Striga Research Methods
— A manual

2nd Edition
(June, 1997)

Edited by
D.K. Berner, M.D. Winslow, A.E. Awad,
K.F. Cardwell, D.R. Mohan Raj, and
S.K. Kim

Prepared by
The IITA *Striga* Research Group
for
The Pan African *Striga* Control Network (PASCON)

International Institute of Tropical Agriculture
PMB 5320, Ibadan, Nigeria

About IITA

The goal of the International Institute of Tropical Agriculture (IITA) is to increase the productivity of key food crops and to develop sustainable agricultural systems that can replace bush fallow, or slash-and-burn, cultivation in the humid and subhumid tropics. Crop improvement programs focus on cassova, maize, plantain, cowpea, soybean, and yam. Research findings are shared through international cooperation programs, which include training, information, and germplasm exchange activities.

IITA was founded in 1967. The Federal Government of Nigeria provided a land grant of 1,000 hectares at Ibadan, for a headquarters and experimental farm site, and the Rockefeller and Ford foundations provided financial support. IITA is governed by an international Board of Trustees. The staff includes around 180 scientists and professionals from about 40 countries, who work at the Ibadan campus and at selected locations in many countries of sub-Saharan Africa.

IITA is one of the nonprofit, international agricultural research centers currently supported by the Consultative Group on International Agricultural Research (CGIAR). Established in 1971, CGIAR is an association of about 50 countries, international and regional organizations, and private foundations. The World Bank, the Food and Agriculture Organization of the United Nations (FAO), and the United Nations Development Programme (UNDP) are cosponsors of this effort.

© 1997
International Institute of Tropical Agriculture

Oyo Road, PMB 5320
Ibadan, Nigeria

Email: IITA@CGNET.COM
Telephone: 234-2-2412626
Satellite telephone (INMARSAT): 871-1454324
Telex: 31417 or 31159 TROPIB NG
Cable: TROPFOUND IKEJA
Fax (INMARSAT): 871-1454325

Note

The mention of product or company names in this publication is merely to ensure factual reporting; it is not intended, and does not imply, that these products are endorsed or recommended by IITA over other similar products.

Foreword

It is with a certain sense of excitement that I present this *Striga* Research Methods Manual to our various colleagues in Africa and elsewhere. It is a clear sign of the excellent progress made in the research for control options for one of sub-Saharan Africa's most important plant health management problems. *Striga* is typically linked to more intensive cereal production and as such, the potential production improvements resulting from the use of improved varieties and production techniques, were often not realized due to the losses caused by this parasitic plant.

Some ten years ago the enormous losses caused by *Striga* to small farmers agricultural production were well recognized. However, the prospects for finding practical solutions seemed very limited. However, since that time, research efforts at various institutions, in particular IITA, have demonstrated that real progress can be made in reducing the devastating effects of *Striga*. This will have to take the form of an integrated pest management package combining host plant resistance, chemical seed treatment, biological control and crop rotations. Details of these are presented in this research manual which I am convinced will be a very useful source of information to all concerned with *Striga* control.

L. Brader
Director General,
The International Institute of Tropical Agriculture

Preface

Striga spp., particularly *S. hermonthica*, are increasingly a menace to crop production in the savanna regions of sub-Saharan Africa (Berner, *et al*., 1995; M'boob, 1986; Musselman, 1987; Parkinson, 1985). To meet this threat, scientists from national and international organizations have been working on various aspects of the problem.

Striga is under control in mechanized, high-input agricultural systems such as in southeastern USA (Sand, *et al*., 1990), but the pest has not been conquered yet in sub-Saharan Africa. In Africa, farmers lack the resources to purchase and apply inputs that are common in developed countries: high fertilizers, herbicides, mechanical tillage equipment, etc. These farmers still await discoveries that will be relevant to their needs and capacities.

Scientists at the International Institute of Tropical Agriculture (IITA) began working on *Striga* control strategies appropriate to the smallholder in 1982, with the initial efforts focussed mainly on breeding (Kim, *et al*., 1985). During 1989, this effort was broadened by adding studies on the biology, ecology, physiology, and genetic variability of the pest; its natural enemies; agronomic control practices; and modeling of the integrated system in which the pest proliferates (Cardwell, *et al*., 1991). This work is conducted by a group of scientists called the *Striga* research group.

Both national and international research institutions have expressed the desire to strengthen African *Striga* research through collaboration. In response to this need, IITA convened a small but representative group in October 1990 in a workshop to discuss research methodologies and suggest priorities. This manual attempts to build on some of the most useful research techniques discussed at that workshop, drawing from available knowledge and adding insights from more recent research.

Workshop participants from national programs, who contributed to the initial effort, are listed in the acknowledgments overleaf. Members of the IITA *Striga* research group, who hold principal responsibility for coordinating inputs into the various sections of this manual, are also so acknowledged, as are our principal resource persons. Published sources of information are listed in the references section followed by a glossary of terms. Details on *Striga* training at IITA are provided at the end of the manual.

The techniques outlined in this manual were developed for *Striga*, but they can also be used with other parasitic flowering plants, with some experimental modifications as needed. We hope this manual serves to record the most practical and useful techniques available at present in an attempt to help researchers in sub-Saharan Africa and elsewhere. If possible, as regional research on *Striga* progresses, updated editions will be produced through similar collaborative efforts. If you have comments or suggestions to offer, please let us know.

D.K. Berner
IITA *Striga* Research Group

Acknowledgments

This manual was begun as an outcome of a *Striga* training workshop held at IITA in October 1990. From the national programs, the following took part: C.A. Agbobli, pathologist, ORSTOM-DRA, Lome, Togo; A.Y. Akintunde, agronomist, National Accelerated Food Production Program, Moor Plantation, Ibadan, Nigeria; K.A. Elemo, agronomist, and S.T.O. Lagoke, weed scientist, Institute of Agricultural Research, Ahmadu Bello University, Zaria, Nigeria; Mr. T. Ngoumou, agronomist, Institut de Recherche Agronomique, BP 415, Garoua, Cameroun; and P. Y. K. Sallah, breeder, Nyankpala Agricultural Experiment Station, Crops Research Institute, Tamale, Ghana. Thanks are due to the workshop participants for contributing heavily to the content of this manual.

All members of IITA's *Striga* research group contributed to this manual. The composition of this group has changed since the manual was started, but the contributions of each member have been valuable. A.E. Awad, D.K. Berner, K.F. Cardwell, I. Igbinnosa, S.K. Kim, J.G. Kling, S.N.C. Okonkwo, B.B. Singh, J. Smith, G.K. Weber, and M.D. Winslow have all contributed.

Thanks for constructive editorial comments are due to Robert E. Eplee, Director of Whiteville Plant Methods Center, USDA-APHIS, Whiteville, North Carolina, USA, L. J. Musselman, Professor of Botany, Old Dominion University, Norfolk, Virginia, USA, and D. R. Mohan Raj, science writer/editor at IITA. Grateful thanks are also due to H.W. Rossel and R. Zachmann, virologist and training materials specialist at IITA, who reviewed the manuscript and suggested improvements.

D.K. Berner
IITA *Striga* Research Group

Contents

About IITA

Foreward, Preface, and Acknowledgments

1. Phytosanitary precautions

1.1 General guidelines 1
1.2 The case of *Striga hermonthica* 1
1.3 Ensuring clean planting materials 2

2. *Striga*: the genus and life cycle

2.1 Genus 4
2.2 Key to species 4
2.3 Life cycle 6

3. Collection and preservation of *Striga* seed

3.1 Materials needed 10
3.2 Procedures 10
3.3 Harvesting *Striga* seed 10
3.4 Drying, cleaning, and storing *Striga* seed 11

4. *Striga* infestation and field experimentation

4.1 Materials needed 13
4.2 Procedures 13
4.2.1 Site selection 13
4.2.2 Land preparation 14
4.2.3 Infestation with sand/*Striga* mixtures 14
4.2.4 Rate calculations in infestation 14
4.2.5 Soil infestation 15
4.2.5.1 Modifications 16
4.2.6 Infestation with water/*Striga* mixtures 17
4.2.6.1 Modifications 17
4.2.7 Mulch infestation 18
4.2.8 Fertilizer management 18
4.2.9 Legume rotation 19
4.2.10 Weed control 19
4.2.11 Other hazards 20

5. *Striga* seed extraction

5.1 Materials needed 21
5.2 Procedures 21

5.2.1 Sampling 21
5.2.2 Expression 21
5.2.2.1 Volumetric 22
5.2.2.2 Gravimetric 22
5.2.3 Sieving 22
5.2.4 Potassium carbonate separation 23
5.2.5 Sucrose separation 24

6. Laboratory techniques

6.1 Materials needed 26
6.2 Procedures 26
6.2.1 Viability testing 26
6.2.2 Production of germination stimulant 27
6.2.3 Cleaning, conditioning, and germinating 28
6.2.4 Testing plants for stimulant production 29
6.2.4.1 Selecting for hosts producing low levels of stimulant . . 29
6.2.4.2 Selecting for nonhosts producing high levels of stimulant 31
6.2.5 Haustorium formation, attachment, penetration, compatibility 34

7. Axenic culture of *Striga* spp.

7.1 Materials needed 36
7.2 Procedures 36
7.2.1 Sterilization of the work station 36
7.2.2 Preparation of *Striga* seeds 37
7.2.3 Preparation of culture media 37
7.2.4 Transfer of *Striga* seedlings to the growth medium . . . 38

8. Screenhouse techniques

8.1 Materials needed 39
8.2 Procedures 39
8.2.1 Pot trials 39
8.2.2 Eplee bags 41

9. Quantifying *Striga* at stages in its life cycle

9.1 Above-ground stages 42
9.1.1 Numbers of *Striga* 42
9.1.2 Duration of above-ground stages 43
9.2 Below-ground stages 43
9.2.1 Duration of below-ground stages 44
9.3 Measures of vigor 44
9.4 Yield components 44
9.5 Damage due to pests/diseases 46

10. Agronomic experimentation

10.1 General principles 47
10.1.1 Stopping *Striga* seed influx 47
10.1.2 Reducing *Striga* reproduction 47
10.1.2.1 Increasing *Striga* mortality 47
10.1.2.2 Reducing *Striga* fecundity 48
10.1.3 Depletion of the seed bank 48
10.1.4 Cropping systems trials 48
10.3 Experimental design and field layout 50
10.4 Crop loss assessment in farmers' fields 52
10.5 Sampling in field plots 52
10.6 Herbicide trials 52
10.6.1 General guidelines 52
10.6.2 Herbicide trial design 54

11. Breeding maize for *Striga* spp. resistance

11.1 Field layouts for *Striga* breeding nurseries 55
11.2 Preliminary screening — thousands of lines 56
11.3 Advanced screening 56
11.4 Types of resistance 57
11.5 *Striga* syndrome rating 58
11.6 Rating systems 59
11.6.1 Scoring symptoms 59
11.6.2 Using the *Striga* syndrome rating 60
11.6.3 When to rate 60

12. Systems approach to *Striga* research

12.1 Farming systems orientation 62
12.2 Assessing the effectiveness of technologies 62
12.3 Survey techniques 63
12.3.1 Extensive surveys 63
12.3.1.1 Analyzing the data 65
12.3.2 Intensive surveys 65
12.3.2.1 Village characterization 65
12.3.2.2 Field characterization 66
12.3.2.3 Analyzing the data 66
12.4 Systems modeling 67

Selected references 70

Glossary 75

Training in *Striga* research 80

1. Phytosanitary precautions

1.1 General guidelines

As with all plant pathogens and noxious weeds, the movement and management of *Striga* for research purposes must be done with care. It is important to have a careful and clear risk assessment of how the organism can be spread, what are the environmental conditions in which it will survive, and what is the geographical range in which it already exists.

Within the area where an organism is endemic, there is no quarantine risk. Phytosanitary precautions are needed only to minimize spread of excessive contagion from experimental areas.

In some cases, the geographic range of an organism is limited by environmental requirements for growth or reproduction, including factors such as temperature, moisture, and availability of host plants. Where the minimum environmental requirements are not present, it is not risky to do controlled research with the organism, because the organism only survives under artificial conditions provided by the researcher.

In other cases, where there may be no environmental constraints to the development of the restricted organism, and where the host plant is present, the researcher needs to provide quarantine facilities which prevent the organism from escaping into the environment. These facilities are designed based on a knowledge of the organism's growth cycle and mode of dissemination, and special precautions are taken to block these at critical points.

If the geographic range, life cycle, and minimum environmental conditions for an organism are not known, it should be quarantined within its area of origin until a risk assessment can be made and a safe protocol has been established.

1.2 The case of *Striga hermonthica*

In the case of *Striga hermonthica*, the geographic range is extensive and the environmental conditions required for its growth and development are not restrictive, although the humid forest zone appears to be suppressive. Experimentation with *Striga hermonthica* within the geographic area where it is naturally abundant on maize, particularly the Guinea savanna and Sudan savanna, poses no quarantine risk. Basic precautions are advisable to avoid spread to neighboring fields. Surround your *Striga* plot with a 10 m border of a susceptible crop, such as maize, which will not be infested. This border area should be checked frequently and any *Striga* found should be pulled out or sprayed. Surrounding this, a 2–3 m border of a "trap crop" such as cotton, pigeonpea, crotolaria, soybean, etc., should also be sown. These crops will trigger suicidal germination of *Striga*. Also, frequently patrol the areas outside the trial to control escaping *Striga* by hand pulling, herbicide, or rotation crops.

Figure 1. Harvested sorghum plants lying in a field heavily infected with Striga hermonthica*. Sorghum seed contamination is virtually assured by this practice.*

Figure 2. IITA Striga *research group poster warning of dangers of contaminated planting materials. Poster is available in English, French, and Hausa upon request.*

Striga hermonthica is obligately allogamous, requiring insect pollinators for fertilization and seed production (Musselman, 1987). Therefore, it is safe to perform research in areas outside of its normal range if all insect pollinators are kept away. This can be done by working in a screenhouse or laboratory. Waste soil that is infested with *Striga* seed can be sterilized, using steam or a quaternary ammonium compound, such as "Coverage 256" (Norris, *et al*., 1986), or suicidal germination of *Striga* seed can be induced using ethylene gas. The soil should then be either recycled within the *Striga* screenhouse or lab, or buried in a deep pit to ensure that none can germinate in the field.

1.3 Ensuring clean planting materials

Striga species are not efficiently distributed by wind. The most efficient agent for movement of *Striga* seeds is man, particularly by aiding movement of infested soil, contaminated crop fodder, and seeds. Crop seed contamination with *Striga* species is common and every effort should be made to ensure that planting materials are free of *Striga* seeds. The simplest way to ensure this is to avoid contamination during harvest and threshing. If the field being harvested is infested with *Striga*, do not lay the harvested product on the ground within the field (Figure 1). Rather, carry it to a *Striga*-free area before threshing or drying (Berner, *et al*., 1994). If you are unsure of whether your planting materials are contaminated, check where the seeds came from. If they came from outside of an infested area, then the materials should be *Striga*-free as advised in Figure 2. If the source is within a *Striga* infested area, then the seed lot should be cleaned before planting. The simplest way to do this is to wash the seed on hardware cloth or mesh screen for 10 min. The seeds will then have to be redried. This wetting and drying process is not detrimental to the crop

Figure 3. Free-ranging livestock in Striga *infested areas can move parasite seeds to uninfested areas.* Striga hermonthica *infected sorghum in background can be readily grazed by nearby cattle.*

seeds if drying is done promptly. If a suitable area for drying is not available, plant the seeds immediately after washing.

Caution: Do not put the clean dried seed back into the original container/bag after washing. Another measure is to prevent ruminant animals (Figure 3) wandering from infested to uninfested areas. Ensure that plant materials imported from other areas, including those used for building materials or animal fodder (Figure 4), have come from areas free from *Striga* species.

Figure 4. Movement of animal fodder can also result in inadvertent movement of Striga *seeds. Here cowpea fodder is being carried to market, but many parasite plants and seeds are often mixed with the fodder.*

2. *Striga*: the genus and life cycle

2.1 The genus

The parasitic seed plant of most importance in Africa is the genus *Striga* (of the family Scrophulariaceae). Members of this genus are obligate annual hemiparasites; they are chlorophyllous, but require a host to complete their life cycle (Musselman, 1987). Although 30 or more species of *Striga* have been described, only 5 are presently of economic importance in Africa (Ramaiah, *et al*., 1983). These are, in approximate order of economical importance in Africa, *Striga hermonthica* (Del.) Benth., *Striga asiatica* (L.) Kuntze, *Striga gesnerioides* (Willd.) Vatke, *Striga aspera* (Willd.) Benth., and *Striga forbesii* Benth. All except *S. gesnerioides* are parasites of Africa's cereal crops sorghum, millet, maize, and rice. *S. gesnerioides* is a parasite on cowpea and other wild legumes. A simple key is presented.

Key to some* Striga *species*

1a. Calyx ribs 5.....2

1b. Calyx ribs more than 5.....4

2a. Scale-like leaves, short plant stature frequently profusely branched with spiked appearance, flower color variable.....***Striga gesnerioides*** (Figure 5)

2b. Relatively long slender leaves, coarse textured, with relatively long branches, pink or mauve flowers 8–15 mm across.....3

3a. Flower neck clearly longer than calyx and bent well above calyx. Glandular hairs evident on corolla tube. Leaves narrow.....***Striga aspera*** (Figure 6)

3b. Flower neck (corolla tube) about equal to calyx in length and bent immediately above calyx. Corolla tube glabrous in appearance....***Striga hermonthica*** (Figure 6)

Figure 5. Striga gesnerioides *parasitizing cowpea (courtesy D. Florini)*

Figure 6. Striga aspera *and* Striga hermonthica *growing on maize*

Key to* Striga *species continued

4a. Calyx ribs 10 or more, relatively small and delicate appearing plant, variable flower color .....***Striga asiatica*** (Figure 7)

4b. Calyx ribs 1`5, somewhat broad leaves, coarsely toothed, salmon-pink flowers...***Striga forbesii*** (Figure 8)

* Adapted from: Ramiah, *et al.* 1983.

Figure 7. Red-flowered Striga asiatica *on maize. Yellow flower morphotypes are also common.*

Other parasitic flowering plants are also common in Africa. *Alectra vogelii* Benth. (Figure 9) is a vigorous parasite on both groundnut and cowpea, and is also able to parasitize soybean. Yield losses due to this parasite on cowpea are reported to be up to 100% (Riches, 1988).

Figure 8. Striga forbesii *(courtesy L. J. Musselman).*

Figure 9. Alectra vogelii *parasitizing groundnut.*

Buchnera hispida, a facultative parasite, looks a lot like some *Striga* species, and some were initially identified as *Buchnera* (Musselman, 1987). This parasite is commonly found in sorghum, millet, and maize fields, but the extent of damage is generally not considered significant. Do not confuse relatively benign *Buchnera* with *Striga*.

Studies at IITA indicate that the dispersal and perpetuation of these parasites is integrally dependent on the particular cropping system and that control may be affected by management of the system (Berner, *et al*., 1994, 1995, 1996; Cardwell, *et al*., 1991). Of particular concern are shifts toward monocrop systems and concomittant increases in parasite populations. As the frequency of cereal monocropping increases, due to human population pressures for food, the land becomes less fertile and initially small populations of *Striga* gradually build to populations prohibitive for continued cereal production. Management of farming systems, therefore, must include rotations to crops that are not hosts of *Striga* if sustainable productivity is to be maintained. The most desirable rotational crops are legumes which reduce *Striga* populations and improve soil fertility. Some legumes, however, are parasitized by *Striga* and *Alectra*, which complicates the development of integrated control programs.

2.2 Life Cycle

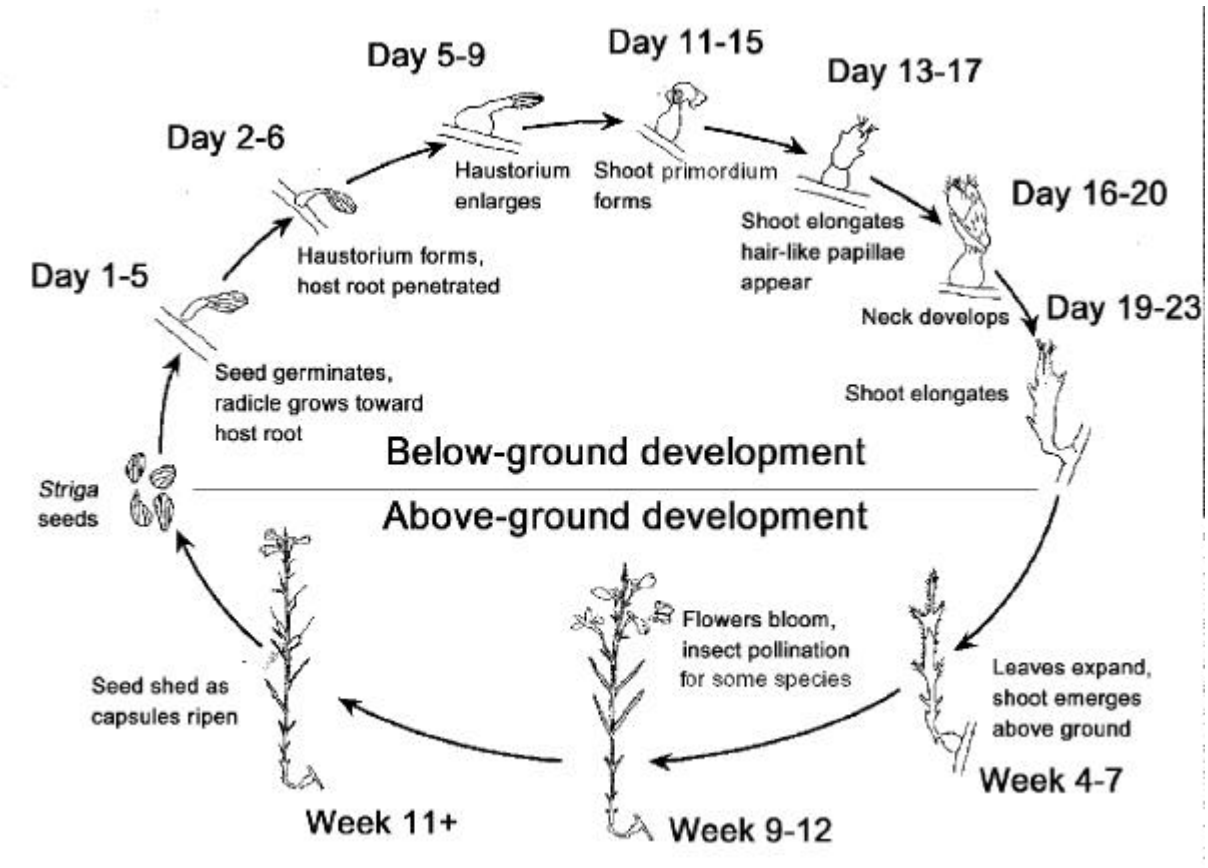

Figure 10. General life cycle of Striga *species (courtesy E. I. Aigbokhan)*

Life cycles and symptoms of *Striga* parasitism are broadly similar, regardless of the host-parasite combination, although there are some minor variations. A general life cycle of *Striga* is shown in Figure 10. Seeds are the sole source of inoculum. They are produced in abundance (roughly 10,000 to 100,000 or more per plant) (Pieterse and Pesch, 1983). Seeds weigh 10^{-5} g each; they are about 200 microns wide by 300 microns long (Figures 11, 12; Saunders, 1933; Worsham, 1987).

Figure 11. Striga *sp. seeds intermixed with seeds of cowpea, maize, millet, and sorghum.*

After dispersal, seeds may remain dormant for several months; during this time, seeds will not germinate even if conditions are ideal. This period is termed after-ripening (Vallance, 1950), and it may be an evolutionary adaptation to prevent germination during the last rains of the season, when there are no hosts around. Studies have indicated that the length

Figure 12. Magnified Striga *spp. seeds showing characteristic ridges on seed coat (courtesy G. Goergen).*

of the after-ripening period is different for different *Striga* species and for seed samples collected from different geographical areas (Saunders, 1933; Van Mele, *et al*., 1992, Wilson-Jones, 1955). It may be anywhere from a few days to 2 years.

After this period, seeds will germinate only under conditions of favorable moisture and temperature (free moisture adequate for seed imbibition and at temperatures between 20 and 33 C) and only in the presence of a germination stimulant, usually exuded from plant roots.

Figure 13. Growing radicles of germinated Striga *sp. seeds.*

The first of these two steps is termed "conditioning or preconditioning" (Vallance, 1950; Worsham, 1987). It is a pregermination requirement of exposure to moisture, combined with temperatures above 20 C for a period of 1 week or more. This requirement is probably a survival adaptation, which prevents the seed from germinating during the irregular first rains, before the rainy season has become well established. At this stage, i.e., before the rains are well established, host root systems will not have had a chance to expand sufficiently to permit maximum successful *Striga* attachments. In the absence of appropriate germination conditions, the seed will become dormant but remain viable in the soil for many years (Bebawi, *et al*., 1984; Saunders, 1933).

Figure 14. Developing Striga hermonthica *seedling on host root.*

The next requirement is a signal by a specific chemical germination stimulant from the proper host root (Saunders, 1933; Vallance, 1950; Worsham, 1987). This stimulation requirement ensures that *Striga*, with its minimal food reserves in the seed, does not germinate until it has received a signal that a host root is nearby.

Once stimulated, the *Striga* radicle (Figure 13) does not just grow in any direction; it grows directly towards the source of the stimulant, i.e. the growth is chemotropic (Chang, *et al*., 1986). This further increases the likelihood that it will encounter a host root.

Figure 15. Numerous attached Striga hermonthica *seedlings on a single sorghum root system.*

Figure 16. The first signs of trouble. Characteristic but non-descript symptoms of Striga hermonthica *infection on maize.*

Figure 17. The first signs of the cause. Newly emerged Striga hermonthica *plants on maize.*

After germination, a series of chemical signals directs the radicle to the host root where it attaches and penetrates (Figure 14). However, if the seedling does not attach to a host root within 3–5 days, the seedling perishes. Once penetration has occurred, an internal feeding structure (haustorium) is formed, and the parasite establishes host xylem connections (Worsham, 1987). The host photosynthate is diverted to the developing parasite, which also utilizes the host root system for water and mineral uptake. Initial symptoms occur while the parasite is still subterranean; they are evident in water soaked leaf lesions, chlorosis, and eventual leaf and plant desiccation and necrosis. Severe stunting and drought-like symptoms (Figure 16) such as leaf margin curling also indicate *Striga* infection (Kim, *et al.*, 1991; Parkinson, *et al.*, 1985; Vasudeva Rao, 1985).

As the host matures, the parasites emerge and begin to produce chlorophyll and photosynthesize (Saunders, 1933). After emergence, host symptom development is intensified. Parasite reproduction can be initiated before emergence. Reproductive schemes vary from autogamy to obligate allogamy, depending on species (Musselman, 1987). *Striga hermonthica* and *Striga aspera* are the only two species known to be obligately allogamous and require insect pollinators (Figure 18). These two species can hybridize and produce viable and virulent offspring. All other species are autogamous.

Figure 18. Adult of Juonia *sp. pollinating* Striga hermonthica *flowers. The larvae of this lepidoptera feed on leaves of* Striga *spp. plants.*

Following reproduction, seeds are dispersed and the cycle is reinitiated. The relative success of each stage of the life cycle governs the volume of seed production. At each stage, there is a potential opportunity for control. However, successful sustained control will depend on eliminating the *Striga* spp. seed reserves in the soil and preventing parasitism at the early crop growth stages.

3. Collection and preservation of *Striga* seed

In order to conduct research on *Striga*, you need to have an adequate amount of viable and germinable *Striga* seed. For field experimentation, appreciably large quantities of seed need to be collected (section 4). Because there are different host-specific strains of *Striga* (King and Zummo, 1977; Wilson-Jones, 1955), store seed collected from different sites and different hosts separately in containers labeled with site, host, and collection date. Since *Striga hermonthic a*is obligately allogamous, a collection area of 25–50 km diam may be a safe estimate of the extent of any one population of this parasite. Bulk and maintain separately seeds collected within such an area.

3.1 Materials needed

Large polyethylene sheeting; paper bags; 250 and 150 micron sieves; vials, bottles, or plastic bags for seed storage.

3.2 Procedures

3.2.1 Harvesting *Striga* seed

1. Once a heavily infested field has been identified, harvest only the floral heads of the *Striga* plants. This will lessen the amount of trash to be screened out later.

2. Harvest only those heads that are mature and have healthy intact (not shattered) capsules. A mature floral head is one on which all florets have completed flowering, with no visible flowers or only the uppermost flowers remaining (*Striga* flowers progressively from bottom to top of the floral head).

3. Avoid harvesting heads that have large, swollen capsules. These capsules contain the larva of a weevil (*Smicronyx* spp.) and have no healthy seed.

4. Collect harvested heads in paper (not plastic) bags for transport to the drying area (Figure 19). A large paper bag full of *Striga* floral heads will only produce 1–5 grams of *Striga* seed. This indicates the amount of harvesting that you would need to do to collect enough seed for field infestation the following year.

Figure 19. Paper bags full of Striga *flower heads hanging to dry in well ventilated screenhouse.*

3.2.2 Drying, cleaning, and storing *Striga* seed

1. After harvesting, if there are only small samples of seed to be collected, hang the bags of *Striga* to dry in a well ventilated covered area.

2. If the harvest required is large, as would be if you need to infest a field, spread the floral heads on polyethylene sheeting in a wind-sheltered area and expose them to the sun to dry (Figure 20). Mix the *Striga* daily to facilitate even and thorough drying.

3. After 10–14 days of drying, tap the floral heads gently on the plastic sheeting to force seed shed. Save the left-over floral head trash to infest "*Striga* sick plots".

4. After "threshing" all of the *Striga*, screen the material on the sheeting by passing it through sieves of 250 and 150 micron openings (Figure 21). Sieving helps remove most of the plant trash in the seed lot and makes subsequent infestations with the seed more accurate. In addition, the seed will store better and be less susceptible to fungal spoilage once the trash is removed. Most of the *Striga* seed will be collected on the 150 micron screen.

Figure 20. Drying large volumes of Striga *flower heads requires daily mixing and exposure to the sun.*

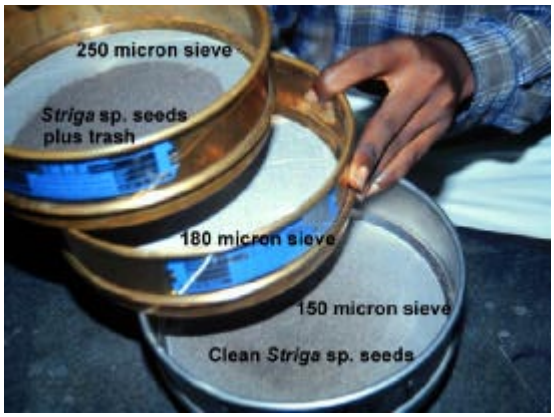

Figure 21. Freshly collected and dried Striga *seeds need to be cleaned of trash by passing through a series of sieves.*

Figure 22. After sieving the dried Striga *seeds, the clean seeds can be stored for years in waterproof containers.*

5. Save all the material remaining on the 250 micron screen separately as trash. Since this trash contains some *Striga* seed, it can be used as an inoculum if no clean material is available. If quantitative infestation is desired, then you will have to give particular care to adjusting the weight of seed needed for the amount of trash (see section 4).

6. Seeds can be stored in plastic containers (Figure 22) at room temperature for long periods of time (years). Storage conditions should be dry. Examine the seed lots periodically for fungal/insect

damage. If damage is present, resieve the seed and redry. We are experimenting with seed treatment chemicals to reduce fungal and insect damage to stored *Striga* seed.

7. Be sure to clean all the sieves, plastic sheets, and bags properly to avoid future contamination.

4. *Striga* infestation and field experimentation

Field and pot experiments with *Striga* frequently have a great deal of variability due, in part, to unequal *Striga* infections (Ransom, *et al*., 1990; Berner, *et al*., 1996). Such variability in infection needs to be controlled. It may be due to differences in age and quality of inoculum, hosts, growth conditions, and in levels of infestation. Some of the variability can be minimized by using *Striga* seed that is at least 4 months old and that has been collected from the same site and host in the same year. Uniform conditioning of this seed will also help, and this is discussed in section 6.

Selection of homogenous host germplasm and control of growth conditions in pot culture will also minimize variability. However, the most manageable source of variability is the level of infestation. Many field experiments have relied solely on natural *Striga* infestations, and they have failed because of the extreme variability in infection.

In the long term, a more viable and economical approach is to infest artificially. An additional advantage in artificial infestation is that infested plots can be grown adjacent to noninfested plots. This provides a means of assessing yield loss and observing differences in host growth that are solely due to *Striga*. General guidelines for field and pot infestation follow.

4.1 Materials needed

Fine sand; 212, 150, or 180 micron sieves; at least 4–month–old *Striga* seeds; materials for germination test (see section 6).

4.2 Procedures

4.2.1 Site selection

1. Choose your field sites carefully, keeping in mind that you probably want each research site to be valuable and permanent. It must be in a locality where the specific *Striga* species is found. Do not contaminate *Striga*-free areas.

2. Look for a level, well-drained, uniform field, with a minimum of other problems such as weeds, termites, variable fertility, variable soil type, soil erosion, etc.

3. Access to the site should be such that researchers and technicians can make frequent visits. Weekly or more frequent visits would be necessary for collecting many types of *Striga* data.

4. Protect the site from animals and from pilferage, by making a fence or posting guards. Ideally, it would be on a national program research farm so that laboratory facilities and a meterological station are nearby.

4.2.2 Land preparation

1. If the field is sloping, have terraces or ridges put in so as to prevent future erosion. The steeper the slope, the closer spaced the ridges will have to be. Your farm manager should advise on how to do this (Couper, 1991). Plant a well-adapted grass on the ridges so that they don't erode during intense rains.

2. Good seedbed preparation is important in achieving uniform *Striga* infection. Ridging is commonly used by farmers on medium to heavy textured soils. It provides a moist but well aerated seedbed and avoids waterlogging. IITA's experience has been that trials on ridged land give better results than does flat planting. However, flat planting and minimum tillage may be desirable on sandy soils, which are subject to sheet erosion.

4.2.3 Infestation with sand/*Striga* mixtures

Because *Striga* seeds are so small, soil infestations are most easily accomplished if the *Striga* seeds are mixed with a carrier material to increase volume. Sand is a good material, but it should be sieved so that only particles of the same size as *Striga* seed are used. If the sand particles are larger than the *Striga* seeds, then the seed will settle out and not be uniformly distributed within the sand. A 212, 150, or 180 micron sieve should be used for sieving.

4.2.4 Rate calculations in infestation

After you prepare the sand, calculate the amount of *Striga* seed that you will need to infest your field/pot.

1. Because it is easier to work with volumes than weights, make a small scoop that will hold approximately 5 ml of water. To make the scoop, measure 5 ml of water into a plastic vial or cup (which will then become the scoop). Mark the water level carefully on the scoop. Then pour out the water, cut the container slightly above the mark, and file it down exactly to the mark. Attach a piece of wire to make a handle for the scoop. Make enough scoops so that there is one for each field worker, so that the whole field infestation can be completed at one time. Next, weigh the amount of sand contained in one level scoop that you have prepared. For this example assume
Sand/scoop = 0.94 grams

2. Pour a small amount of *Striga* seed that you have sieved onto a piece of paper. Examine the sample under the dissecting scope and visually estimate the amount of trash present in the seed sample. Record your estimate, e.g.,
Trash = 25%

3. Always base your calculations on the amount of *Striga* seed that will germinate. How you determine % germinable seed is outlined in section 6. In this example we will assume that you have determined
Germinability = 40%

4. Next, decide what infestation level to use. A level of 3000 to 5000 germinable seed per planting hole gives a good level of infestation and it is economical in the use of *Striga* seed.
Desired infestation = 3000 germinable seed/hole(scoop)

5. Based on the percent germinability, calculate the actual number needed to achieve 1000 germinable seed:
3000 / 0.40 (percent germinability) = 7500 seed/hole

6. Calculate the weight needed. Each *Striga* seed weighs 5 X 10^{-6} grams. So,
$7500 \text{ X } (5 \text{ X } 10^{-6}) = 0.0375$ *grams/hole*

7. Adjust the weight needed for the amount of trash present to arrive at the amount of seed (1 – (% trash/100)). Since your estimate is based on volume, this will not be exact but it should be close.
0.0375g / 0.75(1– % trash = % seed) = 0.05 grams/hole

8. Next, calculate the number of hills (holes) to be infested. This is based on the area to be planted, the between row spacing, and the within row spacing. If the row spacing is 75 cm and the within row plant spacing is 25 cm then each hill will occupy
$0.75 \text{ m x } 0.25 \text{ m} = 0.1875 \text{ m}^2\text{/hill}$
If the area that you wish to plant is 0.5 ha (5,000 m^2), then, with the above spacings, there will be
$5000 \text{ m}^2 / 0.1875 \text{ m}^2\text{/hill} = 26667$ *hills*

9. Calculate the total weight of sand needed to infest this amount of hills at one scoop of *Striga*-sand mixture per hill:
0.94 g/scoop X 26667 scoops = 25067 g sand

10. Calculate the total weight of *Striga* needed:
0.05g/scoop X 26667 scoops = 1333 grams Striga seed

Given the above steps, you should be able to calculate the amount of sand and *Striga* seed needed for artificial infestation of an area of any size. The next sections will outline procedures that you can follow for infestation.

4.2.5 Soil infestation with sand/*Striga* mixture

1. After calculating the amounts of sand and *Striga* seeds needed, divide the total amounts into smaller amounts that can be conveniently worked with. From calculations in section 3.2.4, perhaps into 20 equal amounts of
1254 g sand + 22.65 g Striga seed

2. Start with small and equal volumes of *Striga* seed and sand. Mix thoroughly. Repeat the procedure until all of the *Striga* seed is mixed with an equal amount of sand. Next, add a volume of sand equal to the amount of mixture and thoroughly mix. Repeat until all of the sand is used. By mixing small amounts and gradually increasing the volume of the mixture, you will achieve a very homogenous mix, which is necessary for *Striga* infestation.

3. After the mix has been prepared, you will have to condition it to satisfy the *Striga* germination requirement. Typically, this is done by infesting the hills to be planted 7 to 14 days in advance of planting the crop. To do this, mark the hills that will later be planted, in accordance with the spacing used in the *Striga* infestation calibration. A jab type maize planter is a good tool for this.

Figure 23. Infesting a planting hole using a scoop containing Striga *seed and sand mixture.*

4. Dig out an area of soil about 10 cm in diam and 8 cm deep in the center of each hill.

5. Sprinkle a scoop full of *Striga*-sand mixture into the depression. Lightly cover the dispersed mixture with a thin layer of soil. The objective is to cover the mixture while still leaving a depression that can be seen and planted into at a later date.

6. The *Striga* seed should be conditioned after 7 days and the field or pot can then be planted.

7. The procedure just described is for individual hill infestation. To infest a continuous row, use the same amount of *Striga* calculated for the number of hills in the row. However, the amount of sand used should be increased. Twice the amount is reasonable. If 0.94 g sand had been calculated per hill and there are 20 hills in the row then: (0.94 x 20) x 2 = 37.6 g sand per row. The amount of *Striga* at 0.017 g/hill would be: .017 x 20 = 0.34 g *Striga*/row. This mixture can be sprinkled in a furrow that has been opened in the center of the row. Again, the seed will have to be conditioned for 7 days before planting, as described earlier.

4.2.5.1 Modifications

The methodology just discussed necessitates planting twice, once with *Striga* seeds and again with the host seeds after 7–14 days have lapsed for *Striga* conditioning. This is an effective technique but it requires relatively high labor. Alternatives are to (1) eliminate completely the conditioning period and plant the host seed at the same time as the *Striga* infestation; (2) condition the *Striga* seeds in the laboratory prior to planting. In case 2, the conditioned seeds in sand mixture are used for infestation, followed immediately by planting of host seeds.

1. To precondition in the laboratory, fine nylon mesh (90 micron opening or less) cloth and a bucket with holes in the bottom are needed. Simply line the inside of the bucket with the nylon cloth, fill with the *Striga*-sand mixture to be used, and moisten the sand to field capacity. The cloth will keep the *Striga* seeds from seeping out of the bottom of the bucket.

2. After moistening, allow the sand mixture to set undisturbed for the desired length of conditioning (7–14 days). On the day that you infest and plant, remove the sand mixture wrapped in the cloth, tie the cloth 'bag', and remix the sand. You can now use this mixture, as above, for infestation simultaneous with host planting. When you eliminate dual timing for parasite and host planting, you also ensure that the host and parasite seeds are placed in the same planting hole.

4.2.6 Soil infestation with water/*Striga* mixtures

To avoid sieving sand for use as a *Striga* seed carrier material, water can be used instead.

Figure 24. Infesting a planting hole using a syringe filled with Striga *seed and water.*

1. To do this, you will need a bucket, stirring stick, and several large volume (60 cc) syringes.

2. To calculate the amount of *Striga* seeds needed, follow the steps outlined in section 4.2.4. In the example given there, 1333 g of *Striga* seed was needed to infest 26667 hills (0.5 ha).

3. Next, calculate the amount of water needed. This will depend on how dilute you wish the water-*Striga* mixture to be. It is convenient to use 10 ml of mixture/hill.
Amount of water needed = number of hills X amount/hill. From section 4.2.4: 26667 hills X 10 ml = 266670 ml or 267 liters of water.

4. After the water needed has been measured simply mix the *Striga* seeds with the water, stir thoroughly, fill the syringe(s) with the suspension, and apply 10 ml of the mixture to each planting hole. To ensure even distribution, stir the water mixture regularly. Several syringes can be filled at once, but, before applying to each hole, agitate the suspension in each syringe to ensure good mixture.

4.2.6.1 Modifications

As with the sand infestation techniques, there are several alternatives: (1) the *Striga* mixture can be applied before planting and allowed to condition for 7–14 days, or (2) the conditioning period can be eliminated or (3) the water-*Striga* mixture can be conditioned in the laboratory. To carry out (3) with precision,

1. Calculate both the amount of *Striga* seeds and water needed as shown above. Divide the amount of *Striga* seeds needed into equal batches (approx 50 g each) and place in containers of convenient size (1 liter).

2. Next, add 500 ml of water for each batch of *Striga* seeds. Allow the seeds to condition in the water for the time desired.

3. On the day that you infest and plant, add the mixture in each container to the appropriate amount of water minus the 500 ml in the container. From section 4.2.6 it was calculated that a total of 1333 g of *Striga* seeds and 266670 ml of water were needed to infest a field. For convenience you should divide this amount of seed into 9 batches of 148 g of seeds. Condition each batch in 500 ml of water.

4. The total amount of water needed should similarly be divided into portions corresponding to the number of batches of seeds: *266670 ml / 9 portions = 29630 ml per 148 g batch of Striga seeds.* Since 500 ml had already been added for conditioning, subtract that amount: *29630 ml – 500 ml = 29130 ml (29.13 L).* Add this amount of water to a bucket in the field and introduce the conditioned water-*Striga* seed mixture. After stirring, apply 10 ml of the final mixture to each hill.

4.2.7 Mulch infestation

This method saves labor and is simple, but it gives less uniform infestation. It is less controlled and, therefore, more prone to human errors. The advantage of adjacent noninfested plots is also lost. The method entails collecting *Striga* floral heads and distributing them as uniformly as possible over the experimental field. In order to do this effectively, you should

1. At the end of the rainy season, mow or burn off any plant growth on the *Striga* trial site.

2. Travel to nearby farms several times per week during October through December, collecting mature, seed-bearing *Striga* floral heads from farmers' fields (collect from any crop: sorghum, maize, millet).

3. Place the floral heads in large plastic bags and immediately take them to the experimental field. Spread the floral heads as evenly as possible over the entire field. This "mulch" is loaded with *Striga* seed, which will naturally ripen during the dry season.

4. Repeat 2 & 3 as often as necessary, adding additional layers so that there is a uniform layer of *Striga* mulch over the field.

5. Leave the field untouched over the dry season. Do not allow the field to burn; this will kill the *Striga* seed.

6. At the beginning of the next rainy season, just before planting, operate a shallow disc to about 10 cm depth. Do not till deep; this would put the *Striga* too deep to survive. Run the disc over the field several times in different directions to spread the *Striga* seeds out over the field as uniformly as possible. Establish the host crop according to your usual experimental procedure.

4.2.8 Fertilizer management

Nitrogen is essential for healthy maize growth, but it may also lead to conditions inhibitory to *Striga* (Pieterse and Pesch, 1983). To reduce this possibility use a relatively low nitrogen application rate of 60–90 kg N/ha depending on the innate soil fertility. Use the higher rate on more sandy soils and the lower rate on fertile soils. Apply one half of the fertilizer at planting and top-

dress the other half two or three weeks later.

It is important that micronutrient deficiencies do not cause unwanted variability in *Striga* trials. Arrange to obtain the needed fertilizers well in advance of land preparation/planting time. Zinc, magnesium, and sometimes sulfur, deficiencies are common in the savanna. Consult soil fertility experts to identify problems at your site.

4.2.9 Legume rotation

Rotation with a legume cover crop is recommended for *Striga* screening sites. Such rotations increase the vigor and uniformity of the following cereal crop, by increasing soil organic matter and nitrogen content and by preventing buildup of difficult weeds, soil pests, and pathogens. Additionally, some legumes suicidally germinate leftover *Striga* seed (Wild, 1948; Robinson and Dowler, 1966; Parkinson, et al., 1988), cleaning up the plot so there is less spatial variability in the infestation the following season—particularly if uninfested control plots were accidentally contaminated. Some legumes suited to the purpose in the savanna are perennial pigeonpea (*Cajanus cajan*), *Crotolaria*, *Calopogonium*, soybean (*Glycine max*) and varieties of cowpea (*Vigna unguiculata*). Ideally, legume rotation is done annually.

Do not worry that legume rotations could raise soil fertility too much, suppressing the *Striga* infestation. At the high *Striga* seeding rates recommended (see later), good infestation will still be obtained. Meanwhile, a more vigorous and uniform stand of the cereal crop will be achieved, which will increase the breeder's ability to see differences in resistance among genotypes.

4.2.10 Weed control

Plots must be kept as weed-free as possible, particularly during the first 6 weeks when they do the most damage to the crop. A late-weeded trial results in weak, variable maize plants, gaps in the stand and high coefficients of variability. Weeding should be carried out continuously to avoid weed interference with *Striga*. Hand pulling is preferred because it poses little risk to the *Striga* plants developing just under the soil surface. In any case, mechanical tillage should not be used after the time *Striga* is expected to emerge, about 6 weeks after planting.

If frequent handweeding is not possible, there are several preemergence herbicides that have only minor effects on *Striga* (Eplee and Norris, 1987; Sand, *et al*., 1990). They should be used at the minimum recommended rates. Mixtures of triazine herbicides, alachlor, or butylate can be used. Metolachlor and atrazine at high rates affect *Striga*. To control postemergence weeds, use carefully directed sprays of gramoxone, but be sure to stop well before *Striga* begins emerging, about 6 weeks after planting maize. Avoid herbicides, such as glyphosate, imidazolinones, and sulfonylureas, that inhibit amino acid biosynthesis and may interfere with successful *Striga* parasitism.

4.2.11 Other hazards

Armyworms, cutworms, termites, and birds can attack at germination/emergence stages; it is important to check the site daily to spot these hazards and act quickly against them. Armyworms can wipe out a trial in one day. Immediate spraying will kill armyworms (use malathion, endosulfan, or trichlorphon) and cutworms (use carbaryl, thiodan, or trichlorphon). The insecticide and sprayers must be on hand before the season starts, because these pests destroy trials within a few days. Carbosulfane seed treatment, at labeled rates, can control termites in early growth stages of maize. Only the posting of human scarers can prevent serious damage by birds.

Protect the trial from animals and from human pilferage by making a fence or posting guards. If the trial site is surrounded by grassy bush or forest, there is a high risk of damage by the grasscutter rodent, particularly during the stem elongation phase. Fencing the area with chickenwire, setting traps, and rat poisons can help. Clearing a path (at least 2 m wide) down to bare soil all around the trial will also help. Grass cutters are timid and avoid such cleared areas, where they could be spotted by predators.

5. *Striga* seed extraction

In the preceding sections, artificial infestation of *Striga* and field management were discussed as ways of manually reducing variability in *Striga* trials. With the extremely high variability found under natural infestation conditions and poor trial management, it is often difficult to statistically separate treatments that have an influence on *Striga* parasitism. Artificial infestation and good trial management, combined with experimental designs that block out known variability, usually produce repeatable results and give good statistical separation of treatments. The difficulty arises in the further testing of treatments under natural infestation conditions in farmers' fields. Here artificial infestation is no longer an option and frequently the trial management is poor. The result often is extreme variability that masks treatment differences. Under these conditions, it is helpful to know what the infestation level is in the trial plots. By knowing preplant seed levels, *Striga* ratings can be adjusted for different initial *Striga* levels, either directly or by using the different levels as covariates in statistical analyses. The amount of *Striga* seed in the soil at the conclusion of an experiment can be used in the same way.

Being able to assess the quantity of *Striga* seeds in soil or other samples can also be useful in parasite dispersal studies and studies examining *Striga* population dynamics (Eplee and Norris, 1987). The following section outlines procedures for extracting *Striga* seeds from soil or other materials, such as animal dung or crop seeds.

5.1 Materials needed

Soil probe, augar, or shovel; plastic bags; 250, 212, and 106 or 90 micron sieves; balance; graduated cylinder, graduated beaker; source of running water; potassium carbonate; hydrometer; 1 or 2 liter separatory funnel; small PVC mounted 90 micron mesh screens; dissecting microscope (at least 20X magnification); index cards, colored markers, sucrose, wash bottles, magnetic stirrer, petri dishes, filter papers, funnels, forceps, and 1 liter beaker.

5.2 Procedures

5.2.1 Sampling

The first step in obtaining an accurate measure of the numbers of *Striga* seeds present in the soil is to take a soil sample that is representative of the plot/treatment area. The best way to ensure this is to take a large number of small samples throughout the plot area and then to bulk them together for seed extraction. A zig-zag pattern back and forth across the plot is a good method of sampling and will help in obtaining a representative sample. Samples should be taken to a depth of 15 cm. A small shovel will do, although a soil probe/bore/augar is the best tool.

5.2.2 Expression

Once you have taken the samples, mix the bulked soil from each plot thoroughly. At this point, before extracting the *Striga* seed, decide on how to express the concentration of seed. Two methods are suitable: volumetric or gravimetric.

5.2.2.1 Volumetric

1. Measure the amount of soil in the sample by placing the soil in a graduated beaker or cylinder. Do not 'pack' the soil in.

2. Extract all of the seeds in the sample and express as # *Striga* seed/cc of soil. This method is not very precise because different soil types have different bulk densities and will occupy different volumes. The amount of moisture in the sample as well as the degree of packing will also influence the concentration when expressed volumetrically.

5.2.2.2 Gravimetric

1. Take a small subsample of the mixed soil (15–20 g), weigh the soil, and place the sample to dry. Drying is best done in an oven at 70 C for 48 h. However, samples can also be air dried in the sun for 5 days. This latter method is far less accurate.

2. After drying, reweigh the soil and calculate moisture content of the sample:
moisture = ((wet weight dryweight)/wet weight)

3. Next, weigh the remaining sample and calculate dry matter:
dry matter = sample weight x (1– moisture content)
For example, if the subsample contained 25% moisture and the remaining sample weighed 115 g, then the dry-matter content would be *115g x (1 – .25) = 86.25g*

4. The amount of seed extracted from the sample can then be placed on a 'per gram dry soil' basis. If the sample contained 100 seed, then this would average
100 / 86.25 = 1.16 seed / gram dry soil or 116 seed/100 g dry soil.

5.2.3 Sieving

The next step is to actually separate the seed from the soil. This is done sequentially, by first sieving the *Striga*-size particles from both larger and smaller particles.

1. Stack a series of sieves down from 250, 212, and 90 microns. Over the 250 micron sieve, place a small sheet of coarse screen to remove the largest particles.

2. Once stacked, pour the sample onto the coarse screen. The maximum sample size for the following procedure is 200 g soil, and larger samples should be divided accordingly.

3. Place the stacked sieves and soil under flowing tap water, and wash the particles sequentially through the sieves. After washing the soil on the coarse screen, remove this screen and continue washing the particles on the 250 micron screen. Repeat this procedure until the soil on each of the sieves has been washed. A general rule of thumb is to spend 5 min washing per sieve. All of the *Striga*-size particles will be collected on the 90 micron sieve.

5.2.4 Potassium carbonate separation

Once particles of different size have been sieved out, the *Striga*-size particles can be separated by weight to further reduce the amount of trash in the sample and facilitate subsequent counting. An effective way to do this is by employing specific gravity in a separatory funnel (Figure 25).

1. First a solution heavier than both water and *Striga* seed should be prepared. A potassium carbonate solution of specific gravity 1.4 is ideal (Eplee and Norris, 1987). You should mix roughly 700 ml of solution and can achieve the specific gravity of 1.4 by adding 500 g K_2CO_3 to 700 ml of tap water. Mix and check the specific gravity with a hydrometer, if available.

2. Mount a 1 liter separatory funnel in a ring stand and pour in 400–500 ml of solution. Pour the remaining solution into a wash bottle and wash the contents of the 90 micron sieve into the separatory funnel.

3. Next, add a layer of water over the K_2CO_3 solution by squirting a stream of water down the inside of the separatory funnel and washing any adhering soil particles into the solution. Do this slowly so the water and the solution do not mix but rather form two layers. The water layer should be 200-300 ml thick.

4. Allow the soil/*Striga* to settle for 20 min, after which the heavier particles would have precipitated to the bottom of the funnel, while the lighter particles would be floating on the surface of the water. Any empty *Striga* seed coats will be found in this upper layer, while the intact *Striga* seeds should be at the water/K_2CO_3 interface.

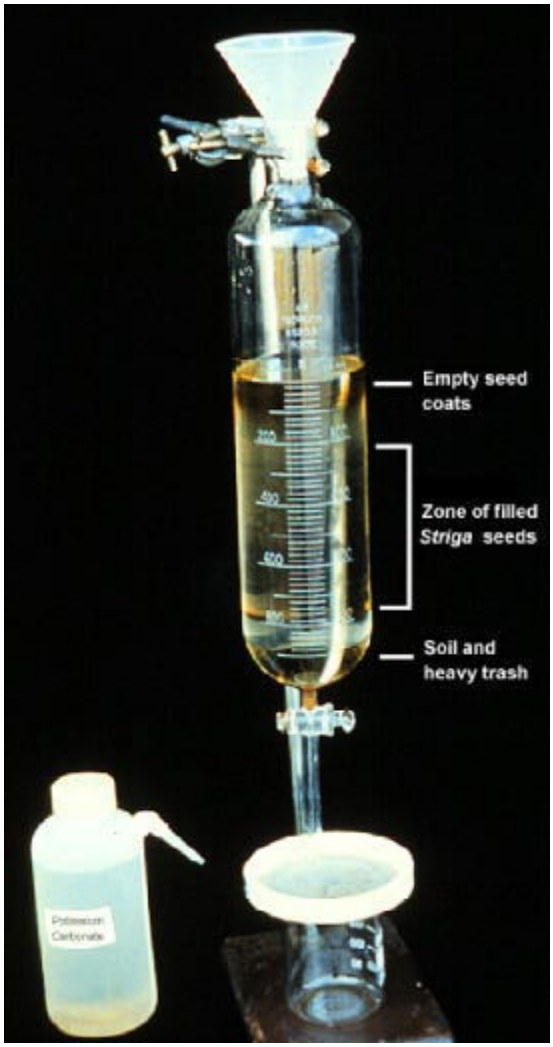

Figure 25. Separatory funnel setup for extracting Striga *seeds from soil using a potassium carbonate solution.*

5. To conserve the potassium carbonate solution and recycle it for future use, place a nylon screen of 60 micron opening over a 500 ml beaker. Place this under the separatory funnel and open the stopcock, draining off the lower potassium carbonate layer and recollecting it in the beaker. Stop draining when there is 10 cm of solution left below the water layer. Wash the trash off of the nylon screen (there should not be any *Striga* seed in this lower layer) and replace it over the beaker.

6. Continue draining until 1 cm of K_2CO_3 remains. If a lot of particles have collected on the screen, then replace with a clean screen. Do not throw away the contents of these screens as they

Figure 26. Layer of empty Striga *seed coats floating on the potassium carbonate solution at the top of the separatory funnel.*

contain *Striga* seed. Save and replace the screens as frequently as necessary. The more often screens are changed, the easier it is to count the number of seed per screen.

7. Once there is only 1 cm of the K_2CO_3 layer left, remove the beaker and replace it with an empty one. Save only the solution collected up to this point and discard the water contaminated solution. Continue collecting *Striga* seed for the next 5–10 cm of water/solution. Discard the rest of the water unless the empty *Striga* seed coats are of interest. In that case collect the material in the upper 1–2 cm of water separately.

8. After collection, count the *Striga* seed on each screen under a dissecting microscope at 20–30X magnification.

9. To make the counting process easier, make a grid on a large index card with two different colored markers, one color vertical and the other horizontal. A workable grid size is 1 x 1 cm. Section off the card and have it laminated so the wet screens can be placed on the card. The grids will serve as reference points and make counting easier.

10. After you have collected the seeds and seed coats it may be of interest to categorize the sample as follows: Total number of seed, number of seed coats, number of intact seeds, number of germinable seed, number of viable but nongerminable seed, number of nonviable seed. The numbers of seed coats and intact seeds can be determined from where in the separatory column the seeds came from. The seed coats will be in the uppermost water layer. Testing seed viability and gerrminability is outlined in the next section.

Figure 27. Extracting Striga *seeds using the potassium carbonate method in a national program laboratory in Ferkessedougou, Cotê d'Ivoire.*

5.2.5 Sucrose separation

An alternative to potassium carbonate separation is separation by floatation on sucrose (Hartman and Tanimonure, 1991), for which you would follow these steps:

1. Prepare a sucrose stock solution by adding 855 g of granulated sugar into 1 liter of boiled water. Stir till all the sugar dissolves and leave to cool. This gives a solution with specific gravity of 1.20.

2. Use 80 ml sucrose solution in a wash bottle to backwash the soil *Striga* mixture from the 106 or 90 micron screen into a beaker. *Striga* seeds float in this dense solution.

3. Into this slurry, drop a stirring bar and stir on a magnetic stirrer for 5 min.

4. Remove the stirring bar by dragging another one along the outside of the beaker to lead it to the top.

5. Gently rinse the stirring bar with a little sugar solution to recover some seeds that may attach to it.

6. Allow it to settle for 2 h.

7. Decant the floating debris, which includes the *Striga* fraction, into a 106 micron sieve; wash off the excess sugar solution carefully under running tap water.

8. Using water, wash the floated debris to a side of the sieve and rinse it into a funnel lined with a 15 cm filter paper.

9. Allow the water to drain and then transfer the filter paper carefully to the cover of a 15 mm c petri dish.

10. Under a dissecting microscope, use a forceps with sharp pointed tips to pick up the seeds and gather them on one side of the filter pap

6. Laboratory techniques

Laboratory methods in *Striga* research are an important aspect. They allow us to observe the individual processes of *Striga* seed germination, radicle formation, attachment, penetration and haustorium production, and establishment of compatibility (Okonkwo, 1987). In vitro techniques enable hosts to be screened for resistance to *Striga* establishment, at any of the above stages (Lane and Bailey, 1991). They also allow nonhosts and chemicals to be screened for their ability to stimulate *Striga* seed germination, and for the efficacy of control treatments to be evaluated (Parker, *et al*., 1977; Vasudeva Rao, 1985).

Since these techniques take little space and can be carried out in the laboratory in relatively short time, a lot of results can be generated quickly. In addition, screening in the laboratory allows much more material to be evaluated than would be possible with limited field space.

However, you should realize that screening germplasm in the laboratory only reduces the amount of material that needs to be further screened in the field and does not eliminate the necessity for field evaluation. A further caution: whereas some characters can be screened for in the laboratory, others, like crop yield and grain quality, require field screening. Since it is desirable to combine these characters, simultaneous field screening for all characters of interest may, in some cases, prove the more efficient method. It is necessary to closely evaluate the objectives of your laboratory work, considering the time and resources available and the ultimate desired outcome, before embarking on intensive laboratory research. This section outlines some basic in vitro techniques for *Striga* research that frequently need to be employed whether your central research focus is the laboratory or the field.

6.1 Materials needed

Salt of 2,3-5 triphenyl tetrazolium chloride; sodium hydroxide; pH meter; graduated cylinder; petri dishes, filter paper, glass fiber filter paper, paper punch; 1% sodium hypochlorite solution; small paired pots or styrofoam coffee cups; host seed; *Striga* seed; aluminum foil, large disposable petri dishes; small funnels; Tween 80 (or other surface tension breaker); vermiculite; synthetic germination stimulant; acetone; small paint brush, fine tipped forceps; dissecting microscope (20X or more); nutrient solution: 0.02M KNO_3, 0.002M $MgSO_4 \cdot 7H_2O$, 0.004M KH_2PO_4, 0.0008M $Ca(NO_3)_2 \cdot 4H_2O$

6.2 Procedures

6.2.1 Viability testing

The tetrazolium red test is used to detect the presence of a dehydrogenase enzyme, which indicates that the *Striga* seed is alive (Eplee and Norris, 1987). When treated with tetrazolium red, viable seeds produce a red to pink coloration in both the embryo and aleurone layer. Nonviable seeds do not show this color change. Tetrazolium red is a more useful indicator of *Striga* seed viability than germination stimulants, because viable seeds that have not after-ripened and/or not conditioned will not germinate even when treated with a stimulant.

1. To test *Striga* seed viability dissolve 1 g of 2,3-5 triphenyl tetrazolium chloride salt into 100 ml of water. The pH of the resulting solution should range between 6 and 8. Neutralize acid solutions (those with a pH below 7) with sodium hydroxide.

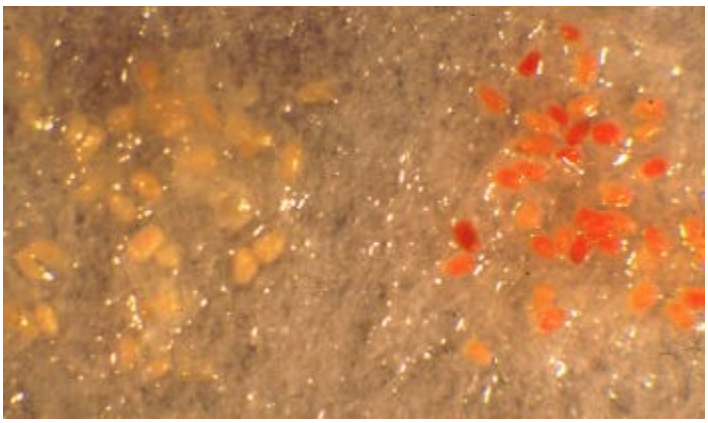

Figure 28. Red stained viable Striga *spp. seeds (right) and nonviable seeds (left) as a result of tetrazolium chloride staining.*

2. After mixing, cover the container with the solution entirely in aluminum foil to exclude light; this solution is very light sensitive and will deteriorate rapidly in light. Once covered, refrigerate the solution.

3. To test viability, place 50-100 *Striga* seed in a petri dish covered with aluminum foil to exclude light. Add enough tetrazolium solution to barely cover the *Striga* seed.

4. Place the dish in a warm (40 C) dark place for 48 h; 7 days are required for staining at 28 C. So, without an incubator, staining period will range from 2–7 days depending on ambient temperature.

5. After this, pour the mixture into a funnel lined with a 9 cm filter paper. Use water to rinse the petri dish and carry any remaining seeds into the funnel. Allow the solution to drain.

6. Place the filter paper in a clean petri dish and and add enough 1% NaOCl solution to barely cover the seed. This will bleach the seed coats and allow the red-stained endosperm beneath to be seen. Examine the seed through the microscope, looking for the red-stained endosperm indicative of viable seed (Figure 28).

7. If the coloration is not clear, mash the seed with a blunt implement to expose its interior. Very lightly stained seeds may be considered non viable. Either the viable nongerminated or the non viable non-germinated seed can be counted depending on which category appears to have fewer, more easily countable seed. The remaining category can be obtained by subtracting from the total number of seed: Total – germinated – viable nongerminated = nonviable seed. Calculate the percent viability which you can then use in studies on *Striga* seed dormancy.

6.2.2 Production of Germination Stimulant

Striga seed will not germinate in the absence of a chemical germination stimulant. These stimulants can be classified as host root exudate, nonhost root exudate, natural leachates/compounds, and synthetic germination stimulants. Stimulants are frequently screened for their relative ability to stimulate *Striga* germination (Parker, *et al*., 1977; Vasudeva Rao, 1985).

1. To prepare stimulants of root exudate, the simplest way is to grow plants in a 'double pot' system (Figure 29). This requires two tapered pots of the same size.

2. Perforate the bottom of one pot and fit it into a second unperforated pot. The upper pot is then filled with sand and the desired plant seed is planted.

3. Water will percolate through the sand and the holes in the upper pot to collect in the bottom pot. After growing the seedlings for 7 -14 days, discard the water in the bottom pot, refill with 25 ml of water and collect the subsequent leachate.

4. This root exudate should be refrigerated and can be used to stimulate *Striga* seed germination.

5. In addition to this root exudate, synthetic germination stimulants (any of the strigol analogs —GR24, GR7, etc.) can be used as checks, since properly conditioned *Striga* seed should be stimulated to germinate with these compounds (Cook, *et al*., 1966; Hsiao, *et al*., 1981)—note that seeds of *S. gesnerioides* do not respond to strigol analogs. A 10 mg L^{-1} solution in water should be used but these compounds first have to be dissolved in a small volume of acetone since they are water insoluble. After dissolving them in acetone, the compounds can be mixed with the final desired volume of water.

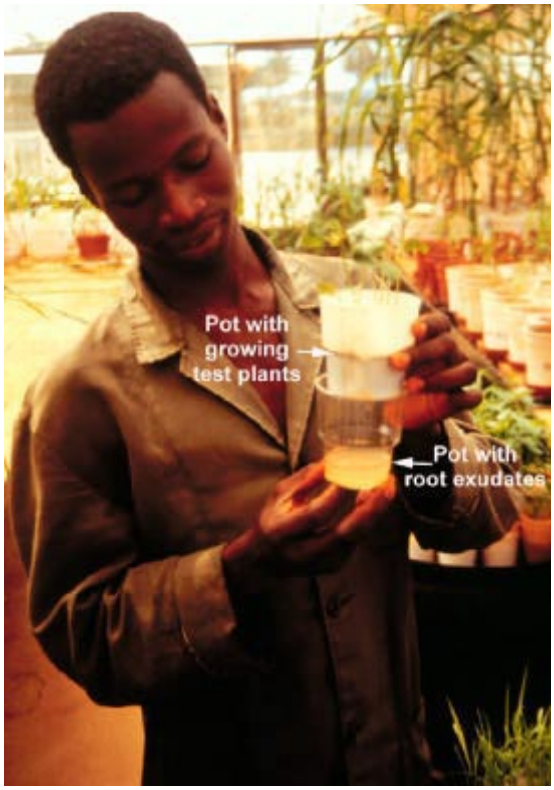

Figure 29. Double pot setup for collecting root exudates to test in stimulating Striga *spp. seed germination.*

6.2.3 Cleaning, conditioning, and germinating

While *Striga* seeds will not germinate in the absence of a chemical stimulant, they will not germinate either unless they have been conditioned, i.e., are no longer dormant and are exposed to the right environmental conditions for germination. After harvest of the *Striga* seed, there is a period of 4–6 months when the seed are truly dormant and generally cannot be conditioned to germinate. After this time period, it takes 7–21 days of exposure to moisture to precondition the seeds so that they will respond to germination stimulant.

There are several ways of doing this in the laboratory. We discuss two here, but the seed should first be surface disinfested to eliminate microbial contaminants. To do so,

1. Prepare some 1% sodium hypochlorite and decant 30 ml into a large petri dish. Add 1 drop of Tween 80 to break surface tension.

2. Add the desired amount of *Striga* seed and stir for 2 min. Discard floating seeds and debris. Pour the mixture into a funnel lined with filter paper and wash the seed with clean, ideally sterile, water.

After surface disinfestation, two methods of conditioning can be used.

Method 1

1. Use a paper punch to punch out small disks of glass fiber filter paper (used to minimize microbial growth).

2. Take 2 large pieces of regular filter paper, moisten, and place in a sterile petri dish.

3. Remove the filter paper with the *Striga* seeds from the funnel. Open the paper while still wet and, using forceps, dab up small amounts (10–25 seed) of *Striga* seed with the glass fiber disks.

4. Place these disks on the moist filter paper inside the petri dish. Many disks representing different replications and treatments can be put in one petri dish.

5. Place the dish in a dark place for 14 days and then place 1–2 drops of stimulant on each of the glass fiber disks. Wait for 48 h and count numbers of germinated seed out of the total on each disk.

Method 2

1. Place the surface disinfested *Striga* seed in 30 ml of sterile water in a sterile petri dish. Stir the seeds to force them to sink.

2. Put the petri dish in a dark place for 14 days. During this period, change the water every 2 days.

3. After this spread the seed on moist filter paper in another petri dish. A small paint brush works well to spread the seed evenly over the surface of the filter paper.

4. After spreading the seed, add enough stimulant to barely cover all of the seed. Count germinated seeds and calculate the germination percentage after 24–48 hours.

6.2.4 Testing plants for stimulant production

6.2.4.1 Selecting for hosts producing low levels of stimulant

It is often of interest to test different crops and cultivars for their ability to stimulate *Striga* seed germination. You can select for host cultivars producing low levels of stimulant by two different methods:

Method 1

1. Collect root exudates as outlined above.

2. After conditioning the *Striga* seeds by either of the above methods, add drops of the host root exudate that you have collected to cover the seeds (Figure 30). Observe germination percentage after 24-48 hours.

3. Make sure you always include a strigol analog and water as checks in each of these tests. In this way, variability between trials can be better accounted for by expressing germination induced by host exudates as a percentage relative to that induced by the strigol analog and water.

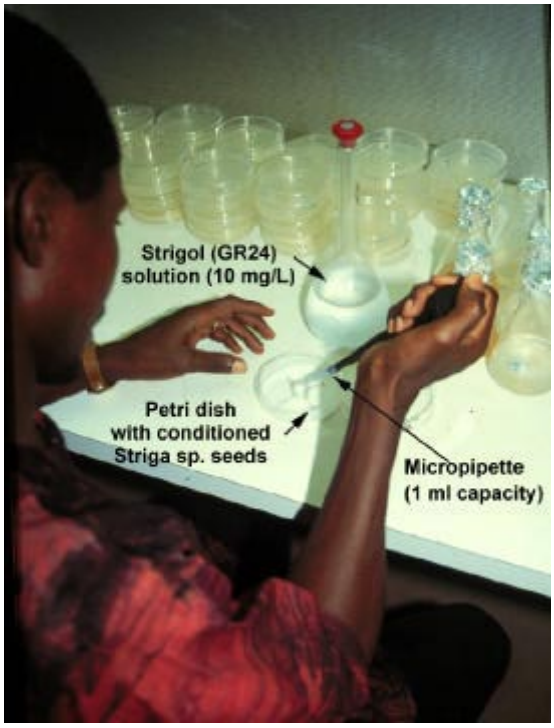

Figure 30. Applying germination stimulant to conditioned Striga *sp. seeds in the laboratory.*

Method 2 (adapted from Hess, *et al*., 1992; Reda, *et al*., 1994)

1. Surface disinfest the *Striga* seeds as previously described.

2. Condition the *Striga* seeds by placing the seeds in 14 ml of sterile deionized water plus 1 ml of 0.015% benomyl. Incubate the seeds in the benomyl solution at 27 C. Change the benomyl solution every 4 days and incubate for a total of 14 days.

3. Surface disinfest seeds of the host cultivars according to the method described for *Striga* seeds.

4. Pregerminate seeds of the host cultivars by first soaking the seeds in sterile deionized water overnight. Next, place the soaked seeds on moist filter paper in petri dishes and incubate for 48 hours at 27 C.

5. Prepare water agar by dissolving 1.05 g of bacto agar in 150 ml of deionized water. Autoclave the medium at 121° C for 20 min. and allow to cool but not solidify.

6. Pipette conditioned *Striga* seeds into a sterile petri dish.

7. Pour 33 ml of the cool liquid agar over the *Striga* seeds in the petri dish.

8. Allow the agar to solidify and transfer one germinated host seed to the petri dish, placing the seed near the edge of the petri dish with the radicle tip pointed toward the center of the dish.

9. Incubate the petri dish with the *Striga* and host seed at 27 C for 48 hours.

10. Measure the furthest distance from the host root to a germinated *Striga* seed. This distance is the measure of amount of stimulant produced by the host. The shorter the distance, the less stimulant produced.

11. All cultivars tested will have to be replicated at least 4 times in separate petri dishes. Mean germination distances can then be analyzed and cultivars compared for low stimulant production.

6.2.4.2 Selecting nonhosts producing high levels of stimulant

Crops that are nonhosts of *Striga* but nevertheless produce germination stimulants of *Striga* seeds have been termed trap crops (Parkinson, *et al*., 1988; Robinson and Dowler, 1966; Wild, 1948). These trap crops are potentially very important as a *Striga* control measure because they can germinate *Striga* seeds that will then die in the absence of a host. The potentially most valuable of these crops are legumes, which could be used in rotation with susceptible hosts to reduce the amount of *Striga* seed in the soil and to improve soil fertility. There is, however, variability in stimulant production among cultivars of trap crops, and high stimulant producers can be identified using in vitro techniques. These cultivars could then be used effectively in *Striga* control.

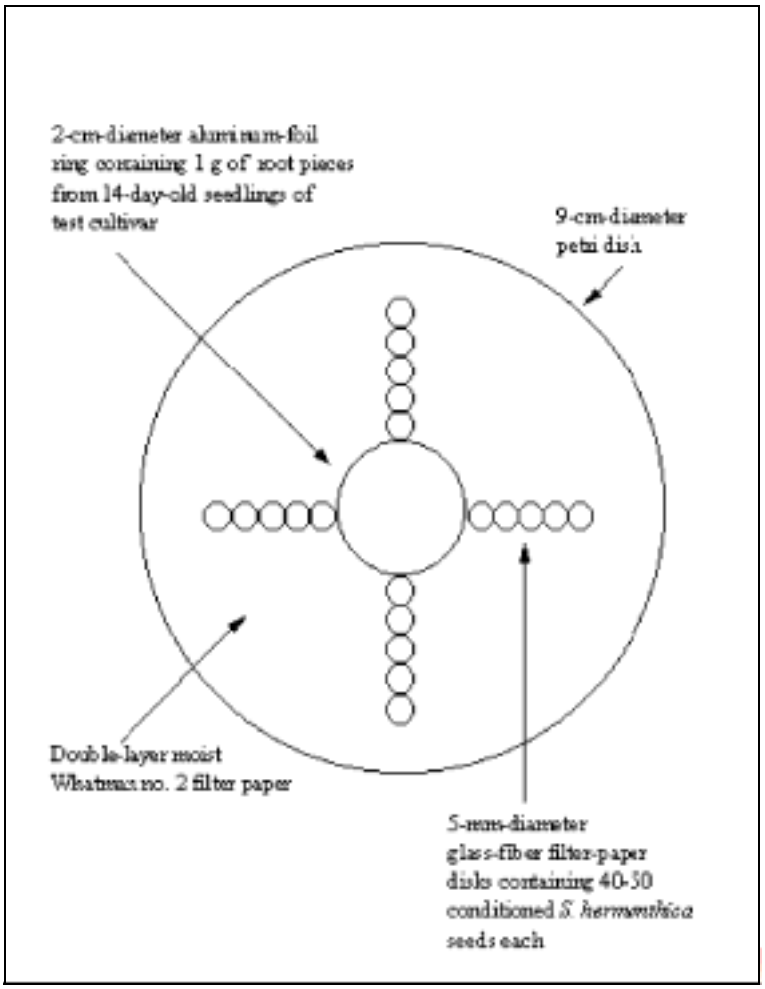

Figure 31. Diagram of setup for testing efficacy of nonhost cultivars in stimulating Striga spp. seed germination

The most efficient method of selecting trap crop cultivars is to initially screen for stimulant production in the laboratory, followed by field screening of the most promising materials. Several means of laboratory screening are available, including the screening of root exudate as outlined in the previous section (Parker, *et al*., 1977; Van Mele, *et al*., 1992; Vasudeva Rao, 1985). However, the root exudate technique does not provide a reliable quantitative measure of each cultivar's ability to produce large amounts of highly active *Striga* germination stimulant. The following technique developed at IITA (Berner, *et al*., 1996) gives excellent reproducible results that account for differences in root weight and distance from the stimulant source.

6.2.4.2.1 Experimental setup

1. Grow the plants to be tested in small pots containing sterile sand. Five to six or more plants can be grown in a single pot depending on the pot size. Don't worry about overcrowding.

2. After 14-21 days growth, gently uproot the plants and wash any sand from the roots.

3. Using a razor blade, cut the roots into 1 cm long pieces.

4. Make a circular hollow ring out of aluminum foil (diameter approximately 1-2 cm, height 1.5 cm). This ring will later be placed in the center of a petri dish, so you should try and fit the well into the petri dish and adjust the height of the well to make it fit (Figures 31 and 32).

5. Weigh the amount of root pieces that will fit into the well. The amount used should be as close to 1 g as possible.

6. Condition the *Striga* seeds according to Method 1 outlined in section 6.2.3.

7. Put two pieces of regular (not glass fiber) filter paper in the bottom of a standard petri dish. Place the aluminum foil ring on the filter paper in the center of the petri dish. Moisten the filter paper with sterile, deionized water.

8. Add the cut root pieces to the ring after weighing. Make sure you record the weight.

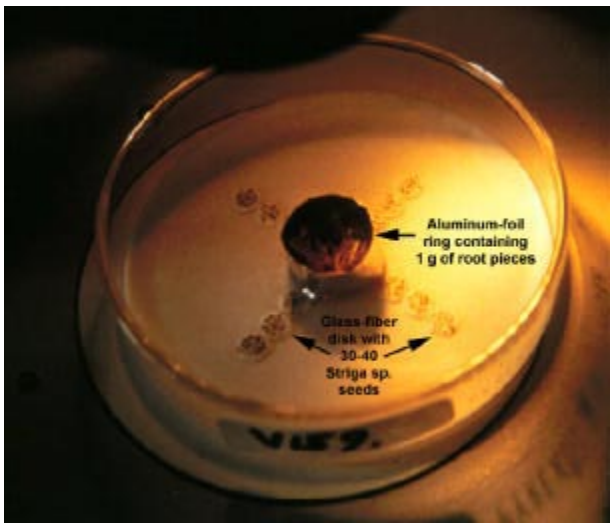

Figure 32. Petri dish technique for testing nonhosts for Striga *spp. seed germination.*

9. Place the glass fiber disks with the conditioned *Striga* seeds next to the aluminum foil well. Make four, or more, radii of glass fiber disks radiate out from the central well (The more radii you make the more work you will have to do in counting, but the results will be more reproducible). This will form a 'cross' of disks with the well as the central point. Each radii of disks should contain 3 or more disks placed edge-to-edge, with the first disk touching the edge of the well. You can thus account for the distance away from the stimulant source in your analysis.

10. Add 300 µL of sterile, deionized water to the roots in the center of the well.

11. For controls, use 300 µL of a strigol analog, or 1 g of root pieces of a susceptible host, in the positive control and 300 µL of sterile water in the negative control.

12. After 48 h, count the number germinated out of the total number of *Striga* seeds on each disk. The disks closest to the well should be considered as 'distance 1', the next disks as 'distance 2' and so on. Make sure to record the distance of each disk as well as the germination.

13. Record the numbers of germinated seeds and total number of seeds on each disk at each distance away from the root pieces (Figure 33). If more than one petri dish is being used as a replicate, average the number of germinated seeds and total number of seeds for each distance away from the root pieces in each petri dish. If plant material and *Striga* spp. seeds are limited and only one petri dish can be used per cultivar, use each row of disks within the petri dish as separate replicates.

6.2.4.2.2 Statistical analysis

To standardize different germination responses over distance away from the root pieces, normalize the data, and evaluate relative amounts of *Striga* spp. seed germination induced by each cultivar, calculate areas under the germination curves, produced by percent germination versus distance.

This can be done using the area command in GenStat™ 5 release 3 (© Lawes Agricultural Trust, 1993, Rothamstead, U.K.) computer program. A sample program is included: You will have to

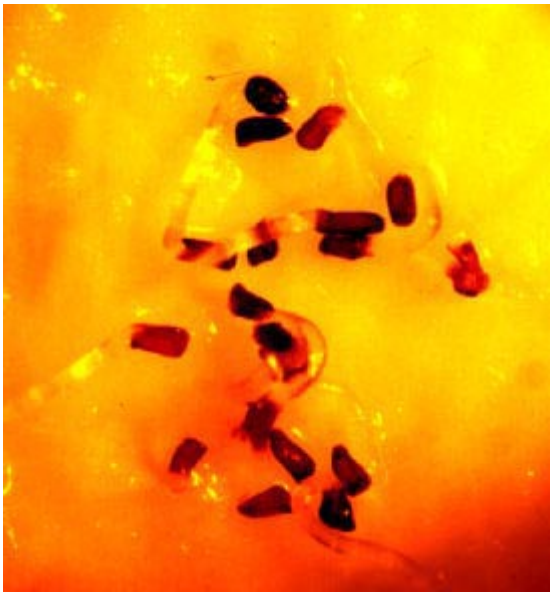

Figure 33. Germinated Striga hermonthica *seeds on a glass-fiber disk, as observed under a microscope.*

GenStat program for calculating area under the curve

```
job "j101"
unit [212]
open 'a:\\yourdata'; file=in; chann=2
open 'a:\\workingfile'; chann=3; file=out

vari [val=0.1,(3,-3,1,*,*,*,*,*)212] fmt1
"
fact [lev=4] row
fact [lev=4] day
fact [lev=33] cult
"
read [chann=2; end=*; form=fmt1] day,cult,row,rtwt

print [chann=3; iprin=*; squash=y; serial=n]
day,cult,row,rtwt;\
        field=3,5,7,9; dec=3(0),4

close chann=2; file=input

open 'a:\\yourdata'; chann=2; file=in
unit [5]
vari [valu=0.1,(-3,3,*)5] fmt

for [ntimes=212]

read [chann=2; end=*; form=fmt] distce,germ,total

calc germ% = 100*germ/total
calc Area = area(germ%;distce)

print [chann=3; iprin=*; squash=y; orient=a] Area;
field=14;\
dec=3
endfor

close chann=2; file=in
close chann=3; file=out
endjob

job "j101u150"
unit [212]

open 'a:\\workingfile'; chann=2; file=input
open 'a:\\yourareas'; chann=3; file=output

read [chann=2; end=*] day,cult,row,rtwt
read [chann=2; end=*] Area

print [chann=3; iprin=*; squash=y]
day,cult,row,rtwt,Area;\
        field=3,7,4,9; dec=3(0),4,3

stop
```

change job names, units, file names, variable statements, and factor names to fit your data set. In the sample program, there were 212 X 5 = 1060 lines of data: one line for each row of disks in the petri dish (row), day of testing (day), cultivar (cult), root weight (rtwt), and distance (distce) of *Striga* seeds away from the root pieces. If petri dishes are used as replicates, the average number of germinated and total number of seeds at each distance away from the root pieces should be used in place of numbers (germ, total) for each distance in each row. The factor 'dish' would then replace 'row' in the analysis.The positive and negative controls (strigol analog and water) should be coded as cultivars, e.g. 'GR24', 'HOH'. Assign a root weight of 1 g to these controls unless root pieces of a susceptible host are used. Then use the actual root weight.

Analyses of variance of areas, representing relative germination induction, for cultivars and controls can be done using either GenStat or Statistical Analysis System (SAS) release 6.04 (SAS Institute Inc., Cary, NC, USA.). In either case, root weight and the germination induced by the controls on each test day should be used as covariates in the analyses. A sample program using SAS to analyze area differences is presented below.

SAS program for analyzing areas from GenStat or total germination per petri dish.

```
DATA ONE;
INFILE 'A:\YOURAREAS';
* DATA FROM GENSTAT OUTPUT;
INPUT DAY CULT $ ROW RTWT AREA;
DATA ONE; SET ONE;
IF RTWT=0 THEN RTWT=1;
* ABOVE STATEMENT SETS ROOT WEIGHT OF
CONTROL SOLUTIONS (GR24 AND HOH) TO 1;
DATA TWO THREE; SET ONE;
IF CULT='GR24' THEN OUTPUT TWO;
ELSE OUTPUT THREE;
PROC SORT DATA=TWO; BY DAY;
PROC MEANS NOPRINT DATA=TWO;
BY DAY;
VAR AREA;
OUTPUT OUT=FOUR  MEAN=GRAREA;
DATA FIVE SIX; SET THREE;
IF CULT='HOH' THEN OUTPUT FIVE;
ELSE OUTPUT SIX;
PROC SORT DATA=FIVE; BY DAY;
PROC MEANS NOPRINT DATA=FIVE;
BY DAY;
VAR AREA;
OUTPUT OUT=SEVEN MEAN=HOHAREA;
PROC SORT DATA=SIX; BY DAY;
DATA CONTROL; MERGE FOUR SEVEN; BY DAY;
DATA ALL; MERGE SIX CONTROL; BY DAY;
PROC GLM;
CLASSES CULT;
MODEL AREA=CULT GRAREA HOHAREA
RTWT/SOLUTION;
LSMEANS CULT/S P;
RUN;
```

Results of the SAS analysis will give relative strength of germination stimulation, adjusted for the controls and root weight, for each of the cultivars tested. This procedure has been successfully field validated with soybean cultivars for *Striga hermonthica* control (Berner *et al.*, 1996).

An alternative to calculating areas under the germination curve is to use total germination within each row of filter paper disks in the petri dish. Total germination can then be averaged for each petri dish and these data entered into a SAS program for analysis. Total germination may not be normal and a log or square root transformation may be necessary.

6.2.5 Haustorium formation, attachment, penetration, compatibility

The lid technique is useful for visually observing haustorium formation, germination, attachment, penetration, and post-penetration compatibility under a dissecting microscope (Lane and Bailey, 1991). Combined with histology, it can give information on varietal resistance to *Striga*.

Differential combinations of various crop hosts with various populations of *Striga* (collections from different locations) can be quickly screened for studies of host-parasite interactions. There is a caution to observe, however. What happens in petri dishes does not always reflect what happens in the field. In dishes, it is possible to demonstrate attachment to hosts that are not attacked in the field. The reasons for this are not clear, but it seems that there may be defensive biotic factors expressed by false hosts in the field, which do not function in dishes.

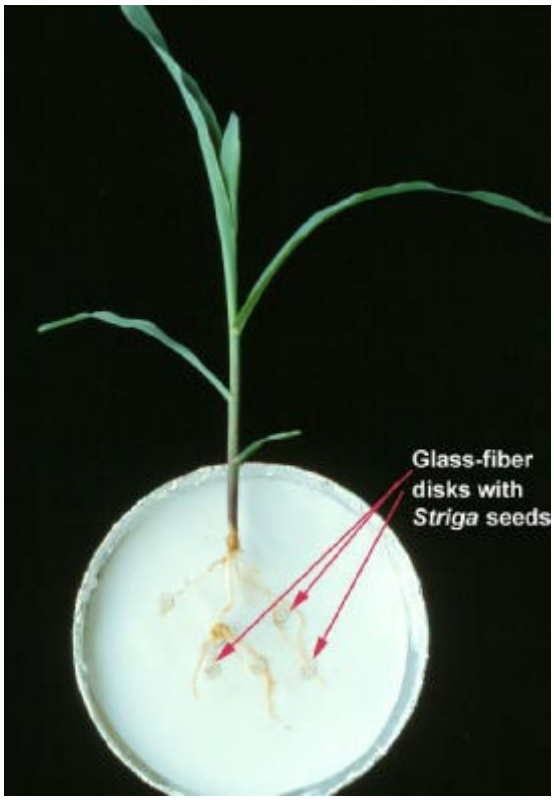

Figure 34. Lid technique for observing haustoria formation and attachment of Striga *spp. seeds to host roots.*

These processes of *Striga* parasitism can be studied by placing pregerminated *Striga* seed near an actively growing host root. To do this,

1. Grow the host in vermiculite for several days.

2. Take a large plastic petri dish and make a cutout on either the top or bottom half of the dish. This is to fit the host plant in.

3. Cover both the top and bottom half of the dish in aluminum foil, to exclude light. Disinfest the inside dish by wiping with alcohol.

4. Gently uproot the host plants and wash the roots. Place the root system on moist filter paper in the prepared plastic petri dish.

5. If prolonged exposure to the parasite is desired, use a nutrient solution (see materials) in place of water to keep the plants moist.

6. Fit the stem and leaves of the host through a cutout in the edge of the petri dish, and tape the plant in place. This will allow the plant to continue to grow.

7. Place glass fiber disks with pregerminated *Striga* seed (previous section) beneath the host roots.

8. An alternative is to place host plant roots on filter paper covered with conditioned seed.

9. Place another piece of moist filter paper over the host roots, and close the dish.

10. Periodically, open the dish to observe the progress of parasitism. In this way, different mechanisms of resistance can be elucidated.

11. After attachment, the infected plant can be transplanted.

7. Axenic culture of *Striga* spp.

Axenic *Striga* culture involves growing *Striga* plants, in the absence of a host, under conditions free from microbial contamination. This technique provides an opportunity to conduct *Striga* research independent of the confounding influence of the host plant can be an important tool in laboratory research and an adjunct to field research. Some studies suited to axenic *Striga* culture are assessment of morphogenesis and effects of fertilizers and other exogenously applied chemicals on *Striga* growth (Okonkwo, 1987).

7.1 Materials needed

Culture chamber or culture work station equipped with (a) UV light; (b) sterile air flow and (c) fluorescent light, laboratory lamp; transfer or mounted needle; dark growth chamber; autoclave; binocular dissecting microscope; pH meter; weighing balance; magnetic hotplate; incubator; cotton wool, aluminium foil, filter paper, sterile syringe with needle; iron baskets (15cm × 15cm × 15cm), culture tubes (25mm × 200mm) with screw cap or bunds/corks made from cotton wool and aluminium foil, glass beakers, glass flasks, spatula (fine and medium), petri dishes (9.5 cm and 4.5 cm diameter), wax glass marking pencils; pipettes, measuring cylinder, forceps, hand lens, scissors; sterilizing agent (clorox), wetting agent, e.g., Tween-20, distilled water; germination stimulant, e.g., GR-24, sodium hydroxide, hydrochloric acid, methylated spirit, absolute ethanol; seeds of *Striga*, vitamins, casein hydrolysate (200 mg L -1), meso-inositol (100 mg L -1), 20 mg L^{-1} sucrose, 9gm L^{-1} Difco Bacto Agar and mineral salts, e.g., Murashige and Skoog (MS) mineral salts, consisting of NH_4NO_3 (1650 mg L^{-1}), KNO_3 (1900 mg L^{-1}), $CaCl.2H_2O$ (440 mg L^{-1}), $MgSO_4 \cdot 7H_2O$ (370 mg L^{-1}), KH_2PO_4 (170 mg L^{-1}), Na_2-EDTA (37.3 mg L -1), $FeSO_4 \cdot 7H_2O$ (27.8 mg L -1), H_3BO_3 (6.2 mg L -1), $MnSO_4 \cdot 4H_2O$ (22.3 mg L^{-1}), $ZnSO_4 \cdot 4H_2O$ (8.6 mg L^{-1}), KI (0.83 mg L^{-1}), $Na_2MoO_4 \cdot 2H_2O$ (0.25 mg L^{-1}), $CuSO_4 \cdot 5H_2O$ (0.025 mg L^{-1}) and $CoCl_2 \cdot 6H_2O$ (0.025 mg L^{-1}).

7.2 Procedures

Properly sieve seeds of *Striga* for aseptic culture studies to remove all debris, so that only clean seeds remain. This treatment reduces the level of contamination during the experiment. Store *Striga* seeds in the desiccator over $CaCl_2$ at 25 ± 2 C until required.

7.2.1 Sterilization of the work station

1. Dip cotton wool in a generous amount of methylated spirit, and carefully wipe the inside of the culture work station.

2. Put on the UV light for 12 to 24 h, and allow the sterile air flow to blow for a further 2 to 5 h.

3. Note that all materials that go into the culture work station must be sterile.

4. In the absence of sterile hand gloves, wash both hands with soap and water, then dab them in cotton wool dipped in a mixture of bleach and distilled water (50:50 v/v).

7.2.2 Preparation of *Striga* seeds

The methods outlined here are similar to those of section 6.2.3, but more care is excercised here to avoid contamination of growth media with fungi and bacteria from infested materials.

1. In the sterile culture work station, surface-sterilize seeds of *Striga* by the method outlined in section 6.2.3.

2. Rinse sterile *Striga* seeds 3 to 4 times with sterile distilled water, to remove chlorine. Decant distilled water until only *Striga* seeds remain in the beaker.

3. Using a sterile spatula, transfer sterile *Striga* seeds into sterile petri dishes (preferably 4.5 cm diameter petri dish), containing 10 to 15 ml of sterile distilled water.

4. Transfer petri dishes containing *Striga* seeds into a dark incubator at 25 C.

5. After 5 to 10 days, transfer each petri dish from the incubator to the sterile culture work station.

6. By gently rotating each petri dish, you can gradually cluster *Striga* seeds at the middle of the dish.

7. Lift slightly the upper lid of the petri dish, and use a sterile syringe with needle to suck out the distilled water.

8. Replace distilled water with autoclaved GR-24, and transfer to the dark incubator at 25 C for another 2 to 3 days, after which germination would have occurred.

7.2.3 Preparation of culture media

The composition of media used for the successful growth of *Striga* beyond radicle emergence varies from species to species. *S. hermonthica* and *S. asiatica* grow until they flower, in media containing only MS mineral salts and sucrose (source of carbon), while *S. gesnerioides* thrives on mineral salts-sugar medium supplemented with vitamins, casein hydrolysate, and meso-inositol. The type of culture media used for research depends on the *Striga* species to be studied.

1. In a 1 liter measuring cylinder, add mineral salts, sucrose, other additives, agar, and make up to 1 liter with distilled water.

2. Transfer medium from measuring cylinder to a 2 liter flask and measure the pH of the culture medium, using a pH meter.

3. Adjust the pH of the medium to between 5.6–5.8, by adding drops of 1N NaOH or 1N HCl. Shake the medium in flask properly, after adding the acid or base to facilitate uniformity.

4. Dispense 20–25 ml of medium into culture tubes. Cover the tubes with screw caps or corks so that it is air-tight, place in iron baskets, autoclave, and slant on laboratory bench.

Figure 35. Striga hermonthica *seedling growing without a host on medium in a culture tube.*

7.2.4 Transfer of *Striga* seedlings to the growth medium

1. Place a binocular dissecting microscope in the sterile culture work chamber, and wipe thoroughly with cotton wool dipped in methylated spirit.

2. Transfer the petri dishes containing germinated *Striga* seedlings (2 to 3 days old) from the incubator to the culture work chamber, and pour the content into a sterile 9.5 cm diam petri dish containing filter paper.

3. Remove the germination stimulant GR-24, contained in the petri dish, by slanting the dish, then slightly lift the upper lid, place the sterile needle of the syringe under the filter paper, and draw out the stimulant.

4. Place the petri dish containing *Striga* under the binocular dissecting microscope and transfer the seedlings individually, using a sterile mounted needle, to the surface of agar medium.

5. Cover the culture tubes tightly with the cap, place in iron baskets, and transfer to the growth chamber under complete darkness or 12 h light-dark cycles at 25 C.

6. Watch for growth and development of *Striga* seedlings (Figure 35).

8. Screenhouse techniques

Various *Striga* trials can be carried out in the screenhouse (Figure 36). For example, screening for varietal resistance, conditioning trials, nutritional interrelationships of host and parasite, effect of herbicides, germination stimulants, and other aspects have been approached effectively through the use of pot and buried seed studies (Eplee, 1975; Eplee and Norris, 1987; Sand, *et al*., 1990; Vasudeva Rao, 1985). One of the major advantages of pot trials is that they can be carried out year-round (Figure 37), while field experimentation is limited to one cycle per year. One must be aware, however, that root growth, a key factor in *Striga* parasitism, is quite different in pots than in the field.

Figure 36. Successful Striga *research can be carried out on the soil floor of a rain and insect excluding screenhouse. Because insects are excluded, species like* Striga hermonthica *and* Striga aspera *will not be pollinated and will not produce seeds.*

8.1 Materials needed

Pots 15–20 cm in diameter, germinable *Striga* seed, NPK fertilizer, 90 micron mesh fabric, twine, materials for germination testing (see section 6.2.3)

8.2 Procedures

8.2.1 Pot trials

1. Place filter paper in the bottom of a 7" or 8" (18 or 20 cm) diam pot. *Striga* seed are so tiny that they can percolate down through the soil and be lost in the drainage. The filter paper will minimize this.

2. Add clean top soil up to a level 8 cm below the desired soil surface.

3. Label and arrange the pots, according to your experimental design.

4. Sprinkle the desired amount of *Striga* seed onto the soil. A rate of 1500 germinable seed per kg of soil gives moderate to good infection. Note that germination should be tested first. Then add the remaining 8 cm of soil.

5. Seven-day conditioning: carefully water pots after application of *Striga*. Do not add so much water that it leaks from the pot.

6. Water again after 4 days. Leave for 3 more days without sowing maize.

Figure 37. Successful Striga *experimentation can also be carried out in pots in a screenhouse. Shown are vigorously growing* Striga hermonthica *plants remaining after the host has been harvested.*

7. Sowing: Use clean and healthy seeds. Plant 3 seeds per pot 2-3 cm deep. This places them 5-6 cm above the *Striga* seed.

8. Calculate the amount of fertilizer to be applied based on the surface area of the pots that you are using, the fertilizer grade, and the recommended rate for *Striga* work (depending on innate soil fertility) between 60–90 kg/ha NPK. For example, if you are using 20 cm diam pots, the surface area of each pot is:
$\pi r^2 = 3.14159 \times 10^2 = 314\ cm^2$ *or* $0.0314\ m^2$.

A rate of
60 kg/ha = 60,000 g/10,000 m^2 *or 6 g/1* m^2.

So you would have to apply
6 × 0.0314 = 0.1884 g of N, P, and K per pot.

If the fertilizer grade that you have is 10-10-10 NPK (note that if the grade reflects P_2O_5 and K_2O, rather than P and K you will also have to calculate for 21.8 % P and 71 % K contained, respectively, in these compounds) then you should apply
0.1884 / 0.10 = 1.884 g fertilizer per pot.

Applying this small amount is most easily achieved by making a fertilizer solution. If you have a total of 50 pots to fertilize, then you would need a total of
1.884 × 50 = 94.2 g of fertilizer.

To make application simple, add this amount of fertilizer to a 1 L flask and fill with water to the 1 L mark. Dissolve the fertilizer, and apply 20 ml of the solution to each pot.

9. Water minimally every 2 days thereafter.

10. Carefully thin to one maize plant per pot, 7–10 days after planting.

11. Allow the crop to grow for 5–6 weeks. If any *Striga* emerges, record the date of first emergence. Before washing soil off the host roots, count the number of emerged *Striga*.

12. Carefully remove the contents of the pot (plant + soil) before submerging them in a series of buckets of water to remove the soil from the host root. Do not squeeze the roots when removing the soil because this will dislodge attached *Striga* seedlings.

13. Carefully count and remove the *Striga* that has emerged (is green), and count the number of underground *Striga*. The sum of the two gives the total number of attached *Striga*.

8.2.2 Eplee bags

These bags of porous material can be filled with *Striga* seed, then inserted into the soil in pots or the field (Figure 38), to create a natural environment for *Striga* seed, allowing water, nutrients, soil organisms, etc. to enter, but preventing seed from leaking out (Eplee and Norris, 1987).

1. Place *Striga* seed on the 90 micron mesh material.

2. Fold the material and tie it to prevent the seeds from coming out.

3. Tie a string to each "bag" ensuring that the string is long enough to reach the soil surface when buried at desired depths. (The bags can be placed at different depths and distances from treatments, and can be pulled out at any time with minimum disturbance to the soil.)

Figure 38. Eplee bag containing Striga *seeds. The bag is placed in the soil in the vicinity of crop plants and removed at periodic intervals to check* Striga *seed germination.*

4. Remove the bags at any desired time and examine the seeds for germination. A viability test can also be performed on nongerminated seeds. The ability to observe seeds under natural germination conditions is a major advantage of the Eplee bag, in comparison to direct extraction of seed from soil.

9. Quantifying *Striga* at stages in its life cycle

Of the many thousands of *Striga* seeds produced and shed onto an infested plot each year, only a very few survive to produce the next generation of mature *Striga* plants. Control methods seek to further reduce *Striga* multiplication, by interfering at different stages in the *Striga* life cycle. To quantify the effects of promising control methods, researchers would do well to measure which stages are affected, and by how much. Stages commonly of interest are the dormancy, after-ripening, preconditioning, secondary dormancy, germination, attachment, underground growth, emergence, flowering, and maturity.

Accurately quantifying *Striga* at various stages in its life cycle is difficult and laborious. Below-ground stages are much more difficult to quantify than above-ground stages. Researchers should consider carefully what types of data they really need and, for those, whether it might be more cost-effective to obtain "rough estimates" than highly accurate measurements. For example, accurate emergence, flowering, and maturity counts require repeat visits to all sampling sites every 3rd day for a 10-week period. However, a single count of the number of emerged *Striga* at the peak period (about 10 weeks after sowing maize) will give a good enough relative comparison of the effectiveness of different treatments in reducing *Striga* emergence.

In the descriptions that follow, both the detailed procedures and the "shortcut" methods are mentioned.

9.1 Above-ground stages

9.1.1 Numbers of *Striga*

Figure 39. Striga asiatica *plants at different growth stages. Many are flowering, but some have yet to reach flowering stage. Counting the number of plants at each stage is often a difficult task.*

The simplest way to get a relative (but not accurate) measure of the numbers of *Striga* at emergence, flowering, and mature stages is to make a single count at the peak period for each stage, which is approximately 10 weeks after maize sowing (depending on local conditions). If all three counts cannot be made, the maturity count is the most important, since it reflects the production of mature seed. The single-count method is not strictly accurate because different *Striga* plants emerge, flower, and mature at different times (Figure 39), so some early and late-developing individuals will be missed in any one count. However, it is often the only practical way to get the minimum essential information, such as relative comparisons of the effects of different *Striga* control treatments in reducing emergence, flowering, and maturation.

A more accurate but very laborious method is to repeat visits to the same sampling area every 3 days from first *Striga* emergence until the last *Striga* matures. (Visits must be this frequent to

avoid missing counts of dying *Striga*, which can deteriorate beyond recognition within just a few days). At each visit, count the number of *Striga* that are found dying in each growth stage (preflowering, postflowering but prematurity, and those dying a natural death at maturity) and remove them so they will not be counted again. Mature plants should be saved for yield component analysis (see section 9.4) and so the seed can be returned to the sampling area. By summing the cumulative totals in each mortality class at the end of the season, the total number of individuals that passed through each growth stage during the season can be calculated.

9.1.2 Duration of above-ground stages

Striga control treatments may affect duration of various above-ground growth stages, so these durations need to be measured. For example, resistant varieties may delay emergence of *Striga* —an indication of low vigor due to "antagonism" of the host against the parasite.

Durations, like total numbers of *Striga*, are easier to measure above ground than below ground. Counts of the total number of emerged, flowered and matured plants are taken at least weekly (preferably every 3 days). They are plotted against maize sowing date, and the peak of the curve is defined as the mean date for each growth stage.

9.2 Below-ground stages

The below-ground stages are also extremely important—dormancy, after-ripening, preconditioning, germination, and attachment. It would also be desirable to estimate below-ground mortality, since many treatments aim to increase this. Making below-ground counts is much more difficult than above ground, however; the problem is compounded by great spatial variability in *Striga* seed concentrations in most soils. Consistently accurate methods for each stage are not known.

The most practical approach is to simply measure the annual change in numbers of seed per unit volume of soil. This is done by making soil extractions at maize planting time each season for a number of seasons (preferably, at least 5). The amount of seed shed measured in the sampled area the previous year is subtracted from the soil count, to separate effects of treatments on seed production from those on the soil seed bank. These data will indicate the comparative rates of depletion in the seed bank over years, although they will not show which specific growth stage was affected.

If you are interested in making more detailed measurements of key below-ground growth stages, the following approximations are suggested.

1. Number of *Striga* that germinated: This is difficult to measure accurately because germination is spread out over a period of a few weeks, and germinating seeds disintegrate quickly if they do not attach. A relative measure can be made by counting the number of germinating seedlings extracted from Eplee bags during the peak germination period, approximately 2–4 weeks after maize planting. This may not give an accurate seasonal total, but it is a useful "index" for treatment comparisons.

2. Number of *Striga* that attached: Attached *Striga* can be counted by excavating the roots at peak attachment time (4–5 weeks after maize sowing), gently rinsing them in water to remove soil, and examining carefully under a hand lens. Evidence of mortality/decay should also be noted and counted; this, for example, should detect the effect of preemergence herbicides, which kill *Striga* seedlings as they grow upward into the surface layer of soil. This method gives only a "snapshot in time", since individuals which were attached earlier may have senesced and other individuals might attach later, but it is acceptable for making treatment comparisons. Care must be taken that the volume of soil excavated is the same for all samples, and the data should be expressed per unit of soil volume.

9.2.1 Duration of below-ground stages

A way to measure durations below ground is by repeated Eplee bag sampling, at weekly or preferably at 3-day intervals. Soil and destructive plant sampling is laborious and destroys plot area, but it can be done if needed. Numbers of seeds/seedlings in various stages can be plotted over time. It is useful to do a few "benchmark" time course studies under local conditions to gain confidence about when the peak periods occur. Once this is known, for most purposes only a single date at the peak period needs to be sampled.

9.3 Measures of vigor

Striga plant vigor influences above-ground mortality and seed production capacity, so researchers want to measure how vigorous the plant is at various stages, and how this is affected by various treatments. For example, larger *Striga* plants, producing more flowers and capsules, on sorghum (Figure 40), as compared to maize, are often noted in the same field. From this comes the hypothesis that sorghum causes a greater buildup of *Striga* than maize.

Figure 40. Sorghum crop destroyed by Striga hermonthica.

The most common vigor measurements include biomass of *Striga*, and height. Above-ground *Striga* can be harvested, dried, and weighed at any stage of interest; 10 weeks after maize planting is commonly used (near the maturity peak for *Striga*). However, this is a destructive sampling, which may not be acceptable in certain experiments. Height of *Striga* is highly correlated with biomass and capsule number per plant and is nondestructive, and it gives adequate discrimination among treatments in many cases.

9.4 Yield components

The annual production of *Striga* seed is the factor which maintains and increases *Striga* infestations. Seed production is a direct result of development of the seed-bearing stalks and organs, which are influenced by a range of environmental conditions as well as by treatments that affect plant vigor. Measurement of "yield components" may give the researcher useful data on the

diminution of reproductive capacity caused by various treatments. Yield components are defined here as the number of plants per unit area, the number of capsules per plant and the number of seeds per capsule.

Striga plants do not all mature at the same time; and they have to be collected just as they mature so that no seed is lost. Therefore, repeated visits to the site (every 3 days) are needed for accurate data. However, as with other parameters described earlier, a single visit can still give data that are useful for making comparisons between treatments. Another labor-saving shortcut would be to simply weigh the total amount of *Striga* seed produced per unit area, without counting the yield components. Such an abbreviation would still indicate the effects of treatments on the most important character: the *Striga* plant's ability to reproduce and multiply.

To measure *Striga* yield components

1. Visit the sampling area on a repeated basis (every 3 days is ideal) beginning when the first mature plants are seen, approximately 10 weeks after sowing the crop. Examine for any mature *Striga* plants. These will be starting to darken, appearing slightly grayish or purplish. Leaves may be yellowing. Do not confuse mature plants with prematurely dead plants. The latter are black and shrivelled, usually the result of disease.

2. Pull or cut the mature plants (if any). Count and record the number found in each sampling area. Also count and record the total number of capsules in the collected plants for each sample area. If you want Smicronyx data, count the number of damaged capsules at the same time (see section 8.5). Then place the plants into a plastic bag, labelled with the plot and subsample number. Keep this bag at the site, in a wind-protected place, awaiting the follow-up counts (see step 3). Open the bags so that any remaining moisture in the plants can evaporate, to prevent rotting.

3. Repeat step 2 each visit until all mature *Striga* plants have been harvested. Add the plants to the same bag designated for that subsample at the first harvest, being sure to record each week's data.

4. Thoroughly dry the *Striga* in a wind-protected place. Thresh and separate as much trash from seed as possible, using sieves. Follow the procedure outlined in section 2.

5. Accurately weigh the cleaned *Striga* seed (in grams). Deduct 10% of the weight as a correction factor for unremovable trash.

6. Divide the corrected seed weight by 5.0×10^{-6}
(the average seed weight of *Striga* hermonthica, in grams) to get the total number of seeds in the sample.

7. Divide the number of seeds by the land area from which it was collected (subsample area) to get the seed rain, expressed as number of seeds per square meter.

8. To calculate yield components,

(a) Divide the season's total number of mature plants (sum the recorded data) by the area, to get mature plants/m^2;

(b) Divide the season's total number of capsules (sum the recorded data) by the total number of mature plants to get capsules/plant; and

(c) Divide total number of seeds (step 6 above) by the season's total number of capsules to get seeds/capsule.

9.5 Damage due to pests/diseases

The most common insect pest of *Striga* is the gall-forming weevil, *Smicronyx* (Bashir, 1987; Greathead and Milner, 1971; Figure 41). This pest lays its eggs in the *Striga* flower, and the larvae bore into the ovary. They feed on the developing ovules, which as a result produce virtually no seed (Figure 42).

Like most pests, damage can be assessed in terms of both incidence and severity. Incidence is the number of *Striga* plants affected. It is measured simply by counting the number of plants that have at least one gall.

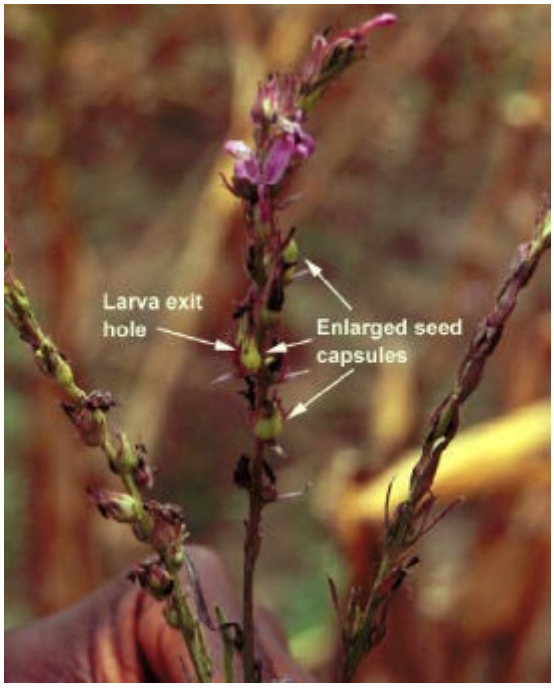

Figure 41. Enlarged seed capsules of a Striga hermonthica *plant. The enlargements have been caused by weevil larvae of the genus* Smicronyx.

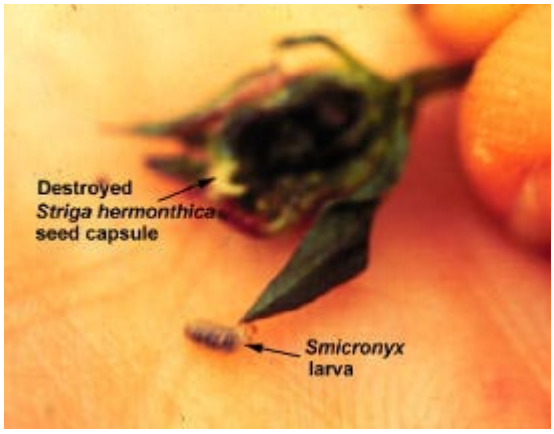

Figure 42. Smicronyx *larva and enlarged* Striga hermonthica *seed capsule. The seeds within the capsule have all been destroyed by the larva.*

To collect data on severity of *Smicronyx* damage, count the number of damaged capsules on mature plants within the sampling area. Count the total number of capsules as well (nondamaged plus *Smicronyx*-damaged), so you can later express *Smicronyx* damage as a percent of the total.

Repeated visits to the sampling area will be necessary since all *Striga* plants do not mature at the same time. Harvest the matured *Striga* as you count, so that they are not double-counted again the following week. *Smicronyx* counting can be done during the repeat visits just described.

10. Agronomic experimentation

10.1 General principles

Crop management techniques are sought which will reduce *Striga* numbers to very low levels and thus relieve crops of stress. A major concern is that these techniques be practical for African farmers.

Reducing *Striga* numbers requires a three-pronged approach:
- stopping influx of *Striga* seeds from other areas
- reducing *Striga* reproduction
- depleting the *Striga* seed bank in the soil

10.1.1 Stopping *Striga* seed influx

Although *Striga* seeds are very small, they are not efficiently dispersed by wind (Berner *et al*., 1994). This is fortunate because efficient and widespread dissemination of these parasites by wind would be virtually uncontrollable; control strategies would then be difficult to devise. From research done at IITA, it appears that man is the primary disseminating agent of *Striga* seeds. Man moves these seeds through the livestock that he manages and through crop seeds. The first step in reducing damage from *Striga* is to prevent further movement of parasites into fields. This implies restriction of animal movement from infested areas to *Striga*-free areas or areas under *Striga* control management. Also needed would be the use of uncontaminated crop seeds.

Because many farmers save crop seeds from season to season, it is important that they prevent these seeds from becoming contaminated with *Striga*. The best means of doing this is to move harvested plants to *Striga*-free areas before laying them on the ground to dry. An alternative would be the yearly purchase of clean crop seeds from reputable seed companies. There is little sense in devoting considerable time and labor to *Striga* control if the parasite is reintroduced to the field on a yearly basis. The use of *Striga*-free planting material is of utmost importance when planting an uninfested area, since prevention is much easier than cure.

10.1.2 Reducing *Striga* reproduction

Striga seed production can be reduced by killing *Striga* plants before they have a chance to produce a seed crop, and/or by reducing the fecundity (reproductive capacity) of the *Striga* plants that do survive.

10.1.2.1 Increasing *Striga* mortality

Striga mortality may be increased by intercropping (shading by the intercrop canopy kills *Striga*), hand weeding, herbicides, and biological control (fungi, nematodes, viruses, etc.)

10.1.2.2 Reducing *Striga* fecundity

Reduced vigor of emerged *Striga* plants, caused by nitrogen application, tillage, and host plant resistance, also reduces *Striga* seed production (fecundity). Fecundity can also be directly affected by biological control agents, such as the gall-forming insect, *Smicronyx*, which attacks developing seed capsules. Techniques for measuring vigor and *Striga* seed yield components have been discussed in section 9.

10.1.3 Depletion of the seed bank

The *Striga* seed bank in the soil is a heterogenous mixture of dormant and nondormant seeds differing in age, confined primarily to the upper 15 cm. Depletion of the soil seed bank is measured by the change from year to year in the number of viable seeds per unit volume of soil. Because of the dormancy factor, any trial on *Striga* seed depletion should be conducted over at least 5 years.

Sophisticated but highly effective treatments to deplete the seed bank include suicidal germination stimulants (particularly ethylene fumigation) (Eplee, 1975), application of toxic fumigants like ethyl bromide, and soil solarization (covering the soil with plastic sheet for about 1 month in the dry season, causing it to heat, killing the seed).

Figure 43. Crop rotation with cultivars of nitrogen-fixing legumes like soybean can be a highly effective means of reducing the amount of Striga *spp. seeds in the soil. To ensure effectiveness of the rotation crop, the cultivars most effective in stimulating* Striga *sp. seed germination should be previously selected in the laboratory.*

Nonhost crops grown for grain, forage, or as soil-building fallows, may also induce suicidal germination. However, the ability of nonhost cultivars to stimulate *Striga* spp. seed germination should be determined in the laboratory (section 6) before recommending the cultivar for use in *Striga* sp. control. Effective nonhosts are not as efficient as ethylene, but they may be more practical for small-scale farmers. If grown for several years, or in frequent rotation with cereals, these nonhosts effectively reduce the seed bank (Figure 43). The capacity of the nonhost to stimulate high numbers of *Striga* seeds in the soil depends on its ability to stimulate *Striga* sp. seed germination, its rooting density (more superficial roots are more effective), and the planting density (more plants produce more roots early in the season when *Striga* attacks).

10.2 Cropping systems trials

One effective way to deal with a *Striga* infestation is to avoid growing the susceptible crop. The simplest case is abandoning the land to a weedy fallow. *S. hermonthica* does not seem to thrive in noncultivated situations, so emerged *Striga* will not be evident. However, *Striga* seed will still

lie dormant under the fallow for 10 years or more, infesting the crop once again when the land comes back out of fallow.

A more desirable option is the use of leguminous nonhost crops which stimulate *Striga* germination, but do not support its growth (Wild, 1948; Robinson and Dowler, 1966; Parkinson *et al*., 1988). These nonhosts can significantly deplete the soil seed bank by inducing suicidal germination of *Striga* (see section 6). Nonhosts may be grown in rotation with cereals, or intermixed with them (intercropping), often intersown into a standing cereal crop (relay cropping).

If nonhost legumes are intersown into the cereal, this will be too late to prevent *Striga* parasitism, which occurs within a few weeks of sowing the cereal. Hence the farmer suffers yield losses in the current season, although the long-term buildup might have been lessened. *Striga* damage in the current season could be minimized if legumes are sown at the same time as the cereal; however, this causes severe nutrient competition between crops, which may reduce yields even more than *Striga* would have. New types and varieties of legumes, cereal varieties, sowing dates, spacings, etc., need to be investigated to solve these problems.

Cropping systems in the African savannas are mainly cereal-based, e.g., sorghum, millet, and maize. Since these cereals are susceptible to *Striga*, they serve to perpetuate the buildup of *Striga* seed in the field. Increasing the frequency of legume crops in the system would contribute greatly towards depletion of the *Striga* seed bank, as well as providing other benefits (nitrogen, disease/pest control, fodder, high-protein grain, etc.).

Given these considerations, the following cropping systems factors might be tested for their efficacy in *Striga* control.

- Different leguminous crops
- Different varieties within a crop
- Different sequences of crops
- Different times of sowing the different crops
- Different spacings and spatial arrangements in intercropping
- Novel nonhosts: agroforestry legumes intersown or alley sown perennial bushy legumes, forage legumes etc.

In all these types of trials, it is essential to monitor changes in the seed bank, and in *Striga* mortality, vigor, and reproduction—not just in *Striga* emergence and crop yield. Emergence counts provide incomplete information about the seed bank, the main target of cropping system treatments.

With the multiplicity of factors involved in these types of experiments, one has to guard against designing trials that are too large, too complex, or too unrealistic. In adaptive testing of potential new cropping systems, it is usually not possible to include all conceivable treatment factors at several levels in factorial designs. Instead, one has to choose a few of the most promising new systems and compare them to a check (the existing common system in that area). Of course, the output of the new system (increased *Striga* control, yield, etc.) can then be explained only as a result of the whole system; the contributions of its components cannot be separated. The effects of the major components can be broken down later in factorial studies, if desired.

In analyzing the potential of any new cropping system for actual adoption, understanding the effect on *Striga* alone is not enough. Any new cropping system entails changes in a variety of inputs to, and outputs from, the system. These must be considered and the socioeconomic viability of the new system must be assessed.

10.3 Experimental design and field layout

Treatments like nitrogen and herbicides, when applied to a *Striga* infested maize field, may increase maize yields and suppress *Striga*. However, it would be misleading to suggest that observed yield increases are wholly attributable to reduction in *Striga*, if the treatments also have yield-boosting effects of their own. For example, nitrogen increases yield even in the absence of *Striga*. In cases like this, one must point out these additional effects.

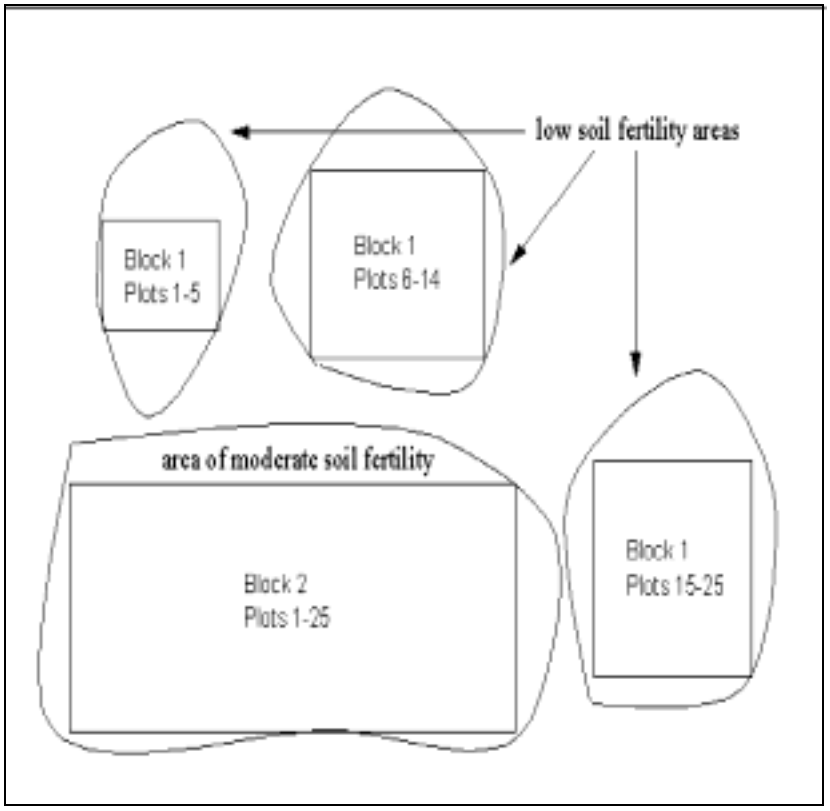

Figure 44. Example of field layout with blocking according to known soil fertility. Note that block 1 is not contiguous, but is divided among areas of low soil fertility.

Field heterogeneity in residual *Striga* infestation, soil fertility, drainage, plant diseases, insects, and other assorted factors result in *Striga* parasitism that is often highly variable from plot to plot. The variability can be reduced by using artificial *Striga* infestation, but an appropriate experimental design and field layout will allow variability to be statistically accounted for or separated out. One of the best means of doing this is to block the field according to the variability that you know to exist. For example, one block of plots can be arranged in a low fertility area of the field while another block is arranged in a higher fertility area. Note that these blocks do not necessarily have to be contiguous, but can be separated based on field heterogeneity (Figure 44).

Another way of accounting for field heterogeneity is the use of covariates. Some examples of co-variates that might be used are preplant *Striga* seed density in the soil, host plant density at harvest, and quantitative measures of soil fertility. You will have to take measurements of these covariates in each plot and include the covariates as regression variables in an analysis of covariance.

A way of accounting for unknown variability in the field is the use of nearest-neighbor statistical analyses. There are different types of these analyses and descriptions can be found in statistics

books. However, the Papadakis 'nearest neighbor' method (Bhatti et al., 1991; Brownie et al., 1993; Papadakis, 1937) is relatively easy to setup and run. Plots should be arranged in a serpentine fashion and row and column numbers assigned. Data are analyzed using the number of surviving maize plants and the Papadakis plot residuals as covariates in each analysis (see text box).

Sample SAS program for Papadakis nearest neighbor analysis

```
data one;
input plot cult tmt $ stand striga;
cards;
* DATA HERE;
data one; set one;
if plot=101 or plot=102 or plot=103 or plot=104 or plot=105 or plot=106 or plot=107 or plot=108 or plot=109 or plot=110 or plot=111 or plot=112 then row=1;
* REPEAT FOR ALL ROWS;
if plot=101 or plot=113 or plot=125 or plot=137 or plot=149 or plot=161 then col=1;
* REPEAT FOR ALL COLUMNS;
proc sort; by row col;
proc glm;
classes cult tmt;
model striga=cult*tmt;
output out=int r=res p=pred;
data lh; set int;
col=col+1;
if col <=12;
lh=res;
keep lh col row;
data rh; set int;
col=col-1;
if col >=1;
rh=res;
keep rh col row;
data under; set int;
row=row-1;
if row>=1;
under=res;
keep under col row;
data above; set int;
row=row+1;
if row<=6;
above=res;
keep above col row;
data all; merge one lh rh above under;
by row col;
nebor=mean(lh,rh,above,under);
proc sort; by row col;
proc glm;
classes cult tmt;
model striga = cult tmt cult*tmt stand nebor/solution;
```

Maintaining *Striga*-free control plots within an infested area is not easy unless the field was only very lightly infested to begin with, and heavy artificial infestations were made on the treated plots. In such cases, care should be taken to mark the plots for re-use in future trials, and complete *Striga* control should be practiced in the control plots. Cultivation equipment or tools should not be allowed to drag soil from the treated plots into the control plots, and any *Striga* emerging in the control plots should be pulled before going to seed. One may wish to arrange noninfested plots systematically (i.e., in strips rather than at random) to make this practical; but for ease of data analysis it is advised that random layouts be used. Research is needed on ways to eliminate *Striga* from control plots, so that *Striga* infested and noninfested plots can be laid out to accord statistically efficient experimental designs. Germination stimulants and certain fumigants such as ethylene, which do not diffuse to neighboring plots over 2 m away, hold promise in this regard.

As a less satisfactory alternative to artificial infestation, natural "random" variation in *Striga* distribution in the field can be taken advantage of; yields in infested and noninfested areas can be compared. However, this leads to possible confounding due to unknown soil factors that may have caused *Striga* to be absent from some areas and present in others.

10.4 Crop loss assessment in farmer's fields

Control plots do not exist in farmer's fields, so the only practical approach is the last one described above, to sample infested and noninfested areas within an apparently homogeneous area. Caution is needed, however, because areas without visible emerged *Striga* may still be heavily infested underground. Symptoms of damage on host crop leaves may be a better measure of *Striga* infestation than number of emerged *Striga*. IITA scientists found good correlations between maize leaf damage symptoms and crop yield in a 1990 on-farm survey in Nigeria, with symptoms evaluated 12 weeks after sowing. Results indicate that yield loss is proportional to yield level in the field: the higher the yield potential of the field, the higher tends to be the yield loss due to *Striga*.

Visual evaluation of *Striga* damage symptoms is done using the 1–9 *Striga* syndrome rating (see section 11). About 40–60 plants per field should be rated individually for damage symptoms, the cobs weighed for yield, and the regression of grain yield on damage symptoms calculated. Numbers of emerged *Striga* plants in a fixed area around each plant should be counted, although as we mentioned earlier this may not always relate closely to yield. If possible, soil samples in a fixed area around the plants should be collected and *Striga* seed extracted from them. The number of seeds in viable, afterripened, and germinable classes are determined, and then used as independent variables in regression analysis. In this way, yield loss can be tested for its relationship to seed bank parameters.

10.5 Sampling in field plots

Sampling *Striga* in field plots has to be planned carefully if the data are to be useful. Detailed field sampling has shown that spatial variability for seed distribution, *Striga* emergence and seed yield components is especially high in farmer's fields.

This problem can be overcome by two methods:

- Taking large numbers of random samples across the plot or field; or
- Repeated visits to fixed sampling areas.

At IITA, the second method is used. Maize is sown on ridges spaced 0.75 m apart, with 0.25 m spacing between plants within a row. Plots consist of six rows, 5 m long. Two or three permanent sampling areas (each 2 m long and 1.5 m wide) are chosen per plot. Ten soil core samples of 3–5 cm diam and 15 cm depth are collected within each sample area, and mixed together for analysis. Subsequent data are collected by repeated visits to the same sampling areas.

10.6 Herbicide trials

10.6.1 General guidelines

Preemergence herbicides against *Striga*, such as oxyfluorfen and dinitroaniline compounds, form a barrier in the top few cm of soil and kill *Striga* as it emerges. They are the most popular class of herbicide in the United States because, (a) application can be done just after the tillage

operation, when tractor access to the field is easiest; (b) herbicide damage to the host is minimal at this early stage; and (c) many other weeds are simultaneously controlled.

Emerged *Striga* can be killed by a variety of herbicides. Examples include glyphosate, paraquat, dimethametryne, 2,4-D, MCPA, Triclopyr, etc. Even a 20% urea solution sprayed on a sunny day will severely burn and kill emerged *Striga*. By directing the spray below the crop plant canopy, crop damage is minimal.

However, pre- and postemergence herbicides do not prevent crop yield loss, because they go to work after *Striga* has already attached and damaged the host (Figure 45). Nevertheless, killing *Striga* that has emerged prevents buildup of seed in the soil, reducing the *Striga* problem in future seasons.

In situations involving resource-poor, smallholder farmers the economics of herbicide use must be carefully analyzed. It may be difficult to convince farmers to invest in a chemical that does not increase yields in the current season.

Attempts are being made to find herbicides that move systemically through the host to the attachment point, killing *Striga* while it is still underground. These may be able to kill *Striga* before it has completely damaged the host plant, allowing the farmer to benefit immediately. Dicamba (Banvel) is a promising chemical in this category. It is safe and inexpensive, about the same cost as 2,4-D. If applied properly, it has little effect on the host. In IITA's experience, the maximum numbers of attaching *Striga* are seen around 5–7 weeks after maize sowing. Therefore, we apply dicamba at this time. Earlier applications might further reduce *Striga* damage, if the residual effect would protect the plant during the main attachment phase, but this has not been studied. Dicamba can also be applied to the soil; this lessens herbicide damage to the host, but it is also less effective in controlling the *Striga*. More work is needed on methods of application, timing, rates, and length of residual effect of dicamba. It should not be applied later than 2 weeks before flowering, because it may impair flowering and grain formation.

Figure 45. Timing of herbicide (either chemical or biological) application is of critical importance to control Striga *spp. and to justify the cost of the herbicide. In this photo, the late herbicide application will produce no yield benefit in this maize crop. Most of the damage to the crop has already occurred, and the only benefit might be a reduction in the amount of* Striga hermonthica *reproduction.*

To be practical for African smallholder farmers, herbicides must be inexpensive and easy to apply. Also, broadleaf herbicides cannot be used in intercropping situations where legumes, cotton, etc. would be exposed to spray drift.

An attractive alternative to conventionally applied herbicides is the use of herbicide seed treatments for *Striga* control. With cowpea, acetolactate synthase (ALS) inhibiting herbicides of the imidazolinone class have been successfully used as seed treatments for *Striga gesnerioides* and *Alectra vogelii* control (Berner *et al.*, 1994). With the seed treatments, parasite seed germination occurs but protection is achieved by postattachment mortality of the parasite. Thus, an additional control benefit is obtained by reducing parasite seed density in the soil. However, many cowpea cultivars are susceptible to ALS inhibitors when applied as seed treatments. To avoid this problem, cultivars that are resistant to the chemical must be selected. This can be done in the laboratory by soaking cowpea seeds in an aqueous solution of 0.35 mg imazaquin ml^{-1}. The seeds should be soaked for only 5 minutes and then dried. After drying, conduct tests in petri dishes to determine the effects of the chemical on cowpea seed germination and radicle growth. Those cultivars that give at least 80% germination and have slightly reduced radicle growth can be further tested in pot and then in field trials.

In maize, phytotoxicity of the ALS inhibitors is a more serious problem. To effectively use ALS inhibitor seed treatments on maize, tolerance to the ALS inhibitor must first be incorporated into high-yielding, adapted African maize cultivars. More work is needed on improving the effectiveness of the seed treatments on cereals, reducing phytotoxicity, and investigating the potential for *Stri*ga spp. to develop resistance to these herbicides, before their potential can be realized in farmers' fields.

10.6.2 Herbicide trial design

Preliminary herbicide trials can be "look-see", testing a large number of materials without replication or sophisticated statistical designs. The crops to be tested are planted in parallel rows, and the herbicide treatments are applied in strips across (perpendicular to) these rows. Different chemicals, rates, timings of application, methods of application, etc., may be tried, each in its own strip.

Once a few promising treatments have been found, replicated trials should be conducted to confirm the preliminary results. If the number of treatments is 20 or less, a completely randomized or randomized block design is recommended, while for larger numbers, a lattice design is needed to help block out the effects of soil heterogeneity. Generally, 4–6 replications are adequate.

The third stage of testing should be on-farm. Herbicide may be only one component of a package at this stage. The package should be compared to the farmer's practice, in large plots. All operations should be carried out by the farmer according to his common practice, except for the treatment variables being studied (e.g., herbicide). Each field is a single replicate, and error is generally high. Therefore, large numbers of replicates (farms) will be needed before definite conclusions can be drawn. Special care should be taken in recording all nontreatment variables, such as farmer's planting date, weeding dates, weeding methods, fertilization dates, remolding of ridges, etc. The effects of these factors can then be removed, using covariate analysis.

11. Breeding maize for *Striga* spp. resistance

Breeding involves challenging crop germplasm with *Striga*, and selecting the best performing genotypes ("screening"). To have effective screening, artificial infestation of a field with *Striga* is necessary. The infestation must be uniform, so there is no doubt that better-performing crop lines are truly better, and not merely chance escapes from *Striga* parasitism. When screening for any stress, such as *Striga*, it is important that it be the only stress in the field, so that observed differences between genotypes are truly differences due to *Striga* resistance. Crop plants must be otherwise healthy, with a uniform stand. *Striga* infestation must also be uniform so the breeder can do effective selection. This is statistically reflected in a low coefficient of variation (CV). For more information on management of *Striga* field plots, see sections 4 and 10.

11.1 Field layouts for *Striga* breeding nurseries

There are many possible types of field layouts useable in *Striga* screening. Some of these are quite complex because of the need to reduce variablility when screening is done under natural infestation. However, if artificial infestation is used a lot of the variability in *Striga* infection is reduced, and relatively simple field layouts can be used. Because plots are artificially infested, it is of great benefit to leave a portion of the plot uninfested for comparison of plant responses with the infested portion. Two general types of field layouts can be conveniently used. In both layouts, half of the plot area is left uninfested while the other half is infested as described in section 4.

If the amount of material to be evaluated is relatively small or if land and labor are available, a layout of 4-row plots can be used, in which two rows are infested and two left uninfested. It is recommended that rows be a minimum of 5-m long. The infested rows within the plots can be chosen randomly or the arrangement can be systematic, infesting either the center two rows or the outside two rows. A systematic arrangement allows for better visual comparison and rating. This type of layout requires considerable space, but provides good information on *Striga* emergence and yield.

If the amount of material to be screened is large or land and labor are limited, a layout of single row plots can be used, in which half the row is left uninfested while the other half is infested. It is recommended that the rows be a minimum of 10-m long, with an alley of 1.5 m between the infested and uninfested sections of each row. Shorter rows can be used, but, with shorter rows, yield differences become much more difficult to detect. Although this layout allows for the screening of more material, there is more variation between infested and noninfested plants. In both layouts, a minimum of 4 replications should be used.

The major value of any of these layouts is to be able to compare infested performance with noninfested. This provides an important correction. For example, height reduction is one symptom of *Striga* damage. However, some varieties are inherently short, and it would be a mistake to rate them as susceptible just because they appear shorter than other entries. With the layouts enabling this comparison, one can rate the relative reduction in height caused by infestation. The greater the relative reduction, the greater the *Striga* susceptibility. Thus, an inherently short variety that nonetheless shows little reduction in height under *Striga* pressure

could still be scored as resistant.

No matter what layout is used, it is important to include check entries. These are baselines for comparison. They should include the most widely grown local variety, and a variety known to be highly *Striga*-susceptible. The susceptible check serves as an indicator plant, confirming that *Striga* pressure really exists in that part of the field. A useful third type of check is an improved variety, normally the best as currently recommended by national authorities. The most *Striga* resistant variety available is another type of useful check.

11.2 Preliminary screening — thousands of lines

These are initial breeding materials, such as exotic germplasm introductions, progenies from recombinations of populations, etc. Ideally, two-row plots are used with 2–3 replications. When seed is limiting, one-row plots are often used. Checks should be sown systematically, e.g., every 20th plot, rather than at random. Plots are laid out in serpentine fashion, for easier movement during note-taking.

Entries are compared to one another, to their control half-plots, and to widely grown check varieties, and those with the least damage symptoms on maize (see section 2) are selected and saved for further propagation. Particular value is placed on entries showing relatively low *Striga* emergence, if the infestation is sufficiently uniform to observe such differences.

11.3 Advanced screening

In advanced screening, breeders take a closer look at the most promising material identified in the preliminary screening, to reconfirm resistance. The same options are available for field layout, namely either the uniform or the half-plot layouts described above. However, plot size should be increased to three or more rows, with three or more replications. Length of half-plots should be increased, so that each half plot is at least 5 m long. Depending on the variable(s) of interest, either randomized complete block designs or completely randomized designs can be used. If *Striga* emergence counts are of interest, then completely randomized designs are somewhat more sensitive. To improve this sensitivity, neighboring plot values can be used as covariates in the analyses (Scharf and Alley 1993). Randomized block designs are especially useful if yield is of primary interest. Entries should be grouped into separate trials according to growth duration (days to flowering). Check entries are randomized just like all other entries. Plots are numbered and laid out in serpentine order.

As in the preliminary screening, *Striga* syndrome ratings should be made on the maize plants (see section 11.5). *Striga* emergence can also be rated visually. If funds are available, *Striga* emergence counts can be carried out for greatest accuracy. The best time for such a count (maximum emergence) is approximately 10 weeks after crop sowing. All the usual agronomic data should be collected, including yields of grain and stover, lodging, flowering date, etc. Data should be collected separately for infested and noninfested areas.

11.4 Types of resistance

The purpose of counting *Striga* numbers in breeding plots is to determine the mechanisms of resistance, i.e., whether a better performing variety is tolerant (supports the same number of emerged *Striga* as the susceptible check), resistant (fewer emerged *Striga* than susceptible check), or immune (no emerged *Striga*, as well as no attachment and no damage symptoms). An awareness of this is important for breeders, because they may want to select for certain mechanisms, and combinations of mechanisms that will give the best end result (Robinson, 1969).

It would appear that immunity is the ideal mechanism, since it completely protects the plant. Breeders must retest any symptom-free plant selections, to ensure that they are not simply "escapes", i.e., plants which happened by chance not to be attacked by *Striga*. Another problem is that immunity is often not "durable" because it frequently involves the function of just a few genes. This creates selection pressure for a corresponding virulence gene to overcome the immunity. Single-gene immunity may thus become ineffective within a few years, cancelling all the progress made. Tolerance and resistance are usually based on larger numbers of genes, so they are more durable.

Striga parasitizes the crop, and propagates itself, through sequential processes: germination, attachment, "intoxication" of its host, subterreanean growth, emergence, flowering, and seed production. Crop plants might be able to resist *Striga* at any of these stages. Combining different resistance mechanisms through breeding may increase overall resistance, and make it more durable.

Figure 46. Zea diploperrenis *(a wild relative of maize) is a promising source of resistance to* Striga hermonthica.

Some genotypes are less efficient in stimulating *Striga* to germinate. Their chemical stimulants may be weak, or their root systems may be less extensive, so that they don't come into contact with much *Striga* seed. These "low stimulators" have fewer attached *Striga*. However, this mechanism often appears variable frome site to site and year to year. When infestation is heavy, or the soil is loose so that roots proliferate and come into contact with more *Striga* seeds, these genotypes may still suffer heavy attachment and may be just as badly damaged as a susceptible genotype.

A second type of reaction is found in genotypes that stimulate *Striga* to germinate and allow it to attach, but slow its growth, delay its emergence, and reduce its vigor. These genotypes are resistant. Progress in breeding for reduced *Striga* parasitism in maize has been constrained by the limited genetic variation for resistance in adapted germplasm. Recently, resistance genes from *Zea diploperennis*

have been incorporated into a maize population adapted to the lowlands of West and Central Africa. A backcross program has been initiated to incorporate resistance genes from *Z. diploperennis* (Figure 46) into an adapted maize population. Progess in improving resistance has been good.

A third type of reaction is found in genotypes that stimulate *Striga* to germinate and allow it to attach, grow, and reproduce normally, but do not suffer much from the intoxication effect. These genotypes are tolerant. The only problem with this reaction is that it does not reduce parasitism or *Striga* seed production, which will attack the following crop. If this mechanism can be genetically combined with low germination stimulation, the result could be quite useful: genotypes that have both reduced *Striga* germination/attachment/emergence and reduced host damage.

Breeders need the backup of laboratory analyses (germination stimulant tests, histological studies of attachment, and measurements of initial growth in vitro and in pots) to learn what mechanisms of resistance are involved in their genotypes. This knowledge is needed so that (a) a range of mechanisms can be identified, saved, and subjected to genetic analysis; and (b) the right crosses and selection procedures can be set up to "pyramid" different mechanisms into one genotype for maximum, durable resistance.

11.5 *Striga* syndrome rating

This is a visual rating of the damage caused to the maize plant by *Striga*. A 1 (no damage) to 9 (maize plants dead or dying) scale is used.

Scientists often ask, what are the discrete, quantifiable measurements that go into making this rating? What exactly is a 3, and a 5, and a 9? They want the rating number to represent one precise phenotype that is the same no matter where or when a crop is grown. They expect it to be like a unit of the metric system, where a meter is a meter no matter where it is measured.

However, this is not the right way to understand a rating scale. A scale is a practical tool for a specific purpose, namely, selection in a breeding trial. In a horse race, what is most important is not the absolute running time, but the relative running time or which horse finishes first. In the same way, the *Striga* syndrome rating is useful in identifying the best among a range of maize genotypes; it is not designed to give an absolute measure of the degree of damage. It doesn't much matter if one breeder rates a certain entry as a "7" while another would rate the same entry as an "8". What matters is that the rating scale enables different people to rank entries in the same order.

The *Striga* syndrome rating sums up several different types of maize symptoms into one number. Some of these symptoms are briefly described here.

Leaf symptoms: these are the easiest to recognize. There are several types:

- "blotching": vague, round whitish spots, reminiscent of bacterial water soaking;

- "streaking": whitish, chlorotic streaks;

‣ "wilting": loss of turgor, as in drought stress; and

‣ "scorching": leaves turn brown and die, starting from tips and edges and progressing downwards and inwards with age.

Ear symptoms: Ears of *Striga* infested plants are shorter, smaller in girth, and lighter in weight. Husks tend to open in severely damaged plants.

Stem symptoms: Stems of *Striga*-infested plants have shorter internodes; in severe infestations, plants apppear to be dwarfed. Stalk diameter is also reduced, and badly affected plants have brittle stalks, which collapse easily under the slightest strain.

11.6 Rating systems

The one to nine scale has several advantages:

‣It gives 9 possible ratings, so there is no need to write down fractions, as often happens with 1–3 or 1–5 scales;

‣There are no double digits, as in a 1–10 scale, which would be wasteful since it would require another column of computer space;

‣It can be adapted easily by those who desire three-number or five-number scales. They can confine their scores to 1, 5, 9 or 1,3,5,7,9, as they prefer. By doing so, their data will still be understandable by other scientists who use all of the nine possible scores. This will improve communication among scientists.

‣It is the system now recommended by the International Board for Plant Genetic Resources (IBPGR) for maize, so it will become more and more universal as time goes on.

11.6.1 Scoring symptoms

1 = Normal maize growth; no visible symptoms.

2 = Scattered small and vague whitish leaf blotches visible. Otherwise normal plant growth.

3 = Blotching and streaking easily noticeable. Mild wilting. Only a trace of scorching, restricted to tips of leaves.

4 = Extensive blotching and streaking, mild wilting. Only a trace of leaf scorching. Slight but noticeable stunting and reduction in ear and tassel size.

5 = Extensive blotching and streaking, wilting. Leaf scorching on a small portion of the leaf area. Moderate stunting; ear and tassel size reduction.

6 = Extensive streaking, now obscuring the blotches, turning to scorching. Leaf scorching

covering about a third of the leaf area. About one-third reduction in height. Reduced stem diam, reduced ear and tassel size.

7 = Extensive streaking/scorching, turning gray and necrotic. About half of the plant's surface is scorched. Severe stunting, about 50% reduction in height. Noticeable reduction in stem diam and in ear and tassel size. Some stalks breaking.

8 = Scorching on most of the leaf area. Stunting resulting in a 50% reduction in height. Stalks look thin and weak; many are broken. Husk leaves are noticeably short and open.

9 = Virtually all leaf area scorched; two-thirds or more reduction in height; most stems collapsing; no useful ear formed; miniature or no tassel; no pollen production; plants dead or nearly dead.

11.6.2 Using the *Striga* syndrome rating

A single score is given for each plot, based on the average performance of all plants in the plot. If the half-plot design is used, the relative change in appearance between noninfested and infested half-plots serves as the basis for the rating. The rating is not calculated as a mathematical average of single-plant ratings, which would be too tedious; rather, it is obtained by mentally integrating the overall appearance of all the plants in the plot, excluding those bordering the alleys. The more uniform the trial, the easier this mental averaging will be.

11.6.3 When to rate

In variety trials, there is the question whether to (a) rate all varieties on the same day, or (b) rate varieties at equivalent growth stages, which may occur on different days for different varieties.

The issue arises because it is sometimes reported that earlier maturing varieties appear to partially "escape" or "avoid" severe *Striga* damage. Since there may be a 2–6 week delay between the time when maize germinates and when the *Striga* attaches (depending on many factors), only a relatively short time remains for *Striga* to do its damage before such early-maturing varieties complete their growth.

Because of this possible "avoidance", an early-maturing variety scored at flowering stage might receive a lower score (less damage) than a later-maturing variety scored at its flowering stage. The physiological tolerance/resistance of their tissues to *Striga* attack might be the same, but the time period of exposure is greater for the later-maturing variety, so it looks worse. More data comparing a wide range of early vs. late materials need to be collected to check on the correlation between earliness and *Striga* avoidance.

A higher priority at the infested site is to search for maize plants which are physiologically resistant/tolerant/immune. These will best be revealed by comparing varieties which have all been exposed to *Striga* for the same period of time. Thus, breeders should score all varieties at the same time (ideally on the same day), regardless of growth duration. This is also the most practical method since it doesn't require multiple visits to the site.

Scoring is effective if done just once during the season, 2 weeks after the mean flowering date for the trial. At this time, symptoms usually reach their peak, yet it is still before senescence begins, when natural leaf death would begin to be confused with the scorching symptom due to *Striga*. Under severe *Striga* attack, scoring may need to be done earlier, because some entries could be killed before flowering. In such cases, do the rating as soon as the susceptible check variety reaches a score of 7, or do both an early rating and a late rating. Use either an average of the two ratings or the more reliable rating. Ideally, symptom rating should be combined with evaluation of materials based on *Striga* emergence (Figure 47).

Figure 47. Maize breeding trial for Striga hermonthica *resistance. Both maize symptoms and abundant emerged parasites make evaluation of germplasm easier.*

12. Systems approach to *Striga* research

12.1 Farming systems orientation

Striga control technologies are meant to be used by farmers, so they have to be suited to their needs, skills, and resources. Technologies must be characterized so their "fit" to various farming systems can be judged.

12.2 Assessing the effectiveness of technologies

One way of assessing the effectiveness of a control technology is the extent to which it reduces yield losses in the current season's crop. This measure is of particular importance to small-scale, resource-poor farmers, who need technologies that give immediate relief from *Striga* damage.

A second way to evaluate technologies is in terms of their long-term effectiveness in eliminating *Striga* from the field, the basic source of the problem. Technologies that deplete the soil seed bank do not necessarily bring economic rewards to the farmer in the current season, however.

Ideally, options are needed that both increase current income and at the same time deplete the seed bank. Examples of options that achieve this are highly resistant varieties; intercropping; rotating with nonhost crops that bring economic returns such as grain legumes and cotton; fallowing with forage legumes that provide feed for livestock; and agroforestry technologies using nitrogen-fixing shrub/tree species that do not host *Striga* and at the same time reduce fertilizer, tillage, and weed control costs.

A good understanding of farming systems extends beyond *Striga*-related and other biological aspects of cropping systems. Socioeconomic factors influence the adoption of any technology, and they must also be well understood. Of the many socioeconomic variables that affect technology acceptability, it is hypothesized that the two which explain the greatest amount of variation are (1) human population density and (2) access to markets. Population density largely determines the cost of labor, the most important agricultural input. Labor is expensive in areas with low population density; conversely, where populations are dense and many are unemployed, labor is cheap. Market accessibility will largely explain farmer's willingness to use purchased inputs, including most of the *Striga* control technologies (seed, fertilizer, herbicide, recurrently supplied biocontrol agents, etc.) In areas with poor access to markets, most food will be grown for subsistence only, since there is no market where the farmer can sell his excess production; if markets are accessible, there will be incentive for cash cropping, and purchase of inputs to increase yield and maximize profits.

Technologies need to be matched with farming systems. *Striga* density is suspected to be a major determinant of which technology will be most biologically effective in solving the problem (see section 10); however, major socioeconomic factors, such as population density and market accessability, interact with *Striga* density to determine what control technologies are likely to be adopted. For example, in areas with low human density and poor market access, farmers are mainly subsistence oriented (will not purchase many inputs), but they have plenty of excess land.

Therefore, fallowing will be a very practical option for them on severely infested land. When human density and market accessability are high, they cannot afford to leave land idle, and will be more willing to go for costly inputs, such as fertilizer and herbicide.

Some cautions are in order. The accuracy and usefulness of technology targeting will be no greater than the accuracy of characterization which has been applied to the technologies and farming systems. With the multiplicity and complexity of factors involved, researchers must be realistic about the degree of uncertainty associated with the recommendations. Also, in a two-dimensional system, not all farming system variables can be represented; there may be situations which are more complex and require a special analysis.

Nevertheless, "systems logic" must be applied in matching technologies with farming systems. We have to remember that, in many cases, technologies promoted at great expense for many years have not been adopted by farmers because they were not appropriate to their needs and resources.

12.3 Survey techniques

Surveys are an essential technique in characterizing farming systems. They can provide data on the extent, spread, impact, and causes of the *Striga* problem, and on farmers' capabilities to deal with it.

However, caution must be taken that surveys are not overdone or inappropriately done. For general advice on surveying, see Horton (1990). Surveys are expensive, so they should only be undertaken after all other sources of information (publications, maps, etc.) have been studied. They should be based on specific written objectives; well planned and focused; questionnaires should be concise; and the methods for analyzing the data should be clear even before data collection starts.

Two types of survey are common: (1) extensive, covering a large region quickly to discover the broad distribution of a problem; and (2) intensive, going into detail in a few selected representative villages to understand the causes of the problem and what farmers are doing about it.

12.3.1 Extensive surveys

Since a large geographical area must be covered, and travel is expensive, it is essential that the survey be efficiently planned, so that a maximum of the right kind of data is collected in minimal time. The most practical approach is to keep to the main roads, using the odometer to stop at precise fixed intervals along the road (e.g., every 20 km). The first field seen after each stop point should be surveyed. This method may not give a representative sampling of a region, since it misses areas distant from the road, but it guards against other kinds of selection bias, such as selecting fields that are unusually high or low in *Striga* infestation, are exceptionally large or small, unusually well managed, etc.

The data to be collected at each stop must be carefully thought out beforehand, and you must

ensure that it is possible to collect within the time allotted for each stop. For example, if you have only 15 min for each stop, including getting in and out of the vehicle, there will not be time to look for and interview the farmer, nor to take detailed measurements. Remember that for this type of survey it is important only to understand the geographical extent of the problem, and to characterize the setting in which it is found. Once you have an idea of the data set you want to collect, it is worthwhile to make a few test runs to be sure the data can really be collected within the allotted time.

Data to be collected in an extensive survey

1. Location: distance (in km) from closest town or from previous stop.
2. Field size: in paces, length and width (see also no. 11 below).
3. Field history: 1= recently cleared from bush (evidence: burned stumps and piles of brush); 2= cultivated for long time (evidence: smooth field surface, no stumps, elaborate ridging, speargrass (*Imperata*) present, *S. hermonthica* present in the cereal intercrop, field near village).
4. Rotation crop: if nearby fields are growing crops like cotton and soybean in rotation, it is likely that the field being sampled operates within a similar rotation. Choose numbers to represent rotation crops.
5. Soil texture: use finger test. Roll soil between fingers. Use rating scale: 1= sandy (grit between fingers); 2= sandy loam (grit between fingers, wet soil makes fingers dirty); 3= loam (very fine grit between fingers, wet soil is plastic but not sticky); 4= loamy clay (no grit, wet soil plastic and slightly sticky); 5= clay (wet soil very plastic and sticky, stains fingers, gray color); 6= organic (dark brown to black color).
6. Cropping system, i.e., sole, mixed with sorghum, mixed with millet, etc.
7. Cowpea growth stage: 1= vegetative, 2= flowering, 3= podding, 4= mature.
8. Cowpea morphotype: 1= *V. unguiculata* type (a= small leaf, b= large leaf, c= lanceolate leaf, d= obdurate leaf); 2= *V. de kintiana* type.
9. Cowpea architecture: 1= trailing, 2= erect, 3= semi-erect.
10. Cowpea grain type: 1= blackeye, 2= white, 3= brown, 4=purple.
11. Incidence of *Striga*: First, walk to the back of the field. (Count paces in so doing to accomplish part of item 2 above). As you return, walk in a zig-zag pattern, randomly stopping 3–5 times. At each stop, count the number of cowpea plants with emerged *Striga* on ten contiguous plants. Express as a percentage (e.g. 6/10= 60%). If there is high variance among the samples, take several more samples.
12. Severity index of *Striga*: multiply the means in 13–15 below. The severity index itself has no intrinsic meaning, but it is used to compare relative damage severity among many fields.
13. Mean number of *Striga* per cowpea plant (count total number of *Striga* in sample and divide by ten).
14. Mean "volume" of above-ground *Striga* biomass per cowpea plant (rate on a 0-3 scale where 0= no emerged *Striga*; 1 = between 1 and 5 stands/clump; 2 = above 5 stands/clump; 3 = above 10 stands/clump. Add ratings for the 10 plants, and then divide by 10). Make 3-5 such mean ratings per field.
15. Mean damage to cowpea plant. Rate on a 1–9 scale (no damage to maximum possible damage), add ratings for the 10 plants, and divide by 10.
16. Note any other parasitic weeds or diseases on cowpea, on the same samples.

An extensive survey of *Striga gesnerioides* distribution on cowpea illustrates this process. The objective of the survey was to define the geographical extent of the problem, and the types of cropping systems in which it occurs. Items are recorded using numerical scales, so that they can be computerized later.

12.3.1.1 Analyzing the data

The data should be numerically coded and analyzed, using correlation and regression analyses, with *Striga* incidence and severity as dependent variables and the others as independent variables. The resultant correlations will give indications of possible causes of infestations, which can then be used as hypotheses to be tested in intensive surveys or agronomic trials.

It should be noted, however, that correlations are considered as "circumstantial" evidence rather than solid proof of the cause of a problem. For example, if low *Striga* incidence was found to be correlated with the occurrence of certain cowpea morphotypes, but not with the other variables, one might suspect that varietal resistance was at work. To confirm this, seed could be collected and screened under controlled conditions.

12.3.2 Intensive surveys

Based on results of the extensive survey, the most interesting areas within the region will have been identified. Villages within these areas must then be characterized, and a few chosen for the intensive survey. Group interviews in villages during the dry season (section 12.3.2.1) will help decide which few villages to focus on. Field visits with farmers from the chosen villages during the wet season (section 12.3.2.2) will then give the detailed data needed.

12.3.2.1 Village characterization

Group interviews with the village head and some farmers from different ethnic or social subgroups are useful for a general characterization. Such interviews normally take 1 to 3 h, and they can be done during the dry season. The following questions are necessary. Answers should be expressed in numerical codes, so they can be computerized.

1. State/province
2. Administrative area (local government area)
3. Village within the local gov't area (use a numbering system)
4. Longitude
5. Latitude
6. Road condition to next market (good, fair, poor)
7. Distance to next market (km)
8. Most important food crops
9. Most important cash crops
10. Percent of fields fallowed each year (ask, how many out of 10 lie fallow?)
11. Percent of fields using fertilizer on maize
12. Which crop is increasing in importance over the last 10 years?
13. Which crop is most attacked by *Striga*?
14. Is *Striga* increasing, static, or decreasing?

12.3.2.2 Field characterization

Within the selected villages, individual fields must be characterized because it is not easy to find a "typical field". Field visits with the farmer are necessary for intensive field characterization. The visits should be made when *Striga* is at its visible peak, around early grain-filling stage for maize. Questions should include

1. Field number within village
2. Field is close (0–200 m), moderate (200–1000 m), or far (> 1 km) from house
3. Soil texture: use finger test. Roll soil between fingers. Use rating scale: 1= sandy (grit between fingers); 2= sandy loam (grit between fingers, wet soil makes fingers dirty); 3= loam (very fine grit between fingers, wet soil is plastic but not sticky); 4= loamy clay (no grit, wet soil plastic and slightly sticky); 5= clay (wet soil very plastic and sticky, stains fingers, gray color); 6= organic (dark brown to black color).
4. Most important crop over the last 5 years on this field
5. Second most important crop over the last 5 years on this field
6. Most important crop this year
7. Second most important crop this year
8. Are the intercrops planted in the same or different rows as the major crop?
9. Maize planting time relative to rains: with first rains; as soon as rains are well established; long after rains are established.
10. Time of first weeding/ridging: in weeks after planting.
11. Time of second weeding: in weeks after planting.
12. Method used in first weeding: chemical; hoeing soil away from crop row; hoeing soil towards crop row; hand pulling; or other.
13. Fertilizer: none; organic manure; NPK; urea; CAN; organic + chemical; combination of different chemicals.
14. *Striga* incidence on maize: average number of *Striga* per maize plant, mean of 5 random samples of 10 plants each (see no. 11, box in section 12.3.1)
15. *Striga* severity on maize: use 1–9 scale to rate visual damage symptoms; mean of 5 random samples of 10 plants each (see nos. 12–14, box in section 12.3.1)
16. *Striga* incidence on sorghum: as in 14
17. *Striga* severity on sorghum: as in 15
18. *Striga* species: *S. hermonthica*; *S. asiatica*; *S. aspera*; *Buchnera* sp.; other
19. Most important *Striga* control method: hoe weeding; handpulling; fertilizer; rotation; fallow; herbicide; resistant variety; early planting; other.
20. Second most important *Striga* control method: as above.

12.3.2.3 Analyzing the data

The method of analysis should be decided upon before embarking on the survey. The objective in the above survey was to better understand which features of the farming system were associated with *Striga* spread and *Striga* control. Therefore, the analysis should test the relation of variation in farming practices to the incidence and severity of *Striga* attack. Correlation and multiple regression analyses are suited to this objective.

12.3.3 Systems modeling

As the saying goes, "what can't be modelled, isn't understood". In its essence, a model is nothing more than a concise way of describing what is going on. It is a tool that aids in capturing or visualizing the overall system, and how the various parts fit into it. It helps researchers to conceptualize problems and decide on priorities among component technologies.

As an example, a model could help bring together the various life cycle stages of *Striga* and the effects that different control treatments have on those stages and on the final *Striga* population. Variables would be chosen to represent the key stages of the life cycle that might be affected by control measures. Technologies can then be characterized as to which specific parameters of the model they affect, and by how much. The stages included in the model can be simple and few, or many and complex, depending on the needs of the researcher.

For example, a simple mathematical model of the *Striga* life cycle can be written thus:

(1) $P_{n+1} = P_n \cdot f \cdot s \cdot g \cdot r$

where:
P_{n+1} = number of mature *Striga* plants in season n+1
P_n = number of mature *Striga* plants in season n
f = average fecundity, i.e., no. of seeds produced per plant in season n
s = fraction of seed that survive the dry season between n and n+1
g = fraction of seed that germinate in season n+1
r =fraction of seed that reach reproductive maturity in season n+1

Equation (1) can be rearranged as

(2) $(P_{n+1}/P_n) = f \cdot s \cdot g \cdot r$

this defines the number of *Striga* plants next year in proportion to the number this year.

Farmers want the number of *Striga* plants next year to be fewer than this year, or mathematically:

$P_{n+1} < P_n$ rearranged as $(P_{n+1}/P_n) < 1$.

Since $(P_{n+1}/P_n) = (f \cdot s \cdot g \cdot r)$, it is evident that if the number of plants is to decline, the product $(f \cdot s \cdot g \cdot r)$ must be less than 1.

(3) $f \cdot s \cdot g \cdot r < 1$ (requirement for *Striga* population to decline each year)

These are simple, concise expressions of what researchers intuitively want to know: exactly how much will reductions in fecundity, dry-season survival, germination, and survival to reproductive maturity affect the *Striga* population. By putting these questions into quantitative terms, the answers will be more precise, and the questions themselves will have been brought into better focus.

Researchers are accumulating data which will eventually give values for these parameters, as affected by different *Striga* control treatments and environments. Once these numbers are available, they can be used to quantitatively compare the effects of different treatments for their potential to control *Striga*. For example, if

$f = 15{,}000$, $s = 0.9$, $g = 0.3$, $r = 0.01$

then $f \cdot s \cdot g \cdot r = 15{,}000 \cdot 0.9 \cdot 0.3 \cdot 0.01 = 40$
so $(P_{n+1}/P_n) = 40$
so the *Striga* population will increase by 40 times in one season.

On the other hand, if "r" is cut by 90% through some control measure
$f = 15{,}000$ $s = 0.9$ $g = 0.3$ and $r = 0.001$
then $(P_{n+1}/P_n) = 4$

The *Striga* population would still increase by fourfold—a much lower rate of increase than 40, but still too high for sustainable cropping. Although reducing the parameter "r" contributes to reducing *Striga*, it appears that (if these values are realistic) fecundity is so high that reductions in other parameters are also needed if population growth is to be stopped.

Research is needed to know the range of values that exist for fecundity, dry-season survival, germination, and growth to reproductive maturity in different natural environments and as affected by *Striga* control treatments. Literature reports about fecundity, for example, vary from 10,000 to 100,000 seeds produced per *Striga* plant. This high variability indicates that fecundity is relatively sensitive to environmental influences. More knowledge about these factors is needed so that they can be utilized in controlling *Striga*.

Seed in the soil undergoes many changes and developmental stages but little quantitative information is available on these. We don't know much about the importance of seed mortality, nor about what percentage of the germinated seed survives to become parasitic, or what percentage achieves reproductive maturity. We need to know how much *Striga* seed set is reduced by varietal resistance, and by other biotic factors such as insects and diseases (Bashir, 1987; Greathead and Milner, 1971; Zummo, 1977). How much does intercropping affect the seed bank in the soil? Does it also have an effect on fecundity? What effects do different types of tillage have on seed mortality? How much do trap crops affect the soil seed bank? How do different rotations compare quantitatively in depleting the seed bank?

The complexity of factors influencing each parameter implies that it will be a long time before the model can accurately predict the *Striga* population of any particular field. As just one example, it is likely that s and g are different for seed of different ages, and we know that *Striga* seed can survive and remain dormant for many years. So these parameters themselves could be represented by complex models. As greater understanding of seed dormancy and survival processes is obtained, models will be refined.

Although the predictive value of a model is limited unless it can take into account complex processes and unless high quality data are available, prediction is not the most important objective in modeling. Models help scientists gain a system perspective. They stimulate us to pose relevant

and precise questions, and to express the answers in quantitative terms. In cases where we find that our understanding of the system is insufficient to be able to do this, the model would still have served its purpose by bringing into focus gaps in knowledge that can be targeted in future research.

Selected references

Agrios, G. N. 1988. Plant Pathology. Third Edition. New York: Academic Press. 703 pp.

Alabi, M. O., D. K. Berner, and G. O. Olaniyan. 1994. Characterization of soybean cultivars for *Striga hermonthica* control. Phytopathology 84:1151.

Allen, D. J. 1983. Pages 268-269. *In* The Pathology of Tropical Food Legumes. New York: John Wiley and Sons.

Bashir, M. O. 1987. The potential for biocontrol of witchweeds. Pages 183-206. *In* Musselman, L. J. (ed.), Parasitic Weeds in Agriculture. Volume 1. *Striga*. Boca Raton, Florida: CRC Press, Inc.

Bebawi, F. F., Eplee, R. E., Harris, E., and Norris, R. S. 1984. Longevity of witchweed (*Striga asiatica*) seed. Weed Science 32: 494-497.

Berner, D. K., Ikie, F. O., and Aigbokhan, E. I. 1996. Methods for soil infestation with *Striga hermonthica* seeds. Agronomy Journal 88:33-37.

Berner, D. K., Awad, A. E., and Aigbokhan, E. I. 1994. Potential of imazaquin seed treatment for the control of *Striga gesnerioides* and *Alectra vogelii* in cowpea (*Vigna unguiculata*). Plant Disease 78:18-23.

Berner, D. K., Cardwell, K. F., Faturoti, B. O., Ikie, F. O., and Williams, O. A.1994. Relative roles of wind, crop seeds, and cattle in the dispersal of *Striga* species. Plant Disease 78:402-406.

Berner, D. K. and Kling, J. G. 1995. Sustainable control of *Striga* spp. through a focussed integrated pest management program. West and Central Africa Regional Maize and Cassava Workshop, 28 May - 2 June, 1995, Cotonou, Benin.

Berner, D. K., Kling, J. G., and Singh, B. B. 1995. *Striga* research and control - a perspective from Africa. Plant Disease 79:652-660.

Berner, D. K., M. O. Alabi, U. Di-Umba, and F. O. Ikie. 1996. Proposed integrated control program for *Striga hermonthica* in Africa. Pages 817-825. *In* Moreno, M. T., Cubero, J. I., Berner, D., Joel, D., Musselman, L. J., and Parker, C. (eds.), Advances in parasitic plant research, Proc. 6th Parasitic Weeds Symposium, April 16-18, 1996, Junta de Andalucia, Direccion General de Investigacion Agraria, Cordoba, Spain.

Berner, D. K., Carsky, R. J., Dashiell, K. E., Kling, J. G., and Manyong, V. M. 1996. A land management based approach to integrated *Striga hermonthica* control in sub-Saharan Africa. Outlook on Agriculture 25:157-164.

Brownie, C., Bowman, D. T.. and Burton, J. W. 1993. Estimating spatial variability in analysis of data from yield trials: a comparison of methods. Agronomy Journal 85:1244-1253.

Cardwell, K. F., Smith, J., Weber, G., and Awad, A. 1991. Framework for orientation of *Striga* research at IITA. Pages 503-507. *In* Ransom, J. K., Musselman, L. J., Worsham, A. D., and Parker, C. (eds.) Proceedings of the 5th International Symposium of Parasitic Weeds. Nairobi: CIMMYT

Carsky, R. J., Singh, L., and Ndikawa, R. 1994. Effect of herbicide and handweeding on current and subsequent season *Striga hermonthica* density on sorghum. International Journal of Pest Management 40:111-116.

Carsky, R. J. and Berner, D. K. 1995. Benefits of crop rotation with soybean and cowpea in savanna cereal based systems. SAFGRAD regional workshop on technology options and transfer systems for food and grain production in sub-Saharan Africa. 26-28 April, 1995, Abidjan, Cote d'Ivoire

Carsky, R.J., Ndikawa, R., Kenga, R., Singh, L., Fobasso, M. and Kamuanga, M. 1996. Effect of sorghum variety on *Striga hermonthica* parasitism and reproduction. Plant Varieties and Seeds. 9: 111-118.

Chang, M., Netzly, D. H., Butler, L. G., and Lynn, D. G. 1986. Chemical regulation of distance: characterization of the first natural host germination stimulant for *Striga asiatica*. American Chemical Society 108:7858-7860.

Cook, C. E., Whichard, L. P., Turner, B., Wall, M. E., and Egley, G. H. 1966. Germination of witchweed (*Striga lutea* Lour.): isolation of a potent stimulant. Science 154:1189.

Couper, D.C. 1991. Use of graded contour banks for soil conservation. IITA Research Guide 6. IITA, Ibadan, Nigeria.

Dejongh, K., P. Van Damme, and D. K. Berner. 1993. Suicidal germination of *Striga* seed using several trap crop varieties. Med. Fac. Landbouww. Univ. Gent 58/3a:983-993.

Emechebe, A. M., Singh, B. B., Leleji, O. I., Atokple, I. D. K., and Adu, J. K. 1991. Cowpea-*Striga* problems and research in Nigeria. Pages 18-28. *In* S. K. Kim, (ed.), Combating *Striga* in Africa. Proceedings, International Workshop organized by IITA, ICRISAT, and IDRC, 22-24 August 1988. IITA, Ibadan, Nigeria.

Eplee, R. E. 1975. Ethylene: a witchweed germination stimulant. Weed Science 23: 433-436.

Eplee, R.E. and Norris, R.S. 1987. Field research techniques. Pages 271-280. *In* Musselman, L. J. (ed.), Parasitic Weeds in Agriculture. Volume 1. Striga. CRC Press, Inc., Boca Raton, Florida.

Greathead, D. J. and Milner, J. E. D. 1971. A survey of *Striga* spp. (Scrophulariaceae) and their insect natural enemies in East Africa with a discussion on the possibilities of biological control. Tropical Agriculture 48:111-124.

Hale, M. G. and Orcutt, D. M. 1987. The Physiology of Plants Under Stress. New York: John Wiley & Sons.

Hartman, G. L. and Tanimonure, O. A. 1991. Seed populations of *Striga* species in Nigeria. Plant Disease 75:494-496.

Hess, D. E. and Ejeta, G. 1992. Inheritance of resistance to *Striga* in sorghum genotype SRN39. Plant Breeding 109:233-241.

Hess, D. E., Ejeta, G., and Butler, L. G. 1992. Selecting sorghum genotypes expressing a quantitative biosynthetic trait that confers resistance to *Striga*. Phytochemistry 31:493-497.

Hess, D. E., Obilana, A. B., and Grard, P. 1996. *Striga* research at ICRISAT. Pages 828-834. *In* Moreno, M. T., Cubero, J. I., Berner, D., Joel, D., Musselman, L. J., and Parker, C. (eds.), Advances in parasitic plant research, Proc. 6th Parasitic Weeds Symposium, April 16-18, 1996, Junta de Andalucia, Direccion General de Investigacion Agraria, Cordoba, Spain.

Horton, D. 1990. Tips for planning formal farm surveys in developing countries. IITA Research Guide 31. International Institute of Tropical Agriculture, Ibadan, Nigeria.

Hsiao, A. I., Worsham, A. D., and Moreland, D. E. 1981. Regulation of witchweed (*Striga asiatica*) conditioning and germination by dl-strigol. Weed Science 29:101-104.

IRRI, 1988. Standard evaluation system for rice. Third Edition. The International Rice Testing Program, International Rice Research Institute, Los Banos, Phillippines. 54 pp.

Kim, S. K., Khadr, F., Parkinson, V., Fajemisin, J., and Efron, Y. 1985. Maize breeding for *Striga* resistance in Africa. Pages *In* Proceedings of OAU/FAO Workshop on *Striga*, 23-27 September, 1985, Yaounde, Cameroon. FAO, Rome.

Kim, S. K. (ed). 1991. Combating *Striga* in Africa. Proceedings, International Workshop organized by IITA, ICRISAT, and IDRC, 22-24 August 1988. IITA, Ibadan, Nigeria. 151 pp.

King, S. B. and Zummo, N. 1977. Physiologic specialization in *Striga hermonthica* in West Africa. Plant Disease Reporter 61:770-773.

Lane, J.A. and J.A. Bailey. 1991. Resistance of cowpea (*Vigna ungiculata*) to *Striga gesnerioides*. Pages 344-350. *In* K. Wegmann and L. J. Musselman (eds.) Progress in *Orobanche* research. Proceedings of the International Workshop on *Orobanche* Research, Obermarchtal 1989. Tubingen: Erbhard-Karls Unversitat.

Levitt, J. 1980. Response of Plants to Environmental Stresses. Vol. I: Chilling, freezing and high temperature stresses. New York: Academic Press.

M'boob, S. S. 1986. A regional programme for West and Central Africa. Pages 190-194. *In* Proceedings of the FAO/OAU All-African Consultation on *Striga* control. 20-24 October, 1986, Maroua, Cameroon.

Musselman, L. J. (ed). 1987. Taxonomy of witchweeds. Pages 3-12. *In* Parasitic weeds in agriculture volume I: *Striga*. CRC Press, Inc., Boca Raton, Florida, U.S.A. 317 pp.

Newman, E.I. 1966. A method of estimating the total root length in a sample. Journal of Applied Ecology 3:139-145.

Okonkwo, S. N. C. 1987. Developmental studies on witchweeds. Pages 63-76. *In* Musselman, L. J. (ed.) Parasitic Weeds in Agriculture, Volume 1: *Striga*. CRC Press, Boca Raton, Florida, U.S.A.

Papadakis, J. S. 1937. Méthode statistique pour des expériences sur champ. Bull. Inst. Amelior. Plant. Salonique 23.

Parker, C., Hitchcock, A.M. and Ramaiah, K.V. 1977. The germination of *Striga* species by crop root exudates: techniques for selecting resistant cultivars. *In* Proceedings Sixth Asian-Pacific Weed Science Society Conference, July 11-17. Jakarta, Indonesia.

Parkinson, V., Kim, S. K., Efron, Y., Bello, L., and Dashiel, K. 1988. Potential of trap crops as a cultural measure of *Striga* control in Africa. FAO Bulletin No. 96.

Pieterse, A. H. and Pesch, C. J. 1983. The witchweeds (*Striga* spp.) - a review. Abstracts on Tropical Agriculture 9:9-34.

Ramaiah, K. V., Parker, C., Vasudeva Rao, M. J., and Musselman, L. J. 1983. *Striga* identification and control handbook. Information Bulletin No. 15. Patancheru, A. P., India: International Crops Research Institute for the Semi-Arid Tropics.

Ransom, J.K., Eplee, R.E., Langston, M.A. and Norris, R.E. 1990. Methodology for establishing witchweed (*Striga asiatica*) in research plots. Weed Technology 4:581-584.

Reda, F., Butler, L. G., Ejeta, G., and Ransom, J. K. 1994. Screening of maize genotypes for low *Striga asiatica* stimulant production using the 'agar gel technique'. African Crop Science Journal 2:173-177.

Riches, C. 1988. *Alectra vogelii* - a parasite of legumes. Botswana Agricultural Research 2:1-2.

Robinson, E. L. and Dowler, C. C. 1966. Investigation of trap and catch crops to eradicate witchweed (*Striga asiatica*). Weeds 14:275-276.

Robinson, R. A. 1969. Disease resistance terminology. Review of Applied Mycology 48: 593-606.

Sand, P. F., Eplee, R. E., and Westbrooks, R. G. (eds). 1990. Witchweed research and control in the United States. Monograph series of the Weed Science Society of America, Number 5, Weed Science Society of America, 309 W. Clark St., Champaign, IL 61820, U.S.A. 154 pp.

Saunders, S. 1952. Studies in the physiology of phanerogamic parasitism with special reference to *Striga lutea*. Science Bulletin Dept. Agric., S. Africa, No. 128.

Scharf, P. C. and Alley, M. M. 1993. Accounting for spatial variability in field experiments increases statistical power. Agronomy Journal 85:1254-1256.

Singh, B. B., and Emechebe, A. M. 1990. Inheritance of *Striga* resistance in cowpea genotype B301. Crop Science 30:879-881.

Vallance, K. 1950. Studies on the germination of seeds of *Striga hermonthica*. I. The influence of moisture treatment, stimulant dilution and after-ripening on germination. Annals of Botany 14: 347.

Van der Plank, J. E. 1975. Principles of plant infection. Academic Press, New York. 210 pp.

Van Mele, P., Van Damme, P., and Berner, D. K. 1992. A new technique to test germination response of *Striga* seed using host plant roots. Med. Fac. Landbouww, Univ. Gent 57/3b:993-999.

Vasudeva Rao, M. J. 1985. Techniques for screening sorghums for resistance to *Striga*. Information Bulletin No. 20. Patancheru, A. P. 502324, India: International Crops Research Institute for the Semi-Arid Tropics.

Weber, G., K. Elemo, S. T. O. Lagoke, A. Awad, & S. Oikeh. 1995. Population dynamics and determinants of *Striga hermonthica* on maize and sorghum in savanna farming systems. Crop Protection 14: 283-290.

Weber, G., K. Elemo, & S. T. O. Lagoke. 1995. Weed communities in intensified cereal-based cropping systems of the northern Guinea savanna. Weed Research 35: 167-178.

Wild, H. 1948. A suggestion for the control of tobacco witchweed (*Striga gesnerioides* (Willd.) Vatke) by leguminous trap crops. Rhodesia Agriculture Journal 45:208.

Wilson-Jones, K. 1955. Further experiments on witchweed control. II. The existence of physiological strains of *Striga hermonthica*. Empire Journal of Experimental Agriculture 23:206-213.

Worsham, A. D. 1987. Germination of witchweed seeds. Pages 45-62. *In* Musselman, L. J. (ed.) Parasitic Weeds in Agriculture. Vol. I. *Striga*. CRC Press, Inc., Boca Raton, Florida, U.S.A.

Zummo, N. 1977. Diseases of giant witchweed, *Striga hermonthica*, in West Africa. Plant Disease Reporter 61:428.

Glossary

after-ripening: generally, the changes that go on within the seed during the breaking of dormancy. For *Striga* there must be a warm, dry period of dormancy after seed shed before it becomes competent to germinate, but the nature of the internal changes is unknown.

allogamous reproduction: production of progeny as a result of cross fertilization. *Striga hermonthica* is known to be allogamous.

allogamy: fertilization resulting from the union of male and female gametes from different individuals, the opposite of autogamy.

appressorium: a swelling on the radicle tip of some *Striga spp.*
at the point of penetration of a host root, from which the haustorium will originate.

autogamous reproduction: production of progeny resulting from self fertilization. *Striga gesnerioides* is known to be autogamous.

autogamy: fertilization resulting from the union of male and female gametes from the same individual.

avoidance: a plant pest control strategy which strives to "avoid" damage by various agronomic practices, such as planting where the pest does not exist, planting at a time of year when the pest is not present, and/or before the pest is infective.

capsule: botanically, a dry fruit that develops from a compound pistil and opens in various ways, allowing seed dispersal.

catch crop: crop plants that have the ability to germinate *Striga* seed, and can also be parasitized.

chemotropism: movement of the *Striga* radicle in response to chemical exudates from roots, as distinct from gravitational response.

coefficient of variation (cv): a quantity that indicates how precise an experiment is. It is computed by dividing the sample standard deviation by sample grand mean and multiplying by 100.

compatibility: in terms of host/parasite interface, a compatible reaction is one in which the parasite is allowed to establish on the host.

conditioning: botanically, gradual change in state of a plant or plant part in response to environmental conditions. The response may not be inductive in that if the environmental conditions are removed, development of the plant (or plant part) response may stop. In the case of *Striga* species, conditioning of the seed requires moisture and a chemical germination stimulant before it is able to germinate.

contamination: generally, to render unfit for a specified use by introduction of an unwanted agent, e.g. to contaminate an experimental control plot, or a previously uninfested field with *Striga* seed.

control: in experimental design, it is a determined or known check or standard by which the treatments are compared; in pest management, it is to prevent or minimize the danger of establishment or the damage due to attack by insects, pathogens, or weeds.

covariance: a statistical measure of the interrelationship of two variables, how they vary together.

covariate : an additional variable related to the primary variable that is being measured in an experiment; e.g., when quantifying the number of *Striga* seed in a unit of soil after seed set in an agronomic trial that lasted one season, the covariate would be the number of seed in the unit of soil before the trial began.

dormancy: botanically, in various types of organs, a period of inactivity (rest) often induced in response to climatic and/or light conditions.

durable resistance: resistance to disease, insects, weeds, and/or physical factors that lasts through the life time of the plant, and/or throughout the economic life time of a plant genotype that is in production.

emergence: the appearance of the growing tip of a plant as it pushes up through the soil.

endemic: occurring locally every year.

escape: a plant that has not become infested with an insect, weed or pathogen due to chance alone, even though the pest is prevalent in the field or experimental plot.

eradication: a plant pest control strategy which seeks to completely remove the pest from a target area. Eradication is a viable control solution as long as there is low probability of re-introduction of the pest. To eradicate *Striga* from a field or region, practices must be used to eliminate all witchweed seed production and to destroy the seed bank in the soil.

exclusion: a pest control strategy which seeks to prevent a weed, pathogen, or insect from entering a given area (see quarantine).

fecundity: the reproductive capacity of an organism measured as number of offspring per individual, or per female.

geotropism: growth in response to gravity, e.g., roots are generally positively geotropic since they grow toward the direction of gravitational stimulus (downward).

gall: an abnormal swelling or growth on or in plant tissues caused by an external factor often *Smicronyx*.

genotype: the genetic makeup (allelic constitution) of an individual, with respect to specific genes

or to the whole genome. Breeding lines which are very closely related or highly inbred are often referred to as genotypes.

germinability: ability of a seed to germinate.

germination: the initial growth and differentiation from embryo to radicle which elongates from the seed.

germination stimulant: generally, natural or synthetic chemical which induces germination of seed. For *Striga*, natural germination stimulants are root exudates, plant hormones such as cytokinins, products of decomposition such as ethylene gas; while synthetic germination stimulants have been developed such as strigol-analogues (GR-24), etc.

germplasm: (1) the part of the cell which holds the genetic materials that are the physical basis of heredity; (2) the sum total of the hereditary materials in a species.

gravimetric: pertaining to measurement by weight.

haustorium: botanically, a specialized outgrowth from the root or stem of a parasitic vascular plant, which penetrates living host tissue and absorbs food or other materials. The *Striga* haustorium forms directly from the radicle, never forming a primary root.

hemi-parasite: parasites which are chlorophyllous, but still dependent for at least part of their life cycle on a host.

holo-parasite: nonchlorophyllous parasites which remain totally dependent on a host throughout their life cycle.

host: an organism that supports the establishment and development of a parasite, by contributing nutrients or water from its own tissues.

immune: completely resistant or non-host interaction of a plant with a potential parasite (see incompatibility).

inbred: the progeny of the cross of two closely related lines.

incompatibility: in terms of host/parasite interface, an incompatible reaction is one in which the parasite fails to establish development on the host due to specific exclusion by the host, or due to failure to meet basic physical or nutritional requirements.

indicator plant: a suitably susceptible plant used to indicate the presence, absense, or relative quantity of a target pest, pathogen, weed, or physical condition.

inoculum: spores, bacteria, insect eggs, *Striga* seed; any part of a pathogen, pest or parasitic weed that can establish infection.

in vitro: generally, in glass; hence in a test tube, beaker, etc.

in vivo: generally, in the body of a living organism.

mortality: the proportion of deaths to population, i.e., death rate. Important to *Striga* population dynamics control strategy is the pre-reproductive mortality.

nonhost: an organism that does not support the establishment of a parasite or pathogen (see incompatibility).

outcross: a genetic cross between two plants of different geno-type. An obligate outcrosser would be a plant that has some physical or biochemical barrier to self-fertilization.

parasite: an organism living in or on a host organism, generally to the detriment of the host. An obligate parasite must have a host to survive.

preconditioning: see conditioning.

quarantine: a plant pest control strategy which seeks to exclude a parasite, pathogen, or insect from an area where it does not already exist by regulatory restriction and prohibition.

radicle: botanically, the basal end of the embryonic axis, which grows into a primary root. In the case of *Striga*, the radicle tip develops into haustorial initial.

root exudate: chemical substances produced by the roots of host or nonhost plants. They are metabolic products of the plant root which certainly affect the living organisms in the rhizosphere, e.g., the stimulation of germination of seeds of parasitic weeds.

resistance: generally, the physiologic, structural, and morphological conditions of a plant that allow it to tolerate, avoid, exclude, be immune to, or recover from, an adverse effect of a parasite, pathogen, pest, weather, or soil problem. By strict definition, parasite resistance is measured by the suppression or retardation of the development of the parasite.

slurry: a thin watery mixture or suspension.

sick plot: an experimental plot that has been systematically infested with inoculum from a parasite, pathogen, or insect pest. Ideally for experimental purposes, there should be uniformity of infestation, and consistent temporal appearance of the parasite, pathogen, or pest.

spatial variability: heterogeneity of distribution of a parasite, pathogen or insect pest in a field or farmer's plot, ranging from normal to patchy.

specific gravity: the ratio of the weight of any volumne of a substance to the weight of an equal volume of some substance taken as a standard unit, usually H_20 for liquids and oxygen or hydrogen for gases.

strigol analog: see germination stimulant.

suicidal germination: in the case of *Striga*, because it is an obligate parasite, if it does not

successfully attach to a host, the seedling will die. Trap crops cause suicidal germination.

survivorship: in terms of population dynamics, the proportion of individuals (seeds) that survive from seed shed to germination.

susceptible: lacking an inherent ability to resist or tolerate disease infection, pest or weed infestation, or physiological damage due to abiotic factors.

tolerant: the ability of a plant to withstand adverse biotic or environmental factors without excessive loss of yield.

trap crop: those crop plants which stimulate the germination of *Striga* seed without being parasitized (non-host, false host).

volumetric: of or pertaining to the measurement of volume.

Training in *Striga* research

Group training

IITA places highest priority on providing training opportunities through group courses, individualized attachments, and graduate research fellowships.

Institute-based training courses

Short (group) courses and workshops are regularly conducted by IITA scientists. Courses and workshops focus on research-related issues reflecting IITA's research mandate and collaboration with national programs.

The IITA *Striga* research group (now incorporated into the project 'Integrated Management of *Striga* and Other Parasitic Plants') has conducted and been involved in various group training courses for research personnel from many African countries. Participants have been from Benin, Botswana, Burkina Faso, Cameroon, Central African Republic, Chad, Cotê d'Ivoire, Ethiopia, Ghana, Guinea, Kenya, Madagascar, Mali, Morocco, Niger, Nigeria, Rwanda, Senegal, Sudan, Tanzania, Togo, Uganda, and Zimbabwe. Some of these courses have been conducted in conjunction with other international agricultural research centers, ICRISAT and CIMMYT. Originally the courses were conducted on the campus of IITA in Ibadan, Nigeria, and were for both anglophone and francophone personnel. Now, IITA and ICRISAT rotate institute-based group training courses in *Striga* research between the two institutes. IITA conducts the anglophone courses with participation of ICRISAT scientists, and ICRISAT conducts the francophone courses with participation of IITA scientists. Courses typically run for 2 weeks and cover the range of topics presented in this manual. These courses are sponsored by the respective institutes.

In-country training courses

In-country group training courses are particularly effective for *Striga* research personnel. IITA has conducted several in-country courses with the goals of training national personnel in research techniques and of establishing basic laboratory facilities in which to conduct *Striga* research. Instead of participants from many countries travelling to IITA for a training course, one to several IITA scientists travel to the country requesting the course. Basic laboratory equipment and supplies are carried to the country by the IITA scientists. Participants for the course are selected by the national agricultural research and extension system. These courses typically run for 5–7 days and cover predominately laboratory oriented topics in this manual. Because of the additional costs of laboratory equipment and supplies, these courses usually require supplemental funding from an external agency before the course can take place.

Individualized training attachments

Individualized training attachments may be developed upon request, depending on the availability of resources. The length of these attachments is variable depending on the extent and intensity of training. Often personal contacts between IITA scientists and national program scientists are the impetus for individualized training of laboratory and field personnel. Numerous national program personnel have had individualized training at the *Striga* laboratory in IITA. These training programs are very beneficial and participants are always welcome. However, with limited funds for such individualized training, national programs may have to finance some individual expenses.

Graduate research fellowships

As part of IITA's training activities in support of national agricultural research systems (NARS), it operates a Graduate Research Fellowship Program (GRFP) which offers a limited number of Graduate Research Fellowships every year to Africans pursuing post-graduate studies at an accredited university.

Fellowships provide support for selected African scholars to conduct the research component of either an MSc or PhD program at a research site in sub-Saharan Africa. To be eligible, applicants must have already completed prerequisite academic work. The IITA Fellowship does not fund academic work.

Application forms for any IITA training activities are available from the Training Program office and must be completed and returned with the recommendation and required supporting documents. IITA does not accept self-nominations for any training, but nominations from employers and sponsoring organizations only.