paul Lurquin's group at Washington State University, USA (Chowrira et al. 1994). This method is based on electroinjection of plasmid DNA directly into meristematic cells of in vivo cultured plants, hence avoiding all in vitro procedures, meristem regeneration, and somaclonal variation. About 8–10% of the electroinjected plants rescued were stably expressing the *gus* gene. Pea, lentil, and soybean plants were obtained from in vivo treatment, and some of their T_2 progenies showed the presence of the introduced gene by Southern analysis. Moreover, cowpea plants stably expressing the *gus* gene were obtained. Some of their progenies were analyzed for the presence of the introduced gene. The presence of the GUS-INT gene, revealed by Southern analysis, confirmed the possibility of transforming cowpea using this system (M.G. Chowrira et al., personal communication). This technique, successful in Pullman, Washington State University, has been applied at the University of Naples to introduce the α-amylase inhibitor gene isolated from bean. The obtained progenies are now under screening at IITA.

Conclusions

It seems clear that very important progress has been achieved in the past few years in the use of biotechnologies in cowpea, mainly in genome mapping through molecular markers and in obtaining transgenic plants. This last achievement opens the road to apply genetic

engineering to this species, with very good prospects of success because the methods are set up and some useful genes are available.

Research should now concentrate on problems related to the step from a transgenic plant to a transgenic crop, which implies the study of the expression level of the inserted genes and the interaction of the new genotypes with different environments.

Globally, we know that cowpea is not a major cash crop and that, therefore, no private biotechnology industry will invest in it, but we have also seen that it can benefit from information and material used for other, better researched crops. This is why an international collaborative action involving leading laboratories should be maintained for making further progress in this effort.

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Molecular markers and genome mapping in cowpea

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Abstract

Molecular markers such as RFLPs exist in almost limitless number in all organisms, and these could be very useful in monitoring the loci of genes that control important traits, as well as in studying genome evolution and structure. In cowpea, a genome map based mainly on RFLP markers has been developed. This map presently has 92 markers and spans 717 cM of the genome. Using this map, quantitative trait loci (QTLs) for seed weight, pod length, and aphid resistance have been identified. Phylogenetic relationship among 44 genotypes belonging to different varieties, species, and sections in the genus *Vigna* was ascertained, following RFLP analysis. A comparison between the genomes of cowpea *(Vigna unguiculata)* and mung bean *(V. radiata)* showed that nucleotide sequences were generally conserved but entire linkage groups were not, although several large linkage blocks were still maintained by both crops.

Introduction

The availability of useful genetic markers in cowpea and other pulse crops is limited in comparison to other groups of crops. A gene list, based on a few morphological markers in cowpea, has been compiled (Fery 1985), and this list has been extended with additional markers, as reported elsewhere in this book (Fery and Singh 1997). Attempts made by researchers to confirm linkages between the identified genes have so far not yielded the desired results. Hence, the linkage orders of these identified genes (markers) have not been ascertained. From available reports, it appears that there is a need to seek additional sources of markers for developing a useful genetic linkage map of cowpea.

Molecular biological techniques provide opportunities for obtaining high frequencies of genetic markers that are useful in developing genetic linkage maps of different organisms. In addition, these molecular markers help in varietal identification and fingerprinting, genetic analysis of agronomically important characters, and in making more effective use of breeding methodologies (Beckman and Soller 1986). Molecular markers such as restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLP), and random amplified polymorphic DNAs (RAPDs) are, like other genetic markers, detected as differences in the DNA sequence of two or more individuals. A marker becomes useful when two individuals carry different forms (alleles)

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that are faithfully transmitted to the progeny resulting from a cross between them. The potential benefits of molecular markers like RFLPs, in crop improvement, have been reviewed by Beckman and Sonner (1986).

RFLP marker technology can be used to gather information about agriculturally important genes. For example, phenotypic and pleiotropic effects of genes, the number of genes influencing the character of interest, the location of genes on the chromosomes, and possibly the influence of the environment on genes can be assessed using this technology. Identification of tight linkages between genes of agricultural importance and molecular markers makes it posssible to select for the latter, thereby indirectly selecting for the genes of importance. RFLPs are ubiquitous, inherited in a Mendelian form, codominant in expression, detectable in all tissues and at all ages, and available in virtually unlimited numbers of probe × enzyme combinations.

DNA markers, especially RFLPs, have in recent times become popular in the production of genetic maps of various organisms, such as common bean (Vallejos et al. 1992), lettuce (Landry et al. 1987), rice (McCouch et al. 1988), and man (Botstein et al. 1980). Other DNA markers, such as RAPDs and variable number tandem repeats (VNTRs), have been found to be useful for generating genetic maps. Because of the potentially large number of detectable DNA markers, saturated genetic maps can be more readily obtained when these types of markers are used. A linkage map is saturated when markers are distributed ~ 5 centiMorgans (cM) apart throughout the set of chromosomes (King 1990). Genetic maps, when saturated, become more useful because all parts of the genome become accessible, thereby facilitating manipulation of individual genetic factors that are associated with traits of economic importance.

Genome mapping using DNA markers takes advantage of the large differences that exist in natural populations, and no two individual organisms are likely to be identical in their DNA base sequence. These differences may be brought about by inversions, recombination during meiosis, deletions, translocations, or transpositions. Maps developed using DNA markers can be effectively used to enhance genetic manipulations of crops and other organisms. Since the locations of these markers in the genome can be identified with a high level of precision, they can be useful for detecting genes of interest which are located near them. Sax (1923) suggested that major genes which can be scored easily should be used to identify the positions of minor genes that are of interest to the breeder. This suggestion by Sax (1923) could not be effectively put to use because identified morphological markers had large effects on phenotypes and masked the effects of linked minor genes (Tanksley et al. 1989). With the discovery of DNA markers which have no deleterious effect on plant morphology and which can be easily detected in very large numbers, those tightly linked to desirable genes can be identified and used by breeders as aids to selection.

Linkage maps based on RFLP markers are being developed for cowpea (*Vigna unguiculata*) and mung bean (*V. radiata*) using a common set of DNA clones from single copy genomic libraries of bean (*Phaseolus vulgaris*), soybean (*Glycine max*), mung bean, and cowpea (Fatokun et al. 1993a). Since a common set of probes was used for generating both maps, it is possible to carry out a comparative analysis of the two genomes (Menancio-Hautea et al. 1993). Using these maps, quantitative trait loci (QTLs) with effects on seed weight were identified on the genomes of both cowpea and mung bean (Fatokun et al. 1992). Additionally, a subset of the probes was utilized to study genetic

variability among several species in the genus *Vigna*, and this enabled the establishment of phylogenetic relationships among several accessions of the different species (Fatokun et al. 1993b).

RFLP map of cowpea

A genetic map of cowpea based on RFLP, RAPD, and some morphological attributes is being developed. The cowpea mapping population comprises 58 F_2 plants, derived from a cross between an improved cultivar IT84-2246-4 and a wild relative TVNI 963 (*V. unguiculata* ssp. *dekindtiana*). Total DNA was extracted from each F_2 plant, digested with up to seven restriction endonucleases, blotted onto nylon membranes, and probed with about 300 single-copy DNA clones derived from bean, soybean, mung bean, and cowpea. Although the two cowpea parents are known to share the same primary gene pool, partial fertility was observed in the F_1 plants. The level of polymorphism between the two cowpea parents that were crossed to generate this mapping population was found to be about 20%, which is rather low. The DNA clones that detected polymorphisms between the two parents were then used to probe the DNA of the F_2 plants. A high proportion of the probes hybridized to both parental and F_2 DNA. The low level of polymorphism observed between the two parents is usually associated with self-pollinated crops. For example, to facilitate the development of a saturated map of tomato, it was necesary to embark on interspecific hybridization to generate a mapping population (Helentjaris et al. 1988) which provided a higher level of polymorphisms than from intraspecies crosses. Like tomato, cowpea is a highly self-pollinating crop.

The cowpea genomic map now has 92 markers distributed among 85 loci (Fatokun et al. 1993a). These markers are made up of 79 genomic, 4 cDNA, 6 RAPD, 2 aphid resistance loci, and 1 seed coat texture locus. The mapmaker computer program was used to determine linkage relationships between adjacent loci and linkage order was inferred when LOD ($\log_{10}$ of the odds ratio) score exceeded 2.0. Five loci have multiple markers (12 markers). The 92 markers are distributed into 10 linkage groups, although cowpea has a chromosome number of $n = 11$. This map spans > 800 cM of the cowpea genome, implying that the mean distance between these markers is < 10 cM. Ten markers have not been linked to any of the existing linkage groups. However, effort is still being made to place more markers on this map so as to develop a saturated map for cowpea, which would facilitate the exploitation of the genetic potential of the crop.

Comparison between the genomes of cowpea and mung bean

A linkage map is also being developed for mung bean, using a similar set of heterologous RFLP markers as for cowpea. Because of the common set of RFLP markers used for both crops, it is feasible to evaluate their genomic relationship. By comparing their genomes, it should be possible to ascertain whether studies on gene action for some desirable traits in one crop can be used to infer gene action for the other crop.

It was observed that ~ 90% of the heterologous clones tested hybridized to the DNA of both crops, suggesting a high level of similarity in the nucleotide sequences of both crops (Menancio-Hautea et al. 1993). Similarly, the high level of hybridization of DNA clones from bean and soybean to the DNA of both crops suggests that all these leguminous crops, to a very large extent, share identical nucleotide sequences. In addition, 53 markers

mapped in common between cowpea and mung bean were used to verify if there were any linkage groups conserved between them. The results showed that although no entire linkage group is conserved, large blocks were retained in some of the linkage groups. Within the blocks that were conserved, the order of the loci were similar in some, whereas in others major rearrangements could be detected. This comparative analysis of the genomes of cowpea and mung bean led Menancio-Hautea et al. (1993) to conclude that insertion/deletion might have played a role in the evolution of the two crops to their respective domesticated forms.

Identification of seed-weight QTLs in cowpea and mung bean

Usually, a number of genes govern agriculturally important traits. Each gene contributes its own quota towards the expression of the trait. Such traits are quantitatively inherited. Individual contribution of the genes tends to be variable, ranging from qualitative to a vanishingly small amount. Each of the several genes affecting a quantitatively inherited trait behaves like those controlling qualitatively inherited traits with regard to the laws of segregation and recombination. Incorporating these multiple genes into varieties is usually not an easy task to accomplish, since these genes may be found in various parts of the genome. Where a linkage map is available, it should be possible to dissect these quantitative traits into discrete genetic factors, i.e., quantitative trait loci (QTLs). Using interval mapping procedures (Paterson et al. 1991a), all parts of the genome can be searched for the presence of QTLs while at the same time accurately estimating their phenotypic effects. QTL analysis allows the identification of individuals with the potential of producing progeny that will express a certain phenotype. It could also be useful in identifying those loci with small effects on the phenotype, i.e., low heritability. However, in such situations, a larger plant population will be required to detect these QTLs than in cases where the loci explain a large amount of the variation for the trait of interest.

The RFLP maps being constructed for both cowpea and mung bean were used to search for the presence of QTLs for seed weight, an important trait in both crops. The mean seed weight of each plant in the F_2 mapping population was determined. By using the computer program Mapmaker-QTL, it was possible to infer the presence of QTLs for this trait in both crops. These seed-weight QTLs, two in cowpea and four in mung bean, explained 52.7% and 49.7%, respectively, of the variation for this trait. It is noteworthy that the regions of the genomes which account for the highest amount of variation in seed weight were spanned by the same RFLP markers in them. These markers were in the same linkage order in the genomes of both crops (Fatokun et al. 1992). It was inferred from this observation that these regions of their genomes have remained conserved in the course of their evolution from the wild to the present forms. In that period, certain traits such as compact/erect plant habit, nonshattering of pods, day-neutral characteristic, and early flowering have become dominant (Smartt 1985). Since seeds represent the economically important parts in these two crops, and since large seed size is preferred by consumers, selection pressure must have been imposed in favor of higher seed weight over the years. Essentially, genes controlling seed weight are being selected along with markers associated with the trait, i.e., the nucleotide sequences of the regions with these particular QTLs for seed weight in cowpea and mung bean must have been selected by farmers growing these crops. In some annual plants, alleles of marker loci that are closely associated with

characters that determine survival and enhanced reproductive capacity tended to increase as the crops evolved from wild progenitors to the present day domesticates (Allard 1988).

The orthologous seed-weight QTLs in cowpea and in mung bean span 14.0 cM and 31.0 cM of their genomes, respectively. This observation suggests a reduction in recombination in cowpea chromosomes as compared to those of mung bean. Bonierbale et al. (1988) compared the genomes of potato and tomato, and found that frequency of recombination was significantly lower in potato. However, nuclear DNA contents of cowpea and mung bean are identical at about $2n = 1.0$ pg (Arumuganathan and Earle 1991).

Quantitiative trait loci for pod number, pod length, plant height, days to 50% flowering, and days to maturity were also detected in cowpea using data of F_2-derived F_3 (F_2:$_3$) progenies. Seed of F_2:$_3$ progenies were sown in the field at IITA, Ibadan, Nigeria, while those of the F_2 were sown in the greenhouse at the University of Minnesota, St. Paul, USA. It was noted that seed-weight QTLs detected in the F_3 population (Fig. 1) were spanned by the same markers (pA509, pO103, pA487, pM185, and pM182) as those in the F_2 population (Fatokun et al. 1992). These observations suggest that the QTLs for seed weight detected in the two regions of the cowpea genome are not particularly sensitive to environment. Paterson et al. (1991b) found that in tomato, 4 of the 29 detected QTLs were not affected by the environment. For purposes of crop improvement, QTLs that remain consistent in their effects irrespective of environment are useful, and where environment specific QTLs are available, they could as well be exploited to enhance the agricultural productivity of the crop. Combining several QTLs with different environment specificities into a genotype might induce an improvement in phenotype that is buffered against environmental vagaries (Paterson et al. 1991b).

Two loci that influence resistance to aphids were detected and mapped on the cowpea genome. One of the two genotypes (IT82 2246-4) that were crossed to obtain this mapping population is an improved aphid resistant variety. A locus that is very tightly linked to an RFLP marker (bg4D9b) on linkage group 1 was detected and a second locus, not tighly

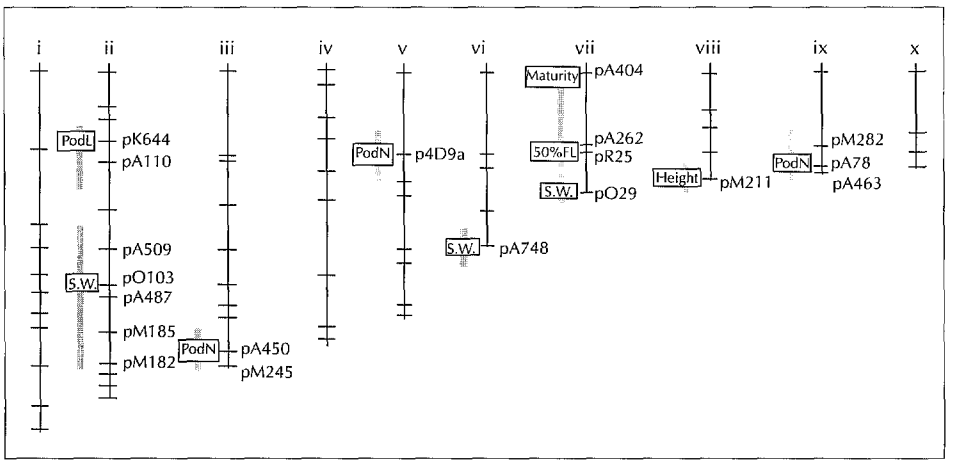

Figure 1. Major QTLs detected for some agronomic traits in cowpea. The traits (PodL = pod length, SW = 100 seed weight, PodN = pod number/plant, maturity, 50% FL = 50% flowering, and height = plant height at maturity) were measured on 58 F_2:$_3$ derived progeny rows.

linked to any of the markers mapped, is on linkage group 8 (Myers et al. 1996). Selection for the marker bg4D9b correspondingly leads to selection for one of the two loci that control aphid resistance in cowpea. Usually, screening for aphid resistance is carried out in the greenhouse or in the field during the seedling stage of cowpea. Situations exist in which plants showing resistance in the seedling stage may succumb to the pest at a later stage of growth. Markers found to be closely linked to the loci conferring resistance to aphids throughout the plant's growth stages can aid breeders in selection. Marker-assisted selection can then be carried out at the seedling stage, which should also identify resistance expressed as the plants mature.

Taxonomic relationships in the genus *Vigna*

DNA markers have been used extensively to study taxonomic relationships between and within species. Morphological attributes have traditionally been employed in establishing phylogenetic relationships among genotypes between and within species. Many of the morphological characters commonly used are prone to environmental influences, thereby reducing the fine resolution required to ascertain phylogenetic relationships. The number of morphological attributes that can be scored is generally limited. DNA markers provide a larger number of characters which are unaffected by environmental influence, and consequently can provide unambiguous character-state assignments (Sanderson and Donoghue 1989).

Morphological attributes along with cytological, phytogeographic, and crossability data have been used to study taxonomic relationships between genotypes belonging to the genera *Phaseolus* and *Vigna* (Verdcourt 1970; Marechal et al. 1981). Isozyme variations among different *Phaseolus* and *Vigna* species, subspecies, and varieties were detected by Jaaska and Jaaska (1988), but the variations were not used to evaluate relationships among the tested genotypes. However, a study on taxonomic relationships among 44 accessions belonging to several species within the genus *Vigna* has been carried out based on RFLP analysis (Fatokun et al. 1993b). All of the random genomic clones derived from soybean, common bean, cowpea, and mung bean hybridized with total genomic DNA from all of the accessions examined. This observation implies that nucleotide sequences of many of the genes are conserved in these leguminous plants so as to permit such a level of heterologous hybridization. In the Graminaea, Hulbert et al. (1990) found that maize (*Zea mays*) DNA clones hybridize very well with DNA of sorghum (*Sorghum bicolor*) genotypes; they suggested that cloned DNA fragments which hybridize to single sites in the genomes of two species can be assumed to have arisen from a single sequence in a common ancestor. Maize and sorghum are both members of the tribe Andropogonae. Cowpea, common bean, mung bean, and soybean are all members of the sub-family Papilionoideae.

For the phylogenetic study based on RFLP analysis, total DNA was extracted from each *Vigna* accession, digested with one endonuclease (*Eco*Rv), and blotted onto nylon membranes hybridized to 40 random genomic DNA clones. A few of these clones were detected as single-copy in cowpea and bambara groundnut (*V. subterranea*), but as multiple copies in mung bean and the *Phaseolus* species. An example of such is a bean genomic clone p_4D_{10}. The genotypes were not scored for any clone that showed multiple bands in any of the genotypes tested in this study. Only 27 clones were eventually scored for phylogenetic analysis. These clones gave rise to 369 RFLP bands, i.e., characters for

which each accession was scored. For each RFLP, a genotype was scored as having (1) or not having (0) a particular band. Data obtained from banding patterns were, thereafter, subjected to the NTSYS-pc program (Rohlf 1990).

The RFLP data obtained from the accessions belonging to different *Vigna* species were subjected to numerical taxonomic procedures, which showed that homology at the DNA level ranged from as low as 62% between soybean in the subtribe *Glycinae* and other test materials in the *Phaseolus-Vigna* complex to 96% between plants of the same accession in some landraces of cowpea, bambara groundnut, and mung bean. The detection of variation as low as 4% at the genome level among members of an accession further attests the robustness of the RFLP technique for genome characterization.

Morphologically, the five plants established from an accession of mung bean could not be distinguished from one another, just as the five plants from an accession of bambara groundnut also resembled each other. However, despite the morphological simililarities among members of an accession in both crops, RFLP markers were able to detect differences between the crops.

The numerical taxonomy of the genus *Vigna* based on RFLP analysis distinctly separated the genotypes into classes that were similar to those already established by conventional classification, which were based primarily on plant morphology. For example, members of sections *Catiang*, *Ceratotropis,* and *Plectotropis* were placed in their natural groups. The classification based on RFLP data also confirmed the existence of a high level of genetic variation among African *Vigna* species.

It is known that polymorphisms detected by the same RFLP probe but more than one endonuclease may not be independent mutational or DNA rearrangement events. Only one restriction endonuclease, *Eco*RV, was used to digest total genomic DNA of the various *Vigna* species tested. Hence, the polymorphisms detected in this study are independent events and the result of this numerical taxonomy should, therefore, be reliable.

DNA markers in cowpea improvement

In the process of crop improvement, the breeder manipulates the genome of the plant of interest, so that the resulting genotype meets his/her set objectives. Accomplishing the objectives will, by and large, depend on the tools at his/her disposal. In recent times, additional tools have become available to breeders in the area of molecular biology with which they can more effectively investigate, among others, the inheritance of complex desirable traits and manipulate the genetic factors associated with these traits. Using the RFLP technique, it is now possible to carry out, based on common probes, comparative studies of the genomes of organisms which cannot be crossed. The genomes of tomato and pepper, as well as those of maize and sorghum, have been compared, following development of their genomic maps based on RFLPs.

Molecular markers available in very large numbers have been found particularly useful for generating genetic maps that may help breeders in their selection work. Markers that are closely linked with traits that are difficult to score can be selected, thereby selecting indirectly for the desired trait. In such situations, selection is carried out for the marker(s) that are tightly linked to the trait of interest. Environmental factors complicate studies of the genetic control of quantitatively inherited characters, and make the selection for such traits rather difficult. Molecular markers are generally independent of environment and

their heritability values are very high. These markers can, therefore, be used to dissect quantitatively inherited traits to their simple Mendelian factors.

Cowpea is susceptible to a number of insect pests that cause considerable yield losses. Low to moderate levels of resistance have been detected in some noncultivated wild relatives of cowpea, and attempts are being made to accumulate in cultivated cowpea the genes conferring the level of resistance available. Even low levels of resistance are often controlled by many genes, i.e., they are quantitatively inherited, and may be distributed into different loci on the genomes. Partial resistance genes, where present, confer durable resistance because they pose very little selection pressure on the pests and diseases organisms (de Ponti and Mollema 1992). Therefore, plant breeders who wish to develop varieties resistant to pests and diseases should aim for this type of resistance. Marker-assisted selection should enable the genes with overlapping effects to be effectively accumulated in cultivated cowpea.

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Developing a transformation system for cowpea (*Vigna unguiculata* [L.] Walp.)

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Abstract

Research has been conducted to develop a cowpea (*Vigna unguiculata* [L.] Walp.) transformation system, using microprojectile bombardment or cocultivation with *Agrobacterium tumefaciens*. A morphogenic system, utilizing embryonic axis and cotyledonary base explants, has been developed that provides a target explant for transformation that can give rise to fertile plants. Besides reporter (*uidA*) and selectable marker genes (*nptII* or *bar*), vectors containing genes encoding either an α-amylase inhibitor (natural insecticidal protein) driven by a 35S CaMV promoter or a Brazil nut 2S albumin (protein with high content of sulfur-containing amino acids) driven by a phaseolin promoter were used in transformation experiments. Transformation conditions were established for optimal delivery of the genes by analysis of transient expression of a ß-glucuronidase reporter gene. Organogenesis induced on medium supplemented with high concentrations (2–20 μM) of N^6-benzylaminopurine (BA) and subsequent shoot culture under kanamycin or bialaphos selection pressure resulted in regeneration of several transgenic chimeras. Introduced genes were detected in genetically modified T_0 cowpea plants by both histochemical GUS/MUG assays and PCR or Southern blot detection of transgenes. Work to obtain evidence of transferred genes in the T_1 progeny (GUS/MUG assays, Biomonitor insect resistance assay, and Southern blot hybridization) is currently being performed.

Introduction

Cowpea (*Vigna unguiculata* [L.] Walp., syn. *Vigna sinensis* [L.] Savi ex Hassk), known also as southern pea or blackeye pea, is one of the world's most important food crop legumes. It is valued as the major source of essential amino acids in tropical diets, which consist predominantly of starchy cereals, roots, and tubers, although the seed proteins are somewhat deficient in the sulfur-containing amino acids (methionine and cysteine) and tryptophan. In the USA, India, Australia, and several countries of southeast Asia, Central and South America, it also has important horticultural, agronomic, and industrial uses.

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In developing countries, cowpea suffers severely from insect pests such as pod borers (e.g., *Maruca testulalis*), pod bugs (e.g., *Clavigralla tomentosicolis*), cowpea weevils (including *Callosobruchus maculatus*), aphids, and flower thrips. During the past two decades, a number of high-yielding varieties and varieties with improved nutritional quality or relatively high resistance to some diseases and pests have been identified by screening cowpea accessions and breeding desirable traits from different genotypes. However, due to difficulties in obtaining fertile progeny after interspecific crosses between cultivated and wild *Vigna* species, conventional screening/breeding methods have not been able to eliminate the major production constraints.

Gene manipulation and plant genetic transformation might facilitate progress in this regard. Further development of plant protection against insects can be achieved by transfer into cowpea genome genes encoding either *Bacillus thuringiensis* endotoxins (Dean and Adang 1992; Feitelson et al. 1992; Peferoen 1992; Fujimoto et al. 1993), protease inhibitors (Hilder et al. 1989; Johnson et al. 1989; Gatehouse et al. 1991, 1992), cowpea trypsin inhibitors, or α-amylase inhibitors (Gatehouse et al. 1986; Ishimoto and Kitamura 1988; Shade et al. 1994), which are known to be effective insecticidal proteins. On the other hand, deficiency in the sulfur-containing amino acids can be alleviated by introducing into cowpea plants genes encoding proteins such as 2S Brazil nut albumin, zein, or the nodulin-21. To accomplish this goal, these genes need to be genetically engineered into plants by transformation techniques.

To date, no transformation system has been reported for cowpea. There are a few reports of obtaining transgenic cowpea calli or chimeric plantlets from leaf discs (Garcia et al. 1986, 1987), axillary buds, or embryos (Penza et al. 1991) by transformation with *Agrobacterium tumefaciens* or embryo imbibition with or without subsequent electroporation (Penza et al. 1992; Akella and Lurquin 1993). Attempts to produce mature transgenic plants, however, failed in all these cases.

Our studies aimed to develop an efficient genetic transformation system for cowpea, utilizing either microprojectile bombardment or *Agrobacterium* cocultivation. Up to now, this study has resulted in the regeneration of several mature, fertile transgenic cowpea plants that were chimeric for the presence of the introduced gene in the genome. Stable transformation of introduced genes in T_0 cowpea plants has been proven by histochemical GUS assay, PCR, and Southern blot hybridization. To overcome the problem of persistent regeneration of transgene chimeras, our research has been focused on developing an efficient morphogenetic culture system and efficient selection procedures utilizing *bar* (bialaphos resistance gene) and *nptII* (kanamycin resistance gene) selectable markers. Results of this experimentation are presented in this paper.

Materials and methods

Cotyledon segments and embryonic axes from immature embryos of cowpea (*Vigna unguiculata* [L.] Walp.) were used for the transformation experiments reported here. Based on morphogenic response studies (data not shown), two genotypes, TARS-36 and CB5, were selected from eight genetically divergent genotypes and used for these experiments.

Media. All media (pH 5.8) contained MS macro- and micronutrients (Murashige and Skoog 1962), BA, and NAA (α-Naphthaleneacetic acid) at concentrations listed in Table 1.

Table 1. Media composition.

	CP1	CP2	CP3	CP4	MS0
MS macronutrients	1X	1X	1X	1X	1X
MS micronutrients	2X	1X	1X	1X	1X
N^6BA	10 μM	5 μM	5 μM	–	–
NAA	0.2 μM	0.2 μM	0.05 μM	0.05 μM	–

In addition, media were supplemented with modified B5 vitamins (Gamborg et al. 1968) (100 mg/L inositol, 12 mg/L thiamine HCl, 0.5 mg/L pyridoxine, 1.0 mg/L nicotinic acid), 30 g/L sucrose, and 7.0 g/L agar.

Primary explants and tissue culture. Green cowpea pods collected from the plants grown in the greenhouse were surface sterilized (70% ethanol for 30 sec, 30% commercial bleach for 30 min) and rinsed with several changes of sterile distilled water. Embryos were excised from seeds, and the embryonic axes and cotyledons were separated. Explants were plated on agar solidified CP1 medium. After microprojectile bombardment by means of helium biolistic gun or *Agrobacterium* cocultivation, explants were cultured for 3 weeks in the dark at 26 °C and plated onto the CP2 (callus maintenance) or CP3 (shoot elongation) in light (16 h photoperiod, 20–25 $\mu mol \cdot m^{-2} \cdot s^{-1}$ "cool white" fluorescent illumination) to promote shoot development. Developing shoots were recultured on the fresh CP3 medium at 2-weekly intervals. Shoot regeneration was conducted under continuous selection pressure (kanamycin or bialaphos). Shoots that survived on selection medium were transferred to CP4 rooting medium. When 10–15 cm tall, plants were transferred to soil, grown in a growth chamber, and subsequently in the greenhouse until pod maturity.

Transformation vectors. For microprojectile bombardment, pML112 and DP532 plasmids were used. The pML112 plasmid contained the *nptII* coding region (kanamycin resistance) under control of the *mas* promoter, the *uidA* gene driven by the CaMV 35S promoter, and the α-amylase inhibitor (α-AI) coding region under control of the CaMV 35S promoter. The α-AI gene encodes a protein that has been shown to be an efficient insecticidal agent against *Callosobruchus maculatus*. The DP532 plasmid contained *uidA* reporter gene and *bar* selectable marker, both driven by CaMV promoter and the coding region for Bex protein (Brazil nut 2S albumin) under control of phaseolin (PHAS) promoter. To optimize bombardment parameters, the plasmid pPUR—carrying the *uidA* gene under control of the CaMV promoter—was also used.

For *Agrobacterium* cocultivation, pMCP-3 plasmid was used containing CaMV::*uidA* reporter gene and *nptII* and *bar* selectable markers under control of *nos* and CaMV promoter, respectively, and α-AI coding region driven by PHAS promoter.

DNA coating and microprojectile bombardment. Gold (1.5–3 μm in diam, Aldrich no. 32,658-5) or tungsten (M25, 1.67 μm in diam, DuPont no. 75056) particles were coated with plasmid DNA, following a procedure described by Bio-Rad. Fifty μL aliquots containing 3 mg of prewashed in ethanol gold or tungsten particles were mixed with

plasmid DNA (0.1–0 μg), 50 μL $CaCl_2$ (2.5 M), and 20 μL spermidine (0.1 M) in 1.5 mL microcentrifuge tube by vortexing. The mixture was pulse centrifuged and the supernatant discarded. Particles were washed twice with 250 μL absolute ethanol and resuspended in 60 μL absolute ethanol by dipping in a water sonicator (Sonicor, Sonicor Instrument Corporation) for 2–3 sec. For each bombardment, 10 μL of particle suspension was spread onto the macrocarrier.

Some 15–20 primary explants were arranged in a circle of ~ 3 cm diam in the center of plastic petri dish (15 × 60 mm, Falcon no. 1007) onto CP1 medium 1 day before bombardment. Bombardments were conducted by means of the Biolistic PDS1000/He particle delivery system (Bio-Rad). Following bombardment, the explants were transferred onto fresh medium and cultured as described above.

***Agrobacterium tumefaciens* mediated transformation.** *Agrobacterium tumefaciens* strain LBA4404 (Clontech) containing the binary plasmid pMCP-3 was used for transformation. *Agrobacterium tumefaciens* infection of explants was performed by cocultivation, following mechanical injury of explants by either micromanipulation with a needle or by vortexing with carborundum. After 5 min incubation, explants were removed from *Agrobacterium* solution and placed on solidified CP1 medium at 30 °C in the dark to complete infection. After 72 h infection, explants were repeatedly washed with liquid CP1 medium, blotted, plated onto solidified CP1 medium supplemented with carbenicillin and either kanamycin (50 mg/L) or bialaphos (1 mg/L), and cultured in the dark. After 3 weeks, explants were plated onto the CP3 medium and transferred to the light. Plant regeneration was performed as described above, under continuous selection pressure. Kanamycin or bialaphos resistant shoots, after rooting on CP4 medium, were subsequently transferred to soil and grown in the greenhouse until pod maturity.

Histochemical GUS assay. Transient expression of the *uidA* reporter gene was evaluated 48 h after bombardment using 5-bromo-4-chloro-3-indolyl-ß-D-glucuronidase (X-Gluc, Biosynth AG, Biochemica, and Synthetica, Switzerland), as described by Jefferson et al. (1986a,b). Samples for histological analysis of *uidA* gene expression were prepared as per Kononowicz et al. (1992).

Histological analysis. Samples of explants grown on induction medium were harvested at 5-day intervals and fixed in FAA (formaldehyde + acetic acid + ethanol, mixture) at 4 °C for 12 h, washed in 95% ethanol, and dehydrated in ethanol series. After dehydration in the ethanol series, samples were cleared in toluene and embedded in Paraplast embedding wax. Sections, 10–15 μm thick, were cut, deparaffinized, stained with Hematoxylin stain, and analyzed under the light microscope Optiphot (Nikon).

Southern blot analysis. For DNA blot analysis, genomic DNA was isolated according to Dellaporta et al. (1983). DNA (15 μg) was digested with *EcoRI* endonuclease, fractionated on a 0.8% agarose gel, transferred to nitrocellulose, and hybridized with ^{32}P-labeled probe (Sambrook et al. 1989). The probe for detection of the *bar* gene was a 0.8-kb fragment from DP620 plasmid (provided by Pioneer Hi-Bred International) that contained the entire *bar* coding region and the proteinase inhibitor II (PinII) terminator.

Results and discussion

Tissue culture and plant regeneration system

Shoot meristem regeneration. In the absence of a defined morphogenic (organogenic or embryogenic) system for cowpea regeneration, we have directed our early efforts to transform meristem initial cells that are progenitors of the germ line cells (data not shown). Methodology of cowpea plant regeneration from shoot apices and axillary buds from 6–7 day old seedlings developed by Pompimon Suriyajantratong (personal communication) was utilized with some minor modifications in our initial transformation efforts. Basically, two different media (shoot elongation CP3 and rooting CP4) were necessary to regenerate plants in a relatively short time. Plants ready to transfer to soil can be obtained within 2 months while cultured in the absence of a selectable agent.

Morphogenesis. Cotyledon explants and embryonic axes of immature embryos isolated from green pods were chosen as another primary target explant for transformation. The culture conditions (mostly BA concentration and the presence or absence of auxin) for maximal adventitious shoot initiation were tested with these explants (Barwale et al. 1986a,b; Gulati and Jaiwal 1992). Our study showed that 2–3 week old culture of these explants in the dark on high BA medium (CP1 medium) followed by culture in light on low BA medium (CP2 or CP3) results in the formation of adventitious shoots via callus intermediary tissue. Developing shoots do not result from the growth of axillary buds on an already existing shoot, but are formed adjacent to one another in a *de novo* fashion. Organogenic culture can be maintained for several months by subculturing on CP2 medium and adventitious shoot production occurs as long as explants remain on this medium. Over a period of 8–10 weeks, up to 15 shoots can be regenerated from a single primary explant, making the system very suitable for transformation. Shoots obtained from cotyledon segment and embryonic axis cultures can be easily elongated on CP3 or MS0 medium, and subsequently rooted on CP4 medium. Developmental or morphological abnormalities in plants regenerated from these explants occurred with low (~ 5%) frequency; however, they increased significantly (up to 20%) among plants produced from organogenic callus after prolonged (> 3 months) culture.

When cultured on high BA medium in the dark, primary explants produce white, compact, undifferentiated callus masses. After culture on CP2 or CP3 medium in light, the morphology and structure of the vigorously growing callus changed significantly; due to chloroplast development increasing in size, green sectors started to appear on the periphery of callus pieces. Further, microscopical analysis of the histological sections showed the presence of numerous meristematic centers, consisting of relatively small cells with prominent nuclei and dense cytoplasm (Fig. 1B). The absence of meristematic centers at earlier stages of callus growth (Fig. 1A) indicates that they are formed de novo, 3–4 weeks after culture initiation. Anticlinal and periclinal cell divisions followed by cell growth and differentiation proceeds in a highly organized manner, and result in the appearance of small protuberances, which later form structures resembling shoot apical meristems and leaf primordia (Figs. 1C–F). The polarity of the longitudinal axes of developing organs can be seen. After further development, multiple shoots are produced. Axillary buds developing in the leaf axis can be detected in the expanding shoots (Fig. 1G). The first burst of the organogenesis results in the production of up to 15 shoots from each primary explant.

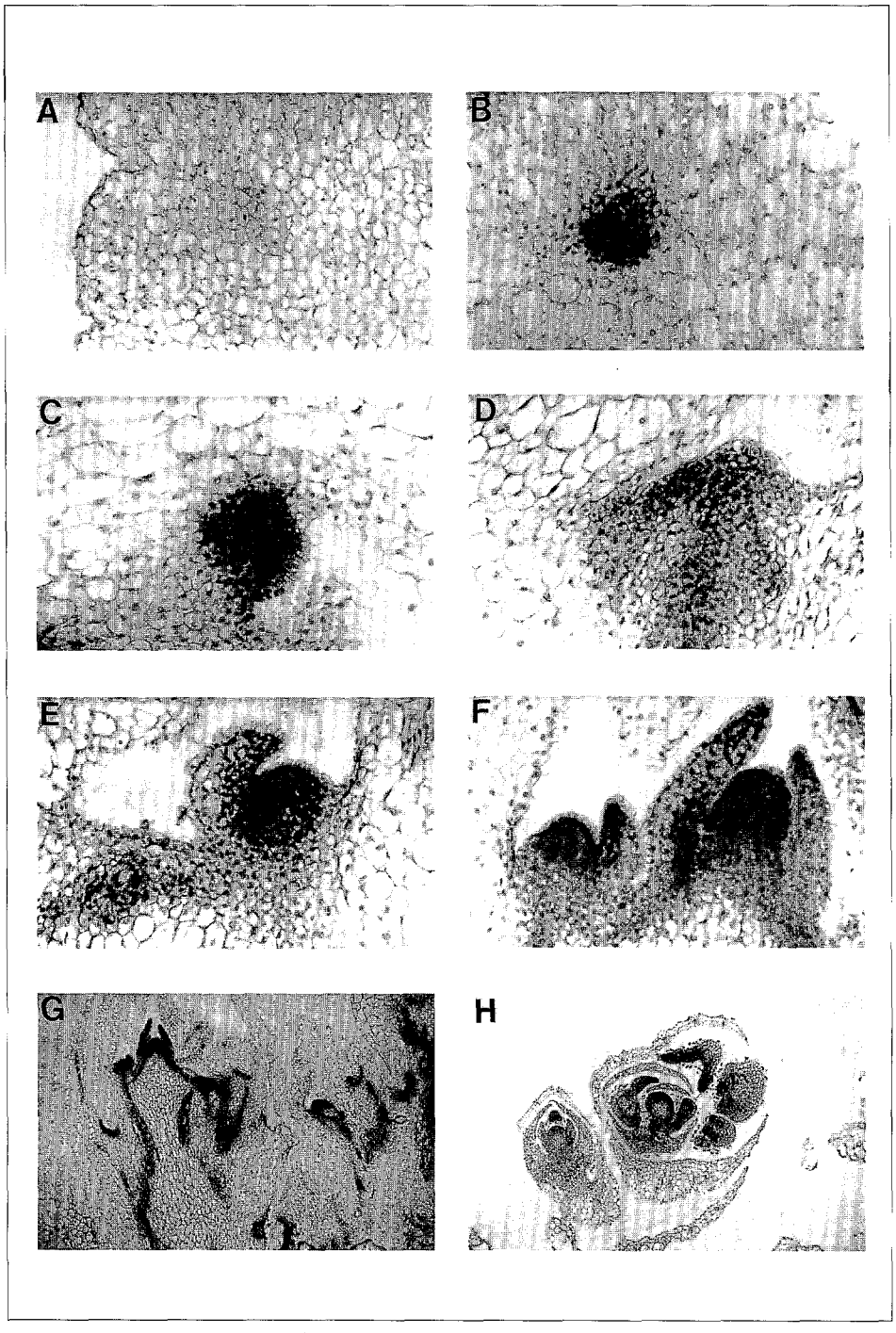

Figure 1. Histology of adventitious shoot regeneration from cowpea morphogenic culture induced by high BA medium (see text).

Since it is anticipated that T_0 plants from morphogenic cultures will have a tendency to be more homogeneously transformed than those regenerated from shoot apex and axillary bud meristems, our transformation attempts have been focused on primary explants that contain morphogenically competent cells and morphogenic callus.

Analysis of different cultivars of cowpea has shown that morphogenic response to high BA induction medium is genotype-dependent. Since the highest frequencies of morphogenic callus and adventitious shoot formation have been found for CB5 and Tars-36 (data not shown), we focused our transformation efforts on utilizing explants from these two cultivars.

Optimization of bombardment parameters. Conditions for microprojectile bombardment have been established for optimal delivery of DNA to primary explants, based on transient expression assay using histochemical techniques (Jefferson et al. 1986a,b; Sanford et al. 1993). Reporter gene activity was evaluated by determining the expression of a chimeric gene construct (pPUR) consisting of the CaMV 35S promoter-*uidA* coding sequence in primary explants 2 days after bombardment. Only minor differences were found, based on the type of microprojectile (gold or tungsten) used, gap, and macroflight distance; however, the size of microcarriers, microflight distance, and helium pressure significantly affect transient expression of reporter genes (data not shown). The optimal bombardment parameters for DNA delivery to cotyledon segments and embryonic axes are presented in Table 2.

Table 2. The optimal bombardment parameters for DNA delivery.

Amount of DNA/bombardment	0.2 µg
Target distance	9 cm
Helium pressure	1550 psi
Particle size	1.7 µm
Macroflight distance	0.6 cm

Selection and molecular analysis of putative transformants

Shoot meristems. Experiments have been conducted to evaluate kanamycin and bialaphos as a selection agent for cowpea. High concentrations (100–200 mg/L) of kanamycin are required to obtain a high degree (80–90%) of lethality of cowpea explants. On the other hand, 50 mg/L kanamycin was able to cause significant bleaching (chlorosis) (Wilmink and Dons 1993) in developing nontransformed shoots. This phenomenon has been utilized in our experiments for "visual" selection of kanamycin-resistant shoots. Treatment of explants with kanamycin within a few days after DNA delivery resulted in very low survival of shoots, without clear evidence of an enrichment of the population in terms of transgenic tissues. This is probably because only minute portions of the initial meristems are transformed, and these cells are not able to survive immediate exposure to a high level of the selection agent. Other experiments indicated that some enhancement of selection is obtained by imposing kanamycin after some degree of shoot development has occurred. However, it was found that this selection strategy results in numerous nontransformed "escapes", and only 19 transgenic chimeras (Fig. 2) were obtained when cotyledonary nodes were used as a target explant. Over 8000 seeds from these 19 plants were assayed,

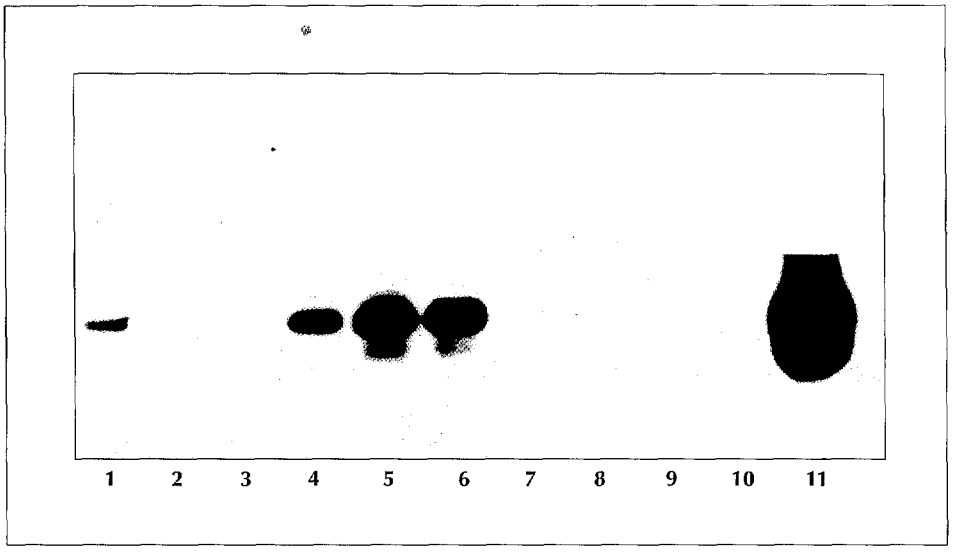

Figure 2. DNA hybridization of PCR-amplified products indicating the presence of neomycin phosphotransferase (*nptII*, kanamycin resistance) gene. Lanes 1–9, template DNA from putative transgenic plants regenerated under kanamycin selection pressure and screened for GUS activity during in vitro culture. Lane 10, DNA from untransformed plant. Lane 11, plasmid containing the *nptII* gene.

utilizing a MUG assay for activity of the *uidA* gene, and more than 20,000 seeds were screened utilizing the Biomonitor, to detect insect resistance as a result of expression of the α-amylase inhibitor gene. Of the 131 seeds (96 MUG-positive and 35 that passed the Biomonitor screening) selected for further molecular analysis, none was Southern positive. It is clear that to obtain T_1 progeny, the L2 layer of the meristem should be the target for the DNA coated particles (Christou 1992; Christou et al. 1993), since this layer gives rise to germ line cells that will produce sperm and egg (Sussex 1989; Iriss 1991; Szymkowiak and Sussex 1992). Histological analyses indicated that in ~ 35% of explants, DNA-coated particles enter the L2 layer cells. However, the results of testing transgenic chimeras so far strongly suggest that L2 layer cells were rarely, if ever, transformed. Alternatively, rearrangement of transformed meristem initials, out of the L2 layer resulting from post-bombardment injury, may occur, precluding transformed cells from giving rise to germ line cells.

Morphogenesis. In our most recent experimentation, 2000 cotyledon bases and embryonic axes were cocultivated with *Agrobacterium* carrying the pMCP-3 plasmid. Kanamycin selection pressure was applied during all induction and regeneration processes. Of the 3249 adventitious shoots induced from explants, only 69 have been identified as putative transformants after 3 subcultures on selection medium (50 mg/L kanamycin), based on the "bleaching" effect. Of these, 15 were found to be GUS-positive. Molecular analysis of these plants has not yet been conducted.

Another selection agent, bialaphos (Wilmink and Dons 1993; Hinchee et al. 1994), has been found to be more efficacious than kanamycin. Dose-response experiments utilizing

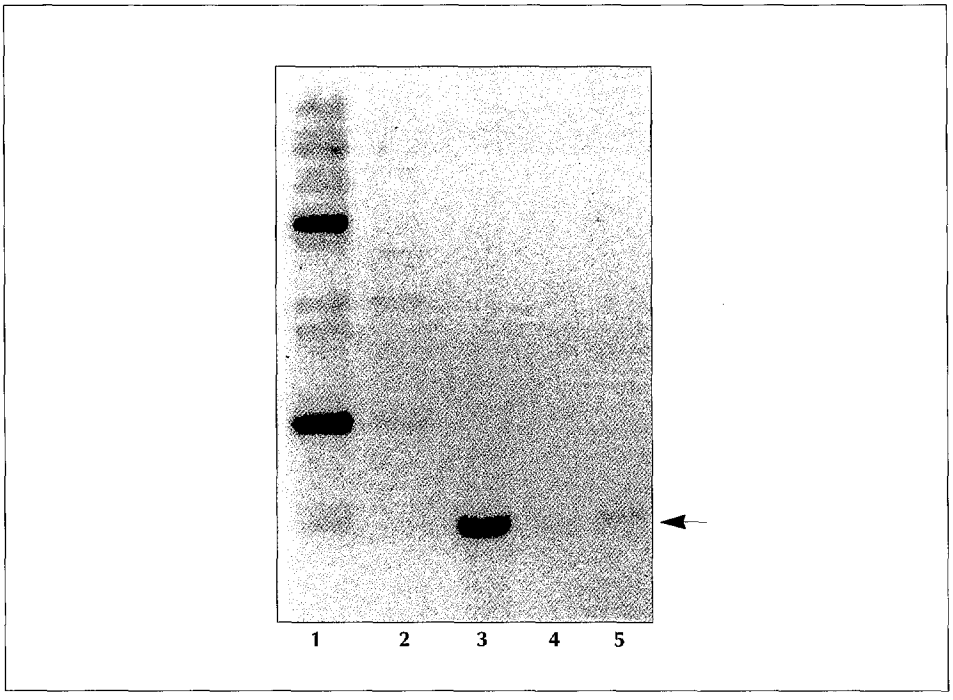

Figure 3. Molecular analysis of putative transformants regenerated under bialaphos selection pressure. Southern blot of putative transgenic cowpea plants regenerated under bialaphos selection pressure. Lanes 1 and 2, positive controls – DNA from transgenic sorghum plants (two different transformation events); lanes 3–5, DNA from putative transgenic cowpea plants. Plant genomic DNA was digested with *Eco*RI and probed with the *bar* inserts.

cotyledon bases, embryonic axes, and plantlets regenerated from morphogenic cultures indicated that bialaphos at a concentration as low as 1 mg/L is lethal for cowpea explants. In the experimentation reported in this paper, 1500 explants (cotyledon bases and embryonic axes) were bombarded with the DP532 plasmid and induced to produce morphogenic cultures in the presence of bialaphos (1 mg/L). Of the 1460 adventitious shoots, > 160 survived the first two subcultures on CP3 medium supplemented with bialaphos. Since the presence of bialaphos during the regeneration process inhibits shoot development, after 3 subcultures those adventitious buds that had survived selection were temporarily subcultured on CP3 medium, lacking a selective agent for shoot elongation. However, selection was applied again during rooting. Of the 10 shoots that regenerated, only 4 of them produced roots in the presence of bialaphos, and these were transferred to the greenhouse. Molecular analyses utilizing the *bar* specific probe indicated that three of them were Southern positive and contained a single insertion of the *bar* gene into the genome (Fig. 3). The position of the *bar* positive band indicated that all of these plants contained the same *bar* insertion and must have arisen as a clone (thus representing a single transformation event) during the culture of lateral shoots. Whether or not these Southern positive plants are able to pass on the *bar* gene to the next generations still needs to be determined.

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Location and organization of major repetitive DNA sequence families in *Vigna unguiculata* (L.) Walp.

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Abstract

Molecular cytogenetic studies are valuable for studying genera with small chromosomes, such as *Vigna*. Extensive information about the structure and organization of the genome comes from methods such as banding to locate heterochromatic chromosome regions, fluorescent staining of chromosomes, silver staining of active nucleolar organizing regions, and in situ hybridization to localize particular repetitive DNA sequences. In cowpea, *V. unguiculata* [L.] Walp. ($2n = 2x = 22$), C-banding showed considerable differences between chromosomes in heterochromatin localization, and enabled the identification of some chromosomes and the study of their evolution. Moreover, using double-target in situ hybridization, the physical localization of ribosomal genes (5S and 18S-5.8S-25S rRNA genes), and of a family of repetitive DNA sequence (pVuKB1) were determined in *V. unguiculata*. Cowpea has 2 pairs of sites of 5S rDNA and five pairs of sites of the 18S-5.8S-25S rDNA. The smallest 18S-5.8S-25S rDNA site is centromeric, while the others are distal. The sequence pVuKB1 was detected around the centromere of all chromosomes. Silver staining of nucleolar organizing regions indicated that all the rDNA sites detected using the 18S-5.8S-25S rDNA probe have active genes. Knowledge about the physical organization of the chromosomes of *Vigna* species is valuable for examining species evolution.

Introduction

Cowpea, *Vigna unguiculata* [L.] Walp. ($2n = 2x = 22$), has a very important position among the pulse crops of West Africa and many other subtropical regions. The potential of this crop is not fully realized because of its susceptibility to several insect pests. Resistance genes to these pests are found in wild species, but their direct transfer to cultivated cowpea has not been possible so far.

Hence, an international project was started to devise a new approach for the nonconventional improvement of this crop, with the goal of transferring useful genes from wild germplasm to the cultivated types through interspecific hybridization or transformation. In any case, a good cytogenetic and molecular background was required to better understand genome organization in the species of interest, and to provide cytological markers to assist genetic work.

Cytological knowledge of this group of species was lacking, mostly as a consequence of the extremely small size of the chromosomes of this genus. DNA measurements for

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Vigna radiata ($2n = 2x = 22$), with a chromosome size similar to *V. unguiculata,* indicate a 1C DNA content of 0.5pg or 480 Mbp (Bennett et al. 1982). The first stage of the research, described here, was to standardize the methods and karyotypes, before further analysis. Achievements to date include the application of molecular cytogenetic techniques to investigate chromosome structure and genome organization in cowpea and related species.

In this paper, we present the cytological characterization of *V. unguiculata* chromatin by means of differential staining techniques, and report on the applications of fluorescent in situ hybridization (FISH) with different probes.

Heterochromatin characterization and distribution

Cytological investigations were initiated to devise reliable methods for studying chromosome morphology and heterochromatin distribution in cowpea and related wild species, through the application of Giemsa and fluorochrome banding techniques (Galasso et al. 1992, 1993). The results obtained allowed the definition of patterns of affinity among some of the species of the sections *Catiang* and *Vigna* (Galasso et al. 1993).

In cowpea *V. unguiculata* ssp. *unguiculata*, in particular, differential chromosome staining using Giemsa (C-banding) allowed the identification of a large number of heterochromatin bands (Galasso et al. 1992). Most of the heterochromatin had a centromeric distribution, although some bands were located at telomeric positions; all chromosomes showed centromeric heterochromatin blocks, but only 4 bands were located at the telomeres on the longer chromosomes.

Chromomycin A3 (CMA) staining produced bright fluorescence of the telomeric blocks (Galasso et al. 1993). This indicates that the subtelomeric heterochromatin is enriched in GC base pairs. This view was confirmed by DAPI staining (AT enhanced), which showed reduced fluorescence in the same areas (Sumner 1990). GC-rich regions of the genome are generally associated with the GC-rich rDNA at the secondary constrictions (Schweizer 1980). In cowpea, only one band appeared consistently associated with the satellite constriction, while the remaining ones appeared to consist of other, GC-rich, subtelomeric DNA sequence families.

The molecular cytogenetic approach

Standard cytological methods are useful for chromosomal analysis, but because of the similarity of the many small chromosomes, and the need for greater understanding of their molecular structure, molecular cytogenetic methods were applied to cowpea. These techniques, including FISH and analysis of specific classes of repetitive DNA sequence, enable the identification of some chromosome pairs and the study of genome organization. FISH is particularly suitable for studying species with small chromosomes (Maluszynska and Heslop-Harrison 1991, 1993; Schmidt and Heslop-Harrison 1994). In situ hybridization using sequences of DNA as a probe to chromosome spreads has now been shown to be widely applicable to many plant species, including cowpea.

A further step in understanding the organization of chromatin in cowpea and its wild progenitors was to localize all rDNA sites and compare them with the already characterized heterochromatin sites. A first attempt was made by means of isotopic in situ hybridization, using the coding portion of total rDNA of *Quercus* sp., labeled with tritium as a probe.

Again, due to the limited size of the chromosomes and to the scattering of silver grains, the results were unclear. As a consequence, nonisotopic in situ hybridization was attempted. The probes used were as follows:

1. pTa71, which contains a 9 kb *Eco*RI fragment from *Triticum aestivum* including the 18S-5.8S-25S rRNA gene and intergenic spacer regions (rDNA) (Gerlach and Bedbrook 1979), was labeled with tetramethyl rhodamine-4-dUTP (TRITC) by nick translation;
2. pTa794, which corresponds to a complete 410 bp 5S gene unit from *Triticum aestivum*, containing the 5S gene (120 bp) and the intergenic spaces (290 bp) (Gerlach and Dyer 1980), was labeled with digoxigenin-11-dUTP using the polymerase chain reaction;
3. pVuKB1, 488 bp *Dra*I fragment isolated from *V. unguiculata* TVx 3236 (Galasso et al. 1995), was labeled with digoxigenin-11-dUTP.

For double target in situ hybridization, 20–80 ng/μL of labeled probes were added to the hybridization mixture; denaturation was performed by heating at 70 °C for 10 min; hybridization was carried out at 37 °C overnight. After washing at a stringency of 85% to remove mismatched molecules, slides were incubated in immunofluorescent reagents to detect digoxigenin sites by fluorescein isothiocyanate conjugated sheep anti-digoxigenin antibody. Chromosomes were counterstained with DAPI.

Hybridization signal from pTa71 probe in cowpea was located on 5 chromosome pairs: three very prominent sites per haploid genome were at subtelomeric positions, one conspicuous pair of sites located at the satellited constriction, and one pair of minor spots positioned at centromeric sites. Major sites of pTa794 probe were located on two pairs of chromosomes. One pair of sites was on the opposite arm to an 18S-5.8S-25S rDNA site, while the other was on a chromosome pair not showing pTa71 signals.

Analysis was done to assess which of the detected ribosomal gene clusters could be associated with active NORs. The Ag-NOR technique applied (Bloom and Goodpasture 1976) uses silver precipitation to cytologically demonstrate the sites of transcription of the r-RNA genes (Hubbell 1985). As a result of this analysis, all the major pTa71 sites displayed a clear Ag-NOR positive reaction.

Hybridization sites of pVuKB1 to cowpea metaphase chromosomes were detected in the heterochromatic regions surrounding the centromeres of all chromosomes. This probe did not give cross-hybridization with the DNA of other *Vigna* species or other Leguminoseae and is, therefore, to be considered a species-specific probe (Galasso et al. 1995).

Conclusion

Cytotaxonomical studies provide information about plant genomes which complements and underpins many other areas of research. The additional information generated from molecular cytogenetics, particularly when combined with the use of molecular and genetic markers, is vital for tracing the evolution in sequence copy number and the position of specific DNA sequences. If such studies are extended to related species, molecular cytogenetics may help to identify the evolutionary patterns of given taxa or groups of taxa and enable chromosomes to be identified and followed in hybrids and breeding lines, thus providing a useful instrument for further interpretation.

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