F I N A L R E P O R T

PROJECT NO. AID/nesa 440 - AGRICULTURAL RESEARCH

AGENCY FOR INTERNATIONAL DEVELOPMENT

WASHINGTON STATE UNIVERSITY

WEST PAKISTAN AGRICULTURAL UNIVERSITY

August 31, 1972

BEST AVAILABLE COPY

TABLE OF CONTENTS

Page

1. INTRODUCTION by Dr. V. N. Bhatia . . . . . .(ii-v)

2. FINAL REPORT by Dr. A. O. Shaw, Resident Advisor 1

3. APPENDICES TO RESIDENT RESEARCH ADVISOR'S REPORT

A. Appendix I WPAU Staff Members Holding Ph.D. Degrees 31

B. Appendix II Map of the Master Plan of the West Pakistan Agricultural University 37

C. Appendix III PL-480 Supported Projects during the Last Two Year Period 38

D. Appendix IV Other Research Grants 43

E. Appendix V Post Graduate Studies at West Pakistan Agricultural University 44

4. CONSULTANTS' REPORTS

A. Proposed Research Program on Gram Wilt and Gram Blight by Dr. C. Gardner Shaw 68

B. Research Report on Gram Wilt and Gram Blight, 1972 by Dr. C. Gardner Shaw 117

C. Proposed Research on Arthropod-Transmitted Viruses of Mung and Related Edible Legumes by Dr. Robert F. Harwood 131

D. Research Report on Arthropod-Transmitted Viruses of Mung and Related Edible Legumes by Dr. Robert F. Harwood 153

E. Programmed Research Report on Gram Wilt and Gram Blight, and a New Foliage Disease on Gram in West Pakistan, 1971 by Dr. Vernon E. Wilson 165

F. Root Rot of Food and Fiber Crops, a Survey in West Pakistan, 1971 by Dr. Stuart D. Lyda 172

INTRODUCTION

In cooperation with AID and its predecessor agencies, Washington State University has been involved in a series of projects of technical assistance in higher education in Pakistan. The effort was begun in 1954; initially, Washington State University's effort involved providing assistance in a variety of fields to the University of the Punjab and affiliated colleges. From 1961 to 1969, the objectives were changed to involve the development of the new institution of West Pakistan Agricultural University (WPAU) at Lyallpur. By August 31, 1969 the majority of the institution building objectives had been completed and this project was phased out. A new three-year contract (July 1, 1969 - August 31, 1972) was entered into between Washington State University and AID (AID/nesa-440). This is the final report of the current contract. The main body of this report consists of the final statement and recommendations prepared by Dr. A. O. Shaw, the Resident Research Advisor at WPAU. Six reports by short-term consultants also are included.

The scope of this contract called for Washington State University to assist and advise the Government of Pakistan through the West Pakistan Agricultural University in developing a sound agricultural research foundation for sustained growth in agricultural production. The operational plan of the contract called for: the achievement of the objectives stated in the scope of the contract by (1) "developing the capacity of the Government of Pakistan and its agencies to administer, coordinate and direct research efforts toward relevant problems; and (2) improving the capacity of the West Pakistan Agriculture University to pursue applied agricultural research activities." It was further stated that in order to achieve the above objective "it was desired to secure services that will

provide technical advice and assistance to the West Pakistan Agricultural University in an interdisciplinary approach to research, focused on problem areas determined to be of high priority by the West Pakistan Agricultural Research Board."

At the start of the project in 1969 the estimated cost was $229,608 (of this amount $55,000 represented the firm budget for the first year and the balance $174,608 represented the projected budget for the two subsequent years). The budget estimates were revised several times and the final revision, effective as of March 10, 1972, made a total commitment of $180,000. The final expenditures for the project will be close to this sum.

As may be noted from the report of Dr. A. O. Shaw, Resident Research Advisor, the work related to the contract has closely followed the operational plan. It can be reasonably concluded that the objectives of the contract have been met to the extent possible in the time period of the grant. Dr. Shaw in his report makes several recommendations; some of these are summarized below:

A. Recommendations of special interest to AID

1. It would be hazardous to abandon WPAU at its present stage without helping it to acquire a more solid base in the area of postgraduate research and teaching.
2. In addition, continued technical assistance could play a significant role by a concerted effort to improve teaching methods. This may be carried out by bringing to WPAU outstanding teachers at the post-graduate level from WSU for a thirteen-week term at a time.
3. WPAU is approaching maturity and needs a different kind of advisory service and participant training. Probably the best results would be obtained from the use of short-term research advisors and re-training

of senior staff members. This would prevent possible regression of the research activities at WPAU.

4. Without an external aid program at the present stage, WPAU scientists could become progressively isolated from their professional colleagues around the world. Assistance directed towards continued updating of knowledge and proficiency is necessary to keep the scientific staff abreast with new developments occurring internationally.
5. In any future projects the Resident Research Advisor should be given a rupee budget to be used at his discretion to help finance a few interdepartmental research projects. The purpose of this would be to promote and demonstrate the importance of interdepartmental research, the importance of proper delegation of authority, to promote the adoption of better sanctioning and purchasing procedures, and to develop administrative control.

B. Recommendations of special interest to WPAU

1. The agricultural faculty and facilities of WPAU should become an integral part of the national research system. It is urgent that WPAU establish strong lines of communication with other Government organizations that need WPAU's research capability.
2. The development of more interdisciplinary research projects should be encouraged. Several interdepartmental projects are currently in progress at WPAU but more should be developed so that each faculty has several of these in progress.
3. The research facilities of WPAU need to be better financed. There have been a few projects funded from non-university

sources that have yielded excellent results, however a much greater flow of such funding needs to be generated. It would be a waste of national resources if the pool of scientific talent at WPAU is not exploited to develop greater research productivity.

4. M.S. and Ph.D. degree programs must be strengthened. This may be achieved by cooperative degree programs with developed institutions and international research institutes.

V. N. Bhatia
Campus Coordinator
Pakistan Project

FINAL REPORT

PROJECT NO. AID/nesa 440 - AGRICULTURAL RESEARCH

US AID-WSU/WPAU

By

Professor A. O. Shaw, S.K.*,
Resident Research Advisor
Washington State University/
West Pakistan Agricultural University
Lyallpur, Pakistan

I. INTRODUCTION

If an agricultural university is to function above the level of a vocational school, if its graduates are to be recognized by universities in the advanced nations, and if it is to be effective in educating leaders in agriculturural research and development, then it must be actively and seriously engaged in research, both basic and applied. Its research program must be oriented to the geographic area it serves so that it stimulates and requires its scientists to study problems and needs and to seek new knowledge that can be applied in improvements and advances that contribute to agricultural development.

Faculty research serves as the backbone of graduate studies. Without research programs, graduate students will fail to obtain the kind of training that is needed. Thesis research for graduate students must grow out of continuing research programs and must be supervised by competent and experienced research scientists.

Many developing agricultural universities operate on very small budgets. A study of these institutions will usually reveal that their post-graduate training is generally mediocre and sub-standard. It is important to recognize

*S.K.--Abbreviation for the "Star of Service" conferred on Dr. A. O. Shaw by the President of Pakistan.

that agricultural research is expensive, though in return it provides food production which makes it an excellent investment. The highly trained scientists must be paid salaries comparable with those paid in other scientific fields. They must have good facilities including land, laboratories, greenhouses and animals for their researches. They require sophisticated equipment that is costly, and they must have adequate operational support.

The leader of a project should be a scientist with other scientists from related areas participating. An example: research for the development of grain legumes in West Pakistan. This project is broad and could be referred to as a major research area. For the project to be successful many sub-projects on specific phases must be investigated. These sub-projects are often used as thesis research problems by graduate students. An example: races of Ascochyta rabiei causing Gram Blight. These sub-projects are designed to ascertain the validity of a hypothesis, to obtain information on a specific phase of a project, or to explore an idea that may lead to a new discovery.

When selecting a major project research area, care should be exercised to insure that it is relevant to the nation. If the project selected tends to duplicate a Government of Pakistan research project but is essential to the educational program, cooperation should be arranged with the government research institute to minimize duplication.

A new agricultural university must concern itself initially with applied research, to develop background information on such matters as crop and varietal adaptation, the fertility status of soils, economic relations for poultry, etc. Also adaptation of research developments from advanced countries should be investigated (e.g., Mexi-Pak wheat). Results from applied and adaptive research will pave the way for the selection of needed basic research studies.

In a developing university there is a tendency for scientists and departments to confine their researches to narrow areas of specialization. It is not uncommon for young Ph.D.'s at developing universities to select the same subject matter research area they used for their Ph.D. thesis research in the USA even though it is not relevant to the country. However, in recent years many technological developments in agriculture resulted from the efforts of a team of scientists. The success of high-yielding semi-dwarf wheat varieties was due not only to the work of plant breeders, but also the contributions of plant pathologists, soil scientists and agronomists. Developing universities with limited operational budgets must be encouraged to use the interdisciplinary approach.

A developing university must seek cooperation not only with the government of the country but also with established research institutions and with international research centers. Foundations and foreign government assistance agencies have agricultural research programs and may provide opportunities for cooperation and research support. Private industries must be contacted to help support research on industrial problems (e.g., handling wastes from textile mills).

There is no substitute in an agricultural research program for highly trained and sincere scientists. Universities in developing countries usually need a faculty improvement program. Promising young scientists should be sent abroad for training at the doctoral level. Senior scientists are in need of retraining in special research techniques.

An agricultural university must have sufficient financing to maintain a regular and continuing research program as a basis for faculty research and

teaching and for graduate studies. If research is of a high standard it will attract grants for research support from outside sources such as private industry and business, foundations, world agencies and governments.

In Pakistan primary responsibility for agricultural research rests in central or provincial Ministries of Agriculture. University faculties of agriculture are considered primarily as educational institutions (under the Ministry of Education) that train agricultural scientists for the ministries and for research institutes. In the U.S. primary responsibility for research is vested in the state university. State departments of agriculture are primarily concerned with regulatory matters other than research. It is not my purpose to argue for one system or the other. The main point I wish to make is that the university faculties of agriculture must be an integral part of the national research system, they must play a significant role, and they must be adequately financed. University involvement with research is particularly necessary for the training of postgraduate students.

II. MAJOR CONTRACT GOALS

According to the "Work Plan" signed in March 1970 the major activities of the Research Consultant are:

(1) Assist WPAU staff to prepare research proposals to be submitted to Government and aid-granting agencies for possible funding.

(2) Assist WPAU to organize a coordinated research program on pulses and a grain legume cell.

(3) Coordinate the services of short-term specialized research consultants.

(4) Assist in the selection of participants to be trained in the United States in reseach administration, procedures, techniques and evaluation.

(5) Assist in selection and purchasing of equipment, seeds, books and journals in support of the project.

(6) Encourage liaison and coordination with other research institutions and bodies.

(7) Improve communication between WPAU scientists and their counterparts in developed countries.

(8) Organize seminars and strengthen postgraduate research.

(9) Encourage the team approach to problem solving.

(10) Assist WPAU to improve its research organization.

Project Intent

Project 391 (296.2) is intended to help West Pakistan develop a sound agricultural research foundation for sustained growth in agricultural production. Efforts at West Pakistan Agricultural University (WPAU) will be focused toward (1) the improvement of WPAU's capacity to train top level agricultural scientists to help man other research facilities, and (2) to help WPAU organize a meaningful agricultural research program focused on a problem(s) of priority.

Improvement of facilities at the WPAU

Nine years of hard work have gone into the finalization of plans and the construction of an adequate teaching and research facility for WPAU. The new campus will consist of academic buildings for five faculties, a library, an administrative block, an auditorium, a student teacher center, a mosque, a health center and student halls.

Since the establishment of WPAU, the Government of Pakistan has allocated the following amounts of rupees for capital outlay.

Year	Sum Allocated in Rupees
1961-62	4,652,000
1962-63	5,842,000
1963-64	6,882,000
1964-65	8,870,000
1965-66	3,855,000
1966-67	4,054,000
1967-68	6,793,000
1968-69	14,654,000
1969-70	9,144,000
1970-71	7,696,000
1971-72	10,078,000

Imported construction materials, and research, and instructional equipment is financed by an IDA loan of $5,900,000 secured in 1964. Expenditures in dollars up to June 30, 1971, are as follows:

	Instructional Equipment	Construction Material	GOP 10%	Consultants Fee
Allocation:	$2,650,000	2,100,000	1,100,000	50,000
Spent Up to June 30, 1971	1,775,000	692,000	468,000	11,000

Current on-going construction includes water, electricity, gas, and sewage supply and installation of air-conditioning systems. These utilities probably will not be completed until December 1973. Some types of experimentation cannot be conducted until these facilities are available.

Construction Largely Complete

Item	Total Cost in Rupees	Physical Progress
Division of Basic Sciences and Arts	43,000	Completed
Faculty of Agri. Engineering and Technology	140,000	Completed
Faculty of Agriculture		
(a) classroom wing	679,000	Completed
(b) central facilities and laboratories	4,664,000	Completed
(c) Glass Houses	336,000	Completed
Faculty of Animal Husbandry and Faculty of Veterinary Science and Clinical Medicine & Surgery	3,748,000	95%
Physical Education	77,000	Completed
Library	897,000	41.1%
Boundary Wall around Campus	100,000	Completed
Four Student Hostels	5,538,000	95%
Staff Residences	902,000	Completed
Utilities	3,075,000	88%
Electricity	1,157,000	80.7%
Faculty of Agri. Economics and Rural Sociology, Auditorium, Administration Block and Student-Teacher Center	4,765,000	Contract signed on June 22, 1971.
Supply and installation of air-conditioning system		Contract tender put out.

Current Approved Construction Budget

Item	Total Cost in Rupees
Faculty of Agri. Economics and Rural Sociology	900,000
Student-Teacher Center	531,000
Auditorium	1,581,000
Administration Building	85,000
Three Students Hostels	4,128,000
Farm Buildings	1,498,000
Mosque	250,000
Dispensary	100,000
Professional Fees	430,000
Land Development	300,000

This new campus will be unpretentious but adequate and highly functional. When complete the research laboratories will be well equipped. Two basic utilities still remain unsolved, namely the development of an adequate supply of potable water and an adequate and dependable supply of electricity. A master plan of the new university building is shown in Appendix II.

Undoubtedly the most promising research building project would be one administered and financed for the most part by the host country. Since this was not possible during the planning of this project it was agreed that some external assistance as well as technical assistance would be required. It was agreed that Washington State University/US AID would provide technical assistance in the form of a Resident Research Advisor, Short-Term Consultants, some participant training abroad for staff and financial assistance for the import of books and equipment.

Since the establishment of the WPAU, the Government of West Pakistan has allocated the following amounts to the WPAU:

Year	Non-Development Allocation in Rupees
1961-62	1,563,000
1962-63	1,189,000
1963-64	1,336,000
1964-65	1,572,000
1965-66	7,009,000
1966-67	7,448,000
1967-68	7,838,000
1968-69	8,121,000
1969-70	8,510,000
1970-71	9,236,000
1971-72	9,641,000

Although these allocations are substantial, the non-developmental allocation (recurring budget) is still not commensurate with the needs of the university. Funds should be allocated to each project and project accounting should be adopted.

During 1971-72, WPAU's non-development budget is as follows:

Major Category	Rupees
Pay of Staff	5,063,540
Recreation Allowance	154,670
House Rent	180,860
Medical charges	185,610
Ad hoc relief	216,770
Provident Fund/Pension Contribution	384,320

Wages	136,090
Furniture, Equipment and Apparatus	11,830
Books	129,050
Repairs & Maintenance	384,500
Suspense	217,000
Scholarships	70,000
Social and Manual work	8,000
Travel	113,420
Supplies Consumable	443,650
Contingencies	1,946,000
Total	9,641,410

Of this total, fixed costs consume all but Rs.2,489,650. Since WPAU does not have project budgeting it is impossible to estimate the amount of rupees available for research support, but consumable supplies for the operation of the university use up most of the approximately 2.5 million rupees mentioned above.

The research scientists at WPAU are handicapped in two areas, (a) paucity of research funds, and (2) centralization of recruitment, sanctioning power, and purchasing procedures. If the Resident Research Advisor was given a quickly expendable rupee budget to help finance two or three interdepartmental research projects it may be possible to demonstrate the importance of proper delegation of authority, adoption of appropriate sanctioning and purchasing procedures and the need to develop administrative control. It is equally important to understand that some procedural changes are only possible provided they are initiated first at the Center, then at the Provincial Government level and finally at WPAU.

During May 1972 a new University Ordinance was finalized which largely eliminates centralization of recruitment and sanctioning power. Most adminis-

trative positions will be elective and rotated. Hopefully the Chancellor of WPAU (Governor of the Punjab) will approve the ordinance in the near future.

Grants and Contracts:

A. PL-480 Grants

These grants are financed with foreign currency accruing to the credit of the United States primarily from the sale of farm products abroad under Public Law 480. The money cannot be converted into dollars for use in the United States.

In the past two years PL-480 grants totalling Rs.6,070,808 have been secured for WPAU. (See Appendix III for detail).

B. Other Research Grants

If it is assumed that in a reasonable length of time a host country should assume the total burden for research funds, then it becomes urgent for WPAU to establish strong linkages with other government organizations that need WPAU's research capability. Currently WPAU has nine research contracts with government departments. These grants total Rs.391,448. The titles and details of these contracts are tabulated in Appendix IV.

Priorities in Planning:

Pakistan cannot work on all development needs at one time. They must choose priorities because of the constraints on resources and other variables. Undoubtedly first priority in development should go to agriculture so that the people can sustain themselves without resorting to the import of food.

It has been stated that in some developing countries 80 per cent of the agricultural problems are man-made, whereas 20 per cent or so could be attributed to natural factors. In order to sustain the agricultural development program

initiated in Pakistan, a number of rather revolutionary changes may have to take place in the organizational support of agricultural development. Administratively it is necessary to develop close coordination between research, extension and education on the one hand and economic planning and policy-making on the other. To sustain an agricultural revolution will require the use of scarce resources like (1) foreign exchange for import of fertilizers and pesticides, etc., and (2) public funds for agricultural education, extension and research. If these inputs are not adequately financed the chance of success is limited.

Japan has triggered agricultural development and at the same time kept the use of scarce resources at a minimum. They were successful because development was based upon previous investments in agricultural education, research and extension. Pakistan could profit by following this example.

On page three in the section, "Revised Phasing, Sectorial Priorities and Allocations"of the Third Five Year Plan the following statement appears:

> The basic element in the agricultural development program is the adoption of improved technology based upon the use of better seeds, application of fertilizers and the availability of insecticide and water. Unlike industry, the adoption of improved technology in agriculture does not require heavy capital investment. The immediate task in agriculture is to motivate the farmer to use better seeds and fertilizer and to insure that these inputs are available at the right place and the right time. He has also to be assured a fair return for his produce.

High yielding, disease resistant, fertilizer-responsive varieties for many crops are unavailable. Data on cropping sequence, rate and time of planting, fertilizer and water requirements and chemical control of insects, etc., are incomplete or lacking. Improved disease-resistant varieties for pulses and oil seeds are unavailable. Agronomic practices for these crops have not been standardized. Without these inputs an accelerated agricultural development plan is doomed to failure.

Administratively Pakistan needs to organize for close coordination between research, extension and education on the one hand and economic planning and policy-making on the other. Planners must examine alternative means to achieve given objectives consistently and efficiently. Sound agricultural research data are needed to meet these objectives.

Since it takes considerable time to find the solution to the problems that present barriers to increasing the yields of food crops and improving their quality, information materials and scientific expertise of developed countries should be used until Pakistan itself can supply the necessary inputs.

Faculty Research at the WPAU

Appendix V provides a summary of postgraduate students, their names, the titles of their thesis research and degrees sought. This summary shows, along with the information in Appendix I, that WPAU has a large pool of qualified scientists and a large postgraduate research program. Certainly it is common knowledge that numbers of Ph.D.'s and numbers of research projects are not a complete measure of research capability. In addition, the staff and postgraduate students must be energetic, motivated, dedicated, and innovative. The staff of most departments at WPAU are trying to resolve problems important to Pakistan. They are also training postgraduate students to be imaginative in using whatever resources are at hand.

Since a considerable part of the research at WPAU is done by postgraduate students and since their success is linked to quality of teaching at the postgraduate level, improving research capability would involve both teaching and research.

An ideal teacher will stimulate his students to search widely for related ideas and implications of the material under study. This view of the function of a teacher is not universal at the WPAU. A part of the staff would, if permitted, revert to the old classical method of reciting orally to a class a given body of information. Many of the younger staff members would like very much to see greater innovation in teaching methods, but they are still somewhat frustrated in their attempts to do so. Part of the difficulty comes from inability to break away from the classical examination system which deprives them of student cooperation in new approaches. Part of the difficulty derives from the continued dominance of seniority over technical capability in some areas.

The role technical assistance could play is to attack teaching methods head on. Bring out an outstanding teacher at the postgraduate level from WSU each term (13 weeks). Give them advance billing among faculty and students and admit only a manageable number of each to the demonstration course. The Resident Research Advisor would make sure all the materials needed to be effective are at hand (but not at a level of affluence in resources that are beyond the reach of WPAU). A continuing stream of such teachers, one per term, over a period of two years could have an enormous impact on the entire postgraduate teaching program.

It would be hazardous to abandon the university in its present stage without helping it to acquire a more solid base in the important area of postgraduate research and teaching.

In research WPAU is handicapped by the restrictions which prevent it from assuming a national role. Most departments register genuine frustration at not being able to bite into the larger problems of the country, and being forced instead to attack piecemeal portions of what they may view as urgently needed action. There have been a few projects funded from extra-university sources that have yielded excellent results. However, there must be generated a much larger flow of such funding. It is a waste of national resources if this pool of scientific talent is not exploited to develop a greater scientific productivity. Clearly the present frustration will give way to serious disenchantment and defection unless additional support is found.

The role which technical assistance can play in helping to generate research support is real and urgent. We can advise and assist in the development of broad-gauge research projects that can compete successfully for funding by interested agencies. During the last two years considerable effort was expended helping work up proposals that were well-conceived and well-documented. As more funds are generated and more sophisticated projects are undertaken, a number of WPAU's scientists will need some retraining in new techniques. In addition, short-term US advisors will be needed to assist in stimulating the total effort.

In the United States it is easy for a scientist to visit outstanding institutions. Consultation about new techniques and new methods of approach is a continuing process that is taken for granted. In Pakistan this is not possible at the present time. US AID could help prevent regression in research by a modest investment in short-term retraining facilities and short-term advisory facilities. Short-term advising can succeed only if the advisor(s) are on campus at WPAU and are available daily for consultation.

Project Approach to Research

The project is the heart of a research program. It details the specific problem to be investigated, the scope, purpose, and method of investigation, the scientists involved, and the estimated cost. Experience has proven that greatest return per unit cost results from an interdisciplinary (team) approach. Several interdepartmental projects are in progress at the WPAU. Every faculty should be encouraged to have one or more such projects underway.

Since WPAU desires additional research funds, a policy was adopted to secure PL-480 research funds for specific projects. Project preparation and submission for PL-480 grants seems to be an ideal tool to accomplish several project goals. Requests for PL-480 support are handled as follows: An individual from a department or individuals from two or three departments prepare a suggested proposal in a prescribed manner. The proposals are sent to the Committee for Advanced Studies and Research. This committee reviews these requests relative to importance of the research and the soundness of the research design. The committee rejects, approves or suggests revision (improving research organization). The research projects are then submitted to the Chairman of the Central Agricultural Research Council. They approve or reject the proposals. If approved, they may be supported with rupees controlled by ARC or sent to the Agricultural Attache, US Embassy, Pakistan (research coordination). The US Agricultural Attache sends them to the Regional Director of PL-480 Programs, New Delhi. This office sends them to Agricultural Research Services (ARS), Beltsville, Maryland, for review. If any section of ARS wishes to sponsor a project, research design and procedures are reviewed. The project is approved or revisions are suggested (this opens channels of communication with US experts and is also excellent training in research design and techniques). ARS then appoints a sponsoring scientist who reviews progress annually and reviews future work plans.

This activity has been very useful to help improve WPAU's capability to do meaningful research and to write statistically sound projects. It is very important that WPAU scientists develop and adapt new technology to meet future needs in their country. The limited number of specialists in the scientific disciplines at a few international centers cannot be expected to furnish the type and scope of adaptive, protective and innovative research required by developing nations located in large geographical areas.

Grain Legume Team:

The "Work Plan" called for the organization of an interdisciplinary team to solve the problems limiting pulse production in West Pakistan. Increased pulse production is a must because of a shortage of protein, particularly balanced protein, in the diet of the people of West Pakistan.

Interdisciplinary Pulse Research Team

Administrative:		
Dr. Muhammad Aslam	-	Project Coordinator
Dr. Abdullah Khan	-	Project Leader
Dr. A. O. Shaw	-	Research Consultant
Plant Breeding:		
Mr. Aqil Khan	-	Research Officer
Mr. Ehsan-ul-Haq	-	Research Assistant
Mr. Mohammad Aslam	-	Research Assistant
Mr. Mohammad Rafique	-	Research Assistant
Agronomy:		
Dr. Riaz Ahmad Khan	-	Advisor

Soil Science:

Dr. Inam-ul-Haq	-	Advisor
Mr. Atta Ullah	-	Research Advisor

Plant Pathology:

Dr. A. G. Kausar	-	Dean, Faculty of Agriculture
Mr. Inam Ullah Khan	-	Senior Research Officer
Mr. Riaz Ahmad	-	Research Assistant
Mr. Mohammad Ahmad	-	Research Assistant

Entomology:

Dr. M. Younus Chaudhry	-	Advisor
Mr. Mohammad Sarwar	-	Research Officer
Mr. Mushtaq Ahmad	-	Research Assistant

The composition of the interdisciplinary team was patterned after similar successful research teams as far as restrictions on funding would permit. The pulse project is funded by funds supplied by both WPAU and PL 480 funds.

Current Status of Pulse Project:

Chickpeas:

(a) Three thousand one hundred and ten chickpea cultivars have been tested for adaptation.

(b) More than two hundred of the most promising cultivars have been tested in a chickpea Wilt Nursery.

(c) Cooperative chickpea variety trials at Rawalpindi, Campbellpur and Lyallpur with the cooperation of Department of Agriculture are in progress.

(d) Chickpea variety trials and seed multiplication at Kallur Kot and Piplan in cooperation with the Agricultural Development Corporation are in progress.

(e) Chickpea trials at Murree, Swat and Lyallpur, in cooperation with PARI are in progress.

(f) CPC Limited of Philippines were supplied advanced chickpea selections for testing.

(g) AUB of Lebanon was supplied advanced chickpea selections to be tested. Attempts to cross some of these selections with a wild chickpea (Cicer lebanonoticum) have been unsuccessful.

(h) Washington State University has multiplied I13 and Bulgarian. These varieties have been crossed with C727 and 12-100-01528. A wild chickpea from Pakistan has been successfully grown. Crosses with C727 have succeeded.

(i) F1 to F4 material being tested.

(j) Promising F2 material and other selections are being screened for Gram Blight and Gram Wilt.

(k) Chickpea cultivars are being tested for resistance to bruchids under storage conditions.

(l) Promising chickpea cultivars are being analyzed for protein and amino acid content.

(m) Three agronomic, irrigation, fertility trials are in progress.

(n) I-13 (Blight tolerant) have been crossed with C727 (Wilt tolerant) and C60 (Wilt tolerant) and AUG 424 (bruchid resistant).

(o) The major activity during 1972 has been to transfer disease resistance to better agronomic strains.

Mung and Mash:

1,640 cultivars have been tested for adaptation and screened for YMM tolerance.

631 new cultivars are being screened for adaptation. They will also be screened for disease resistance.

During the past two years the following conclusions have been reached:

1. Yellow mosaic of mung (YMM) and leaf crinkle (LC) are not seed or soil borne.
2. Thrips (Thysanoptera) are not a vector for YMM or LC.
3. Leaf hoppers (Empoasca) are not a vector for YMM or LC.
4. Aphids (Aphididae) are not a vector for YM and LC.
5. White flies (Aleyrodidae) are a vector for YMM but not LC.
6. Top necrosis (TN) of mung is probably seed borne.
7. YMM cannot be transmitted mechanically.
8. YMM can be transmitted by grafting.
9. Systemic insecticides provide some early protection (March planting) against acquisition of YMM.
10. YMM cannot be transmitted to cowpeas by white flies but can be transmitted to soybeans.
11. White flies taken from cotton and transferred to mung fail to transmit YMM.
12. 115 hybrid lines of mung are under observation.
13. 310 new cultivars are being tested for adaptation and disease resistance.

14. Several varieties of mung with short duration and uniform maturity have been located.

Cowpeas, Pigeon peas, Soybeans and Peanuts:

1. Ten new cowpea cultivars are being tested for yield and adaptation. Five F3 hybrid lines are being indexed for disease.
2. International variety trial on pigeon peas is underway.
3. New soybean cultivars from China have been sown.
4. New peanut hybrid lines from Nigeria are under observation.

Multidisciplinary Research

WPAU is composed of the faculties of Agriculture, Animal Husbandry, Veterinary Science, Agricultural Economics and Rural Sociology, Agricultural Education and Extension and Agricultural Engineering and Technology. A concerted effort has been made to organize a multidisciplinary team in each faculty.

1. A project titled "Derivation of Fertilizer Response and Optimum level of Fertilization on Various Crops in West Pakistan" is underway. This is a joint effort by economists, agronomists and soil scientists.
2. A project, "Comparison of Efficiency of Buffaloes and Cows in the Utilization of Various Feeds", has been funded. Staff from Departments of Nutrition Livestock Management, Animal Husbandry and Veterinary Science are involved.
3. The grain legume project is staffed by plant breeders, plant pathologists, entomologists, agronomists and soil scientists.

4. The Institute of Reproductive Physiology and Artificial Insemination will be a joint effort of the Faculties of Veterinary Science and Animal Husbandry.
5. The enriched chapati project is a cooperative effort between WPAU and KSU. The departments of Food Technology, Nutrition and Economics will be involved.
6. Estimation of economic losses due to livestock and poultry diseases in Lyallpur district. Faculties of Veterinary Science and Agricultural Economics are involved.
7. Evaluation of SCARP IIA with special reference to extension service involves Economics and Extension staffs.
8. Impact of modern agriculture technology on employment patterns of rural areas of West Pakistan. Economics and Rural Sociology staffs are involved.

All but one of these projects are staffed and financed. This interdepartmental approach should demonstrate to administration and staff the value of a competent team effort.

Inter-institutional Cooperative Research:

For a research institution to be most effective it must establish strong linkages with other research institutions both local and international. During the past two years inter-institutional cooperation was encouraged. Cooperative efforts currently in progress are:

a. WPAU is doing contract research for WAPDA and the Central Planning Commission.
b. Seventeen PL480 projects are underway.
c. WPAU is cooperating with Göttingen University on a study of agricultural marketing.

d. A project proposal by the staff of Rural Sociology and CUSUSWASH has been prepared and submitted to ARC for funding.

e. WAPU is screening chickpeas for Gram Blight and Wilt in cooperation with the Gram Breeding Station, Campbellpur.

f. WAPU is conducting a variety trial at Rawalpindi in cooperation with the Department of Agriculture.

g. WAPU is conducting chickpea variety trial at Kallur Kot and Piplan in cooperation with Agricultural Development Corporation.

h. WSU is multiplying and crossing wild gram varieties with exotic varieties supplied by WPAU.

i. Several postgraduate students from WPAU are doing their thesis research at PARI.

Developments:

Two important WPAU goals were accomplished during 1971. The Government of Pakistan amalgamated the Punjab Animal Husbandry College, Lahore, with the West Pakistan Agricultural University, Lyallpur. This is a breakthrough because now it will be possible to standardize veterinary science training. The Agricultural Engineering Workshop was transferred to the West Pakistan Agricultural University. These buildings and shops will be converted into a campus maintenance complex.

On March 15, 1972, Government of Pakistan announced that Faculties of Science and Rural Home Economics would be added to the West Pakistan Agricultural University, Lyallpur.

SHORT-TERM CONSULTANTS

The following short-term consultants have visited the WPAU and spent a total of 14 man-months:

1. Dr. C. Gardner Shaw, Plant Pathologist, Washington State University, concentrated his efforts towards establishing the information needed to develop a research program on Gram Wilt and Gram Blight. A survey of the Gram Wilt and Gram Blight areas was conducted. The causative pathogens were isolated from diseased plants. Procedures for testing gram seedlings for resistance to both diseases were developed. The results of his research and a proposed research program on Gram Wilt and Gram Blight is a part of this report.
2. Dr. Robert F. Harwood, Entomologist, Washington State University, directed his research toward mosaics, and similar pathogens of mung and related legumes. He demonstrated that the biological agent causing the disease known as yellow mosaic of mung is a virus, and is arthropod-transmitted by white flies. The details of a proposed research on "Arthropod-Transmitted Viruses of Mung and Related Edible Legumes" is a part of this report.
3. Dr. Vernon E. Wilson, Plant Pathologist, Washington State University, along with the WPAU staff, concentrated primarily on a search for methods of screening gram varieties for disease resistance. His report is included in a subsequent chapter of this total report.
4. Dr. Stuart D. Lyda, Plant Pathologist, Texas A & M University, helped organize a research program to study Root Rot of food and fiber crops in West Pakistan. A preliminary survey of the Root

Rot regions of West Pakistan was conducted. Dr. Lyda's report is included as a subsequent chapter of this total report.

5. Dr. Floyd J. Williams, Plant Pathologist, and Mr. Shannon Wilson, Entomologist, US AID/Lahore and Dacca visited WPAU and discussed pulse research. During these discussions they made many valuable suggestions.
6. Dr. K. H. Evans, Plant Breeder and Dr. W. J. Kaiser, Pathologist, Regional Pulse Improvement Project, Tehran, and Dr. Floyd J. Williams, Research Coordinator and Pathologist, US AID/Lahore spent four days reviewing pulse research at WPAU. Techniques employed and research results were discussed. Advanced cultivars were exchanged with RPIP.

Participant Training

1. Dr. Manzoor Ahmad Khan, Plant Breeder, US AID participant, studied U.S. research administration for six months.
2. Dr. Mian Mohammad Yosuf, Maize Breeder, Ford Foundation Participant visited Philippines, Mexico and USA to study advanced maize breeding.
3. Dr. Israr-ul-Haq, Vice-Chancellor, WPAU, Ford Foundation Participant visited USA, Philippines, Mexico, England and Lebanon to study research administration and interdisciplinary research. While visiting IRRI, CIMMYT, WSU, KSU and AUB, a proposal was made that WPAU graduate students, after completion of course work at WPAU, be permitted to proceed to the above research organizations to do their thesis research. They would then receive their advanced degrees from WPAU.

4. Dr. Mahmood Ali Khan, Head, Department of Cooperation and Credit, visited West Germany and studied the cooperative movement and recent developments in cooperative organization.
5. Dr. Manzoor Ahmad, Veterinary Physiologist and Dr. Wahab Qureshi, Animal Breeding Specialist, spent six months in Sweden studying the research programs related to reproductive physiology and artificial insemination. They were guests of the Government of Sweden.
6. Dr. Haider Ali Chaundhry, Dean, Faculty of Agricultural Economics and Rural Sociology, participated in a three-country seminar (Japan, Philippines and Pakistan) convened by UNESCO at Manila in connection with a comparative study on the "Historical Development of Agricultural Education in Asia". Also a conference on the Teaching of Soil Science Research Methodology held in Bangkok.
7. Dr. Haider Ali Chaundhry and Dr. M. R. Raza visited Colorado State University to help finalize a proposed research project on "Behavioral Factors Affecting Farm Water Management Decisions".
8. Dr. Tanvir Ahmad Khan Lodi participated in a three-week seminar on "Environmental Education in School Curricula" in July, 1970, in the United States sponsored by UNESCO.

Professionals from U.S. Department of Agriculture

1. Dr. Louise M. Russell, Entomology Research Division, ARS,

reviewed project A17-ENT-26 (Mites), and conducted a seminar on recent advances in entomological research.

2. Dr. R. D. Plowman, Livestock Division, ARS, acquainted himself with the livestock research program at the WPAU, and helped review and rewrite project A17-AH-10.

3. Dr. Jack Meiners, Crops Division, ARS, reviewed pulse research and suggested minor changes in design and techniques for project A17-CR-38. This project has since been funded.

4. Dr. A. W. Monlux, Federal Regional Veterinary Laboratory, Ames, Iowa, reviewed veterinary research in progress by the staff of the Faculty of Veterinary Science and suggested cooperative research on fungal diseases of buffalo.

5. Dr. C. W. Hesseltine, Chief, Fermentation Laboratory, Northern Regional Research Laboratory, Peoria, Illinois, consulted and held discussions with the staff of the Department of Food Technology.

6. Dr. James H. Taylor, Agricultural Engineer, University of Alabama conducted seminar on "Soil Reactions to Traction Forces", and also took part in discussion sessions.

7. Mr. Elvin W. Tilton, M. Q. Laboratory, Savannah, Georgia, discussed irradiation and control of major stored-grain insects both at WPAU and Radiation and Genetics Institute.

8. Dr. James R. Copp, Economic Research Service, Washington, D.C., conducted seminar on socio-economic research on marketing for the Faculty of Agricultural Economics and Rural Sociology.

9. Dr. R. D. Plowman, Chief of Beef Cattle Research, ARS, Beltsville, Maryland, reviewed animal research in progress in U.S. Also procedures for determining research priorities.
10. Dr. Louis Feinstein, Branch Chief of Field Crops and Animal Products Research, Market Quality Research Division, ARS, Hyattsville, Maryland had discussions with Plant Science Departments to explore possibilities of developing research projects for support under PL 480.
11. Dr. Albert C. Trouse, National Tillage Machinery Laboratory, Auburn, Alabama, outlined soil physics research that needs investigating and explored the possibilities of developing cooperative research with Agricultural Engineering Department at the WPAU.

Other Specialists:

1. Dr. Ewert Aberg, Professor and Head of Department of Plant Husbandry, Uppsala 7, Sweden, inspected crop research being conducted by the Departments of Plant Breeding and Agronomy.
2. Dr. W. R. Schmehl, Colorado State University, consulted on land and water management.
3. Dr. E. W. Spraque, Director of the Maize Program, CIMMYT, was involved in consultation and supply of advanced inbred lines of maize.
4. Team of Ford Foundation Specialists evaluated post-graduate studies program.

III. RECOMMENDATIONS

The continuous increase in productivity of both food grains, fiber and livestock in the U.S.A. and Japan was possible because of a highly integrated system of institutionalized agricultural technology. Similar developments are needed in Pakistan.

WPAU has reached the stage of maturity where they should pay specific attention to problem-oriented interdisciplinary research. US AID/WSU efforts for the past two years have concentrated on this goal.

If Pakistan moves into a science-based agriculture, they must depend not only on external aid but they must also invest larger amounts of their own money. Only then will a national research capability result. What is needed now is external aid to ensure in-service training in applicable experimental methods for field and laboratory research on problems of high priority. M.S. and Ph.D. degree programs must be strengthened. Cooperative degree programs with developed institutions and international research institutes must be expanded. Also a program whereby graduate students of developing countries under US training can do their thesis research in their own country on problems of their own country is needed. Some senior staff must be retrained in advanced or specialized techniques. Specialized research equipment and commodities will be needed in the future.

In the author's opinion WPAU is approaching maturity. As an institution matures a different kind of advisory service and participant training is needed. Probably the greatest return on investment would result from the use of short-term advisors and the retraining of senior staff. Careful structuring and planning for short-term consultants and senior staff retraining is a must.

Thesis research by WPAU graduate students at developed institutions and international institutes until WPAU can improve facilities will pay high dividends. Meeting the needs for specialized research equipment and commodities would be a good investment.

Section 406 of PL 480 would seem to make it possible to award outstanding scientists in US universities and USDA to carry out research on special problems in cooperation with scientists in WPAU. Such a program coupled with in-country training would certainly help speed up the development of research related to agricultural development.

An external aid program as detailed above would help keep WPAU scientists from becoming progressively isolated from their professional colleagues engaged in similar pursuits around the world. It would also help them to update their knowledge and proficiencies, and permit them to keep abreast with new developments occuring internationally.

APPENDIX I

WPAU STAFF MEMBERS HOLDING Ph.D. DEGREES

1.	Dr. Riaz Ahmad Khan	Agronomy Ph.D. (Calif.)
2.	Dr. Mohammad Aslam	Agronomy Ph.D. (Calif.)
3.	Dr. M. Youns Chaudhry	Entomology Ph.D. (WSU)
4.	Dr. M. H. Chaudhry	Entomology Ph.D. (WSU)
5.	Dr. Mohammad Akhtar	Entomology Ph.D. (UK)
6.	Dr. M. A. Halimi	Entomology Ph.D. (UK)
7.	Dr. M. Rafiq Khan	Entomology Ph.D. (UK)
8.	Dr. Daud Ahmad Khan	Horticulture Ph.D. (Calif.)
9.	Dr. Niaz A. Chaudhry	Horticulture Ph.D. (Oregon)
10.	Dr. M. Yaqub Chaudhry	Horticulture Ph.D. (Calif.)
11.	Dr. M. N. Malik	Horticulture Ph.D. (Calif.)
12.	Dr. Mohammad Hassan	Horticulture Ph.D. (UAR)
13.	Dr. Mohammad Aslam	Plant Breeding Ph.D. (Minn.)
14.	Dr. M. Yousaf Chaudhry	Plant Breeding Ph.D. (Minn.)
15.	Dr. A. Rehman Chaudhry	Plant Breeding Ph.D. (WSU)
16.	Dr. Mohammad Iqbal Khan	Plant Breeding Ph.D. (UK)

17.	Dr. Manzoor A. Khan	Plant Breeding Ph.D. (UK)
18.	Dr. Khurshid Alam	Plant Breeding Ph.D. (WPAU)
19.	Dr. M. Abdullah Khan	Plant Breeding Ph.D. (WPAU)
20.	Dr. Inam-ul-Haq	Soil Science Ph.D. (N. Carolina)
21.	Dr. Shah Mohammad	Soil Science Ph.D. (UC Riverside)
22.	Dr. Riaz H. Qureshi	Soil Science Ph.D. (UK)
23.	Dr. M. F. A. Khan	Soil Science Ph.D. (Canada)
24.	Dr. Altaf Hussain	Soil Science Ph.D. (Czech.)
25.	Dr. Haider Ali Ch.	Rural Sociology Ph.D. (Mass.)
26.	Dr. M. Rifq Raza	Rural Sociology Ph.D. (Cornell)
27.	Dr. A. H. Chaudhry	Rural Sociology Ph.D. (Georgia)
28.	Dr. Mahamood Ali Khan	Cooperation & Credit Ph.D. (Germany)
29.	Dr. Dilawar Ali Khan	Cooperation & Credit Ph.D. (Wisc.)
30.	Dr. Ahmad Saeed Khan	Agri. Economics Ph.D. (Wisc.)
31.	Dr. A. Sajjad Haider	Agri. Economics Ph.D. (Wisc.)
32.	Dr. Ali Mohammad Ch.	Farm Management Ph.D. (Ohio)
33.	Dr. Nasir-ud-Din	Agronomy Ph.D. (Calif.)

34.	Dr. A. Rashid	Agri. Marketing Ph.D. (UK)
35.	Dr. M. S. Chaudhry	Cereal Technology Ph.D. (Kansas)
36.	Dr. A. A. Malik	Food Technology Ph.D. (Ill.)
37.	Dr. H. M. Chaudhry	Meat Technology Ph.D. (Iowa)
38.	Dr. R. A. Gill	Dairy Technology Ph.D. (Wisc.)
39.	Dr. M. Afzal Kazi	Theoretical Mechanics Ph.D. (Wisc.)
40.	Dr. G. S. Khan	Agri. Extension Ph.D. (Cornell)
41.	Dr. Ansar A. Khan	Agri. Extension Ph.D. (Ohio)
42.	Dr. Tanveer Lodhi	Agri. Education Ph.D. (Ohio)
43.	Dr. Khalid Bokhari	Agri. Education Ph.D. (Ohio)
44.	Dr. Jafar A. Shah	Agri. Education Ph.D. (Penn.)
45.	Dr. Mohammad B. Sial	Animal Nutrition Ph.D. (WSU)
46.	Dr. M. Asghar Toor	Veterinary Anatomy Ph.D. (WSU)
47.	Dr. Manzur-ud-Din	Animal Breeding Ph.D. (Iowa)
48.	Dr. Waheed Ahmad	Animal Breeding Ph.D. (WSU)
49.	Dr. Bakht B. Khan	Livestock Mgt. Ph.D. (Colo.)
50.	Dr. M. Jamil Qureshi	Livestock Mgt. Ph.D. (WPAU)

51.	Dr. M. Sial	Nutrition Ph.D. (WPAU)
52.	Dr. A. H. Gilani	Nutrition Ph.D. (WPAU)
53.	Dr. M. R. Chaundhry	Poultry Science Ph.D. (WPAU)
54.	Dr. M. Irfan	Veterinary Pathology Ph.D. (UK)
55.	Dr. Israr-ul-Haq	Reproductive Physiology Ph.D. (UK)
56.	Dr. A. H. Cheema	Veterinary Pathology Ph.D. (WSU)
57.	Dr. Manzoor Ahmad	Veterinary Physiology Ph.D. (WSU)
58.	Dr. Mushtaq A. Khan	Veterinary Physiology Ph.D. (WSU)
59.	Dr. M. Ajmal	Veterinary Microbiology Ph.D. (UK)
60.	Dr. Sikander Hayat Ch.	Veterinary Parasitology Ph.D. (WSU)
61.	Dr. Berjees Rehman	Veterinary Parasitology Ph.D. (WPAU)
62.	Dr. A. G. Kausar	Plant Pathology Ph.D. (Minn.)
63.	Dr. J. H. Mirza	Plant Pathology Ph.D. (Canada)
64.	Dr. A. H. Hanif	Fiber Technology Ph.D. (UK)
65.	Dr. Naseer Beg	Botany Ph.D. (UK)
66.	Dr. Feroza Beg	Botany Ph.D. (UK)
67.	Dr. M. A. Beg	Zoology Ph.D. (Mont.)

68.	Dr. A. H. Sheri	Zoology Ph.D. (Canada)
69.	Dr. A. Z. Memon	Math & Stat. Ph.D. (Iowa)
70.	Dr. Ali Asghar	Food Technology Ph.D. (Australia)
71.	Dr. M. Amin Khan	Plant Breeding & Genetics Ph.D. (Japan)
72.	Dr. Fateh Mohammad Ch.	Agronomy Ph.D. (Japan)
73.	Dr. M. Aslam	Agri.Marketing Ph.D. (Michigan)
74.	Dr. S. Sirajuddin Shah	Plant Breeding & Genetics Ph.D. (WPAU)
75.	Dr. Shafi Nazir	Agronomy Ph.D. (Moscow)
76.	Dr. Wali Mohammad	Agronomy Ph.D. (Moscow)
77.	Dr. Alauddin	Animal Husbandry Ph.D. (Moscow)
78.	Dr. Altafur Rehman	Parasitology Ph.D. (Moscow)
79.	Dr. Ejaz Rasul	Botany Ph.D. (WSU)

Ph.D's on the staff of WPAU and country of training.

USA - - - - - -	46
UK - - - - - - -	13
USSR - - - - - -	3
Germany - - - -	1
Czechoslovakia -	1
UAR - - - - - -	1
Pakistan - - - -	7
Japan - - - - -	2
Australia - - -	1
	79

During the past two years advanced training supplied by US AID was drastically reduced. Since WPAU will need training facilities for several years other aid giving agencies were contacted.

WPAU staff under training is listed below: -

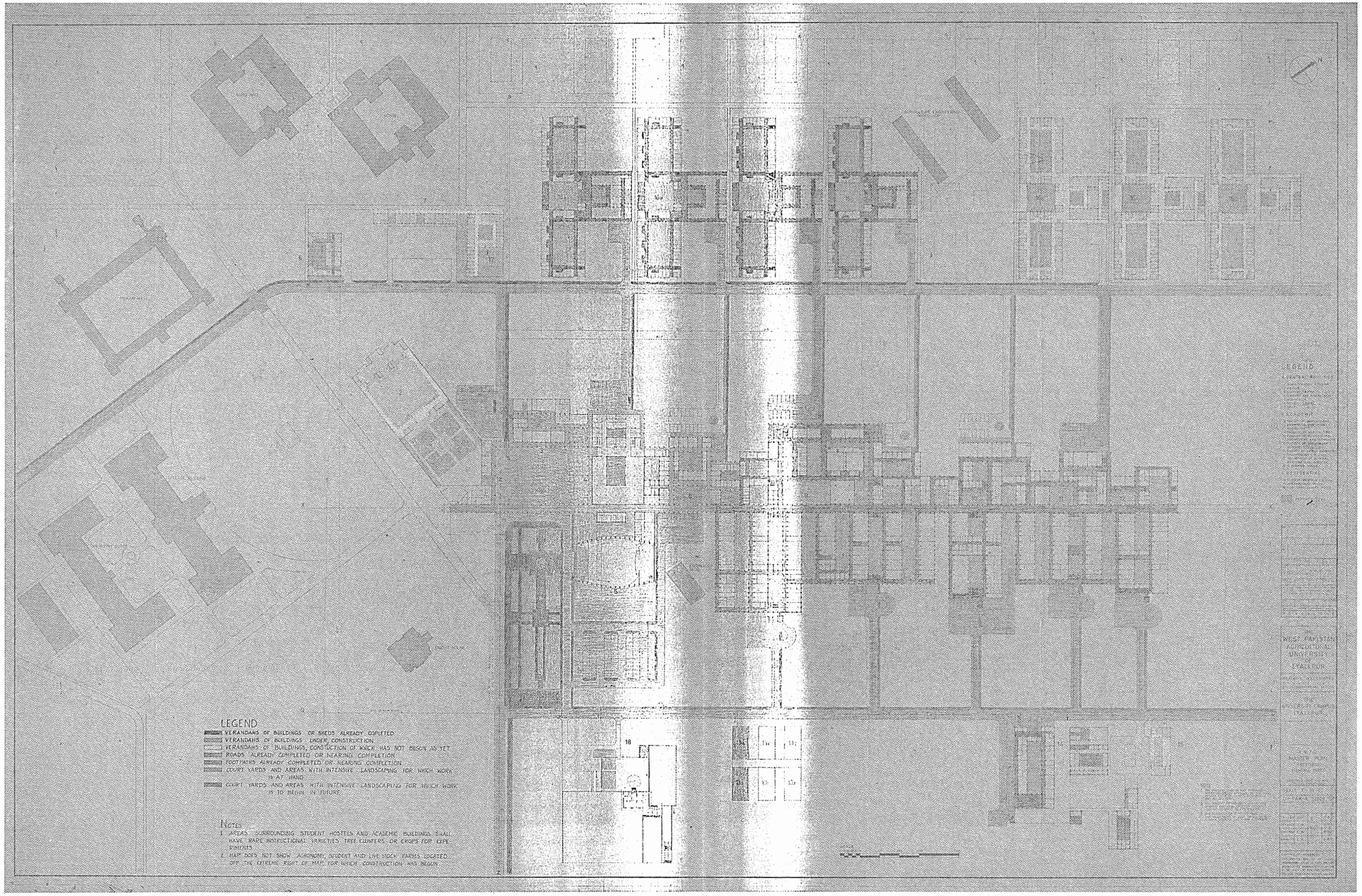
USA	2 (Ph.D.)
USSR	4 (Ph.D.)
UK	8 (Ph.D.)

APPENDIX II

MAP OF THE MASTER PLAN

OF THE

WEST PAKISTAN AGRICULTURAL UNIVERSITY



BEST AVAILABLE COPY

APPENDIX III

PL-480 SUPPORTED PROJECTS DURING THE LAST TWO-YEAR PERIOD

A major effort was directed toward rupee support from PL-480. PL-480 projects submitted and approved are detailed below:

Faculty of Agricultural Economics & Rural Sociology:

A-17-ERS-7

Determination of feasibility models of cooperatives under different socio-economic conditions.

Grant: FG-Pa-160 Duration: Three years

Amount: Rs.213,384

Principal Investigator: Dr. Mahmood Ali Khan

Sponsoring Scientist: Mr. Savage Federal Cooperative Service Washington, D. C.

A17-ERS-15*

Derivation of fertilizer crop response and optimum level of fertilization on various crops in West Pakistan.

Grant: FG-Pa-172 Duration: Four years

Amount: Rs.400,800

Principal Investigator: Dr. Ahmad Saeed Khan

Sponsoring Scientist: Dr. David Harrington Economic Research Service Washington, D. C.

*Inter-departmental Project

A17-ERS-14*

Impact of Land Reform on Agricultural Productivity.

Grant: FG-Pa-173 Duration: One year

Amount: Rs.33,335

Principal Investigator: Dr. Ahmad Saeed Khan

Sponsoring Scientist: Dr. G. L. Wunderlick
Economic Research Service
Washington, D. C.

A17-ERS-11

Type and Extent of Marketing Losses in Agricultural Products in Developing Areas.

Grant: FG-Pa-177 Duration: Four years

Amount: Rs.432,591

Principal Investigator: Dr. Abdul Rashid

Sponsoring Scientist: Mr. Fred Poats
Economic Research Service
Washington, D. C.

A17-ERS-9*

Selecting the Most Profitable Cropping Patterns for Various Ecological Regions.

Grant: FG-Pa-174 Duration: Three years

Amount: Rs.440,480

Principal Investigator: Dr. Ali Mohammad Chaundhry

Sponsoring Scientist: Dr. A. Barry Carr
Agricultural Research Service
Washington, D. C.

Faculty of Agriculture:

A17-ENT-26

Toxonomic Studies of the Mites Belonging to the Families Tetranchidae, Phyloseiidae, Tenaipalpidae, and Stigmaeidae.

Grant: FG-Pa-144 Duration: Five years

Amount: Rs.233,201

Principal Investigator: Mr. Wali Mohammad Chaudhry

Sponsoring Scientist: Dr. E. W. Baker, Entomologist, ARS, Beltsville, Maryland

Dr. Louise M. Russell, Entomologist, ARS, Beltsville, came and reviewed progress on this project. She also presented a seminar to the staff on recent entomological research.

A17-MQ-6(b)1

Scheme for Research on Stored Grain Pests in West Pakistan.

Grant: FG-Pa-161 Duration: Three years

Amount: Rs.192,755

Principal Investigator: Mr. H. Abdul Qayyum

Sponsoring Scientist: Mr. Gaelen D. White
Marketing Quality Research
Manhattan, Kansas

A17-ENT-29

Insect and Mite Pests of Cotton in West Pakistan.

Grant: FG-Pa-178 Duration: Three years

Amount: Rs.208,100

Principal Investigator: Dr. M. Yunus Chaundhry

Sponsoring Scientist: Dr. Sloan E. Jones, Entomologist, Beltsville, Maryland

A17-UR-(10)-12

Collection, Isolation and Classification of Molds in Pakistan Belonging to the Order Mucorales as Potential Agents for the Conversion of Farm-Produced Raw Material into Useful Products.

Grant: FG-Pa-170 Duration: Three years

Amount: Rs.228,826

Principal Investigator: Dr. J. H. Mirza

Sponsoring Scientist: Dr. C. W. Hasseltine
Northern Marketing & Nutrition
Peoria, Illinois

A17-CP-28*

Research for the Development of Grain Legumes in West Pakistan.

Grant: FG-Pa- Duration: Three years

Amount: Rs.783,900

Principal Investigator: Dr. Mohammad Aslam

Sponsoring Scientist: Dr. Jack Meiners, Crops
Beltsville, Maryland

Dr. Jack Meiners visited WPAU and reviewed the pulse research project.

Division of Basic Science:

A17-CR-27

Biology of Farm Weeds.

Grant: FG-Pa-159 Duration: Three years

Amount: Rs.129,777

Principal Investigator: Mr. Akhtar Saeed Syed

Sponsoring Scientist: Dr. R. B. Taylerson, Crops
Beltsville, Maryland

Dr. J. T. Holstun, Crops, Beltsville, reviewed progress.

He gave a seminar to the staff on weeds.

Faculty of Animal Husbandry:

A17-AH-10*

Comparison of Efficiency of Buffaloes and Cows in the Utilization of Various Feeds.

Grant: FG-Pa-176 Duration: Three years

Amount: Rs.205,700

Principal Investigator: Dr. M. B. Sial

Sponsoring Scientist: Dr. Chester H. Gordon
ARS, Beltsville, Maryland

Dr. R. D. Plowman, ARS, visited WPAU and helped develop the above project.

APPENDIX IV

OTHER RESEARCH GRANTS

	Name of Scheme	Total Value of the Scheme Rs.	Expenditure	Controlled By	Aid Giving Agency
1.	Study of Farm Mechanism	4,000	3,995	Dept. of Agri. Economics	Planning Commission
2.	Research study of Economics of Plant Protection and Modern Poultry Production	78,800	25,460	Dept. of Agri. Economics	Planning Commission
3.	Research in Agri. Marketing in Sargodha	15,000	5,555	Dept. of Agri. Economics	Commissioner Sargodha
4.	Primary Agri. Credit and Cooperative Societies	6,000	60	Dept. of Cooperation and Credit	Cooperative Department
5.	Biological Gall Forming of Insects	65,648	25,020	Dept. of Entomology	A. R. C.
6.	Genetic Analysis of Rust Resistance in various varieties	58,000	6,308	Dept. of Plant Breeding and Genetics	A. R. C.
7.	Case studies on capital formation in relation to agricultural production in West Pakistan	44,100 (2 Years)		Dept. of Agri. Economics	A. R. C.
8.	An economic survey and analysis of factors affecting farm production and income in a selected area in West Pakistan.	99,900 (3 Years)		Dept. of Agri. Economics	A. R. C.
9.	Advisory Services to Mona Reclamation Project	20,000	Nil	Dept. of Farm Management	WAPDA

APPENDIX V

POSTGRADUATE STUDIES AT WEST PAKISTAN AGRICULTURAL UNIVERSITY

A. POSTGRADUATE STUDENTS

Year	Students Admitted
1962	142
1963	212
1964	326
1965	421
1966	516
1967	527
1968	455
1969	475
1970	613
1971	662

B. RESEARCH PROJECTS COMPLETED BY GRADUATE STUDENTS

Year	Research Projects
1962	73
1963	55
1964	57
1965	47
1966	111
1967	108
1968	165
1969	130
1970	114
1971	169

LIST OF RESEARCH PROJECTS
COMPLETED BY THE GRADUATE STUDENTS 1969 and 1970

S.No.	Name & Degree	Title of the Thesis
	AGRONOMY	
	1969	
1.	Mohammad Akram Sadla, M.Sc.	A study to see the effect of different levels of nitrogen, phosphorus and dates of sowing on the growth and yield of spring maize.
2.	Abdul Latif Bhatti, M.Sc.	Effect of different doses of nitrogen and their application time on wheat varieties after green manuring.
3.	Sheikh Abdul Latif, M.Sc.	Effect of different levels of NP fertilizers on the growth and yield of three wheat varieties, i.e., Mexi-Pak-65, Indus-66 and C-273.
4.	Anwar Ahmad Ghuman, M.Sc.	Effect of plant population and fertilizer rates on the growth, yield and quality on double cropping of maize crop under Lyallpur conditions.
5.	Ghulam Mohammad Sarwar, M.Sc.	Effect of different rates of potassic fertilizer and dates of transplanting on the yield and quality of 'Hukka' Tobacco.
6.	Narongsak Senanarong, M.Sc.	Study of some morphological and physiological characteristics of three inbred lines of maize presumably differing in drought resistance.
7.	Mohammad Azhar, M.Sc.	Effect of different seed rates and nitrogen levels on the yield of Mexican wheat under Lyallpur conditions.

S.No.	Name & Degree	Title of the Thesis
8.	Mohammad Aslam Gill, M.Sc.	Effect of nitrogen and potassium on the yield and quality of jute fibre.
9.	Khurshid Ahmad, M.Sc.	Effect of sowing date and seed rate on the yield of soybean.
10.	Rashid Ahmad Shad, M.Sc.	Effect of different levels of nitrogen and seed rates on the yield of Mexi-Pak-65.
11.	Mohammad Munir Bajwa, M.Sc.	Effect of nitrogen levels and plant spacings on the yield and quality of spring maize J-1.
12.	Fazal Karim, M.Sc.	Studies on the yield and quality of Mexi-Pak-65 wheat as influenced by different nitrogen levels and irrigations frequencies.
13.	Sakol Sutheesorn, M.Sc.	Effects of soil types, planting depths, environmental temperature on seedling emergence, coleoptile length first internode growth and seedling height, in a maize single-cross hybird.
14.	Shaukat Hussain Hashmi, M.Sc.	Effect of Gibberellic Acid during seed maturation on dormancy and seedling growth in barley.
15.	Abdul Rahman Saleemi, M.Sc.	The effects of stage of maturation and size of seed on germinability, seedling growth and yield of wheat.
16.	Mohammad Riaz Hussain, M.Sc.	Effect of different levels of nitrogen and seed rates on the yield of Mexican Wheat (Penjamo) under local conditions.
17.	Mohammad Shabbir Rauf, M.Sc.	Salt concentrations affecting the germination of barley seed.
	1970	
18.	Maghmoom Mohammad Iqbal, M.Sc.	A study on the effect of different nitrogen levels and stages of cutting on the quality and yield of Bajra fodder.

S.No.	Name & Degree	Title of the Thesis
19.	Mohammad Rafiq Sabir, M.Sc.	Salt concentrations affecting the germination of wheat seed.
20.	Mohammad Akmal, M.Sc.	Micro-nutrient cum irrigational studies on maize, Jl.
21.	Bashir Ahmad, M.Sc.	Studies into the deterioration of two sugarcene varieties under different storing conditions harvested at different times.
22.	Mohammad Ashraf, M.Sc.	Effects of number or minimum tillage and row spacing on gram.
23.	Mohammad Nawaz, M.Sc.	Comparative field studies on wheat varieties in composite versus pure stands.
24.	Mohammad Arshad,	Analytical yield studies on wheat blends versus pure stands.
25.	Mohammad Anwar Javed, M.Sc.	Effect of different densities of preparatory foliage and NP levels on the growth and yield of wheat Maxi-Pak. 65.
	ENTOMOLOGY	
1.	Mohammad Ali Shah, M.Sc.	Evaluation of sacred wheat losses caused by *Trogoderma granarium* Everts and *Phizopertha dominica* (F).
2.	Rahmat Ali, M.Sc.	Rearing of silkworm, *Bomby mori* Linn on artificial diets and alternate host plants.
3.	Mohammad Razzaq Ahmad, M.Sc.	Effect of different temperatures and relative humidities on the bionomics of *Callosobruchus maculatus* (F).
4.	Ejaz Munawar Siddique, M.Sc.	Taxonomic studies of tenuipalpid mites (*Acarina*: *Tenuipalpidae*).
5.	Haider Ali Shah,	Studies on the bionomics and natural enemies of *Hanosepilachna* chrysomelina F. at Lyallpur.
6.	Abdul Quddus, M.Sc.	Temperature effects on the toxicity of some insecticides against *Rhizopertha dominica* (F.).

S. No.	Name & Degree	Title of the Thesis
7.	Mohammad Sarwar Butt, M.Sc.	Effect of environmental heterogeneity on the development of *Chilo Zonellus* (Swinhoe).
8.	Mohammad Mushtaq, M.Sc.	Taxonomic studies of Hupopi (an additional stage in the life cycle of certain mites). (Acarina: Anoetidat and Tyroglyphidae).
9.	Abdul Wahid, M.Sc.	Nutritional requirements of sugarcane top borer, *Scripophaga nivella* Fab.
10.	Asif Abdullah, M.Sc.	Bioessay of effective residues of malathion dimencron and endrin on radish and tobacco plants.
11.	Jameel Javed, M.Sc.	Determination of residues of ethion, aldrin, phosdrin, petkolin, folidol M, EPN and Diazinon on cauliflower and radish plants by biological method.
	1970	
12.	Mohammad Moosa, M.Sc.	Biology of Maize Jassid Zygina sp. at Lyallpur.
13.	M. Akram Khan, M.Sc.	Studies on the haemocytes of different post-embronic stages of the normal and moribund *Acheta domesticus* L. reared under semi-natural conditions.
14.	Mohammad Wahid, M.Sc.	Control of cotton pests with some modern insecticides.
15.	Mohammad Anwar, M.Sc.	Life history and chemical control of wax moth (*Galleria mellenella*).
16.	Taj Mohammad, M.Sc.	Biology of *Rhizofortha dominica* under different environmental conditions.
17.	Mohammad Bashir, M.Sc.	Residual analysis of dimecran (2-chloro-2-ethyl cabamoyl-I-Methyl vinyl dimethyl phosphate) on cotton rice and bhindi foliage.

S.No.	Name & Degree	Title of the Thesis
18.	Salah-ud-Din, M.Sc.	Further studies on biology and control of *Leucinodes orbonalis* Guen.
19.	Abdul Majid Hussain, M.Sc.	Further taxonomic studies of Lyallpur.
20.	Mohammad Akram, M.Sc.	Chemical control of leaf-hoppers attacking maize.
21.	Mohammad Sarwar, M.Sc.	Determination of residues of Malthion, Dimecron, and Endrin on cabbage and radish plants by biological methods.
22.	Mohammad Younus, M.Sc.	Control of Beber-mite with modern acaricide /insecticides.
23.	Mohammad Akram Zafar, M.Sc.	Quantitative determination of Dimecron residues on citris brinjal and sarghum by chemical method.

HORTICULTURE

1969

1.	Mohammad Arshad Zahid, M.Sc.	Studies on the fruiting behaviour of mango tree, (*Mangifera indica* L); comparative evaluation of various tree sectors for fruit setting, drop, yield and fruit composition.
2.	Mohammad Aslam, M.Sc.	Studies on the influence of five different rootstocks on vegetative growth, productivity and flower quality of four cultivars of roses.
3.	Mohammad Ibrahim, M.Sc.	Effect of managanese and zinc sprays on the seasonal changes of these elements and some biochemical factors in the leaves of pineapple orange, (*Citrus sinesis* Linn.).
4.	Karamat Ali, M.Sc.	Effect of different cultural treatments on flower sex-ratio and fruit production in mango.
5.	Ghulam Sarwar Khan, M.Sc.	Studies on the propagation of olive from stem cuttings.

S.No.	Name & Degree	Title of the Thesis
6.	Malik Mohammad Usuf, M.Sc.	Evaluation of some exotic cultivars of tomato in respect of yield and certain growth characteristics.
7.	Mohammad Amin Shakir, M.Sc.	Effect of manganese and zinc sprays on fruit growth and chemical composition of pineapple orange.

1970

8.	Akhtar Jalis, M.Sc.	Description and evaluation of some grape hybrids *Vitis vinifera* L.
9.	Nerma S. Marasigan, M.Sc.	A study on the anatomy of graft union in mangoes.
10.	Noor Ahmad, M.Sc.	Effect of methods and time grafting and number of scion-buds on the success and growth of guava grafts.
11.	Ashfaqun-Nabi, M.Sc.	Hetresis and combining ability in Pea *Pisum sativum*.
12.	Mohammad Iqbal, M.Sc.	Effect of NPK on the growth and yield of potato (*Selanum tuberesum L.*) crop.
13.	Nazir Ahmad Sahid, M.Sc.	Effect of *Tiba* & *Nea* sprays on alternate bearing in Kinnow Mandarin as related to time of application and concentration.
14.	Mahmood Ahmad, M.Sc.	Performance of some important varieties of pea under Lyallpur conditions.

PLANT BREEDING & GENETICS

1969

1.	Mohammad Sarwar, M.Sc.	Relationship of grain weight and shelling percentage with the hybrid vigour in flint-dent crosses of maize.
2.	Maqbool Ahmad, M.Sc.	Cytogenetic studies in brasica species.
3.	Karim Baksh Malik, M.Sc.	Drought resistance studies in sugarcane.

S.No.	Name & Degree	Title of the Thesis
4.	M. Anwar Mirza, M.Sc.	Genetic studies in some intra-specified crosses of *G. hirsutum L.*
5.	Mohammad Ramzan, M.Sc.	Drought resistance in wheat.
6.	Mohammad Iqbal, M.Sc.	Evaluation of transgressive variation for plant height, spike length, spike density and tillering capacity in some crosses of local and Mexican Wheats.
7.	Ata-ur-Rehman Anwar, M.Sc.	Heritability and inheritance of plant height heading date and grain yield and their association with seedling height in some wheat crosses involving Mexican semidwarfs.
8.	Mohammad Ghayyur Khan, M.Sc.	Genetic analysis of some physiological and economic characters in *Gossypium hirsutum L.*
9.	Mohammad Sharif, M.Sc.	Pollination anthesis and seed setting studies in sunflower crop (*Helianthus annuus*).
10.	M. Siddique Sadiq, M.Sc.	Response of inbred lines and their hybrids to population pressure in corn.
11.	S. D. Shah, M.Sc.	Genetic analysis of yield and components of yield in wheat (*T. aestivum L.*).
12.	Bashir Ahmad Naseem, M.Sc.	Comparative inbreeding depression and heterosis in self and cross combination of various lines in sunflower *Helianthus annuus L.*
13.	Rashid Anwar, M.Sc.	Genotypic competition in wheat for grain and straw yield.
14.	Abdul Rashid Gill, M.Sc.	Genetic analysis of some physiological and economic characters in wheat (*Triticum vulgare*).
15.	Mohammad Rafiq, M.Sc.	Cytogenetic studies in interspecific crosses of cotton involving *G. hirsutum L.* and *G. barbadense L.*

S.No.	Name & Degree	Title of the Thesis
16.	Mohammad Sadiq, M.Sc.	Morphogenetic responses in some economic characters of six promising varieties of wheat (*T. vulgare*) to different environments.
17.	M. Hanif, M.Sc.	Effect of plant population and phosphorus fertilization on grain yield and plant composition of spring maize.
18.	A.R.M. Ihteshamur Rehman, M.Sc.	Studies on intra and interspecific crossability and pollen-tube growth in *Oleiferous brassicae*.
19.	Mohammad Aslam, M.Sc.	Heteresis and inheritance studies in watermelons.
20.	Mohammad Afzal, M.Sc.	Heterotic development of plant characters in some inter and intraspecific crosses of *Oleiferous brassicae*.
21.	Abdul Rauf, M.Sc.	Irradiation effect on gram *Cicer arietinum L.*
22.	Fida Hassain, M.Sc.	Genetic studies on salt tolerance in wheat *Triticum vulgare*.
23.	Mohammad Ibraheem, M.Sc.	Morphogenetic studies on three important cotton varieties (*Gossypium hirsutum L.*) under different growth condition.
24.	Ghulam Sabir, M.Sc.	The reliability of individual plant selections in F2 generalization for genetic advance of some quantitative characters in wheat.
25.	Naazar Ali, M.Sc.	Heterosis studies in some intraspecific crosses of *G. hirsutum L.*
26.	Mansoor Ahmad Akhtar, M.Sc.	Heterosis studies in some intraspecific crosses of *G. hirsutum L.*
27.	Sujawal Khan, M.Sc.	Heterosis studies in some intraspecific crosses of *Gossypium hirsutum L.*

S.No.	Name & Degree	Title of the Thesis
28.	Mahmoodul Hassan M.Sc.	Genetic mechanism controlling response to nitrogen in F2 population of some crosses in wheat (*Triticum aestivum* L.).
29.	Manzoor Ali Akhtar, M.Sc.	Genetic mechanism controlling expression of quantitative characters in F2 generation of some crosses in wheat *Triticum aestivum* L.
30.	Mohammad Saeed, M.Sc.	Performance of maize synthetics in relation to hetrozygosity level.
31.	Mohammad Saleem, M.Sc.	Inheritance of kernel number in maize (*Zea mays* L.).
32.	Abdus Samad, M.Sc.	Genetic mechanisms controlling response to spacing in wheat (*Triticum aestivum* L.).
33.	Mohammad Sarfraz Khan, M.Sc.	Combining ability for yield and its components in a diallel crosses in *Sorghum vulgare* Pers.

PLANT PATHOLOGY

1969

1.	Mohammad Ashraf, M.Sc.	Nematodes infesting the roots and rhizosphere of citrus nursery plants.
2.	Sultan Mahmood, M.Sc.	Taxonomic studies on the mucorales of Lyallpur.

1970

3.	Mohammad Aslam, M.Sc.	Infection process, cytology and genetics of *Fedvessica indica* Mimdhur.
4.	Shafqat Ali, M.Sc.	Infection process, cytology and genetics of *Tolypesperium enrenbergii* (Kuhn) Pat., the cause of long smut of sorghum.
5.	Miss Aisha Khatoon, M.Sc.	Morpho-cytological studies in *Sordaria humana* (Fuckel) Winter.
6.	Safdar Ali Anwar, M.Sc.	Relation between Nematodes and cotton wilt.

S.No.	Name & Degree	Title of the Thesis

SOIL SCIENCE

1969

1.	Mohammad Ashraf Cheema, M.Sc.	Effect of phosphorus, zinc and plant population on corn yield and leaf composition.
2.	Wyclifee Simiyu, M.Sc.	Effect of nitrogen, phosphorus and potassium on the wheat grain composition of zinc, copper, iron and manganese.
3.	Mohammad Bashir, M.Sc.	Effect of biuret content of urea on the germination, growth and quality of maize.
4.	Atta Ullah Shah, M.Sc.	Effect of saline irrigation water on the yield and chemical composition of maize variety J-I.
5.	Abdul Ghaffar, M.Sc.	Soil phosphorus forms-determination of different forms of phosphorus in some W. Pak. soils having different pH, organic matter and texture.
6.	Ghulam Sarwar, M.Sc.	A study of the mineralogy of the layer-silicates in West Pakistan soils.
7.	Mohammad Hanif, M.Sc.	Effect of plant population and phosphorus fertilization on grain yield and plant composition of spring maize.
8.	Mohammad Sharif Zia, M.Sc.	Nitrogen uptake by Irri Pak. rice as affected salinity and various sources of nitrogen.
9.	Abdul Rashid, M.Sc.	Effect of phosphorus and molybdenum application to berseem on the yield of J/I maize.
10.	Maqbool Ahmad, M.Sc.	Salt tolerance of some adapted and imported soybean varieties.
11.	Mohammad Ramzan M.Sc.	Relationship between electrical conductivity and total dissolved salts determined gravimetrically.

S.No.	Name & Degree	Title of the Thesis
12.	Habib Ahmad Habib, M.Sc.	Combined effect of boron, copper and zinc on yield and quality of maize.
13.	Riaz Ahmad Riaz, M.Sc.	Composition of maize plant at various stages of growth as affected by phosphorus fertilization.
14.	Abdul Bari, M.Sc.	Effect of nitrogen, phosphorus and potassium on the yield and composition of maize varieties.
15.	Mohammad Zaman, M.Sc.	Effect of different sources and levels of nitrogen, on germination growth and chemical composition of rice under different saline conditions.
	1970	
16.	Mohammad Bashir, M.Sc.	Effect of nitrogen and phosphorus on the uptake of micronutrients at various growth stages of rice.
17.	Mohammad Hanif Kausar, M.Sc.	Effect of different rates of nitrogen phosphorus and potash fertilizer on the yield and composition of Maxi-Pak. 65 wheat variety.
18.	Barat Ali, M.Sc.	Effect of N, P and K. on oil and protein contents of sunflower seeds.
19.	M. Latif Khan Niazi, M.Sc.	The role of cycocel in inducing draught and salinity tolerance in wheat.
20.	Mohammad Iqbal Makhdoom, M.Sc.	Effects of salts on the yield and quality of sunflower (*Helianthus annuus* L.).
21.	Mohammad Islam Gill,	Effect of application of nitrogenous fertilizers at various times on yield and protein content of wheat grain.
22.	Mohammad Mukhtar, M.Sc.	Effect of different insecticides on nitrification process in some soils of Lyallpur District.
23.	Nazir Ahmad, M.Sc.	Evaluation of fertility status of salt-affected soils.

S.No.	Name & Degree	Title of the Thesis
24.	Mohammad Sarwar, M.Sc.	Effect of phosphorus fertilization upon the uptake of zinc in two rice varieties.
25.	Mohammad Iqbal Khan, M.Sc.	Effect of Azotobacter on maize.
26.	Mohammad Mukhtar, M.Sc.	Effect of time of application of ammonium sulphate and urea on yield quality and growth characteristics of wheat Maxi-Pak. 65.
27.	Khalid Hussain, M.Sc.	Phosphorus fixation capacity of soil as affected by fertilizer source, soil texture and salts.

AGRICULTURAL ECONOMICS

1969

1.	Manzoor Ahmad, M.Sc.	Proportions of farm products marketed by farmers by months and the differentials between farm-level and wholesale prices in southern West Pakistan.
2.	Mohammad Aslam, M.Sc.	Proportions of farm products marketed by farmers by months and the differentials between farm level and whole-sale prices in Northern West Pakistan.
3.	Mohammad Zahid-ur-Rahim, M.Sc.	An evaluation of the Government monopoly procurement scheme of rice.
4.	Abdul Salam, M.Sc.	The problems of excess capacity adjustment and source of financing of private tubewell farmers in Sahiwal Tehsil.
5.	Abdul Majid Buzdar, M.Sc.	Contribution of farm labour to the national income of Der Ghazi Khan District 1966-67.
6.	Shoukat Rashid Sethi, M.Sc.	Derivation of fertilizer crop response functions and optimum level of fertilization on various crops in Sialkot Tehsil.

S.No.	Name & Degree	Title of the Thesis
7.	Nazar Mohammad Shah, M.Sc.	Economics of fertilizer use in Multan District.
8.	Mohammad Arshad Chaudhry, M.Sc.	Effect of different modes of land-rent payment on productivity in canal-irrigated areas of Sahiwal District.
9.	Mohammad Sarwar Anjum, M.Sc.	Effect of different modes of land-rent payment on productivity in canal-irrigated areas of Sahiwal District.
	1970	
10.	Mohammad Siddique, M.Sc.	Economics of custom work tractor farming on small farms in Shahkot, District Sheikhupura.
11.	Ghulam Nabi Chudhry, M.Sc.	Past production and future production potentials of wheat crop in West Pakistan.
12.	Noor Ahmad, M.Sc.	Past production and future production potentials of maize crop in West Pakistan.
	COOPERATION & CREDIT	
	1969	
1.	Mohammad Safdar Jhan Khan, M.Sc.	Lending and repayment frequency for Agricultural Development Bank Loans in Lyallpur District.
2.	Mohammad Anwar Khan, M.Sc.	Marketing credit needs of peasant properties in Lyallpur Tehsil.
3.	Faiz Mohammad Tahir, M.Sc.	Short and medium term credit needs of vegetable growers in Lyallpur.
4.	Miss Kausar Parveen, M.Sc.	Consumption and savings analysis of farm household.
5.	Mohammad Ashiq, M.Sc.	The evaluation of cooperative farming societies in Multan and Sahiwal Districts.
6.	Manzur Ahmad, M.Sc.	A study into the working of tubewell Cooperative Societies.

S.No.	Name & Degree	Title of the Thesis
7.	Ahmad Saeed Khan, M.Sc.	Capital formation on mechanized farm in Sheikhupura Tehsil of Sheikhupura District.

FARM MANAGEMENT

1969

1.	Abdur Rashid Nadeem, M.Sc.	A study into the economics of local and short-statured wheat varieties in Toba Tek Singh Tehsil.
2.	Nazir Ahmad Saleemi, M.Sc.	Economic comparison between cooperative and individual farms in Multan Tehsil.
3.	Mohammad Mansha, M.Sc.	Economic rise of holding for mechanized farms in Sahiwal Tehsil.
4.	Bashir Ahmad, M.Sc.	Developing farm plans for some selected farms of Lyallpur Tehsil.

1970

5.	Bashir Ahmad, M.Sc.	A study into economic comparison of bullock farming versus tractor farming in Lyallpur.
6.	Mohammad Zafar Iqbal, M.Sc.	An economic appraisal of combine harvesters, threshers and conventional methods in the central zone of West Pakistan.

RURAL SOCIOLOGY

1970

1.	Fida Mohammad, M.Sc.	Leadership structure in Umar Khel Community, Tehsil Swabi and District Mardan.
2.	Riaz Hussain, M.Sc.	A comparative study of health practices in two rural communities in Tehsil Toba Tek Singh, District Lyallpur.
3.	Manzoor Ahmad, M.Sc.	A sociological survey of a village in the Punjab.
4.	Mohammad Iqbal Khan, M.Sc.	Socio-economic impact of watershed management.

S.No.	Name & Degree	Title of the Thesis
5.	Nazir Hussain, M.Sc.	Influence of reference group in the acceptance of improved agricultural technology.

FIBER TECHNOLOGY

1969

1.	Mohammad Ashraf Salimi, M.Sc.	Some technological studies in fiber fineness, strength and other characters of promising cotton varieties.
2.	Abdul Wadood Bhatti, M.Sc.	Studies in chemical constituents of cotton fibre and its relationship to physical properties and spinning performance in some Pak. Cottons.
3.	Abdul Ahad, M.Sc.	Some technological studies in variations of fibre quality in LSS, AC 134, AC 307 and AC 321 under different treatments at Lyallpur.
4.	Noor Mohammad, M.Sc.	Some technological studies in fibre quality of long and extra long staple cotton as influenced by season and other factors.
5.	Mohammad Nawaz, M.Sc.	Some technological studies in spinning performance of AC-134 and some other cottons, and the effect of mercerization and parchmentizing on yarn quality.

1970

6.	Abdul Khaliq, M.Sc.	Some studies in fibre quality and spinning performance of new strains of cotton at Lyallpur.
7.	Nisar Ahmad Jamil, M.Sc.	Some studies in cellulose contents of khip fibre (*Lepladenia pyrotechnica*).

FOOD TECHNOLOGY

1969

1.	M. A. Karim, M.Sc.	Canning of mango pulp; effect on canning and storage on the quality.

S.No.	Name & Degree	Title of the Thesis
2.	Abdul Majib, M.Sc.	Comparison of butter from cow and buffalo stored at room temperature and at 40^{0} F at different humidity levels.
3.	S. Aulad Hussain, M.Sc.	Effect of packing techniques on the quality of dehydrated powdered garlic as assessed by chemical and organoleptic tests.
4.	Asghar Ali Shah, M.Sc.	Determination of adulteration in milk by lactomotric and golding's beads methods.
5.	Mohammad Usaf, M.Sc.	Effect of different additives on the quality of bottled mango juice.
6.	Ikramul Haq Amjad, M.Sc.	The utility of soybean oil as a food product.
	1970	
7.	H.A.H. Mahmood Mosselly, M.Sc.	Studies of the effect of different methods of dehydration and storage conditions on the quality of tomato powder.
8.	M.A.H.M. Anims Aslam, M.Sc.	Effect of various additives on bottled preserved orange juice.
9.	Abdul Halim M.Sc.	Studies on the evaluation of important tomato varieties for the preparation of tomato ketchup.
10.	Tariq Rashid, M.Sc.	A study on the enrichment of Nans with Lysine, certain Vitamins and Minerals.
11.	Ghulam Mohyuddin, M.Sc.	Study of the blending mango and mango juices.
12.	Mohammad Akram, M.Sc.	Effect of pH and temperature on the reducing sugars contents during clarification of cane juice.
13.	Naeem Haider, M.Sc.	Effect of replacement of sucrose by liquid glucose on quality of mango juice.
14.	Mian Abdul Malik, M.Sc.	Curing of dates.

S.No.	Name & Degree	Title of the Thesis
15.	Mohammad Rafiq, M.Sc.	Some studies on the blended mango and orange squash.
16.	Pervez Kausar, M.Sc.	Some studies on the characterization and determination of various protein factors in some important wheat varieties in Pakistan.
17.	Khalid,	The development and evaluation of a protein rich snack food for school children.

ANIMAL BREEDING AND GENETICS

1969

1.	Rashid Javaid, M.Sc.	Heritability estimates of age at first calving and calving interval in Nili-Ravi buffaloes.
2.	Khadam Hussain Malik, M.Sc.	Heritability estimate of birth weight in Nili-Ravi buffaloe calves.
3.	Hashmat Ali Khan, M.Sc.	Preservation of bull semen at room temperature.

1970

4.	S. M. Akhtar, M.Sc.	Evaluation of dilutors for preservation of bull semen at 40°F under local conditions.
5.	Naseem Ahmad Cheema, M.Sc.	Influence of extenders containing skim milk powder, glucose, fructose, and glycerol on bovine semen stored at 50°C.

LIVESTOCK MANAGEMENT

1969

1.	Mehmood Ahmad, M.Sc.	Preservation of fat tailed rams spermatozoa in various diluents and at different dilution rates.
2.	Mohammad Aslam, M.Sc.	Influence of age on population, type and diameter of wool follic in Kajli Sheep.

S.No.	Name & Degree	Title of the Thesis
3.	Mohammad Afzal, M.Sc.	Histological studies of wool follicle in Lohi Sheep.
4.	Fazal Din, M.Sc.	Bullock power (effect of environmental temperature on body temperature, pulse, respiration and work efficiency).
5.	Shabbaz Ali Butt, M.Sc.	Bullock power requirements in West Pakistan.
	1970	
6.	Afzal Ahmad Yousaf M.Sc.	Cost of milk production in cows and buffaloes in and around Lyallpur.
	NUTRITION	
	1969	
1.	Mohammad Laiq Khan, M.Sc.	Comparative feeding value of cottonseed cake, til cake and ground-nut cake in broiler's room.
2.	Letty June Baurico Pasco, M.Sc.	The influence of the plane of nutrition on the growth and feed efficiency of broiler chicks.
3.	Wheeda Akhtar Qureshi, M.Sc.	To determine losses of nutrients during preparation and cooking of lamb mutton and poultry meat.
4.	Mohammad Yousaf, M.Sc.	Nutritive value of cereals as affected by blending with fish flour; skimmed milk powder and dried beef.
5.	Mohammad Siddiq Tahir, M.Sc.	Comparative utility of different rations, with and without added urea; comparative utilization of urea by buffaloes, cows and old bullocks.
6.	Miss Ameena Nuzhat, M.Sc.	Losses of nutrients during cooking of cow, buffalo and fish meat.
7.	S. Ibrar Hussain Gilani, Ph.D.	Availability of protein nitrogen in the rumen ingesta of fistulated buffalo bulls fed single or mixed sources of nitrogen with and without added grain.

S.No.	Name & Degree	Title of the Thesis
8.	Nisar Ahmad Sial, M.Sc.	Influence of varying levels of protein, energy and calcium on the performance of broiler chicks.
9.	Munawar Ahmad Sial, Ph.D.	Determination of economical and efficient rations for dairy cows in West Pakistan.
10.	Rashid Ahmad, M.Sc.	Nutrient losses in milk of various species of animals during processing in Pakistan.

POULTRY HUSBANDRY

1969

1.	Masood Ahmad Sheikh, M.Sc.	Study on the hatchability of White Leghorn eggs stored in polyethlene bags for varying periods.
2.	Taimur Shah Khan, M.Sc.	Effects of dextrose on the growth rate of Lyallpur Silver Black.
3.	Mohammad Altaf Ali, M.Sc.	Effect of supplementation of vitamin 'C' during summer on egg production of White Leghorn layers.

MICROBIOLOGY

1969

1.	Hameed Afzal, M.Sc.	Study on the cause of infection of foot and mouth disease virus type 'O' in buffaloes in West Pakistan.
2.	Mohammad Arshad Khan, M.Sc.	Epizootiology of New Castle disease in wild birds.
3.	Mukhtar Ahmad Khan, M.Sc.	Studies on the antigenic variants of New Castle (Ranikhet) disease virus in West Pakistan.
4.	Mohammad Bashir, M.Sc.	Salmonellosis in poultry.
5.	Abdul Haq Awan, M.Sc.	A study of the surface microflora of the udder of buffaloes with emphasis on identification, antibiotic sensitivity and effect of selective disinfectants.

S.No.	Name & Degree	Title of the Thesis
6.	Mohammad Khalid, M.Sc.	Studies on the isolation and characterization of trichophyton species causing ringworm in animals in West Pakistan.
7.	Munir Ahmad Tariq, M.Sc.	A study on the microflora of wild birds and their epizootiological importance.
8.	Shahida M. Qureshi, M.Sc.	Studies on colibacillosis in chicks in West Pakistan.
9.	Abdul Hamid Arshad, M.Sc.	Studies on the vaginal microflora of buffaloes and their relationship with infertility.

1970

10.	Noor Ahmad Khan, M.Sc.	Investigations on the bacterial infections of the respiratory tract of sheep.
11.	Laique Ahmad, M.Sc.	Studies on the microflora of cows and buffaloes.
12.	Hazoor Ahmad Khan, M.Sc.	Studies on the isolation and characterization of micro-organisms causing wound infections in cattle and buffaloes.

PARASITOLOGY

1969

1.	Abdul Raoof Kazam, M.Sc.	Taxonomy and life-cycle of the species of genus *Heterakis Dujardin*, 1845 in domestic fowl in West Pakistan.

PATHOLOGY

1970

1.	Munawaruddin Dhamial, M.Sc.	Studies on the haematology and pathology of *Avian spiro* chaetosis in chicks.

S.No.	Name & Degree	Title of the Thesis

PHYSIOLOGY AND PHARMACOLOGY

1969

1.	Mohammad Niaz, M.Sc.	Effect of seasonal variation on body temperature, respiration and semen characteristics of dairy bulls.
2.	Makhdoom Ali, M.Sc.	The study of reticulo-rumen movements in sheep and the factors affecting their activity.

AGRICULTURAL EXTENSION

1969

1.	Mohammad Idress, M.Sc.	A study of the farmers attitude toward the use of fertilizers for increase in crop yield in Lyallpur Tehsil.
2.	Ali Ahmad, M.Sc.	A study of the opinions of Chairman of Union Council towards agricultural extension programs in Lyallpur District.
3.	Zahur Ahmad, M.Sc.	A study of the effectiveness of different sources of information regarding selected improved agricultural practices in Toba Tek Singh Tehsil of Lyallpur District.
4.	Taj Mohammad Khan, M.Sc.	A study of effectiveness of "Grow More Maize Programme" in Sargodha Tehsil.
5.	Bashir Ahmad, M.Sc.	Studies into the appraisal of Agricultural Extension activities in Phalia Tehsil of Gujrat District.
6.	Mohammad Rafique Saroya, M.Sc.	A study to determine the attitude of farmers toward Mexican Wheats in Sheikhupura Tehsil.
7.	Mohammad Shafi, M.Sc.	A study of the effects of Agri. Extension Activities on the development of Farming Community in Campbellpur Tehsil.

S.No.	Name & Degree	Title of the Thesis
8.	Mohammad Munsha, M.Sc.	A study of the land use patterns of the farmers of Tehsil Chunian District Lahore.
9.	Abdul Rahman, M.Sc.	A study of the adoption of Agricultural Extension recommendations and their effectiveness in Dera Ghazi Khan Tehsil.
10.	Abdus Samad, M.Sc.	A study of the plant protection measures adopted by the farmers against pests and diseases of potato crop in Sialkot District.
11.	M. Shafique Khan, M.Sc.	Evaluation of existing extension methods in the department of Agriculture.
12.	Hameed Ahmad Chaudhry, M.Sc.	A study of the factors which in adoption of improved agricultural practices by the farmers of Wazirabad Tehsil of Gujranwala District.
13.	Mukhtar Ahmad, M.Sc.	The study of effectiveness of improved land reclamation practices and their adoption by the farmers of Chiniot Tehsil Distt. Jhang.
14.	Ghulam Mohammad, M.Sc.	To study the effectiveness of extension teaching methods in disseminating information on plant protection measure and use of fertilizers in Lyallpur Tehsil.
15.	Abdul Hussain, M.Sc.	The study of the opinion of the Chairman & B.D. Members of Union Council regarding the Agricultural Extension Programme in Daska Tehsil of Sialkot District.

AGRICULTURAL EDUCATION & TEACHER TRAINING

1970

1.	M. Akram Butt, M.Ed.	A study of the effectiveness of communication between the teacher and the taught.

S.No.	Name & Degree	Title of the Thesis
2.	Mohammad Jamil M.Ed.	Impact of internal system of examination on the achievements of students in the West Pakistan Agricultural University, Lyallpur.
3.	Mohammad Sadiq M.Ed.	Development of a criterion for evaluating teachers.
4.	Kh. Mahfooz Hassan, M.Ed.	A study of the problem of overcrowdedness in the Secondary Schools of Lyallpur.

PROPOSED RESEARCH PROGRAM ON GRAM WILT AND GRAM BLIGHT

by

C. Gardner Shaw, Consultant
Professor and Chairman, Department of Plant Pathology
Washington State University
April 1970

I. Introduction:

The normal project outline has been modified in this presentation since: (1) a statement fully justifying increased emphasis on grain legumes, particularly gram, and on the diseases of these crops has previously been prepared by Dr. A. O. Shaw; (2) Dr. A. G. Kausar has summarized through 1960 previous work on pulse diseases in Pakistan ("Fifty years of Investigations on Plant Diseases at Agricultural College and Research Institute, Lyallpur". Published in 1960 by Department of Agriculture, West Pakistan). His publications since 1960 (subsequently cited) summarize the important work done on gram diseases in Pakistan in the past ten years. Progress Reports of the Regional Pulse Improvement Project USDA, ARS, and US AID and cooperating agencies and universities in India and Iran report the other significant research being performed on pulses and applicable to Pakistan (the most recent report available is #6). The mimeographed report of the Pulse Improvement Project Seminar held at Karaj Agricultural College, University of Teheran, January 7 to 9, 1969, also contains excellent summaries of recent research. These reports cite references to other pertinent literature. A summary of the author's research findings during the two months of his appointment is appended (Appendix V).

II. Selection Versus Breeding

Selection for disease resistance, at first unintentionally and more recently as a result of a direct search, has played a major and significant role in the

development of the gram varieties currently grown in Pakistan and other countries. In each country, and even in each distinct agricultural area, selective processes have resulted in the varieties of gram currently grown having fair to good productive potential, desirable season of maturity, and some tolerance to the two most important diseases. For thousands of years the process of selection for disease resistance was "natural", i.e., seeds for the next crop was of necessity that produced by plants which survived previous disease epidemics. During this period selection for high yield, particularly in non-epidemic years, undoubtedly involved more objectivity than did selection for disease resistance.

Only in recent time (actually since 1930) has there been a planned program in Pakistan aimed at developing varieties with disease resistance. In the early thirties work to evolve Blight resistant types was started at Rawalpindi and Campbellpur. Simultaneously gram seeds were imported from Italy, USA, Mexico, Australia, Iraq, Bulgaria, Egypt and Morocco. These selections were extensively tested for Wilt and Blight, and consequently F8, a yellow seeded, rough surface and bold-grain type was selected, multiplied and released in 1938-39 for cultivation in Blight affected areas.

Hybridization among the local and exotic materials in different combinations was attempted on an extensive scale. PB 7 was crossed with F8 and from the subsequent generations a higher yielding, Blight resistant and relatively early ripenning strain, C12/34 was approved in 1942 for areas of Rawalpindi, Campbellpur, Jhelum and Mianwali. Further efforts led to the evolution of a famous gram variety, C612 (F8 x C144), which was approved for general cultivation in 1952. Since then a new strain, 93 C727 (F8 x PB 7), was approved (1963) for general cultivation. This strain is said to be Blight resistant. Dr. A. G. Kausar, Chairman, Department of

Plant Pathology at WPAU has played a significant role in the selection of gram varieties with tolerance for each of the two main diseases of gram, Blight and Wilt. Mr. Abdullah Khan of the Department of Plant Breeding has played a vital role in the breeding work done to date.

In the past twenty-five years (since partition) this program has, however, involved for the most part material already available within Pakistan. Various impediments have prevented free exchange and receipt of gram germ-plasm from other gram-producing countries.

One comes to the conclusion that continued selection by itself within and among varieties originating in Pakistan offers little hope of providing much better sources of resistance to either Gram Blight or Gram Wilt than have been found to date.

In 1968 and 1969, Dr. A. O. Shaw obtained a part of the world collection of gram. Obviously with the world collection now available, selection must continue and should be even more productive. However, one important aspect of any productive breeding program appears to have received less attention than it deserves to date in the search for disease resistance in gram.

With a self-pollinated crop such as gram, selection alone almost certainly will not provide the final solution to the problem. Much more emphasis must be given in the program to crossing. Particularly in Root Rot diseases factors for resistance have repeatedly been demonstrated to be cumulative. Some varieties currently being grown at Lyallpur are obviously more tolerant to Root Rot (Wilt) than are other varieties. Similarly, there is tremendous variation in tolerance to Blight in the varieties planted at Campbellpur. As soon as possible an extensive program involving the crossing of those varieties now available and known to have tolerance to the two important diseases must be initiated. Hand pollination

of gram is a most tedious and delicate process and the product of each successful cross is frustratingly small (one or two seeds). I challenge each and every pathologist involved in this program to become a proficient pollinator of gram. Mr. Abdullah Khan has indicated his willingness to instruct any and all interested in the proper procedure. Only by the active participation of all can the crosses that need to be made be accomplished. It is the responsibility of pathologists to be able to make crosses in the crops with which they work. In programs aimed at developing sources of disease resistance the plant pathologist customarily carries his material through two to five generations before turning it over to the plant breeder. It is the pathologist's responsibility not only to find, but to concentrate, the factors for resistance to a particular disease in the lines he makes available to the plant breeder. Only by cross pollination can the diversity of recombination, obtained naturally with a cross pollinated crop, be obtained in a self-pollinated crop such as gram. The plant breeder's task is a difficult one because frequently disease resistant strains may be low yielding and may have many other undesirable characteristics. The plant breeder must transfer this resistance by making crosses to locally adapted, high yielding, short-season strains.

Much emphasis to date on developing resistance to both Gram Wilt and Gram Blight has been placed on selection. I urge henceforth a balanced and coordinated program in which both crossing and selection receive equal emphasis.

I sincerely believe the search for suitable disease-resistant material can only proceed rapidly if henceforth the actual crossing of different lines receives just as much emphasis, effort, and attention as has selection during the past twenty-plus years. Breeding for disease resistance in any crop must involve a proper balance between both crossing and selection. If anything, crossing is even

more important in a self-pollinated crop than in one which is cross-pollinated. Perhaps I beleaguer this point excessively, but I consider it the most important aspect of this entire report.

III. Handling of the world collection of gram

Unfortuantely the portion of the world collection received in 1969 arrived so late that it could not be planted at the optimum time (approximately October 10). Planting was completed by November 7, 1969. Some lines were lost and others produced very few seeds. Once harvest of the world collection has been completed and the lines that have been lost can be identified, every effort should be made to obtain seeds of the missing lines. Any of them may be a source of resistance to either Wilt or Blight; equally important some of the lines lost because of late planting may be an excellent source of early maturity and short season - exactly the characters needed in a gram variety to be planted following rice in large areas of West Pakistan.

Introduction of the world collection obviously necessitates the continuation of an extensive and intensive selection program, in an attempt to find additonal and better sources of disease resistance.

This selection program, however, must not depend - as has been for the most part true in the past - only on natural infection.

Because of varying climatic factors, epidemics of Gram Blight develop in some years, in other years (fortunately more numerous) the incidence of Gram Blight is low. Years of low Blight incidence result in many lines which actually have little or no resistance being continued in the trials until they are subjected to a year of severe epidemic. Similarly Wilt is more severe in years of low rain fall than in years with higher precipitation.

To speed up the selection process for resistance to both Wilt and Blight, natural incidences of both diseases must be supplemented with artificial inoculations. Suggested procedures are presented under the separate discussions of each disease (Appendices I and II).

Obviously it is most important to maintain and increase seeds of the different selections represented in the world collection.

1. Each line should be harvested separately. Within each line any obviously different plant or plants should also be harvested separately. There is no assurance that any given line is homogeneous. Sources of good characteristics may be missed unless plants deviating from the norm of a given line are selected. Any new lines added to the collection, whatever their source, should be separately numbered and a record kept of their origin. There has been two years of testing of the irradiated seeds. This material is an excellent source of new lines for the world collection. The 2.5 acres of bulk irradiated seeds should be harvested and seed saved. Assuming that considerable of this will have one or two genes difference, and also since much of it seems to have some resistance to Wilt, it should be planted at two or three locations in the Campbellpur area to test its resistance to Blight. Possibly some of the mutations may have resistance not only to Wilt but also to Blight.

2. A sacred reserve (say 25 seeds) of each individual line should be preserved under the best possible storage conditions. If less than 25 seeds exist of any line, first emphasis should be put on its increase.

3. Next a minimum of 50 seeds of each line should be planted in October, 1970, at Lyallpur solely for seed increase. The land selected for the seed-increase plot should be of excellent fertility, free of salinity, and preferably a site known from past history to be relatively free of Gram Wilt. If the site was in gram

during the 1969-70 year, all gram debris should be cleared from the field at the time of harvest in May of 1970. It is also recommended that it be deep plowed. It would be better to use land not planted to gram the previous year for seed increase. I would suggest that a soil analysis be made of the field selected for seed increase and fertilization be based on the soil analysis.

4. Only seeds beyond that needed for reserve and seed increase should be used for disease experimentation. Judicious decisions, based on the number of seed available will be necessary as to what experiments for determining disease reaction can be performed with each line. I suggest that no experiment involve less than 50 seeds (100 seeds would be preferable). The actual number of seeds to be planted of each line in each experiment should be decided in advance.

5. If I interpret the available literature correctly, length of row, rather than number of seeds planted has been the basis for most experiments in the past. Accurate determinations of germination, emergence, and disease incidence, can only be made if the number of seeds planted and the number of plants surviving at each significant stage of growth (or after each treatment) are determined and recorded.

6. The standard row spacing for gram is 1½ to 2 feet, the standard distance between plants within the row is apparently six inches but usually is not specified. These details must be recorded and be uniform within any given experiment. I am not yet sufficiently familiar with the crop to make judicious recommendations in regard to row and plant spacing. I can only point out that the stand density employed in commercial plantings may or may not be the best for (a) development of disease epidemics or (b) recording and interpretation of results. Indeed, these two objectives may themselves dictate different planting densities and thus necessitate compromise. For studies of the two major disease problems different planting densities may be desirable. A dense planting would be most favorable

for Blight; a less dense planting might well facilitate recording data in Wilt plots and should not reduce Wilt incidence as it might reduce Blight incidence. I am inclined to recommend a 18 inch spacing of rows and a six inch spacing of seed for experimental plots on Blight, and 24-inch row spacing and a six-inch spacing of plants within rows for Wilt.

IV. Plan for experimental testing of lines for resistance to Blight: The Gram Blight Nursery.

1. Planting to be at Campbellpur. Planting to be completed by October 15, 1970. Plots to be on land planted to susceptible varieties in 1969-70. Debris from the previous crop not to be removed from the field and not to be plowed under. At time of planting all living volunteer plants resulting from seeds in the debris left in the field are to be pulled up to avoid confusion in subsequent recording of data.
2. Plant at least 50 seeds (preferably 100) of each line to be tested. If less than 50 seeds are planted, it may be necessary to retest that line another year.
3. Plant one row of a variety known to be susceptible to Blight (e.g., C-7, C218/1, or BR 786 or some other equally susceptible variety) between each line to be tested. Also plant border rows of a susceptible variety across the ends of test plots.
4. Since this test should be as severe as possible, additional inoculum should be introduced subsequent to planting. Three steps are recommended for this.
   A. As previously recommended, plant in a field which had infected susceptible gram during the 1969-70 season and in which the debris from the previous crop was left unplowed in the field.

B. At harvest time this spring (late April or early May 1970), a large amount of gram debris from susceptible varieties currently being grown at Campbellpur should be collected and stored. It should be dry when harvested and kept as dry as feasible - store preferably in a shed or at least under canvas or plastic. Approximately one month after emergence of the test planting (i.e., November 20-25) this accumulation of debris should be spread evenly over all test plots (on both the lines to be tested and the intervening rows of susceptible varieties). This spreading of debris should be in addition to the debris left in the field at harvest time. This debris should be placed carefully so as not to break stems of growing plants. Indeed, if the debris permits, and the procedure is not too time consuming, dead infected gram stems should be placed upright among the living plants. This would greatly facilitate spread of inoculum.

Note: The introduction of additional debris will also introduce seeds from which volunteer plants probably will develop. The experimental plots should be rogued carefully several times to remove all such volunteer plants. Failure to do so may make it difficult to take accurate records on the test lines at the time of harvest. If rogued several times starting two to three weeks after spreading the debris, there should be no difficulty distinguishing between voluntary plants and plants under test.

C. In addition to post harvest debris and introduced debris, spore inoculum should also be applied to the Blight disease planting. Large amounts of inoculum can be obtained from a few stock cultures in a relatively short time. (See Appendix II-A for procedure). A back-pack sprayer is available with which to apply the inoculum. Limited experiments in April and May 1970 indicate that a continuous surface film of moisture for 18-24 hours is essential for infection. In April to May at Lyallpur, at least, these moisture conditions were obtained in the field only as a result of natural precipitation which occurred during one of the experimental inoculations. Spraying inoculum in the evening - followed by application of additional water by the back-pack sprayer the next morning - either did not provide sufficient total moisture or continuity of a moisture film for a sufficient consecutive period to result in appreciable infection unless this procedure was augmented by natural rainfall.

Therefore, it is suggested that inoculum be held ready from one month after emergence of the gram in the disease nursery and that, at the time of actual precipitation, spore suspensions be prepared and applied. Sufficient inoculum should be prepared in advance so that it can be applied both at the beginning of a rainy period, and at its termination. The entire plot, including inter-spaced susceptible rows, should be inoculated completely and uniformly. At that time of year (December) the stage of growth should be such that each plant can be sprayed individually to the drip-off stage.

The approximate concentration of spores per mm. in the spore suspension should be calculated with a bacterial counting chamber and viability of the inoculum confirmed by a germination test on agar medium.

If, on the basis of the spore germination test referred to above and the appearance of symptoms 8 to 12 days after spraying, the artificial inoculation is successful, it need not be repeated at more than approximately monthly intervals. But, it should be repeated at least four times during the growing season with the viability of the inoculum being checked, and its concentration determined each time. If any inoculation, particularly those in December, January and February, does not result in appreciable infection on the susceptible varieties, the inoculation should be repeated at the next rain.

5. Records and Data

Record:

(a) Percent emergence of each line (three weeks after planting).

(b) Date of initial symptoms of Ascochyts rabiei of each variety.

(c) Date of each artificial inoculation.

(d) Number of living plants in each line showing any sporulating lesions on December 15, January 15, February 15, March 15, at harvest.

(e) Total number of living plants in each line on December 15, January 15, February 15, and March 15.

(f) Overall disease rating at mid-season, one month before harvest, and at harvest for each line.

Note: Tag any individual plant obviously resistant in an otherwise susceptible line so that it can be harvested separately.

The following is a suggested rating scale:

(4) All plants dead.

(3) Multiple sporulating lesions on stems, pods and leaves.

(2) Sporulating lesions sparse.

(1) Non-sporulating lesions only.

(0) No indication of infection.

Note: Only experience can determine if this is a realistic rating scale. The intermediate categories (1, 2, 3) should be modified as experience dictates. Whatever rating scale is employed should be employed consistently throughout the season.

Additional intermediate ratings (e.g. 3+, 3, 3-) may be employed if deemed useful.

6. Crossing: Most Important!

As soon as flowering is under way cross pollinations between the lines showing the lowest incidence of Ascochyta blight should be initiated. Since a 10-40% "take" is the most that can be expected from hand pollination, I suggest a minimum ten pollinations be attempted for each cross. This should give five to six seeds. Crosses should be made in all combinations progressing on a priority basis from crosses between the varieties rated to have the highest resistance to crosses between those with somewhat less resistance.

Crosses should also be made between the most resistant lines and the standard recommended varieties.

Exchange of information between those responsible for the Gram Blight Nursery at Campbellpur and those in charge of the Wilt Nursery at Lyallpur is essential. Each team should independently make crosses between the varieties reported most resistant to Wilt and most resistant to Blight. This will double the chances for successful crosses between sources of resistance to both diseases.

The Seed Increase Nursery and both disease nurseries should be toured by agronomists and crosses also made between lines selected by the agronomists and those selected for Blight or Wilt resistance by the plant pathologists. There must be a complete exchange of information and material and good and continuing cooperation. The successful accomplishment of these crosses is the most important aspect of the total program. Rapid development and release of improved gram varieties cannot be accomplished unless these crosses are made.

7. Harvesting of F 1 seeds

Each series of crosses must be harvested separately, and completely identified as to origin (i.e., parents).

It must be remembered that the seeds and subsequent plants resulting from the original crosses are the F 1 generation. They represent new hetergeneous combinations of germ plasm. They are to be used to produce the seeds of the F 2 generations in which segregation will occur. The F 1 generation is not itself to be tested for resistance to Blight or Wilt. Seeds produced from the original crosses should be planted in the best possible site, i.e.,

where Blight will not be a factor and in land where Wilt is not known to be a problem.

The sole objective is to get maximum seed increase from the F 1 plants.

If the conditions under which a summer crop of gram could be produced were known, valuable time could be saved in the program. However, the environmental factors influencing flowering (e.g., temperature and particularly day length) are apparently not known. Until they are determined it would be too risky to attempt to grow seeds of the F 1 generation in the off-season and perhaps lose it. However, experiments should be started this April and May with standard varieties (of which plenty of seeds are available) to see if gram can be brought to flower and maturity between May and October.

Suggested approaches are:

a. Planting at higher cooler elevations (5-8,000 ft.) in West Pakistan, (e.g., Murree).

b. Even though growth chambers are not available, experiments could be conducted with potted plants in dark rooms. This would require a daily shifting from dark to light. In view of the normal time of flowering of gram, a day light period of ten to eleven hours and darkness for thirteen to fourteen hours is suggested.

c. Prompt shipment of some seeds of any crosses of which there is some extra to other countries (e.g., Iran or Washington State in U.S.A.) so that it can be increased there. Australia or New Zealand offers the opportunity

to reverse seasons and perhaps eliminate the problem of photoperiodicity.

8. Handling of F 2 Seeds

F 2 seeds produced by the F 1 plants should be divided into three units; one for testing in the Wilt Nursery, one for testing in the Blight Nursery and one for seed increase. Segregation will by no means be complete in the F 2. Therefore, disease readings should be taken on an individual plant basis. New lines (i.e., individual plants) must be evaluated at the time of flowering and crosses again made between promising individuals. In the F 2 and from this stage forward there must be a rigorous screening of all material. Only 10 to 20% at most should be saved; otherwise the numbers of lines involved will become impossibly large.

V. Plan for experimental testing of lines for resistance to Wilt: The Gram Wilt Nursery

1. Planting to be at Lyallpur and to completed by October 15, 1970. Planting to be on land where Wilt is known to have occurred in previous years, and where gram was grown the previous year. Debris from previous crop is to be left, and additional debris from Wilt-infected fields is to be added and disced into the soil.
2. Plant at least 50 (preferably 100) seeds of each line to be tested.
3. Half the seeds of each line are to be inoculated with seed inoculum of Fusarium isolates. (Directions for preparing Fusarium inoculum on gram seed and spore suspensions are in Appendix I A.) Each seed of gram is to have two Fusarium infested seeds planted with it, one above and one below the seed under test.

4. Percentage of emergence is to be recorded separately in the inoculated and uninoculated portions of each line.

5. One, two, three, and four months after emergence (approximately December 1, January 1, February 1, and March 1) a spore suspension is to be sprayed on the ground at the crown of each plant. Spray until standing water is obvious. The objective is to actually wash Fusarium spores into the root zone. Directions for preparing spore suspensions of Fusarium are contained in Appendix I A.

6. Records and Data

   Record:

   (a) Percent emergence in the seed inoculated and seed uninoculated portions of each line. These records are to be taken one month after emergence (approximately December 1).

   (b) On January 1, February 1, March 1, and April 1, record number of living, unwilted plants, number of wilted (yellowed) plants, number of dead plants, and number of "missing" plants. The latter to be based on count of living plants as of December 1.

   Note: Wilt will occasionally occur laterally in one side or branch of a plant while the rest of the plant is green and healthy. Count such plants as wilted. Also, the final reading on Wilt must be taken two to four weeks prior to maturity. Once the entire planting begins to brown due to maturity it is too late for an accurate determination of the percent of Wilt.

7. Crossing. Most Important!

   As discussed under the Gram Blight Nursery, the disease readings for Wilt taken at the time of flowering are the ones which must be the

basis for selecting parents for crosses. Crosses should be made between (1) lines showing obvious resistance or tolerance for Wilt; (2) Wilt tolerant lines and Blight resistant lines; (3) Wilt tolerant lines and standard varieties; (4) Wilt tolerant lines and lines recommended by agronomists because of their yield potential, length of season, etc. The amount of crossing is sufficient to keep all involved busy - pathologists exclusively with disease aspects, agronomists and plant breeders with the incorporation of disease resistance into lines with good agronomic characters.

8. Harvesting F 1 seeds, and growth of F 1 generation:

The same points made in regard to the F 1 of the Blight Nursery also apply here. Increase, not testing, is the objective of the F 1 generation. Development of procedures for producing an "off-season" increase of the F 1, are equally important for Wilt as for Blight.

9. Handling of F 2.

The points made under this heading in regard to the Blight Nursery are equally applicable here.

APPENDICES

I. SPECIAL ASPECTS IN REGARD TO GRAM WILT

A. Preparation of Mass Inoculum of Fusarium. (Seed inoculum and spore suspensions.)

Most cultures of Fusarium produce abundant spores on a variety of media. One means of producing mass inoculum of Fusarium is outlined here.

Starting with a stock test tube culture of Fusarium, make transfers to fresh test tube slants. Use malt, PDA or gram agar. While the sub-cultures are developing (which takes six to seven days), prepare sterile gram seeds in the following manner:

1. Use wide mouth quart jars (canning jars) if available. Ehrylmeyer flasks or beer bottles can be used if necessary, but wide mouth jars are best as they permit easy removal of the final inoculum.
2. Fill one-fourth full with dry, whole gram seeds. Cover seeds with water. Allow to stand 12 to 16 hours replenishing water as it is absorbed by the seeds.
3. Plug with cotton, cover plug with newspaper and tie newspaper around neck of bottle or flask.
4. Sterilize (15 lbs. pressure for 15 minutes) on each of three successive days.

   If, after the first sterilization, there is not some obvious free water (actually a decoction) present (approximately one-fourth to one-half inch) in the bottom of the jar or flask, add some sterile water. After the third sterilization there should still be some free water (to the depth of one layer of seed

5. Let the jars of sterilized seeds stand for two days. If there is any evidence of bacterial action in any of the jars (frothiness in the free water decoction), sterilize those jars again.
6. By the time you are sure the jars of gram seeds are sterile (six to seven days) the subcultures of Fusarium will be ready. Flood each subculture with 10 ml. of sterile water, shake, remove one drop aseptically to a microscope slide and check for the presence of abundant Fusarium spores. Use only sporulating subcultures to inoculate the sterile gram seeds. Pour the spore suspension from one subculture into one of the jars of sterile gram seeds. Shake to distribute the spore suspension.
7. Reshake each inoculated jar of gram seeds each morning and night. After three to four days each seed will be coated with abundant mycelium of Fusarium. Each seed may be used as inoculum, the individual seeds being planted with sterile gram seeds or placed in the soil, two to three inches deep in the root zone of developing gram plants. Or the entire jar can be filled with sterile water, shaken, and the water poured off to give a massive spore suspension. If kept sterile the same jar may be a continuing source of spores on successive days. However, discard any mass culture upon any indication of contamination.

For inoculation experiments in the Wilt Nursery, it is recommended that spore suspensions from at least three different original isolates be combined. Records should be kept of the source (i.e., location) of all cultures used for inoculation.

B. Methods of Inoculating with Fusarium.

1. The use of debris, Fusarium grown on gram seeds and spore suspensions (to flood the soil around the crown) as inoculum has been discussed in connection with the Fusarium Wilt Nursery.
2. Should flooding the surface of the ground around the crown of each plant not result in infection from either the December or January inoculations (if these inoculations are negative, it will be obvious by the February 1 reading), it is suggested that a pointed steel bar (one-fourth inch diameter) be pressed to a depth of six inches along side each plant (two to three inches to the side of the crown of each plant). Weld a two to three inch disc on the steel bar six inches from the tip so the depth of each hole will be uniform. Then pour the inoculum into this hole. The penetration of the bar will injure and break some roots and provide a suitable portal of entry if wounding is necessary. (Be sure the metal disc does not touch or injure the lower stem of the plant).
3. Spore suspensions can be injected either into the soil next to the crown or into the plant itself with a hypodermic needle. Injection of individual plants is a much more time-consuming procedure than flooding and should first be attempted with potted gram to determine its effectiveness before resorting to extensive field tests. As gram matures its lower stem and upper main root

become woody and successful insertion of the hypodermic needle without injury is very difficult. Controls in which sterile water only is injected must be run with each experiment, so that death from injury alone can be distinguished from wilting and death due to Fusarium inoculation.

4. Fusarium inoculum grown on gram seeds can be used to inoculate at the time of planting gram in either pots or in the field (see discussion of this technique under Gram Wilt Nursery).
5. Another technique for testing small numbers of plants is to start them in sterile soil in pots. When approximately one month old remove carefully the entire soil ball from the pot, wash the soil from the roots, dip the roots in a spore suspension and repot. Even careful removal of plants from pots will injure sufficient small roots to provide portals of entry for the pathogen. No obvious attempt to injure should be necessary. A control consisting of replanted but not inoculated plants should be run to determine the survival of transplants in the absence of inoculation.
6. Inoculation in conjunction with nematodes is discussed under the heading of Nematodes. (See Appendix III).

C. Influence of Irrigation on Gram Wilt.

It has long been recognized that the occurrence of Gram Wilt is inversely correlated with rainfall (A. G. Kausar, Pak. Jour. Agri. Sci. 5:264-237, 1968). Where average rainfall is consistently high

during the growing season, Wilt is of minor importance; where rainfall is low, Wilt is serious. In any one location the same relation holds--in years of low rainfall Wilt is serious, in years of high rainfall it is of minor importance. Furthermore, as long ago as 1922, (Milne, Report of the Economic Botanist to Govt. Punjab for year ending 30th June 1921. Report on the operations of the Dept. Agr. Punjab, Part II. Govt. Print. Press. Lahore) it was reported that the disease was less severe alongside water channels.

In spite of these observations, no investigations have been undertaken to study the influence of irrigation practices on Gram Wilt or gram production. Lacking field data on the effects of irrigation, it is not surprising that no recommendations on the irrigation of gram have been made. Gram is not normally irrigated at all even where water is readily available. A single field trial in the current season indicates that such investigations could produce significant results. Of three adjacent fields all planted to the same variety (C 727) at the same time, one received no irrigation, another one irrigation in early March, and the third two irrigations (one inadvertantly a few days after the first). Wilt was more prevalent, and showed up earlier in the field that received one irrigation. There was approximately 5% Wilt in the field that received two irrigations.

R. J. Cook (1969) working on the influence of soil moisture on germination of propagules of Fusarium "culmorum" has shown that spore germination can occur over a relatively wide range of soil moistures. However, at high moisture levels (approaching 100% relative humidity) other microorganisms, particularly bacteria, are also very

active. As result of the biological activity of these other micro-organisms germinating propagules of Fusarium are apparently lysed. Thus, at high moisture levels although Fusarium germinates readily, infection is low. At lower relative humidities (90-92 RH) Fusarium propagules still germinate, but the activity of other components of the soil microflora is markedly reduced. Fusarium germ tubes are not lysed and infection of susceptible crops is much higher at low levels of moisture.

A series of experiments on the influence of irrigation on Gram Wilt could result in the development of significant recommendations for those areas where water is available for the irrigation of gram.

The following series of experiments is suggested. These should be conducted in fields where Wilt is known to be prevalent during dry growing seasons. The irrigations employed should be replicated at least once. These experiments should be cooperatively planned and conducted by both plant pathologists and agronomists.

1. Irrigation for three to four days approximately two weeks after harvest.
2. Irrigation for three to four days approximately two weeks before planting.
3. Combination of 1 and 2 above. (These first three schedules are aimed at ascertaining if flooding can be used to reduce Fusarium inoculum in the soil).
4. Irrigation on single dates after planting.
    a. December 1
    b. January 1

BEST AVAILABLE COPY

c. February 1

d. March 1

5. Irrigation on two, three, four, and five dates in all combinations. If this comprises too large an experiment for a single year, I would suggest combinations of two dates and one plot which received all five irrigations. The same experiment could be conducted with and without the addition of *Fusarium* inoculum, but I would suggest postponing a combined irrigation plus inoculum experiment until additional information on the success of various field inoculation techniques with *Fusarium* and initial results of the first irrigation experiment have been obtained.

D. Variation in Pathogenicity of *Fusarium* Isolates and Their Identity

Fusarium has been the predominant fungus isolated by the author from roots of wilted plants. It has been obtained consistently and uniformly. No other pathogenic fungus has been identified in the isolates obtained. There are, however, obvious cultural differences in the *Fusarium* isolations made. Additional isolations probably would provide even greater variety.

The pathogenicity of various isolates must be determined. Those demonstrated to be pathogenic (and only those) should be sent to Dr. William Snyder, Department of Plant Pathology, University of California, Berkeley, California, for identification. There may be more than one species or variety, and very likely several races involved.

BEST AVAILABLE COPY

Precise determination of pathogenicity of different isolates must await the initial selection of series of gram varieties showing various degrees of resistance, tolerance, and susceptibility to one pathogenic isolate. Once such a series is designated all isolates can be tested against this series to determine if races differing in pathogenicity exist.

BEST AVAILABLE COPY

II. Special Aspects in Regard to Gram Blight

A. Preparation of mass inoculum of *Ascochyta rabiei*.

Gram, oatmeal, and malt agars have proven very satisfactory from abundant sporulation of *Ascochyta rabiei*. It has, however, proven very difficult to keep gram agar free of contamination. I, therefore, recommend that malt agar be used during the 1970-71 season for the growth of mass inoculum of *Ascochyta rabiei*.

Malt agar has the following composition:

Defco Malt Extract	50 grams.
Agar	20-30 grams.
Lactic Acid	10 drops.
Water to	1000 ml.

The ingredients are melted and poured into test tubes (10-20 ml. each) flasks (2/3 full), or beer bottles (40 ml.), the containers plugged with cotton and then sterilized (15 minutes at 15 lbs. pressure per square inch). Tubes should be slanted and bottles laid on their sides when removed from the autoclave. While still hot, malt agar can be poured from flasks into petri dishes to provide a broad surface for inoculation. However, beer bottles are even more effective than petri dishes for mass cultures (less chance for contamination) and far less expensive. Quart soft drink bottles would provide an even greater surface for growing inoculum. About 40 ml. of a medium in a beer bottle laid on its side gives a broad surface for cultural growth and is not so much that the cotton plug is wetted by the agar. Prepare bottle or tube and plug all media one to two weeks before actually needed. This

provides time for excess moisture to evaporate and permits any containers that become contaminated to be identified and discarded. Test tube stock cultures of some ten different isolates of Ascochyta rabiei have been prepared and are available.

From one of these stock cultures make two subcultures by transferring to two other tubes of sterile malt agar. Keep one to replace the original stock culture in case it becomes contaminated. After ten days abdundant pycnidia should develop on the surface of the subculture. This will be apparent by a darkening of the surface and the development of minute "pimples" on the surface of the culture. Flood one subculture with 10 ml. of sterile water. Let stand for two to five hours and agitate it gently several times. Using aseptic techniques remove a drop of the water to a microscope to be certain abundant spores are present.

Then aseptically pour the spore suspension from the original subculture successively into as many (three to ten) bottles as you need for abundant inoculum. Drain off the excess spore suspension from each bottle and replug. In seven to ten days each bottle will provide massive numbers of spores. These are removed from the culture by flooding each bottle with 10-20 ml. sterile water and again gently agitating several times during two to four hours.

If all steps are done carefully, mass cultures can be retained and reused. New crops of spores are produced in 24 to 48 hours. However, any contaminated cultures should be discarded. The spore concentration in any suspension prepared for spraying should be checked. The final dilution in the spray tank should always contain 200 to 500 spores per ml. as determined with a bacterial counting chamber. Final dilution in the spray tank does not require the maintenance of sterile conditions. The viability of the inoculum

should also be checked--either on a glass slide in a petri dish moist chamber or by using the spore inoculum to inoculate a test tube, petri dish, or another bottle containing media.

Note: If facilities are not available at Campbellpur for preparation and sterilization of media, these steps will have to be performed at Lyallpur and mass cultures taken to Campbellpur as needed (approximately once per month). Lack of the required facilities at Campbellpur will be an inconvenience and will require careful scheduling but is not cause for not undertaking spore inoculation.

Once mass cultures have started to sporulate they can be held in a sterile condition until actually needed (one to three months). After prolonged storage the viability of spores obtained from the culture should be checked. It may be possible to revitalize old cultures by flooding them with sterile water for three to five hours, discarding the initial spore suspension, and reflooding them with sterile water after 24 to 48 hours.

B. Inoculation of single plants (or potted plants) with *Ascochyta rabiei*.

In some instances it will be desirable to inoculate potted plants or individual plants in the field. A hand atomizer should be more effective for such inoculations than the much larger back sprayer. Spore suspension should be prepared as described elsewhere. Soil in the pots or around plants in the field should be well watered. The spore suspension should be applied to the point of dripoff, and the individual plant or the pot enclosed with plastic for 24 to 48 hours. Pots should be placed in the shade so that the

plants will not become excessively hot under the plastic. In the field the plastic should be brought to the ground level and held in place. Field inoculations during the winter months should be successful using this procedure.

This procedure will permit the testing of lines of which but few seeds are available and the testing of some material at Lyallpur even though the sprayer is committed for most of the growing season at Campbellpur.

This technique can also be used to ascertain the minimum time free moisture must be maintained on plants in order to obtain infection. The number of times during a growing season free moisture persists long enough for infection to occur may be far more important than total rainfall. Seven pots can be inoculated and covered and the plastic removed from each pot at two-hour intervals from 12 to 24 hours. To facilitate rapid and uniform drying at the end of the inoculation period pots of inoculated plants should be placed in front of an electric fan for one hour immediately after removal of the plastic cover. This experiment should be performed once per month (November through April) both in the greenhouse and out of doors to ascertain (1) the influence of temperature on the infection process and (2) differences in susceptibility at different stages of growth. Temperature, relative humidity, and rainfall should be recorded at field sites.

Potted plants should provide better material for microscopic study of the germination and infection processes than field material, since material can be removed from plants inoculated as described above at known time intervals after initial inoculation. Also there should be less dust on the vegetation of potted plants grown in the greenhouse than on field grown plants. The infection process should be studied on leaves, pods and stems. Both

epidermal strips and cross sections should be studied. The collodin-strip technique should be useful in studying germination.

Inoculum used for study of the inoculation process should be of uniform age. Cultures should be flooded from three to five hours, the initial spore suspension discarded, and the cultures reflooded after 24 hours to obtain the inoculum actually to be used for inoculation.

Good infection has been obtained with water suspension of spores. However, the addition of wetting agents and/or surfactants might increase infection by more uniformly wetting and dispersing the inoculum. It might also reduce the amount of spore suspension required to obtain run off and permit the use of more concentrated spore suspensions at low volume. Inoculation of potted plants offers an ideal means of testing the effectiveness of such additives to the inoculum.

If the number of seeds of a given line is extremely small and if, as the work progresses, there are indications of races of Ascochyta rabiei, a single plant can be tested simultaneously for resistance or susceptibility to several races. Enclose different branches of single plants within polyethylene bags and tie the mouth around the base of each branch. Rig a hand atomizer with a hypodermic needle, puncture the bag and spray the foliage of each branch with a different spore suspension. Or, without a hypodermic needle, simply insert the nozzle of the atomizer into the mouth of the bag and spray with the mouth of the bag held closely around the nozzle. Use a different atomizer for each culture of Ascochyta rabiei. Run a water control on each plant (using a separate atomizer for this also).

Both Mr. Bashir and Mr. Khaleel Ahmad are investigating the germination and infection process of Ascochyta rabiei. Each is competent to undertake aspects of work proposed here.

C. Development of preferential Medium for *Ascochyta rabiei*.

Oatmeal, gram decoction and gram flour agar, and malt agar, as normally prepared all provide excellent sporulation of *Ascochyta rabiei*. Mr. Khaleel Ahmad is attempting to correlate the concentration of malic acid in foliage and in pods with relative susceptibility to *Ascochyta rabiei*. Using the normal concentrations found in susceptible varieties as a median pH, the effect of adding both greater and lesser concentrations of malic acid to these three media on growth and sporulation should be determined. This could well be the first step in the development of a selective medium for *A. rabiei*.

This worker had no difficulty in making tissue isolations of *Ascochyta rabiei*, but experienced great difficulty in making spore dilutions. Even with well-dried agar plates, bacterial contamination was constantly a problem even with spore dilutions made from fresh pods. Contamination would be an even greater problem in isolations attempted from material left in the field to determine the longevity of *A. rabiei* in debris. Thus, the development of preferential media would be a most valuable research tool.

Malic acid concentration is the place to start.

One medium may be best for isolation, another best for sporulation. Gram seed decoction, gram flour (not more than fifty grams per liter), oatmeal, and malt agar in different concentrations and even in mixture should receive different concentrations of malic acid.

The reaction of *A. rabiei* to the addition of Rose Bengal, streptomycin and other antibiotics at various concentrations should be determined. Any

antibiotic found not to materially reduce the rate of growth of A. rabiei should be tested extensively against other fungi, especially normal contaminants.

D. Study of Races of Ascochyta rabiei.

Work in Iran indicates there are races of Ascochyta rabiei. There are obvious cultural differences in isolates of A. rabiei made by the author. A variety resistant to one race may be very susceptible to another race. It may be necessary to find sources of resistance to several races and to combine these sources of resistance by crossing.

Based on the results obtained from the field trial at Campbellpur this year some six varieties might be selected (e.g., two very susceptible varieties, two resistant varieties, and two intermediate), and the pathogenicity of the ten isolates (made by the author) of Ascochyta rabiei tested against those six varieties. This test can be conducted with potted plants and can initially involve whole plants since adequate seeds of the varieties suggested should be available.

This initial test should indicate if distinct races can now be differentiated. Even if the initial test is negative, it must be kept in mind that new races may exist or may appear at any time.

The program of testing, selection, and crossing proposed herein should in two to three years time provide material with a high degree of resistance. Once different lines representing a broad range of susceptibility, resistance, and even immunity are available, they should be challenged with isolates from

a wide variety of locations - or tested in numerous locations in and outside Pakistan.

E. Experiments on the influence of Sanitation on the Occurrence of Gram Blight.

Based on research already performed a comprehensive program of sanitation could go a long way toward reducing the severity of Gram Blight. For maximum success each step would need to be employed on an area-wide basis.

1. Production of all gram seeds in non-Blight areas.

There are many areas in Pakistan where Gram Blight either does not occur or is very rare. Mohindra, forty years ago, (Annual Report of the Mycologist to Government Punjab, Lyallpur for the year ending 30th June 1930. Govt. Print. Press, Lahore, 1931.) demonstrated that Ascochyta rabiei is seed borne. He concluded that infected seed was a major source of primary infections and that these primary infections are responsible for secondary spread.

Production of disease-free seed in areas where diseases do not occur is a standard procedure for many crops, particularly legumes. Seeds of both peas and beans are grown in irrigated areas of Idaho and Washington in the United States where rainfall is very low during the growing season. Seeds from these production areas is shipped to all other areas where peas and beans are grown. By this means bacterial Blights of beans and of peas are almost never serious diseases in the United States. England and other countries also obtain pea and bean seeds from Idaho

and Washington. Furthermore, before harvesting each seed crop, planting is inspected by government inspectors. In the seed-growing areas any seed crop found to be infected must be destroyed. Only seeds from disease-free fields are certified. Farmers growing peas and beans commercially have learned from sad experience that it just isn't worth the risk involved to plant anything but certified seeds.

Admittedly it would take time, training, education and undoubtedly government subsidy and regulation to initiate such a program in Pakistan. But it could be done, indeed it has been done in the past!

2. The second and only other source of primary inoculum of Ascochyta rabiei is debris from previous crops. Again work done long ago by Luthra, Sattar, and Bedi (Agri. and Livestock in India 5: 489-498. 1935.) elucidated the salient points to be considered in a control program involving sanitation.

   a. Rotation: One-year old debris causes virulent infection, whereas the infective capacity of two-year old debris declines considerably. Three-year old debris causes little or no infection. Therefore, plant gram only once in two years or once in three years on the same land.

   b. Remove gram debris and destroy it by burning or otherwise.

   c. Bury diseased debris in the soil by furrow plowing. Burial renders debris harmless for subsequent gram crops, especially if there is plenty of moisture in the soil. Indeed, a program of debris removal, plowing, and flooding immediately after harvest where irrigation water is available, might reduce the rotation period or even eliminate the necessity of rotation.

Note: Such a program would have to involve an entire gram-growing area. It would do no good for one farmer to employ all these practices and then have his gram infected with gram debris blown from his neighbor's fields onto his.

d. Exactly such a program was conducted in 1933 and 1934 in Attock District with excellent results. However, it was not continued primarily because it was difficult to convince farmers that all these steps were essential.

With gram the debris is usually concentrated in a few locations during harvest and the threshing operation. Hence, a majority of it would easily be removed or burned.

The following experiments are suggested to demonstrate the influence of sanitation on the incidence of Gram Blight. However, since these experiments in the main would only confirm published results, I assign low priority to them.

During harvest of a field which has high incidence of Blight in 1969-70, remove as completely as possible all gram debris from one field, actually pull crowns and roots of any stubble. In another field which was similarly infected in 1969-70 leave <u>all</u> the debris on the surface. Indeed, the debris from the cleared field can be added to the uncleared field. Then combine a plowing experiment: on half of each of the debris and non-debris fields employ only the standard methods of cultivation in preparation for planting the next (1970-71) gram crop. In the other halves furrow plow as deeply (six inches or more) as feasible shortly after harvest. The objective should be to bury the surface layers including surface debris to a depth of at least six inches.

Seed used to plant this experiment should be surface sterilized (dip thirty seconds in a 50-50 mixture of 5-6% sodium hypochlorine and 95% ethyl alcohol or dip one minute on 0.1% mercuric chloride). This experiment should be replicated if possible. Records should be kept on the initial occurrence of Blight in each subplot, amount of infection at different times of the season, and the direction of spread within plots. Alleys as wide as eight to ten feet between subplots would be desirable if feasible.

A second series of experiments could be performed to demonstrate again that Ascochyta rabiei is seed borne. From a single infected planting of gram collect 1,100 normal-sized seeds from pods bearing several large obvious, typical fruiting lesions of Ascochyta rabiei. After shelling the seeds store in the normal manner (take precautions against insects). Prior to planting surface sterilize 550 seeds, do not surface sterilize the other 550 seeds. Use the 50 extra seeds from each lot to run a germination test for viability, plant the remaining 500 seeds in each lot.

From the original infected planting collect another 1,100 seeds from pods having absolutely no blemishes. Again prior to planting surface sterilize half of these seeds and do not sterilize the other half. Run germination tests on lots of 50 and plant lots of 500. A third collection of 1,100 seeds should be made from a non-Blight area and again half the seed surface sterilized and half not.

These six lots of 500 seeds each should be planted in an area where Blight normally occurs but in a field where gram was not grown the previous year and, if possible, in relative isolation from other gram plantings and from each other. The soil should be deep plowed prior to planting to bury any surface debris, so that it will not be a factor in the experiment.

Additional lots of 100 seeds should be collected from pods with characteristic lesions. Individual lots should be soaked in sterile distilled water (just sufficient to cover the seeds in a small [250 ml.] flask) for one hour with occasional rigorous agitation. The wash water should then be drained off, a dilution series run and the various dilutions plated on agar medium to ascertain if any propagules of Ascochyta rabiei are present. If the presence of Ascochyta rabiei on seeds can be demonstrated in this manner, additional lots of seeds should be stored and tested after 3, 6, 12, 18, and 24 months to ascertain how long inoculum on the seeds remains viable.

Dilutions of washing from seeds (and any debris contained therein) harvested in the normal manner from fields known to be heavily infected with Blight should also be plated. Seeds may be surface contaminated by spores from lesions on pods and stems even if not infected by direct growth of the fungus through the ovary (pod) wall and into the seed coat.

Seeds collected from heavily infected pods should be surface sterilized and (together with a non-sterilized control) plated to ascertain if fungus mycelium actually penetrates the seed coat.

A longer-term experiment on survival of Blight on debris should also be initiated. This experiment would confirm the results of Luthra, Sattar, and Bedi (Agriculture and Livestock in India 5:489-498, 1935).

Collect heavily infected debris--whole plants and individual pods with lesions. Wrap in cheese cloth and expose at least two sites (one in a Gram Blight area; one in a non-Gram Blight area). Rainfall and temperature records should be available for each site. Expose some material above ground (one to two feet) on the surface and at two, four, and six inch depths. Remove individual portions at 3, 6, 12, 18, and 24 months and attempt isolation of Ascochyta therefrom. Stake and flag (and keep a plot map of) each burial

site. Select sites where loss or disturbance can be prevented. I would suggest the initiation of such an experiment now, even though with the media now available isolation of Ascochyta from weathered and buried debris may be difficult. Successful isolation may have to await development of a preferentially selective media. However, Mr. Khaleel Ahmad has successfully inoculated potted gram; and a susceptible variety of the the natural host may well prove the very best selective medium available. Material from which isolations are to be attempted should be washed in running water for fifteen minutes to two hours then placed in petri dishes on well moistened filter paper for 24, 48, and 72 hours (water drops of condensation should appear on the debris), removed to sterile water blanks for two to three hours (with agitation) and the washings then either plated in dilution series or used in an atomizer to spray susceptible gram plants.

Results of these experiments should confirm that a sanitation program, if rigorously adhered to, is feasible. The results would also be a basis for determining whether chemical seed treatment would be of any value in a Blight control program.

III. Nematode Experiments

Nematodes not only cause direct damage to agricultural crops, but also are of great significance in providing portals of entry for pathogenic fungi causing Root Rots and Wilts.

The role of nematodes in the Wilt complex of gram must be ascertained by a series of experiments both in pots and in the field involving inoculation with Fusarium isolates and nematodes isolated from gram roots. Mr. Riaz Ahmad, a competent Nematologist, has commenced to isolate and purify the nematodes associated with gram. In cooperation with the pathologist assigned responsibility for the invesitgations of Gram Wilt, experiments should be planned and conducted that will elucidate the role played by nematodes.

Varieties of gram currently rated tolerant and susceptible to Gram Wilt should be inoculated with single species of parasitic nematodes found on gram, with and without simultaneous inoculation with Fusarium isolates. These inoculations should be made prior to planting and at various stages of growth. In other series of experiments the introduction of nematodes and Fusarium sequentially after various time intervals should be tested. Finally the influence of inoculation with several Nematode species with and without Fusarium should be investigated. These experiments must be performed in the field and in pots. Pot experiments should involve non-sterilized soil and soil sterilized in various ways.

If it is demonstrated that nematodes are involved in the development of Wilt, those species proved pathogenic (or significant because they provide entry for Fusarium) should be identified. Studies as to the nutritional requirements of these Nematodes should receive lower priority than studies of their pathogenicity.

(with and without Fusarium inoculation) to determine the influence of fertilization on Wilt. In a third year optimum fertilization could be combined with the optimum irrigation schedule to ascertain the interaction of these two factors on Wilt.

C. Experiments on Nodulation:

The comments made here are suggestions only and should be evaluated by both competent agronomists and soil microbiologists before any experiments are planned or undertaken.

One thing that should be done this summer during the scheduled visit to the native gram areas is to collect both roots and soil surrounding roots. Root systems should be excavated carefully and examined for root nodules. These should be brought back and attempts made to establish the bacteria on cultivated gram.

A competent bacteriologist or soil microbiologist should obtain Rhizobium from as many sources (locations and varieties) as possible and, by inoculating them on a single variety of gram in sterile potted soil and sterilized seed, ascertain if any differential responses result.

IV. Miscellaneous Aspects

A. Pathological Observations on Native Gram:

Native species of gram should be examined carefully for any evidence of Ascochyta rabiei. If lesions are found, material should be collected and isolations and inoculations (on potted gram) attempted at Lyallpur.

Should any plants be found showing symptoms of Wilt, their roots should be excavated and, together with some surrounding soil, brought to Lyallpur so that isolations can be attempted. In any case, both roots and some soil should be collected so that isolation of nematodes can be attempted therefrom.

Any plants showing any indication of other possible diseases should be collected and studied by Pathologists and/or Mycologists.

B. Experiments on Fertilization:

Gram is characteristically grown without any fertilization whatsoever. It may well be that none is needed. However, of the major nutrients, phosphorous may be deficient in many locations. A simple replicated experiment with two standard rates of a complete fertilizer, with P only, and with P and N should be performed by an agronomist. Soil analyses should be run prior to planting and after harvesting. A site at Lyallpur where Wilt will be a minimal influencing factor should be selected for the initial fertilizer trials. If a definite response is obtained to any rate of fertilization, an experiment should be conducted the next year in a known Wilt-infected area

V. Summary of research performed by C. G. Shaw between February 23 and April 28, 1970, on Diseases of Gram in West Pakistan

Upon my arrival at West Pakistan Agricultural University, Lyallpur, Dr. A. O. Shaw took me on a comprehensive tour of the gram plantings. These included: (1) 2,600 varieties and lines obtained from the world collection of gram; (2) a varietal testing nursery consisting of ten varieties being tested simultaneously at some ten different, worldwide locations; (3) varietal trials being conducted by the Department of Plant Breeding (under supervision of Mr. Abdullah Khan); (4) a 2½ acre planting derived from irradiated seed; (5) a Wilt Nursery established by Dr. A. G. Kausar, Chairman, Dept. of Plant Pathology, WPAU; (6) various plantings established by the Departments of Agronomy and Plant Breeding for agronomic studies. These various plantings were examined regularly during the eight weeks of my stay.

Gram Wilt was obvious in many of the plantings and in varying degrees of severity in different varieties. After preparation of various media, isolations were undertaken from obviously wilted plants in different plantings at Lyallpur. Based on information in the literature and obtained in personal discussion I expected a great variety of fungi to develop. This was not the case. In the first series of 200 isolations: 45% produced only isolates of Fusarium; 25% produced both Fusarium and a bacterial contaminant; 10% produced Fusarium plus another fungus and/or bacteria; 10% produced bacteria only; and 3% produced no visible growth. Essentially the same results were obtained in two other series of 200 isolates. One additional series was from material also collected at Lyallpur; the second series of isolates was made from material collected at Dokri (visited March 21, 1970).

The consistency with which Fusarium was isolated indicated that it may be a significant factor in Wilt development in West Pakistan, whether it is important in other countries or not.

Since Fusarium was obtained so consistently, the method of isolation is detailed.

1. Isolations were made only from obviously wilted, but not yet dead, plants. Plants were collected at the ideal time for observing Wilt symptoms; between March 1-21st gram is at the height of vegetative growth and most varieties are in full bloom, with pod set commencing.
2. Roots and lower stems were swished vigorously in tap water, cut into one-inch sections and then washed in dripping tap water (from a five gallon demijohn) for twelve to fourteen hours.
3. Medium sized roots were then scraped to remove epidermis and cortex, dipped for 30 seconds in a 1:1 mixture of 5-6% sodium hypochlorite and 95% ethyl alcohol and plated on various media.
4. Larger roots and basal stem portions were scraped, dipped in the surface sterilant and then aseptically dissected so as to obtain uncontaminated discolored fragments from the central stele. This procedure gave the highest percentage of pure isolates of Fusarium.
5. Smaller roots were plated after surface sterilization without scraping. Contamination, particularly bacteria, was higher; but Fusarium was still obtained in a high percentage of the isolations.

Isolations were attempted on Streptomycin agar and on malt and oatmeal agars. Contamination was less and bacterial growth was slower on streptomycin agar than on malt or oatmeal agar, but all were satisfactory for isolation.

To test, in the short time available, the pathogenicity of these isolates, four series of inoculations were made. Inoculum for these experiments was produced

as follows: Soaked (twelve to fourteen hours) gram seeds were steam sterilized (fifteen minutes at fifteen lbs. psi pressure) on three successive days, and then inoculated with a spore suspension from a six-day old culture of Fusarium. In five to six days every gram seed was coated with abundant sporulating mycelium of Fusarium.

A. Sixteen crocks were filled with soil, and flooded with formaldehyde solution (three pints 40% formaldehyde in 25 gallons water) and capped with tied-down, plastic sheeting. After four days the crocks were emptied, the soil aerated for one week, and then the crocks were refilled with the treated soil. In each crock six seeds of a Wilt-tolerant variety (C 727) and six seeds of a Wilt-susceptible variety (Punjab 7) were planted. All seeds were surface sterilized (one minute in 1:1 sodium hypochorite [5.25%] and 95% ethyl alcohol). In eight crocks two Fusarium infested seeds were planted with each healthy seed. Seeds in the eight other crocks were not inoculated. Seeds were planted April 1, 1970. Inoculum was Lyallpur isolate #3.

On April 16, 1970:

	Inoculated crocks	Uninoculated crocks
Tolerant variety (C 727)	24/48	42/48
Susceptible variety (Punjab 7)	6/48	35/48
Total	30/96	77/96

Reisolation from the obviously decayed roots of wilted seedlings is being attempted.

B. Two to four healthy plants of six varieties considered susceptible to Wilt in the world collection were inoculated with a mixed spore suspension of Fusarium (Lyallpur isolates #1 + 3). With a steel rod (¼ inch diameter) four

three-inch holes were made one to one and a half inches from the crown of each plant. The holes were sufficiently close to the crown to penetrate the root zone and injure roots. Fusarium spore suspension (10 ml.) was poured into each hole. Inoculations were on March 28, 1970. On April 14, 1970, all inoculated plants were dead. Uninoculated healthy plants in the same rows of these varieties continued to develop normally.

C. On April 2, 1970, gram plants approximately one month old planted in pots in unstelilized soil were inoculated with Fusarium only (four pots); Nematodes only (four pots); and Fusarium plus nematodes. Four pots were not inoculated. A concentrated spore suspension (a mixture of Lyallpur isolates #1 and 3) was used in these inoculations. A suspension of nematodes isolated and cultured by Mr. Riaz from gram roots was used for the nematode inoculum. Inoculum was introduced with a hypodermic needle (5 ml.) into the root zone (one and a half inches deep) of each plant. No attempt to wound (or to avoid wounding) the roots was made. As yet no clear-cut differences are apparent in this experiment.

D. Again using a concentrated spore suspension of Lyallpur isolates #1 and 3 of Fusarium, six plants were inoculated by hypodermic injection. The hypodermic needle was pushed directly down into the crown of the plant and 1 to 2 mm. of spore suspension was injected. Inoculations were performed on six plants on April 3, 1970. On April 16, 1970, two plants showed obvious Wilt symptoms.

It is very difficult to inject gram plants with a hypodermic needle; the tissues of the crown and upper top root are very woody. Since seed inoculum planted with seeds the introduction of spore suspensions into the root zone in one-fourth inch holes three inches deep both appear satisfactory and give quick results, I recommend use of these procedures for testing the Pathogenicity of isolates and the resistance or susceptibility of varieties.

Five Fusarium isolates from gram grown at Lyallpur and seven isolates from gram grown at Dokri have been left in the Department of Plant Pathology (duplicate cultures left with Dr. A. O. Shaw and Mr. Abdullah Khan).

Initial attempts to isolate Ascochyta rabiei from infected pods stored in an open laboratory for one year were unsuccessful. Spore suspensions were attempted. Contamination was the major problem. Since freshly collected material then became available, subsequent isolations were attempted only from lesions produced during the current season.

An old contaminated culture of Ascochyta rabiei was available. I started (half-heartedly) to attempt purification by spore dilution, streaking and direct transfer from apparently uncontaminated portions of the culture. Pure cultures were obtained by all three methods.

This culture was increased on both malt agar and gram decoction agar. A pure test tube culture was flooded to obtain a dense spore suspension and this poured over the surface of sterile gram and malt agar (in petri dishes and beer bottles). In five to six days dense mats of pycnidia containing abundant pycnidiospores had developed. These mass cultures were used to prepare a spore suspension for inoculation purposes. A gasoline powered, back-pack sprayer was used to disperse the inoculum. One block of gram (variety C 727) was sprayed at dusk on two successive evenings, March 17 and 18, 1970. During both nights it rained. On March 30, 1970, good infection (50% of plants with one to fifty lesions) was found in this experimental plot. Ascochyta rabiei was reisolated from typical lesions on pods. stems, and leaves.

Two other blocks of gram, including several varieties, were sprayed on three successive evenings (March 25 to 27 or March 26 to 28, 1970). No natural precipitation

occurred during this period. Each following morning the inoculated plots were sprayed with water in an attempt to prolong the period of favorable conditions for infection.

On April 12, 1970, typical lesions of Ascochyta rabiei were found on some varieties included in these plots, but the incidence of infection was extremely low (only a trace - less than 0.1%). The results of these inoculations lead to the conclusion that water must actually be present continuously for 16 or more hours for infection of gram to occur. This condition occurred when natural precipitation followed artificial inoculation. The moisture applied in the inoculum was insufficient by itself to maintain a continuous moisture film long enough for infection to occur. The high temperatures prevalent in Lyallpur may also have been a factor. However, artifical infection can be obtained in the laboratory or green house at this time of year. Mr. Bashir successfully inoculated potted gram in the laboratory by enclosing the entire plant in a polyethylene bag so as to maintain a constant film of moisture on the vegetation.

On March 30-31, 1970 the area where Gram Blight occurs most severely was visited - Haripur to Cambellpur to Talagang. Collections of Ascochyta were made at many points. At Campbellpur A. rabiei was collected on a number of different varieties of gram. The Ascochyta nursery to test susceptibility and resistance at Campbellpur was well designed and an effective test was obtained this year. There were obvious differences in susceptibility and resistance in the varieties under test. While no variety proved immune, analysis of the data from this nursery will identify varieties with considerable resistance.

Extensive isolations were attempted from the collection of A. rabiei made on this trip.

The most successful technique was to wash pods and stems having sporulating lesions in tap water for two to three hours. The surface of the lesions were then scraped, and the lesions cut into small sections. These were dipped fifteen seconds to surface sterilize them, shaken (while held with tweezers) to remove excess sterilant, and plated on malt agar. Isolations were obtained from eleven locations and/or varieties. Most interestingly, these isolates fall into two obvious categories. On malt agar the majority of isolates (seven) produce tan- to cream-colored, slow growing colonies on which pycnidia are obvious after seven days. A smaller number (four) produce olive black, faster growing colonies on which pycnidia are not obvious after seven days. The original culture used in the inoculation experiments (now designated Lyallpur Reisolate) belongs to the latter category. Microscopic examination of all cultures confirmed their identity.

The existence of obvious cultural differences suggests that different isolates from Pakistan may also vary in pathogenicity as has been found in other countries.

Subcultures of all isolates have been left with the Department of Plant Pathology, Dr. A. O. Shaw and Mr. Abdullah Khan.

Spore dilutions were again attempted, this time with limited success. The following procedures reduced contamination markedly.

1. Use well-dried agar plates. Agar concentration should be 2.5 to 3%; petri dish plates should be poured well in advance of use and allowed to dry until all moisture or condensation has evaporated.
2. Wash pods and stems bearing sporulating lesions for two to three hours in tap water. Then blot off free moisture and place in a petri dish moist chamber of 24 hours to permit production for a fresh crop of pycnidiospores.
3. Scrape pycnida from several lesions and place in sterile water (10 ml.).

4. Let stand for two to three hours with occasional gentle agitation.
5. Prepare dilution series (1/10, 1/100, 1/1000) and pour each dilution (and original suspension) onto a sterile, well-dried malt agar plate. After two minutes drain off all water not absorbed by the agar.

Graduate students and junior staff members of the Department of Plant Pathology were involved in all aspects of this work. Their training has been a major objective of my program. I particularly express my appreciation to Mr. Javed Iqbal who worked with me continuously. Mr. Iqbal is competent to continue the Fusarium Wilt investigation. Mr. Riaz Ahmad isolated, cultured and started identifications of the nematodes associated with gram. Mr. Bashir and Mr. Khalil Ahmad Khan are investigating various aspects of Ascochyta Blight and should be encouraged to undertake the research proposed herein on that disease.

I appreciate the help, cooperation, and encouragement received from Dr. A. G. Kausar, Chairman of Plant Pathology, West Pakistan Agricultural University. To Mr. I. U. Khan my thanks for sharing your laboratory with me.

Finally, I sincerely appreciate the help and interest of Mr. Abdullah Khan (Department of Plant Breeding and Genetics), and Dr. Mohammad Aslam, Chairman of the Department of Plant Breeding and Genetics. The role of the breeder is vital to the success of this pulse program. The participation of such a competent, dedicated, and productive worker as Mr. Abdullah Khan augers well for its success.

RESEARCH REPORT ON GRAM WILT AND GRAM BLIGHT, 1972

by

C. Gardner Shaw, Consultant
Professor and Chairman, Department of Plant Pathology
Washington State University
May 1972

I. Introduction

Two years ago, at the completion of a similar duty assignment, the writer submitted both a report of research accomplished and an extensive "Proposed Research Program on Gram Wilt and Gram Blight" (April 1970). There is no need to restate that program herein. This report will summarize the progress that has been made in the intervening two years and report on my activities during the two-month period (March 16 to May 15, 1972).

II. Progress in the past two years

When, because of health, I was unable to return in 1971, Dr. Van E. Wilson, Plant Pathologist with the ARS USDA, permanently stationed at Washington State University, Pullman, Washington, came to Pakistan as a consultant to US AID. Dr. Wilson effectively continued this consulting program and submitted a report, "Programmed Research Report on Gram Wilt and Gram Blight and a New Foliage Disease on Gram in West Pakistan, 1971."

Dr. Wilson reported no conclusive evidence proving the inducement of Gram Wilt by *Fusarium* species. As had I the year previously, Dr. Wilson consistently isolated *Fusarium* spp. from wilted plants; but he was unable, except in one experiment, to obtain either Wilt symptoms or to kill plants with *Fusarium* inoculum. In one experiment Dr. Wilson added 2% by volume of chopped root tissue from

diseased (wilted) plants to soil in which gram seeds were then planted. In this experiment the seedlings died soon after germinating. This experiment by Dr. Wilson was similar to one performed by the author in 1970. When Fusarium seed inoculum was planted with healthy seeds, two thirds of the healthy seeds either did not germinate or the seedlings which did develop died (damped off) within sixteen days.

Experiments which combine Fusarium inoculum with various types of wounding and which continue throughout the normal growing season are essential to determine whether there is a causal relationship between Fusarium and Gram Wilt. The consistent association of Fusarium with the symptoms of Wilt, as repeatedly demonstrated by all who have attempted isolations, argues strongly for a causal relationship. The inability to obtain infection and symptoms comparable to those that develop in the field are probably due to some factor in the environment which has not yet been consistently introduced in the infection experiments conducted to date. At present we have no knowledge concerning the water stress conditions existing in the soil at the time the Fusarium invades the root system. For Fusarium species attacking cereals it has been demonstrated that a moisture stress of eight to fifteen bars (90 to 92% RH) is more conducive to infection than is either a wetter or a drier soil.

Mr. Dost Salahuddin, an M.Sc. student in the Department of Plant Pathology, has successfully fulfilled Koch's postulates and demonstrated conclusively that Fusarium oxysporium causes Gram Wilt. Mr. Salahuddin's experiment is briefly outlined here; a more complete report will be contained in his M.Sc. thesis.

Soil was sterilized with formalin, covered with polyethylene sheeting for 48 hours and then aerated for six days with daily turning. Crocks were sterilized with methyl alcohol and fire, and then filled with the sterilized soil. To each crock a spore suspension obtained by washing the surface of 750 ml. culture flask

with 100 ml. of sterile water was added and mixed thoroughly with the soil. A little water was then added to each crock to wash the spores into the soil. Twenty-four hours later ten seeds of each of four varieties of gram (Pb 7, F 8, C 612, and C 727) were planted in different crocks. The experiment was replicated four times, and included one series of pots that were not inoculated (control). Ten days after planting the germination counts were:

	Number of Seedlings	
Variety	Control	Infested soil (Average of four reps.)
Pb 7	10	6.0
F 8	10	4.5
C 612	9	7.0
C 727	10	6.75

Thus, in the inoculated pots some seeds did not germinate, or the small seedlings damped off in less than ten days. When the remaining seedlings in the infested crocks were six inches high, they commenced to wilt. At the end of three months all seedlings in all replications of the infested crocks were dead.

Roots of the dead seedlings of each variety were cut into small pieces, surface sterilized with 0.1% mercuric chloride ($Hg\ Cl_2$) for one minute, and then washed sequentially three times in sterile water for one to two minutes. The root pieces were then plated on Richard's agar and incubated for one week. *Fusarium oxysporium* was isolated from these petri dish cultures and compared with the original isolate used for the inoculations. This particular isolate turns Richard's media wine-red in color. Both the original isolate and the reisolates stained the media. On microscopic examination, no differences between the original culture used for inoculation and the reisolations could be determined. *Fusarium oxysporium* was reisolated from each of the four varieties of gram (Pb 7, F 8, C 612, and C 727).

To my knowledge, Mr. Salahuddin's experiment represents the first time that anyone has demonstrated the causal relation of Fusarium oxysporum with Gram Wilt. Mr. Salahuddin reports that the plants in his experiment were watered once per week, but he does not state the amounts of water added. Because soil water relations are so important in germination, growth, and infection by Fusarium spp., I urge that an experiment of the type conducted by Mr. Salahuddin be performed in which the amounts of water in the soil be the only variable. Seed should be planted in soils of varying moisture contents from air-dry soil to saturation and sufficient water added daily to keep the moisture content of each crock constant. The 1972 issue of Annual Review of Phytopathology will, when published, contain an article by Dr. R. James Cook on the relation of soil water to germination of and infection by soil fungi. The techniques described in this paper, and the references contained therein should be the basis for such an experiment with Fusarium oxyspiorium and Gram to determine as precisely as possible the soil moisture levels most conducive and least conducive to the development of Gram Wilt.

Dr. Wilson conducted several experiments which demonstrated that Gram Blight (Ascochyta rabiei) can be induced by inoculation with spore suspensions: (1) by spraying plants growing in the field and enclosing them in plastic bags for 72 hours; (2) in the greenhouse by enclosing potted gram plants in plastic bags for 24 or 72 hours. The bags were left in place for the periods indicated to assure 100% humidity during the infection period. Dr. Wilson's results confirmed the field inoculation experiments performed by the author in 1970.

The most encouraging development during the two year interval between my two visits was the cooperation evident among the several departments at the

university and governmental agencies, all of which are mutually interested in and involved in the Gram Improvement Program. Most encouraging of all was the abundant evidence that a gram breeding program is now in operation. In spite of the low success ratio of cross pollination attempts with gram (from 1-2% to no more than 15-25% even when performed by the best qualified) crosses are being attempted on a massive scale.

That the program had been in progress since my previous visit, when a breeding program had been my strongest recommendation, was evidenced by the F_2 and F_3 material currently being tested in the field. Crosses were being made not only by members of the Department of Plant Breeding and Genetics, but also by personnel at the Punjab Agricultural Research Institute. Cooperation was also evident in the excellent testing program underway at Campbellpur, where personnel of the Department of Agriculture and West Pakistan Agricultural University had a variety of experiments on Gram Blight underway.

III. Summary of Accomplishments by C. G. Shaw on Diseases of Gram during the period between March 16 and May 15, 1972

After arrival in Lyallpur on March 21, 1972, all gram plantings on the University Farm were inspected with Dr. A. O. Shaw. One day was also spent at the Punjab Agri. Research Institute where Dr. Iqbal acted as our guide.

The week of March 25 to April 2, 1972, was spent in a trip to Rawalpindi to Campbellpur to Mianwali to Kallurkot and Piplan and return. While in Rawlpindi I conferred with Dr. Floyd J. Williams and with Dr. Leon F. Hesser, Assistant Director, Agricultural Policy, AID. At each of the locations named gram plantings were inspected. In addition farmers' plantings were checked.

At the time of our visit we did not see Gram Blight (*Ascochyta rabiei*) at any location except at Campbellpur. Even there Blight was severe only where,

by spreading debris in one of the experimental plantings, an attempt to establish Blight had been made. This attempt had been very successful and had provided an excellent varietal test. Plot records taken on April 15th by Professor I. U. Khan and Mr. Muhammad Riaz indicated that the following varieties had been completely killed:

> FAO-16, FAO-41, FAO-14, FAO-7. A few plants of FAO-49 were still surviving. C727 and L.S.-22 were rated fairly tolerant. Varieties L.S.-8, L.S.-2 and L-15 were rated resistant. L.S.-21, L.S.-20 were rated very resistant. All varieties, however, had some Blight infection.

In the Ascochyta Blight Nursery, debris from fields infected with Gram Blight the previous season (1971) had been spread over the planting. Between each two-row planting of the varieties being tested the very susceptible variety Punjab-7 had been planted. Because, as reported by Dr. Van Wilson, the previous season (1971) had not been conducive to an epiphytotic of Gram Blight, the amount of diseased debris that could be collected was limited. Even though the weather through March 31, 1972, had not been conducive to an epidemic in farmers' fields in the Rawalpindi-Campbellpur area, there had been enough rain to give an excellent test in the Blight Nursery where the debris had been spread.

It was particularly striking that the heavy incidence of Blight had been restricted almost entirely to the bund where the Blight Debris Nursery had been established. Ascochyta rabiei is spread by wind during periods of rain. That Blight had spread hardly at all from the bund where the debris experiment had been established to adjoining bunds was additional evidence that through March 31, 1972, conditions had not yet been favorable for the development of an epiphytotic of Blight in 1972 anywhere except where large amounts of infected debris had purposely been spread.

The high incidence of Blight where the debris had been spread and its absence (or presence in minimal quantities) even in other plantings at Campbellpur confirms the important and effective role that a strict sanitation program would play in the control of Ascochyta Blight. Blight persists from year to year in only two ways: (1) on infected plant debris left on the surface of the field from one year to the next; and (2) on or in infected seed. Seeds harvested from fields where even a small amount of Blight was present provide an initial source of inoculum which can rapidly build up to an epiphytotic of Gram Blight if weather conditions are favorable (frequent light rains).

The common method of plowing results in any infected plant debris left in the field remaining in the top two inches of soil precisely where it can serve as a source of inoculum for the next crop. If, after harvest, as much debris as possible were removed from the fields and the remainder turned under to a depth of six inches or more, there would be no chance of infection of the next year's crop from debris. Such a sanitation program, combined with clean (certified) seed would essentially eliminate Gram Blight. To be effective the program would have to involve a large area with all farmers in the area cooperating in both aspects of the program - field sanitation and the planting of disease-free seeds.

The opportunity to initiate such a program currently exists. There is available seed from 1,800 acres planted at the ADC farm at Kallur Kot which can be planted in the barrani area next year. This will yield about 9,000 maunds of clean disease-free seeds. If farmers can be induced to practice sanitation in their fields and the disease-free seeds are made available to farmers who have practiced sanitation, there would be little likelihood of a Gram Blight seed epidemic in the area so treated next year.

These aspects of Gram Blight were discussed with the Director of Agriculture for the Punjab, Masahib-ud-Din Khan, who agreed to issue an announcement of such a program.

While Blight was severe and prevalent only in the Gram Blight Nursery at Campbellpur, there were small (trace -5%) amounts of Blight in some of the other plantings. No Blight was seen elsewhere on the entire trip. During the period between March 31, when my trip ended, and April 15, 1972, conditions were ideal for Blight to develop and spread - light and frequent periods of rainfall with gusty winds. The rapidity with which Blight can develop if there is any source of inoculum is indicated by observations made by Mr. Inam Ullah Khan on April 15, 1972. Professor Khan found Blight in Trial #2 at the Rawalpindi Agriculture Farm. Mr. Khan stated that at this location only a few patches were found and "the individual patches were not more than a few square feet in area and the overall incidence of the disease was insignificant." Nevertheless, Blight was present where it had not been observed two weeks earlier.

Observations by Professor Khan on April 15, 1972, in farmers' fields were quite different than ours only two weeks earlier. "At Campbellpur, the situation in the farmers' fields was not very encouraging. The Blight disease was fairly common and the pods were mostly without seeds. The weather on 15.4.72 was perfect for another round of infection. The sky was overcast with heavy clouds; light drizzle was accompanied by winds of medium velocity."

In spite of the condition in farmers' fields where obviously some inoculum had been present, "In almost all the trial plots overall conditions of the crop was very good. Few plants here and there showed symptoms of Blight disease". This indicates that on the Agricultural farm where sanitation had been practiced the incidence of Blight was still low.

On April 3, cultures of Fusarium and Ascochyta on hand were examined. All cultures of Fusarium were found to be contaminated. It was considered easier to reisolate Fusarium than to attempt to clean up the cultures available. Also a graduate student, Mr. Dost Salahuddin, had pure cultures of Fusarium isolated from gram which he kindly made available.

Two Research Assistants, Mr. Muhammad Riaz and Mr. Mohammed Ahmed Khan, were assigned to work with me. While each was charged with learning all the techniques and procedures involved in working with both diseases, Mr. Riaz was assigned primary responsibility for working with Gram Blight (Ascochyta rabiei) and Mr. Ahmad was assigned primary responsibility for Gram Wilt (Fusarium).

Preparation of media was initiated including media containing antibiotics; new isolations were attempted of both Fusarium and Ascochyta; and transfers were made from the old cultures of Ascochyta.

On April 3, in Mr. I. U. Khan's absence I delivered a lecture to his graduate class on the importance of Wilt and Blight in the culture of gram in Pakistan.

As was true two years ago initial attempts at dilution series as a means of isolating Ascochyta were plagued by contamination. For success by this method it is essential that agar plates poured at least a week in advance be available. Only then is the agar firm enough to prevent the spreading of bacteria to contaminate the whole plate.

By repeated attempts employing different techniques Mr. Ahmad Khan became adept at the isolation of Fusarium. Similarly Mr. Muhammad Riaz became proficient at isolating Ascochyta rabiei. The training of these young pathologists in the techniques of isolation, purification of cultures and inoculation of gram was the most rewarding and valuable result of my visit.

On April 13, 1972, we inoculated ten healthy gram plants in the field with Fusarium. Gram seed inoculum of Fusarium was placed in each of three, three-inch holes within the root zone of each healthy plant, and then the soil around the crown of each inoculated plant was flooded with water. Three, three-inch holes were made around another ten healthy plants, but not inoculated; and their crowns flooded with water as were the inoculated plants. Twenty healthy plants were selected as an unwounded control. On April 24, six of the ten plants wounded and uninoculated plants showed Wilt symptoms; two of the twenty unwounded controls also showed Wilt symptoms. This simple experiment, performed in a field known to be Wilt infested, indicates that Wilt symptoms continue to develop in the absence of added inoculum or wounding, that with intentional wounding there is sufficient inoculum in the field for some plants to become infected, and finally that with wounding plus inoculum a large number of plants become infected even in an eleven-day period. Far more valuable, however, than any attempted interpretation of the results of this experiment was its value as training in methodology for Mr. Riaz and Mr. Ahmad. The agars first prepared (Potato Dextrose, Malt, and Peptone) were all mistakenly made without lactic acid. While this delayed the progress of attempts to isolate Ascochyta and Fusarium, it effectively demonstrated to Mr. Ahmad Khan and Mr. Muhammad Riaz the absolute necessity of including some material antagonistic to bacteria in any medium to be used for the isolation of fungal pathogens. Subsequently Mr. Riaz and Mr. Ahamd prepared standard media with lactic acid, Pentachloronitrobenzene (PCNB), Streptomycin sulfate, or Penicillin G added. Any and all of these media proved satisfactory for the isolation of either Ascochyta rabiei or Fusarium sp.

On April 15, 1972, indices for mung yellow mosaic were taken in a large varietal planting of Mung beans. The plants were in the three to four trifoliate

leaf stage. There was tremendous variation in the amount of mung yellow mosaic present in the 293 varieties. For example, large blocks of equal size (approximately 1,200 plants each) of varieties C 10, C 127, and C 233 were found to have 78, 7, and 87 infected plants, respectively. One or more infected plants were found in 135 of the 293 varieties on April 15, 1972.

Surprisingly when reindexed on April 28, 1972, when the plants were in the six to seven leaf stage (first flowers on some) fewer infected plants could be found than in the first survey. In varieties C 10, C 127, and C 233 only 22, 3, and 28 infected plants, respectively, could now be found. In only 87 of the 293 varieties could one or more infected plants now be found. The disease had not only not spread from plant to plant in the field, but also had not moved from the lower leaves up into the new foliage. Thus, the new symptomless foliage hid most of the lower leaves on which, when seen, typical symptoms were still present. Remission of symptoms was particularly evident in lines previously selected for mung yellow mosaic resistance characteristics. For example, in variety C 10 on April 15 there were 78 YM plants; on April 29 there were 29 YM plants, and only two YM plants on May 11.

On April 28, far more common than mung yellow mosaic, was another virus-like symptom best described as leaf crinkle. This condition was still much more prevalent than yellow mosaic on May 11.

On April 18, 1972, inoculum of Ascochyta rabiei was prepared as follows: Fifty pods infected with sporulating lesions of Ascochyta rabiei (selected from infected plants collected at Campbellpur) were covered with sterile water and shaken at fifteen-minute intervals for three hours. Additional inoculum was prepared by flooding one beer bottle culture (eight days old) of Ascochyta rabiei with sterile water. The surface was scraped to release the conidia. The two spore

suspensions, one from infected pods and the other from a sporulating culture, were both checked for the presence of conidia; conidia were abundant in each. The two spore suspensions were strained and mixed and used to inoculate one-month old seedlings of gram planted in five-gallon crocks. Half the seedlings in each crock were inoculated; half were not. Plastic bags were placed over both the inoculated and the non-inoculated plants and left in place for forty hours at approximately 70°F. and then removed.

The inoculations were performed with a hand atomizer. One drop of the spore suspension was placed in a moist chamber overnight and examined for spore germination the next day. Spore germination was 95%+. Data on the results of the inoculations were collected on April 24, 1972.

Variety	Inoculated Plants			Non-inoculated Plants		
	Diseased	Healthy	Total	Diseased	Healthy	Total
C 7	0	8	8	0	8	8
I 13	6	5	11	0	10	10
424	4	3	7	0	7	7
Pj 7	19	0	19	0	19	19

The experiment was conducted in a small, air-conditioned growth chamber. It demonstrates that varietal testing for resistance to Ascochyta rabiei can be conducted in pots and crocks during the hot weather of April with the facilities currently available. Every effort should be made to restore the greenhouses to usable condition as soon as possible so that more extensive experiments on a larger number of varieties can be conducted.

I wish once again to thank Dr. A. O. Shaw for his help, support and encouragement throughout the course of both my visits to West Pakistan. Mr. Mirza had extended to me the hospitality of the Department of Plant Pathology. Again

Professor Inam Ullah Khan has shared his facilities with me; this time his office served as my laboratory while I was here.

I extend every encouragement to the graduate students and research assistants who have worked with me: Mr. Mohammad Ahmed Khan, Research Assistant; Mr. Muhammad Riaz, Research Assistant; and Mr. Dost Salahuddin, Graduate Student. Only by their efforts and those of their colleagues will the disease problems, not only of gram but of all agronomic crops, be solved.

IV. Recommendations

1. Any future consultants on the diseases of gram should come for the major portion of the growing season--a period longer than two months. I would recommend the period from December 15 through April 15. If only periods of two months can be approved, these periods should terminate not later than April 15. The weather is too hot and the crop too far advanced after this date for much effective research to be accomplished.
2. The next consultant on gram diseases should be a specialist on Root Rots, Wilts, and the *Fusarium* diseases of pulses and legumes. An excellent person to work on Gram Wilt would be Dr. William Haglund, Northwestern Washington Research and Extension Unit, Washington State University, Mt. Vernon, Washington. The precise cause and the means of infection can best be determined by an individual with such prior experience.
3. The program of cooperative breeding of gram involving both university and governmental research workers should continue. It holds the greatest promise for the development of long-term solutions to the two major diseases of gram--Wilt and Blight. Because of the limited facilities currently available at WPAU, it is recommended that a portion of the

breeding program be conducted at a cooperating institution such as Washington State University. This would permit a continuous year-round program.

4. The program of producing seeds for the barrani area in regions where Blight does not occur should be continued and expanded. The availability of disease-free seeds, combined with an educational program to inform farmers of the importance of removing as much debris as possible from their fields then plowing to a depth of at least six inches to bury any remaining debris, could essentially eliminate the occurrence of Blight over a major portion of the area where Gram Blight is often so serious.

5. The testing of varieties for resistance to Gram Blight in a Gram Blight Nursery should continue. The dramatic results obtained this year in the Gram Blight Nursery at Campbellpur are evidence of the value of such a varietal testing program.

PROPOSED RESEARCH ON ARTHROPOD-TRANSMITTED VIRUSES OF MUNG AND RELATED EDIBLE LEGUMES

by

Robert F. Harwood, Consultant
Professor and Chairman, Department of Entomology
Washington State University
September 1970

I. Introduction

Statements justifying research on increasing grain legumes will not be made here as the situation regarding these crops has been covered in the proposed research program developed by C. G. Shaw on diseases of gram (April 1970). Since production of mung (*Phaseolus aureus*), mash or urid bean (*P. mungo*), moth (*P. aconitifoluis*), and Lobia or cowpea (*Vigna sinensis*) are main objectives within the overall program, and since these crops might be planted from February through August to potentially yield two crops each year following wheat, the need for concentrating on these pulses is obvious. Mung is the most utilized of these crops in West Pakistan, comprising some 257,000 acres annually; it also has high acceptance in the diets of the area. As similar viruses attack mung throughout the region, mung was the crop concentrated on by this investigator. A further reason was that plant breeders of West Pakistan Agricultural University, Lyallpur, had already made preliminary selections of mung from 400 local lines, plus 620 lines from other areas, and a half acre of irradiated seeds. The germ plasm was obtained from as diverse areas as, Iran, Turkey, the Soviet Union, the Philippines, and the United States. Additionally some 350 lines of *P. mungo* were at hand.

The etiological agent causing the disease known as yellow mosaic of mung (hereafter referred to as YM) is presumably a virus, is exceptionally prevalent in West Pakistan, and affects mung and mash (Williams, F. J., J. S. Grewal, and K. S. Amin. 1968. Serious and new diseases of pulse crops in India in 1966. Pl.

Dis. Reptr. 52: 300-304). Mung plants at least six weeks old at Lyallpur, and particularly those planted in June, are virtually all affected by YM symptoms. It must be stated, however, that closer observations suggest more than one YM or yellows entity is present, and YM symptoms were often seen in conjunction with leaf crinkle symptoms (see Williams et al., 1968).

Despite the prevalence of YM in the Pakistan-India subcontinent very little is known about the disease; only two references are known to the writer on how this pathogen enters virus-free plants. Both papers state that the pathogen is transmitted by the white fly Bemisia tabaci (Nariani, T.K. 1960. Indian Phytopath. 13:24-29. Original not seen but cited by Williams et al., 1968; Maramorosch, K. 1963. Ann. Rev. Entom. 8), but the second reference is a general review stating this insect transmits a number of viruses affecting pulses though no documenting reference is cited. Because firm evidence is lacking on the possibility of virus transmission to mung by insects or mites, it was decided to concentrate on such a line of investigation. This proposal is based on the assumption that at least YM is so transmitted, and the experimental approach pursued by the writer will be discussed first because the program proposed is based upon findings during mid July to early September, 1970, and not on extensively published information.

At the outset it must be stated that the research conducted thus far, as well as the future of this program, requires an interdisciplinary approach involving plant breeders, entomologists, plant pathologists and agronomists. The interest of Dr. A. G. Kausar, Dean of the Faculty of Agriculture, West Pakistan Agricultural University, is sincerely appreciated. Likewise Dr. Muhammad Aslam, Head of the Department of Plant Breeding and Genetics, gave encouragement and advice, and within the same department Mr. Abdullah Khan provided potted plants of desired varieties while Dr. Khurshid Alam collaborated in setting up field experiments

testing varieties, and systemic insecticide plots. Many observations were made in mung plots of different aged plants maintained by the Department of Agronomy. From the Department of Entomology Dr. Muhammad Yunus, Head, was most helpful in obtaining necessary supplies, Mr. Ejaz M. Siddiqui and Dr. Mukhtar A. Halimi worked at my side; additionally Mr. Manzoor Ahmad provided advice and materials in establishing systemic insecticide plots. Dr. Inamullah Khan, Department of Plant Pathology, was most gracious in assisting in the interpretation of pathogenic sysmptoms. Dr. A. O. Shaw, WSU/AID program, collaborated in the design of experiments, and implemented the construction of needed items. To all of the people and disciplines named I extend my sincerest appreciation, and urge the continuation of collaboration because such an united approach is essential for solving the fascinating problems associated with arthropod transmission of pathogens affecting mung and related legumes.

II. Experimental Procedures

The initial experiment was designed to determine whether arthropods transmit YM pathogen. At the same time it was assumed that such is the case; therefore, the insects and mites of mung were sampled extensively and further experiments were conducted to determine which insects transmit YM and related pathogens.

Furthermore, assuming insect transmission, the effect of insect control procedures upon YM acquisition by mung plants was studied. In all of these experiments rather obvious differences were sought and practical solutions attempted, the feeling being that this program should progress from general to specific conclusions, and the finding should be of such a nature that they can be readily utilized by local farmers.

1. Validating arthropod transmission

Insect exclusion cages were used to run these tests. These cages consisted

of large wooden-framed glass structures with some cloth panels, that could hold several crocks containing plants; cloth cages with clear plastic tops and wire frames that fit over one crock; wooden-framed cages with cloth sides and clear plastic top, fitting over two feet of two rows in the field.

Nine crocks, with approximately five plants per crock were set up as follows: (a) autoclave-sterilized soil (250° for one hour), (b) soil from a field where mung infected with yellow mosaic had been growing, (c) ordinary soil with no recent history of mung culture. In these three soil conditions seed of an intermediately resistant mung variety (S64) was planted using, (d) seeds from an apparently virus-free parent, (e) seeds from a parent with YM symptoms, and (f) seeds identical to (e) except that it had been subjected to dry heat just below the threshold affecting germination (94°C. for five minutes).

None of these plants developed YM or leaf crinkle symptoms, nor did any other plants from which insects were excluded, including plants in field plots. At time of writing this report at least 160 plants of intermediate susceptibility, plus at least 52 plants of intermediate susceptibility, have grown as far as or beyond the second trifoliate leaf group stage and have no virus symptoms. In a few of the exclusion cages covering these plants thrips, flea beetles, and in one instance white flies, were observed, meaning that they were not entirely vector proof in every instance.

The only reasonable conclusion from the above results is that YM of mung is transmitted by insects or mites, and not by soil or nematodes. If any seed transmission occurs, it must be at an incidence below 0.5%.

2. <u>The insect and mite complex of mung</u>

General collecting was accomplished by use of a Malaise trap (Marston, N. 1965. J. Kansas Entom. Soc. 38: 154-62) and by sweeping vegetation with a net.

Suffice it to say that the Malaise trap provided excellent general sampling of larger flying insects, particularly Hymenoptera and Diptera, but was of no value in sampling insect vectors of phytopathogens. This trap might prove useful in the future investigations involving pod borer of gram, or similar larger flying insects.

General observations as well as sweep collections indicate that among potential vectors there are commonly at least three species of thrips (Thysanoptera) present, particularly on young plants; one or more species of very small white flies (Aleyrodidae); and at least two species of leafhoppers or jassids (Cicadellidae). On occasion a species of fulgorid (Fulgoridae), and spider mites (Tetranychidae) were also observed. Samples of all of these arthropods were taken for species determinations, but identification must await the opinion of appropriate toxonomic experts.

The smallest of these insects, namely thrips, white flies, and leafhoppers, were most frequently associated with mung and, therefore, most suspect as vectors of YM. It was necessary to devise a reliable index of abundance of these forms as they are too small and active to sample accurately by sweeping. After some experimentation a sticky-stake technique was devised; it is strongly urged that this method be utilized in further investigations of vector populations.

The sticky-stake technique consists of driving a flat wooden stake, covered with paper, into the row of growing plants. The bottom of the paper should be at about the top height of the plants, and the paper coated with an adhesive material. The propietary product called "Ostico", available locally for mango mealybug control, was a satisfactory adhesive. By always utilizing an identical surface area, standard capture conditions are possible, and by using lined paper the stakes can be readily scanned under a dissecting microscope. The stakes were left in

the field plot rows for 24 hours. They showed good uniformity of capture within a location at any particular time. The principle drawbacks to the sticky-stake capture method are that rain and dust storms adversely affect results, and insects captured cannot be used for any other than population estimates.

The question of aphid (Aphididae) transmission naturally occurs because these insects are known vectors of several viruses affecting legumes. Aphid transmission of pathogens to mung can be categorically eliminated at Lyallpur during the term of this study. At no time were any aphids observed on mung, or collected by sweeping or the use on four occasions of Moericke yellow pan traps (Moericke, V. 1957. Z. Pflanzenbau u. Pflanzenschutze 64: 507-14).

3. Insect transmission experiments

All of the transmission experiments used potential vectors captured in YM-infected mung fields. Test insects, therefore, were of variable age and the proportion of infective specimens present was unknown. Nonetheless, because of the very widespread occurence of YM in plantings, the chances of obtaining transmission by this method should be high if feeding by vector species can be accomplished.

Insects for vector experiments were captured by sweep collecting fields and placing the catch in a box having one clear plastic side and a black cloth sleeve opposite. Insects released into this box crawled against the plastic in an escape response towards light. By placing one's head inside the box through the black sleeve and using a small straight-tube aspirator, the specific kinds of insects desired were readily captured for transfer to uninfested plants.

Initially specific kinds of insects were introduced into the large wooden-framed glass cages in each of which was a crock bearing at least five uninfested mung plants. It was observed that very little establishment of potential vectors

took place on plants under these conditions, probably because the principal behavior of introduced insects was to escape towards light, and the small proportion host plant surface to overall space was too great, causing death of the insects with practically no feeding. Very clearly transmission experiments, to be successful, must be conducted in isolation cages of dimensions not much greater than total plant size, or even cages covering only one or a few leaves.

Results with transmission experiments were as follows:

Thrips. Using single crock isolation cages, thrips were readily established on mung plants of first trifoliate leaf development or larger. Predominately a black robust species of thrips was present, causing extensive feeding damage (also observed on young plants in the field), but there was no evidence of pathogen development.

One condition prevalent in the field, and believed to be thrips-associated, was a stippled black spotting of the first-leaf pair, particularly of the under surface, as well as other leaves. It was felt that this spotting was due to a saprophytic fungus growing on excreta and sap exudate associated with thrips attack. The same spotting was readily obtained with caged thrips in the absence of other insects, and the black spotting was determined to be due to Capnodium species sooty mold (identified by Dr. Inamullah Khan).

To one caged crock of LP III mung plants three collections of thrips totaling 111 specimens were introduced. Additionally, some caged groups of plants became heavily infested with thrips, probably due to their entering by way of the somewhat open fabric or through other small openings. None of these thrips infested plants developed YM. Despite the great abundance of thrips on very young mung plants in the field, these experiments do not implicate thrips as vectors of any pathogen to this host. Maramorosch (1963), in reviewing insect transmission

of viruses, states that thrips are only known to vector tomato spotted wilt virus.

Leafhoppers. Leafhoppers were readily established on mung plants in single crock isolation cages, even producing nymphs in time. Better acceptance of the plants by newly introduced leafhoppers was obtained by covering the clear plastic top of such cages with leaves or other light diffusing objects, and setting the crocks in shade for the first couple of days after introduction.

The predominant leafhopper on mung at this time of year was a small green species, (probably an Empoasca), though two tannish apparent species were also found in about ten per cent of the total leafhopper catch. Mung plants fed on by leafhoppers began to assume a characteristic appearance, first indicated by the tips of leaves curling over. This was then followed by some vein clearing and a diffuse yellowing that was confined to certain areas of the leaf, not the whole leaf surface.

Very likely the vein clearing and yellowing are local responses to leafhopper salivary toxins, termed "hopperburn", and frequently seen on potatoes, cotton, and other crops. It must be noted, however, that similar diffuse yellowing was seen occasionally on plants in the field. It is recommended that leafhoppers be placed in leaf or branch confinement cages on isolated plants--presumably if only leaves directly attacked by leafhoppers show the symptoms described, this is due to hopperburn and not to a systemically circulated pathogen.

White Flies. By elimination of thrips and leafhoppers as vectors of YM virus, and also based on fragmentary reports in the literature, white flies must be vectors of yellow mosaic of mung. However, to this point it has not been possible to demonstrate white fly transmission under the experimental conditions attempted. The basic difficulty seems due to peculiarities of white fly behavior--it was not

possible to achieve survival of more than two days during transmission experiments, indicating that the methods employed failed to achieve much actual feeding on mung and, therefore, the possibility of transmission was minimal. White flies introduced into single-crock cages, or confined in single-leaf cages, constantly attempted escape wherever the most light entered. Under confinement the escape reaction seems to predominate over the necessity to feed.

White flies brought directly from YM-infected mung fields were introduced into a single-crock cage over insect-free mung plants of second trifoliate leaf group development or older. The plants were LP III, a highly susceptible variety. Within a period of one week, and on three days, a total of 85 white flies from YM-infected mung flies was introduced to this cage. The top of the cage was covered with leaves and the crock placed in shade, yet only once were a few live white flies seen on the day following introduction.

Single leaf cages were developed and white flies were introduced in the hope that closer confinement to leaves would increase the chances of feeding. These cages were constructed of cardboard. On two occasions a leaf cage with clear plastic top was used, with 10 and 25 white flies from infected fields introduced. Live insects were observed the next day but not the day after, and predominate behavior observed was continual walking over the clear plastic surface. To date four leaf-cage experiments have been conducted in which white flies were confined in a dark chamber, with light entering only through the leaf. After two days the cages were opened but no insects, live or dead were observed, suggesting they had escaped through minute openings between the cage wall and the leaf. In these latter experiments it is doubtful that sufficient time has elapsed at this writing for YM symptoms to develop even if transmission occurred.

Other possible vectors. The possibility remains that some arthropod vector of YM pathogen has simply not been detected as yet. It hardly seems likely that insect

vectors have been overlooked, therefore the remaining possibility would be a very small mite. Tetranychid mites cannot be involved, because although they were seen they were not common. Microscopic mites in the family Eriophyidae are a possibility that should not be overlooked, though if they are involved and are abundant it would seem likely that at least one plant under insect exclusion cages would have developed yellow mosaic simply because microscopic mites could hardly be entirely excluded. It is worthwhile mentioning that the eriophyid Aceria tulipae transmits wheat streak mosaic virus, and the vector of sterility mosaic virus of pigeon pea (Cajanus cajan) has been stated to be Aceria cajani Channabasavanna, though there is some doubt that this is the case (Williams et al; 1968).

4. Sticky-stake indexing of mung varieties.

Plant breeders at WPAU have tested many lines of mung for YM resistance, with quite high levels of partial resistance identified. When plant resistance to an arthropod transmitted pathogen is noted it is likely that intrinsic resistance to this pathogen within the plant line selected is the primary mechanism (Carter, W. 1962. Insects in relation to plant disease. Interscience, N.Y.; 705 pp.). Additionally, however, lower rates of transmission may occur in such resistant lines because they are not very attractive to insects. The latter possibility was sought in this experiment.

Plots of three lines, replicated three times each, were planted on August 10. The lines were comprised of LP III (highly susceptible), S 64 (intermediate resistance), and C 602 (high partial resistance). One sticky stake was placed near the center of each plot on August 19. To date only this single index was taken because these plots were mistakenly treated with endrin on August 24, but that

single index showed the susceptible variety to have the lowest sticky-stake count of thrips and white flies, and both other varieties to have higher numbers. The differences were not very great, but the indexing should be repeated several times to see if a consistent trend is present, provided insect populations again build up to sufficient levels after the endrin treatment.

It might seem a paradox that the lowest count could occur in plots containing a preferred plant variety. This may be as expected, however, because the stakes only measure insects impinging on them. If a plant variety is not very suitable for insects they will spend more time searching for suitable plants and less time actually feeding, thereby becoming more likely to contact the stakes.

5. Effects of early protection on seed yield.

Generally speaking, the earlier a plant pathogen is acquired the more effect it has on seed production of the host. To test the feasibility of concentrating upon early insect control, three insect-exclusion cages were placed over two feet of two rows within the susceptible variety LP III on August 11, the day after planting. These cages are to be removed one at a time at two-week intervals, the last one remaining for six weeks. The plants are to be marked and a comparative estimate of yield obtained.

Two days after planting a very heavy rain occurred and one cage had to be moved to a new site one week after original placement because the first portion was in a low spot where very little stand developed. On August 23 it was observed that at least two plants in the new location were exhibiting YM symptoms, and when the cage was removed on August 25, three of seventeen plants enclosed had symptoms. To date none of the plants under the undisturbed exclusion cages show symptoms. By inference one can deduce that a significant amount of insect transmission of YM virus occurs within the first week, and the incubation period before

symptoms appear (under the temperature and humidity conditions within the cage) is around twelve days.

6. Systemic insecticides to provide early insect control.

On the theory that early protection from arthropods will delay YM occurrence and thereby reduce its effects, five systemic insecticides that were locally available were applied as the granular formulation in the furrow along with the seeds. The high YM-susceptible variety of mung, LP III, was used. Each treatment, as well as the untreated control (check), consisted of a single plot 25 X 25 feet. In the belief that vector insects would succeed in transmitting YM to the border rows, only all the plants in the center of each plot out to eight feet from each border were counted, and indexed for YM symptoms 15 and 24 days after treatment. This indexing should be carried out at least two more times, and final analyses of mung seed production should be made for comparative purposes.

Each systemic insecticide was applied at a rate of one pound actual per acre. This rate is probably higher than would be feasible, but such a rate was chosen to see if satisfactory results could be obtained when plants were heavily protected. All materials consisted of 10% granules, except Bidrin which was formulated as technical material applied to sand.

The results obtained thus far are as follows:

Insecticide	Thrips[1]	White flies	Leafhoppers	% Plants with YM[2]
None (Control)	10	3	1	9.4; 15.0
Thimet[3]	10	5	0	4.2; 22.8
Bidrin	7	4	0	4.7; 19.3
Disyston[3]	13	6	0	1.0; 17.4
Temik	10	5	2	6.5; 17.7
Furadan	4	0	0	2.6; 19.9

BEST AVAILABLE COPY

1. Analysis from a single sticky-stake placed in each plot for 24 hours on August 18, eight days after planting. No further analysis has been made to date because all these plots were mistakenly treated with endrin on August 24. A visual estimate of thrip populations on the plants on August 19 suggested that all materials but Bidrin had about 1/6 the population of the check, Bidrin had about 1/4. On the same date those plots for which seeds were treated with Brassicol has 1% of presumed damping off, untreated check plants had 13%.
2. First indexing took place on August 25, 15 days after planting and two days after symptoms of yellow mosaic were clearly present in the plants; second indexing 24 days after planting. From 149 to 254 total plants were counted due to poor stand resulting from excess rain; perhaps also due to phytotoxicity.
3. Treatment showed some phytotoxicity at the rate of insecticide applied. Phytotoxic symptoms consisted of drying of the tip and edges of the first leaf-pair as well as slight early retardation of total plant growth.

It can be concluded that systemic insecticides provide some protection against early acquisition of YM pathogen, but later YM rates are higher. This same phenomenon has been seen when certain insecticides were used to control aphids transmitting leaf roll virus of potatoes in the state of Washington. Apparently in the presence of insecticides, if vectors are not rapidly killed, irritation from the pesticide causes restless behavior that results in more feeding probes on larger numbers of plants. In the present situation this suggests that the vector(s) can transmit YM pathogen during brief feeding probes. At time of writing the plants within the Furadan plot look the healthiest.

Conclusions

Though the experimental approaches used are not completed at time of this writing (September 2), certain conclusion can be made about yellow mosaic and other pathogens of mung. In summary these are:

1. Yellow mosaic pathogen is arthropod transmitted, and not transmitted by infected soil or nematodes. If any seed transmission occurs, infected seed is present at a rate of less than 0.5 per cent.
2. Yellow mosaic pathogen is probably transmitted by white flies, however, transmission by eriophyid mites may also need to be investigated.
3. Very significant amounts (17-35% of all plants in a stand in this analysis) of transmission of yellow mosaic take place within the first week after planting.
4. In very young plants, under conditions of high temperature and humidity, the incubation period before first appearance of symptoms is about 11 days.
5. Sticky stakes can be used to satisfactorily index populations of small insects of mung, especially thrips and white flies.
6. Thrips cause more damage to newly emerged young plants than has been previously suspected. The overall effect of this early damage is not known.
7. A commonly observed black spotting, particularly to the underside of young leaves of mung and mash, is due to the development of sooty mold (*Capnodium* sp, and perhaps other fungi) on the excreta or sap exudate associated with thrips attack.
8. The feeding of leafhoppers resulted in somewhat diffuse yellowing symptoms and vein clearing in mung leaves, but it must be determined whether this is due to salivary toxins or to transmission of a pathogen.

9. Protection of mung plants from insect attack in the field, by the use of insect exclusion cages, results in plants entirely free from yellow mosaic symptoms. Such plants have much more vigorous growth than surrounding unprotected plants, no doubt due to a "greenhouse" effect, but also possibly due to the complete absence of thrips and other insect attack, as well as the absence of arthropod-transmitted pathogens.
10. Systemic insecticides, applied as granules in the furrow at the time of planting, can provide some protection from thrips, and probably other insects as well. Such treated plants also do not acquire yellow mosaic symptoms as rapidly as untreated plants.
11. Observations suggest that the systemic insecticide Furadan, applied as granular formulation at time of planting, provides better very early insect and yellow mosaic protection than Temik, Thimet, Disyston, or Bidrin applied at identical rates in an identical manner. However, it is doubtful that this method is of any significant use.
12. A combination of presently available lines with partial YM resistance, plus the development of good early insect control programs, can undoubtedly radically increase present average yields of mung, and possibly of related legumes. Such a program could be developed very soon, and could be recommended while desirable highly resistant lines are being bred.

Additional Program.

During the term of this consultation I presented six informational seminars within the field of entomology. The subject of these talks, as well as the audience participating was as follows:

1. "The current DDT situation in the United States: implications for world agriculture." WPAU Entomology faculty and graduate students; personnel from many disciplines at Punjab Agricultural Research Institute.

2. "Research program on mosaics and related problems of mung." Entomologists, plant pathologists, and plant breeders of WPAU and PARI. (A review of the research program started at Lyallpur).

3. "Insect photoperiodism." Entomology faculty and graduate students, WPAU.

4. "Epidemiology from the entomologist's viewpoint." Faculty and students, veterinary medicine, WPAU.

5. "Pest management." Entomology faculty and graduate students, WPAU.

6. "Entomological research in the state of Washington." Entomology faculty and graduate students, WPAU.

RECOMMENDED RESEARCH APPROACHES

Plant Breeding.

Yellow mosaic of mung is exceptionally prevalent in the Lyallpur area. It is, therefore, readily screened for in any plant breeding program conducted at this location, assuming the same pathogen or pathogen complex is characteristic throughout mung, mash, and lobia-growing regions of West Pakistan. Since lines of great genetic diversity are already at hand, and different levels of resistance to mosaic have been recognized in these lines, plant breeding is the most promising means of solving yellow mosaic. Disease resistant lines should be selected further, and if these do not have sufficient other desirable qualities they should be crossed with lines having desirable characters, and the progeny further screened for favorable attributes.

Since it is now recognized that arthropod transmission is necessary for the development of yellow mosaic, entomologists should work closely with the plant breeders in determining insect resistance; especially plant resistance to vector species of arthropods once such vectors are determined. By this is meant that entomologists should go into the field and index lines for resistance to vectors at the time principal transmission occurs. Plant pathologists should also study the pathogen entities present, develop reliable means of determining which entities are in the field, and learn to index lines. Once this has been achieved they should join the plant breeders in selecting resistant lines.

Even when the details of arthropod transmission of yellow mosaic and related viral pathogens become known, development of lines resistant to such vectors as well as lines resistant to the pathogens holds great promise. Control by the use of insecticides should be further attempted, but this approach does not fit well into the economy of the region, and such technology is beyond the ability of the average small farmer at the present time. Furthermore, there is no present assurance that adequate control of yellow mosaic by the use of insecticides can be accomplished.

Entomology

There are important tasks for entomologists that must be completed to bring this research program to a satisfactory conclusion. I urge that at least one entomology faculty member and two to four graduate students at WPAU be assigned to devote major effect on this program. The tasks to be completed cannot be solved from behind a desk; therefore, each faculty member assigned must have good powers of observation, ingenuity, and sufficient conviction and dedication to the task. He must agree to spend time in the field observing, and directing the research program of graduate students.

Mung and other virus-affected pulse plants must be readily available for making observations and conducting research. I specifically recommend that pot cultures of host plants, and insect isolation cages, such as were used in this research investigation, be constantly at hand. Perhaps most important of all a nearby plot area, which need consist of no more than ten to twenty rows of host plants, should be maintained as near as possible to the research center (for instance near the insectary) in order that research faculty and graduate students can readily observe transmission occurring under natural conditions, and can easily obtain infectious vectors for transmission experiments. This small plot area should have a continual supply of susceptible plants in different stages of early development, and after one month to six weeks such plants can be removed and the area replanted. By this means vector arthropods can be observed with the least difficulty, and insect-exclusion cage experiments can be easily conducted.

Before any further research is conducted the greatest care must be taken, by dissecting and observing a large number of young mung and mash plants under a dissecting microscope, to eliminate the possibility that an eriophyid or other small mite is not the vector sought after. After this, top priority must be given to studying potential transmission of YM pathogen by white flies. This means that techniques for feeding white flies, to the exclusion of other insects, must be developed to the point where it is definitely known that such feeding takes place. Furthermore, the species of white flies on susceptible hosts must be accurately determined, to be certain that these species are concentrated upon. This is no simple task, because accurate determination can only be made from pupal cases. The local fauna consists of this *Bemisia tabaci*, as well as *B. hancocki*, and at least three other species. Those species present in mung fields must be concentrated upon.

Since it is now known that a high production of transmission occurs on young host plants, it may be desirable to concentrate attempts at transmission on very young plants. The mechanism will only be truly verified when it is possible to obtain transmission via uninfected vectors from infected to uninfected hosts. By this means, such details as time necessary for pathogen acquistion and transfer by vectors can be determined.

White fly behavior must be carefully studied, once transmission can be repeatedly achieved such behavior of vector species can be analyzed under natural conditions. These analyses can be accomplished by a graduate student, who must determine for vector species the details of their life history, host plant preference, feeding behavior (time of each feeding probe, number of different host plants fed on per unit of time, etc.), flight behavior, and so forth. Assuming white flies are vectoring YM pathogen, detailed knowledge of the behavior of the vector species will serve as a valuable adjunct for the control of this disease.

Attack of thrips on very young pulse crops is much more severe and prevalent than had been previously recognized. A careful analysis must be made of this attack to determine what effect it ultimately has on seed yield, and to also determine whether control of these insects would realize economic returns. The leaf spotting caused by thrip attack, and attendant sooty mold, should be studied to determine if this affects seed yield. This can be accomplished by rearing two identical groups of host plants under insect-exclusion cages, but infecting one of the cages with thrips to obtain an attack level typical of natural conditions.

Assuming white flies are the vector of YM and related pathogens of mung, experiments need to be conducted to determine whether these insects can be controlled in order to reduce virus transmission, and particularly whether such control methods will be economical. Present indications are that this cannot be done

solely by applying systemic insecticides in granular form at the time of planting. Furthermore, the systemic insecticide approach seemed to increase transmission--behavior of white flies under these conditions needs to be compared against behavior of white flies on untreated plants.

Pod borers of gram, the lepidopteran Heliothis armigera and other species, could not be studied under natural conditions during the course of this investigation. Nonetheless, it is the writer's understanding that attack by this insect is an important problem. An entomologist should work with the plant breeders to analyze for resistance to such attack. Additionally, an excellent graduate student program could be developed. Heliothis armigera can be continuously reared in the laboratory on artificial diets such as wheat germ diets. To such diets the tissue from pods and young seeds of different lines of gram should be added, and the development of young larvae analyzed. Likewise, pods of different lines should be presented to female pod borers to determine which lines are avoided in oviposition, assuming that this is the site where oviposition occurs. Once resistance to larval growth or female oviposition is found, the physical and chemical characteristics of resistant lines should be determined. When characters conferring resistance are identified the task of selecting for pod borer resistance will be much easier for the plant breeders.

Some four beetle species in the family Bruchidae attack the seeds of gram. A rough texture in the seed coat has been shown to deter oviposition by these insects (1969 progress report, International Pulse Improvement Project). Bruchid resistance experiments should be conducted. These would consist of exposing known lines, provided by plant breeders, to determine oviposition preference as well as rate of larval development under standardized conditions. Once mechanisms of resistance to bruchids are known, the task of the plant breeders in incorporating

bruchid resistance will be greatly facilitated. Additionally, investigations should commence to develop methods of protecting pulse crops in storage, as there will be some differences in the attacking insect and mite complex as compared to protecting wheat in storage.

Plant Pathology.

The services of a plant pathologist, particularly one versed in determining plant viruses, should take high priority in further research on mung and related crops. If such a resource person is not available locally he should be brought in on at least short term consultation, and he should acquaint WPAU professional plant pathologists and their graduate students with methods for distinguishing and characterizing virus entities. Much more needs to be known about mosaics affecting mung and related legumes, as well as entities causing leaf crinkle, top necrosis, and other symptoms. As the pathogens are characterized, many of them will undoubtedly prove to be arthropod transmitted. Therefore collaboration between plant pathology and entomology will prove fruitful.

Agronomy.

Since YM is undoubtedly arthropod-transmitted, various cropping practices will lead to high or low rates of transmission. Once the vector is identified, entomologists should join with agronomists in studying the relationship of management practices in mung to arthropod transmission of YM. For example, useful avenues include the effects of date of planting, management of weeds (especially of possible alternate virus hosts), plant spacing, cultural practices, and fertilization. Proper management of these practices can reduce vector arthropod populations or alleviate the effects of the disease. These approaches cannot be rationally conducted until the entomologists have certainly identified the vector species involved.

In Summary.

Mosaics, and similar pathogens of mung and related legumes, are presently of utmost importance in affecting seed production of these grain legumes. So-called mung yellow mosaic has been demonstrated to be arthropod-transmitted, probably by white flies. The control of this important problem, and of associated pathogens, will be most speedily and economically achieved by collaborative studies among responsible personnel assigned from the fields of plant breeding, entomology, plant pathology (expecially virology), and agronomy.

RESEARCH REPORT ON ARTHROPOD-TRANSMITTED VIRUSES OF MUNG AND RELATED EDIBLE LEGUMES

by

Robert F. Harwood, Consultant
Professor and Chairman, Department of Entomology
Washington State University
May 1972

Introduction

The first report on this subject covered a period from mid-July through the first week of September, 1970. No introduction to the general problem will be discussed here because it is covered in the first report. I was unable to return to this study during the 1971 growing season, but fortunately Mr. Mushtaq Ahmad, a M.Sc. candidate under the direction of Dr. Mukhtar A. Halimi, Entomology Department, continued the program with considerable success.

The present report covers a period of investigation from April 11 through May 26, 1972. This is a time when daily temperatures and white fly populations are characteristically lower than during June through August; therefore conditions were markedly different from my previous investigation. The sum of observations during both periods of study, covering the summer period, has been useful in analyzing the actual and potential problems to be encountered.

As was characteristic of my previous visit, a high degree of cooperation was obtained from several individuals and departments. I particularly acknowledge the assistance of Mr. Mushtaq Ahmad, now appointed to the Department of Plant Breeding and Genetics; likewise Dr. Muhammad Aslam, Head, and Dr. Abdullah Khan of this same department. Dr. Muhammad Yunus, Head, Department of Entomology, and

other members of that department were very helpful on many occasions. Dr. A. O. Shaw, Research Advisor, WSU/AID program, was invaluable in implementing many items during the current period of investigation.

A brief review of past findings will bring the status of this research project up to the present study. During my investigations of 1970 it was clearly shown that yellow mosaic (YM) of mung is transmitted by some arthropod, and by inference the transmitting agents were believed to be white flies, especially Bemisia tabaci. A test with systemic insecticides applied at the time of seeding indicated that this method held no real promise for controlling YM; but from June through August, when thrips become abundant, insecticides might be required on every young plant to prevent excessive thrips damage. Toward the end of this previous study two grafting experiments between YM-affected and non-affected plants were conducted, and one of the healthy plants came down with YM symptoms after my departure.

Experiments conducted by Mr. Mushtaq Ahmad during the summer period of 1971 further confirmed transmission by grafting, and also showed that Bemisia white flies from fields of mung containing high amounts of YM symptoms would pass on YM to healthy mung and soybean plants. Symptoms were obtained in a minimum of ten days.

Review of 1972 Weather Conditions

During the present study carried out in April and May, the weather was unseasonably cool at first. This seems to have had a profound affect on the expression of YM symptoms, with incidence of this disease quite low in the field, and actual reversion of symptoms in many cases, especially in partially tolerant strains of mung.

Present Study Transmission Tests

A number of transmission tests have been run to date using white flies, leafhoppers, and aphids, particularly from mung plantings infected in the field, to mung (*Phaseolus aureus* L.), mash or urid bean (*P. mungo*), moth (*P. aconitifolius*), cowpeas (*Vigna sinensis*), peanuts or groundnuts (*Arachis hypogaea*), soybeans (*Glycine sp.*), fava beans (*Vicia fava*), chickpeas (*Cicer arietinum*), cotton (*Gossypium sp.*), cucumber (*Cucumis sativus*), and tobacco (*Nicotiana tobacum*). Non-leguminous hosts were included in order to determine if these might show a particular kind of response useful in identifying viral entities.

Transmission experiments were carried out with single-leaf cages, usually containing about 20 white flies that before placement on healthy host leaf had been confined overnight on a host plant leaf bearing symptoms. In addition, small isolation cages were placed over all of one or two healthy plants, and several white flies or other suspect vectors were swept from diseased plantings and introduced. For reasons not clear, no transmissions were achieved to date (May 25). However, less than ten days of incubation have transpired since most of the whole plant exposure tests were started. It is my belief that early transmission experiments failed because of temperatures that were too cool, or single leaf cages were frequently placed on the first-leaf pair rather than subsequently developing trifoliate leaf groups that may be more susceptible. Single-leaf cage tests may fail most of the time, because observations lead me to believe that it is the very young unopened leaf terminals that are highly susceptible to YM. I might add that the sudden advent of very high temperature from mid-May on in 1972 destroyed many of the plants confined in cages.

While lack of experimental transmission was a keen disappointment, confining white flies to these various host plants provided good insight into their

suitability as a food source encouraging white fly survival and reproduction. This will be commented on further in a subsequent section.

In an attempt to transmit YM virus using white flies from mash to mash, success was obtained after 13 days.

Nature of YM Virus

At the outset I must make it clear that reference to YM virus infers a single etiological agent, yet there may well be several distinctly separate or separate strains of agent. A recent paper by Indian workers suggests three entities (personal communication from Floyd Williams, Plant Pathologist, AID). From observations during April and early May, I conclude that YM symptomology requires sustained high temperatures, probably averaging over 90° F., and the virus is not very systemic so that characteristic symptomology on most of the leaves from June through August is due to very active and continuous transmission by white flies. I further believe that older leaves do not succumb readily to the virus, and under cooler conditions leaves that started to show early evidence of infection reverted to a healthy appearance. To validate these statements it was found that average maximum temperature in April 1971 at Lyallpur was 95.5° F., this same month in 1972 was 89.9; in April 1972 mung plantings showed less YM infection than in 1971 (personal communication from M. Ahmed); many lines indexed in 1972 actually showed a decline in symptoms (see 1972 report of C. G. Shaw).

One important question regarding YM virus is the nature of its maintenance during times mung and related legumes are not present. In a situation of this type, where leguminous plants are affected, one would be particularly suspicious of wild symptomless, or nearly symptomless, legumes. No good clues were obtained along these lines. Chenopodium weeds were suspected at one point because of a slight twisting of the leaves on these plants, but nothing is certain about that

speculation. I am suspicious of bindweed plants, Convolvulus arvensis, because these are prevalent in any mung plantings. I have observed in several locations, and where I have observed YM-affected plants I have also succeeded in finding some Convolvulus that were yellow instead of green, occasionally with a yellow mottling and twisting of the leaves. During May white flies were seen to preferentially feed on yellowed Convolvulus, and they also like to feed on this plant during the winter period (communication with M. Ahmed and A. O. Shaw). While there may be more than one wild host of importance, a thorough study, including experimental transmission, should be conducted with Convolvulus.

White Fly Behavior

A study of white fly behavior, particularly preference of hosts and color preferences, has provided the most interesting clues to the role of these vectors in YM transmission. Regarding host preference, during transmission experiments the preferred plants in terms of longevity and egg laying appeared to be soybean, cucumber, mung, fava beans, and cotton; tobacco and chickpeas were very unsatisfactory and other plants tested were somewhat intermediate. On preferred plants good survival took place through two days, and even up to six days. Published information states that these white flies will not survive more than six hours without feeding, and our tests revealed no more than four hours survival under such conditions. Tests on different strains of mung and related legumes should be possible to suggest their suitability for sustaining white flies.

Bemisia white flies were found to show a marked preference for yellow and orange color. This was suspected from simple field observations, and clearly confirmed by exposing for 24 hours in mung plantings, sticky stakes of different colors. The accompanying table indicates the degree of preference.

Large size stakes (35 in.^{2} sticky surface)

White Flies Caught On	White	Green	Yellow	Red
May 8	1	9	45	3
May 15	4	1	90	2

Small size stakes (3.75 in.^{2} sticky surface)

White Flies Caught On	White	Yellow	Red	Pale Green	Orange	Pink	Light Blue
May 16	0	25	2	1	24	4	2
May 22	1	15	5	1	21	1	0

This preference for yellow to orange seems to be of great significance. It means that white flies will be very attracted to YM-affected leaves, that are bright yellow in color, later often becoming orange, the flowers are yellow, and the tender new terminal growth of most mung varieties is yellowish-green. This finding would confirm that a high degree of symptomology can occur at proper high temperature conditions, even if the viral agent is not highly systemic, because the large numbers of white flies preferentially acquire virus from yellowed leaves, passing it on to new growth and flowering structures. It also explains why on occasion one can observe YM-affected seed pods, which developed after the yellow flowers, while on the same plant there may be no evidence of YM on the leaves.

A similar attraction to yellow has been noted in various aphids, and studies in Washington State suggest heaviest transmission of viruses to common beans by aphids attracted to varieties with a yellowish leaf color.

Heritable Characters of Mung

The Plant Breeding and Genetics Department has selected out a few strains of mung that appear to have partial resistance, and in addition I was able to

examine 92 lines of F_2 and F_3 hybrids obtained from a USDA collection of crosses where there was some resistance to YM indicated in at least one parent. T^I, a resistant line from south India, was one of the parents in most cases. Some observations on YM resistance, and on heritable characteristics, follow.

The lines selected for YM tolerance show a wider, and more reversible, symptomology than ordinary unselected lines. This was particularly noted in lines 6601 and 6602 (see C. G. Shaw 1972 report). In these lines YM symptoms, when present, never appear as intense as in unselected lines. Instead of brilliant yellow and sharply defined plagues of chlorotic tissues in the leaves, there is more characteristically a fine light-yellow stippling.

The following heritable characters in the hybrid lines seem noteworthy: (a) The same mild stippling indicating partial resistance to YM. (b) Dark stem and leaf petiole, usually accompanied by stiffer leaves protruding out from the central stalk and providing a bushy appearance. Also more dark green in color, including new growth, therefore should be less attractive to white flies. (c) Leaves tridentate, referred to as "okra leaf". (d) Flowers whitish to whitish-green instead of yellow, also should be less attractive to white flies. (e) Early maturity, which should be desirable because pathogens generally affect seed yield more if symptomology sets in before seed develop. (f) Plant height. The shortest plants were most subject to animal feeding on the developing pods and seeds. The type of cutting damage, and attack at lower levels, makes birds suspect though the birds concerned were not identified.

Review of Barrani Conditions

From May 15 through 17, accompanied by Dr. Abdullah Khan, I was able to make a survey trip by car through the barrani (rain fed) areas to the west

and north of Lyallpur. The route was as far west as Piplan, near the Indus river, north to Campbellpur, and back southeast by way of Rawalpindi, Gujrat, Jhelum, and Lahore. This survey was desirable in order to determine the potential for growing edible legumes, other than chickpeas which are a standard crop in much of this area already, in such nonirrigated lands. There was much evidence of peanuts, moth, and some mash often interplanted with pearl millet. Mung plantings were seen as trials near Jhelum and Gujrat. In summary it can be said of all this area that white flies were very scarce. Convolvulus weed was abundant but all appeared green, some mosaic symptoms were seen in low incidence on moth. The experimental plantings of mung were of variety 6601, known to be rather YM tolerant, but in one adjacent planting of local mung a small amount of YM symptoms was seen. This was much less than one per cent incidence. Leaf crinkle symptoms were seen in low incidence at all locations.

It is my belief that YM virus will not be much of a problem in this barrani area, but increased yields are likely dependent on more comprehensive growing in irrigated districts such as Lyallpur, where white flies and YM are abundant.

Likelihood of Further Success

a. Several YM tolerant strains of mung, notably 6601 and 6602, have been developed. These plantings looked very good at Lyallpur during April and May, though there was still much evidence of leaf crinkle. It is not possible to say how well these will hold up in June plantings during the severe July and August YM period, but in any event they will be superior to local varieties.

I believe further success can be achieved by simple selection and by developing hybrid lines, especially as desirable criteria can now be selected.

b. In May there was clear evidence of YM resistance on mash plantings at Lyallpur. The evidence of resistance is much clearer than on mung, and it should be relatively easy to select lines with YM resistance combined with other desirable qualities.

c. Further work on cowpeas, pigeon peas, and peanuts, seems worthwhile. Five lines of cowpeas were seen growing at Lyallpur, though later plantings may be much more subject to virus attack. A leaf crinkle condition observed, especially in white seeded varieties, is apparently seed transmitted because it appeared in plants grown from this same stock where insects were excluded. Peanuts are extensively planted from Rawalpindi across to Jhelum, indicating there is no great problem there. At Lyallpur, peanuts in June through August are severely affected by a yellow condition, and this needs to be further investigated if production is to be increased in hotter irrigated areas.

d. Soybeans should be selected for mosaic resistance. The whole problem of soybean utilization depends on simultaneous development of processing facilities and increased production. It may well be that soybeans can only be produced well in cooler foothills areas in June through August, but some promising selections of this crop were seen at the Punjab Agricultural Research Institute at Lyallpur in early May, possibly again because April and early May temperatures were unseasonably cool.

Recommendations

a. Trials and selections of mung have been primarily carried out at Lyallpur, especially to seek resistance to YM. Very likely any great increase in production will depend on irrigated areas of agriculture, but some varietal

trials should be carried out under barrani conditions to determine desirable agronomic characteristics in nonirrigated production.

b. Further attention should be paid to leaf crinkle, which as stated may be more than one condition. This general condition affects mung, mash, and moth, it never occurred on plants grown where insects were excluded so it must not be seed borne. However, attempted transmission by leafhoppers and white flies failed.

c. A greenhouse facility needs to be developed for winter and spring insect transmission tests. These tests could cover the period of late September through early April. The facility need not be complicated or expensive, merely consisting of about three 10' x 10' rooms within an existing greenhouse, with muslin cloth walls to isolate insects and with the ability to maintain temperatures above 90° F., and perhaps with additional light supplementation in winter. Such a facility would permit greatly accelerated screening of crosses and selections, but it would not be used in summer when full-scale field tests are practiced in any case. At a later period this experimental transmission facility could be used to study insect transmission of pathogens to forage legumes, tomatoes, potatoes, cotton, and other crops.

d. Some members of the WPAU faculty should be sent abroad for specific training in insect transmission of phytopathogens. There are many special techniques in this type of investigation, and no person at WPAU has any formal training along these lines. I suggest Mr. Mushtaq Ahmad be sent for such training, perhaps earning a postgraduate degree at an institution such as the University of Hawaii, or the University of California, Riverside. Mr. Ahmad is young, has good intellectual curiosity and drive, and would be able to provide maximum future service to WPAU.

e. There needs to be a reference insect and mite collection developed for edible legumes. Several types of injury, often minor, were observed on a variety of these plants. There appears to be surprisingly little knowledge available at WPAU on this subject, and particularly on typical injury. A collection of specimens, clearly identifying associated hosts and characteristic damage would be most useful.

f. More critical indexing of selections and crosses of mung is needed. At present, lines of a given length are planted assuming that numbers of plants are similar. Counting all the plants in the hybrid selections of mung revealed that as much as 100 per cent differences in stand occurred. I recommend planting with dibble stick spacing, perhaps one seed per six inches. In this manner the rate of germination, occurrence of damping off, and percentage of disease incidence, can be readily calculated. There should also be more widespread use of tally counters in order to quantify results, rather than relying on general appearances.

g. A rate of transmission study was started but not completed before my departure. This consisted of covering seeds in a new planting with three insect-exclusion cages. When the plants were well up the cages were removed for two, three, and four days each, and then replaced to the same location until symptoms first develop. Shortly thereafter a percentage of YM affected plants can be related to days of exposure, and similarly rates of transmission could be calculated at different times of the year in different locations.

h. In my 1970 report I recommended that a study be carried out for resistance of gram to Bruchus weevils. Mr. Hafiz Qayyum, Department of Entomology, has started some promising investigations along these lines, and these could be extended to include a research for resistance in other edible legumes.

i. For all of the insect or seed-transmitted pathogenic conditions observed the most practical answer to control lies in plant selection and breeding. The use of insecticide for these conditions will often not succeed because of the high numbers of insect vectors encountered, and insecticides are expensive and dangerous for untrained persons to use.

PROGRAMMED RESEARCH REPORT ON GRAM WILT AND GRAM BLIGHT, AND A NEW FOLIAGE DISEASE ON GRAM IN WEST PAKISTAN

by

Vernon E. Wilson, Consultant
Plant Pathologist, Washington State University
April 1971

I. Introduction

Three objectives influenced gram (*Cicer arietinum*) research in West Pakistan, January to April, 1971. These were (1) to review the work completed as outlined in Dr. C. G. Shaw's "Proposed Research Program on Gram Wilt and Gram Blight", printed at Lyallpur, April, 1970 (hereafter called C. G. Shaw's report); (2) to continue the search for methods of screening for disease resistant gram varieties, and (3) to accelerate interdisciplinary participation among West Pakistan Agricultural University (WPAU), Washington State University (WSU), and other research institution members, to hasten gram improvement.

Acknowledgement of cooperative participation goes to many persons for making this assignment possible, administrators of WPAU, WSU, the U.S. Department of Agriculture (USDA), and U.S. Agency for International Development (US AID) in Pakistan. Special acknowledgement belongs to Dr. A. O. Shaw, Chief of WSU Project and Resident Research Advisor, Lyallpur; Mr. Abdullah Khan, Plant Breeder, and Mr. Javed Iqbal, Plant Pathologist, both WPAU.

Justification for this program has been previously established in C. G. Shaw's report. Also, "The Food Legumes", a survey by Dr. Lewis M. Roberts, The Rockefeller Foundation, published in 1970, further underscores the importance of legume research.

Orientation to the gram research program at WPAU was conducted by A. O. Shaw. Administrative personnel and the faculty of the College of Agriculture were introduced. Staff members detailed gram research in the department of Plant Breeding and Genetics, Plant Pathology, Entomology, and Agronomy. A comprehensive tour of gram nurseries included those adjacent to WPAU campus, off-station plots, and a review of the nursery at Punjab Agricultural Research Institute (PARI). Gram under observation included 13 advanced lines growing in seven locations, lines imported from USDA-AID Regional Pulse Improvement Project (RPIP), and miscellaneous lines being grown for disease and agronomic testing.

II. Procedures:

Gram Wilt: By March, 1971, experiments designed to induce Wilt, outlined in C. G. Shaw's report, had been completed and reviewed. Neither field nor greenhouse experiments, including seed inoculation, spore suspensions, and soil infestations, had produced Gram Wilt.

Eighty out of 100 isolations made from roots of chlorotic plants proved to be *Fusarium* spp., substantiating similar isolation work done in 1970 by C. G. Shaw. Chlorotic plants suspected of Wilt-infection and tagged early in the season later developed symptoms of virus diseases. Inspections of the root systems revealed *Fusarium* spp. infection.

Cultures of *Fusarium* spp. were chopped and mixed into greenhouse soil prior to planting gram in pots. Seedlings germinated and developed without symptoms of Wilt. Chopped *Fusarium* spp. was spread around potted gram seedlings, then worked into the soil. This process failed to produce Gram Wilt.

An experiment using chopped root tissue from diseased plants, mixed with soil at two percent tissue by volume, produced discolored roots on seedlings that died soon after germinating. In 1971 all experiments designed to produce Gram Wilt failed.

Gram Blight: A review of Gram Blight studies revealed that although Blight-inducing procedures outlined in C. G. Shaw's report had been closely followed, an epiphytotic failed to occur. Thus, a screening technique to determine disease-resistant varieties had not developed. Techniques were not at fault. An unusually dry growing season prevented development and spread of the causative agent, *Ascochyta rabiei*. Therefore, experiments using moist chambers to offset dry atmospheric conditions were set up.

Experiment I was planned to determine if established plants growing in the field could be infected with Blight by enclosing them in plastic bag moist chambers. Ten plants each of 29 gram varieties, growing in the field since October, 1970, were sprayed with a water-spore-suspension of *A. rabiei*. Plastic bags were slipped over each plant, secured at the base, and left in place 72 hours. Temperatures rose to 28 degrees C between 10 a.m. and 3 p.m. daily.

Readings were made on days 14 and 28 after inoculation. One to five ratings were used, one indicating no Blight symptoms and five indicating dead plants.

Results were: 1 variety rated 2; 7 varieties rated 3; 6 varieties rated 4; 15 varieties rated 5, with no infection on controls. This proved that Blight could be induced on plants in the field, under controlled conditions of high atmospheric humidity and temperatures not exceeding 28 degrees C. This technique indicated gram could be screened for disease-resistant varieties in the field, so long as the weather remained cool.

Experiment II was planned to determine if seedlings could be infected with Blight in the greenhouse, for convenience in selecting disease-resistant varieties. Five varieties were planted in pots: 6104; 6114; 6531; C727, and a control. When seedlings were four weeks old they were sprayed with a water-spore-suspension of *A. rabiei*. Each pot was enclosed in a plastic bag for 72 hours.

Temperatures rose to 35 degrees C between 11 a.m. and 3 p.m. daily. Readings were made on days 14 and 28 after inoculation. One to five ratings were used as previously described.

Results indicated variety 6104 rated 4; 6114 rated 3; 6531 rated 4; C727 rated 2, with no infection on controls. Variety C727 showed strong resistance to Blight. Although results indicated gram could be screened in the greenhouse for disease-resistant varieties, it appeared that high temperatures were not favorable to inducing Blight.

Experiment III was planned to refine the screening technique used in experiment II. Four week old seedlings of the same five varieties were treated as in experiment II. Two exceptions were (1) temperatures were kept under 25 degrees C by shading the pots, and (2) plastic bags were removed after 24 hours instead of 72.

Although readings paralleled those in experiment II, symptoms were more uniform and more severe within varieties. So, Blight can be induced under controlled conditions in the greenhouse in 24-hour tests, to screen for disease-resistant gram.

Experiment IV was designed to distinguish if races of A. rabiei existed among isolates in the C. G. Shaw collection at WPAU. Five gram varieties with one control for each variety were planted in the greenhouse. Water-spore-suspension inoculum was prepared from each of seven A. rabiei isolates. When seedlings were four weeks old they were sprayed. Each variety was tested with all seven isolates. Inoculated plants were placed in a metal almost moist chamber for 72 hours. Temperatures were kept below 27 degrees C by shading the chamber.

Readings on days 14 and 28 were inconclusive. Overwatering had inhibited plant development in many of the pots. Responses were widely varied, but trends

suggested different races of A. rabiei exist in this collection. This experiment should be repeated in the hope of effecting more conclusive evidence.

Experiment V was designed to study pathogenecity of eight isolates of A. rabiei on one gram host. Variety I-13, reported to be highly resistant to Blight, was used in this test. Three-month-old field grown plants were potted and transferred to the greenhouse, where they grew for one month prior to inoculation. Ten branches on each plant were tagged. Eight branches were inoculated with water-spore-suspensions of eight isolates from C. G. Shaw's collection. Two branches were controls. Pots were enclosed in plastic bags for 72 hours at temperatures not exceeding 23 degrees C.

Readings on day 9 after inoculation indicated isolates i and m caused greater infection than b, c, d, h, j, or k. The infections on all of the plants was so severe that all died prior to day 14 readings. Results indicated probably existence of at least two races of A. rabiei in the C. G. Shaw collection.

New Foliage Disease on Gram: This disease was first seen on plants growing in the PARI nursery and in plots adjacent to WPAU campus in February, 1971. A survey of other nurseries in the Lyallpur area failed to locate other incidents of the disease. An occasional plant showing the disease symptoms was found in March. Surveys in Campbellpur and Rawalpindi failed to locate the disease in those areas.

The disease first appeared on seedling plants. Symptoms developed on the underside of the stem at the nodal area. Small elliptical breaks in epidermal tissue continued to develop through cortical tissue. Girdling of the stem was common. Disease symptoms developed along the mid-rib of leaves. Seriously infected plants were brittle. Cankers ranged in color from light to dark brown.

One fungus was consistently isolated from infected tissue. It was cultured in the laboratory, but not identified. The fungus produced conidia which were used to make water-spore-suspension inoculum. Six week old gram seedlings were sprayed with this inoculum and bagged in plastic for 72 hours. Temperatures did not exceed 23° C.

Readings on day 15 after inoculation found typical symptoms of the disease developing on seedling stems. Later observation proved the disease was spreading.

In April, 1971, Dr. F. J. Williams, Plant Pathologist US AID, Lahore, and Dr. W. J. Kaiser, Plant Pathologist RPIP, Teheran, examined this disease during visits to Lyallpur. Neither had seen it before, nor a disease similar to it, in India or Iran.

III. Recommendations

Gram Wilt: Continue experiments using soil-root-mixes prepared from diseased tissue. Isolations should be made from seedlings infected from soil-root-mixes. Attention should be made to isolates, fauna as well as flora, associated with root infections.

Studies should be made to determine if predisposing agents or conditions are associated with Wilt.

Gram Blight: Screening of gram varieties for resistance should be done on salt- and alkali-free land. An overhead fog-nozzle sprinkling system should be used to create a high moisture environment during inoculation and for two weeks afterward. Studies to isolate and identify the races of *A. rabiei* should be continued. Screening gram varieties for resistance to different races of *A. rabiei* should be done in the greenhouse during the cool season from October to March.

New Foliage Disease on Gram: This new disease is mostly an unknown factor. It could easily be mistaken from Gram Blight. Future studies should include (1) identifying the fungus and working out its life cycle; (2) determining the extent of crop damage; (3) finding controlling methods; (4) determining if the disease is seedborne; (5) determining if the disease is systemic; (6) determining how it is spread; (7) screening for resistant varieties, and (8) breeding for resistance.

Studies of viral and fungal diseases of gram in West Pakistan will require full-time attention from a resident plant pathologist before appreciable improvement can be expected.

ROOT ROT OF FOOD AND FIBER CROPS,

A SURVEY IN WEST PAKISTAN

by

Stuart D. Lyda, Consultant
Associate Professor of Plant Pathology
Department of Plant Sciences
Texas A & M University
August 1971

I. Introduction. In the United States of America and Mexico a root rot of food and fiber crops occurs annually that costs farmers millions of dollars. The organism causing the disease is a soil-borne fungus names *Phymatotrichum omnivorum* (Shear) Duggar.

The fungus is found in highly calcereous soils of southwestern United States and northern Mexico. It has been reported capable of parasitizing over 2,000 species of taprooted plants such as alfalfa, cotton, peaches, pecans, and most ornamental shrubs. Practically all dicotyledonous plants are subject to injury by this devastating pathogen.

The fungus has the potential of surviving many years in fallow land. It produces small, seed-like, survival structures in the soil which are called sclerotia. They are produced at varying soil depths, depending upon the soil type and environmental conditions. The majority are produced 18 to 30 inches deep in the blackland soils of Texas; however, some are found at depths to eight feet. The vertical distribution of the sclerotia is such that they are not disturbed by normal tillage procedures, thus they are free to germinate and destroy any roots that enter their ecological niche.

After germination, the sclerotia form mycelial strands which ascend the root analogous to an ectotrophic, mycorrhizal fungus and when the strands approach the air-soil interface a necrotic lesion girdles the root and destroys the

vascular tissues. The plant rapidly succumbs, with leaves still attached. These symptoms are very characteristic of Phymatotrichum root rot.

The disease occurs in localized patches, but in years of abundant precipitation many of the patches coalesce and entire fields are covered with dead plants.

In 1935, Vasudeva published studies on a root-rot disease of cotton in the Punjab, and at this time he estimated the loss from root rot to be approximately 1,575,000 rupees. Vasudeva discussed the similarities between the root rot in Punjab and Texas but he did not believe they were caused by the same organism. Most of his isolations yielded two species of Rhizoctonia, i.e., Rhizoctonia bataticola and Rhizoctonia solani.

Many interesting studies were published by Vasudeva and co-workers in the decade following the initial report in 1935. Scientists that continued work on this disease have had difficulty in producing the disease under controlled conditions using R. bataticola or R. solani as inoculum. This has led to speculation that these fungi may not be the primary pathogens.

Mr. Inam Ullah Khan has studied the disease in Pakistan for several years and he has noted that several plant species are subject to root rot similar to that reported for cotton. He has been successful in producing the disease on cotton with a species of Armillaria.

In view of the similarities between the root rot found in the United States and in Pakistan, and in view of the apparent confusion over the pathogen causing the disease in Pakistan, a quick survey was undertaken during August 1971, to compare the two diseases.

II. Survey of Root-rot Regions in West Pakistan. There are several areas near Lyallpur with cotton plants affected by root rot. On August 7, diseased alfalfa and cotton plants were collected from the West Pakistan Agricultural University farm

(Rajwala) and later from the Punjab Agricultural Research Institute. Both Gossypium arboreum and Gossypium hirsutum were found to have root rot.

A one-day trip to the Sargodha region was made on August 8. A light incidence of disease was found; however, some good specimens were collected from several fields.

The following day a lengthy trip was undertaken which lasted five days, and covered the major root-rot regions of the Punjab and Sind provinces. The first day began at Lyallpur, we drove to Gujranwala and then to Lahore. No root rot was observed in the Gujranwala area; however, on inspecting fields mixed stands of G. arboreum and Gossypium thurberi were observed.

On the second day, we drove from Lahore to Sahiwal, Khanewal and Multan. Several heavy infestations were observed at Khanewal and slight disease incidence was noted on cotton at Multan. We visited the assistant cotton botanist, Mr. Nazeer Chaundhri at the Agricultural Research Station. He accompanied us to visit the Cotton Research Institute and the German Demonstration Farm in the Multan vicinity. Specimens of cotton were collected, but not much disease was found in Multan. The German Demonstration Farm had some nice field tests in progress. They were incorporating sesbania into the soil with a rototiller. The sesbania was approximately four feet tall and was well shredded by the rototiller and incorporated into the upper eight inches of soil. Sesbania is one plant that shows a high degree of tolerance to the root-rot fungus in the United States.

All specimens collected on the second and third days were kept in separate plastic bags and were returned to Lyallpur on the fourth day, where they were refrigerated until isolations could be made.

I flew to Karachi on August 11 from Multan and met an AID driver at 7:00 a.m., August 12. We drove to Nawabshah, Tandojam and Hyderabad. Specimens were collected

at Nawabshah and Tandojam. The disease incidence becomes more severe as you go from the northern cotton-growing regions of the Punjab to the cotton-growing regions of the Sind province. We returned to Karachi on August 12 and the next day I flew back to Lyallpur.

In many of the fields single dead plants could be observed scattered throughout the field. These plants were not killed by "white ants". The roots of all plants inspected with termites had evidently been injured during cultivation and termites became established in these necrotic regions.

Many of the farms are so small, the farmers attempt to avoid the infested soils when planting cotton. The prevalence of cotton root rot was more pronounced in the larger fields.

In alfalfa fields many small localized areas with dead plants were found, but several of these were due to rodents feeding upon the roots. Alfalfa is usually not grown perennially as it is in the United States, so root rot is not a serious problem on this annual crop.

III. Root Isolations. Freshly wilted plants were dug from root-rot infested soils and the tops were immediately removed. The roots were placed in plastic bags and refrigerated until isolations could be made.

Before isolation, each root was washed in a 10% chlorox solution for two minutes, and rinsed in sterilized tap water.

Sections of tissue were cut from either the periderm or the stele and plated on a medium consisting of 2% agar, 0.2% KNO_2, and 200 ppm. each streptomycin sulfate and penicillin G. The plates were wrapped in cheesecloth or placed under a bell jar at room temperature (30-35° C.) for several days until each isolation could be examined microscopically. Transfers were made from colonies

radiating from each section onto potato-dextrose-agar slants fortified with streptomycin sulfate and penicillin G (200 ppm.).

No mycelial strands were observed on any roots. Isolations from cotton roots yielded species of Fusarium, Rhizoctonia, Curvularia, Helminthosporium and from alfalfa these same fungi plus Chaetomium. Several unknown organisms were also observed in some plates.

Phymatotrichum omnivorum was not found in any of the isolations. The fungus may be present, but it does not grow very well in the presence of other organisms. There is need for a selective medium to isolate this fungus.

IV. Search for Sclerotia. Soil was dug from a root-rot infested area at the Rajwala site near Lyallpur. Soil was removed from a hole approximately 3 x 3 x 5 feet and washed through a 16 mesh screen (U.S. Standard Sieve Series No. 18). No sclerotia could be found.

It was extremely difficult to search for sclerotia since there were many small limestone particles about the same size, shape and color of sclerotia. The limestone nodules became much larger with soil depth, however, no sclerotia were found. Nearly two tons of soil were wet sieved through a screen 10 inches in diameter!

Though sclerotia were not found, an extensive search should be made for survival structures. Rhizoctonia bataticola produces astronomical numbers of sclerotia, so why should they be confined to localized spots within a field? It would seem logical that the survival stage is produced deep in the soil and it is not transferred by normal tillage methods.

Pycnometric methods could be used to separate sclerotia from small rocks, mollusks, insects and root debris collected on the sieve.

V. Recommendations.

1. One of the most striking aspects of my tour was to find such poor stands of cotton on the small farms. In many cases there were six to eight feet between plants. Use of high quality seeds would probably increase cotton production by 30 to 50%, without any additional expense to the farmer. I would recommend a seed-testing program be initiated to ascertain the germinability of cotton seed, and only seeds of 90% germination or better would be distributed for planting. An extensive education program would be needed to encourage farmers to purchase new seeds. The seeds should be acid delinted and treated with a fungicide to prevent seedling diseases. Acid treated seeds would eliminate the need to soak the seeds in urine and cow dung, a practice now employed to cause the linters to stick to the seed coat and prevent the seeds from sticking together. Perhaps the farmers should only plant varieties recommended by the West Pakistan Agricultural University, as sometimes one could find fields of *Gossypium thurberi* planted along with *G. hirsutum* or *G. arboreum*.

2. Two or three plant pathologists should be sent to the United States to study root-rot diseases and learn techniques of isolation and verification of pathogenicity. These scientists would profit by a concentrated study of three to six months on root-rot organisms. Upon their return to West Pakistan, they could pursue research and implement greenhouse and field tests directed toward control of the disease.

3. The present facilities for root-rot research at West Pakistan Agricultural University are very poor. In addition to the need for better laboratory facilities, there is a dire need for controlled temperature soil tanks. The existing greenhouses should be repaired, so disease problems could be studied under controlled environmental conditions. Temperature can be a limiting factor for proving pathogenicity of an isolated organism.

4. There is need for a research plant pathologist to concentrate on ascertaining the pathogen involved in this root-rot disease. Koch's postulates need to be adhered to for confirmation of pathogenicity. The plant pathologists at WPAU have heavy teaching responsibilities and do not have sufficient time to conduct meaningful research.

5. An extensive search should be made to determine whether sclerotia can be found in the localized spots in fields where the disease is apparent. Since there are many small limestone particles about the same size as sclerotia, a solution should be made to float the lighter sclerotia from these particles. We have used sugar to make these adjustments.

6. Good students of plant pathology should be given the opportunity of studying for an advanced degree at major universities in the United States, but returning to Pakistan to conduct their research for the dissertation. After their course work is completed, they would return and do their research under the supervision of a staff member at West Pakistan Agricultural University. The staff member could have a temporary appointment to the Graduate College of the American University, and the major professor in the United States should travel to Pakistan at least once during the course of the research to review progress and make suggestions for additional research.

The advantage of this approach should be obvious. Upon completion of the degree, the student would continue working on a problem that would aid agriculture in Pakistan. Many scientists tend to continue working on problems related to the research they were doing for their dissertation long after they have completed their degree.