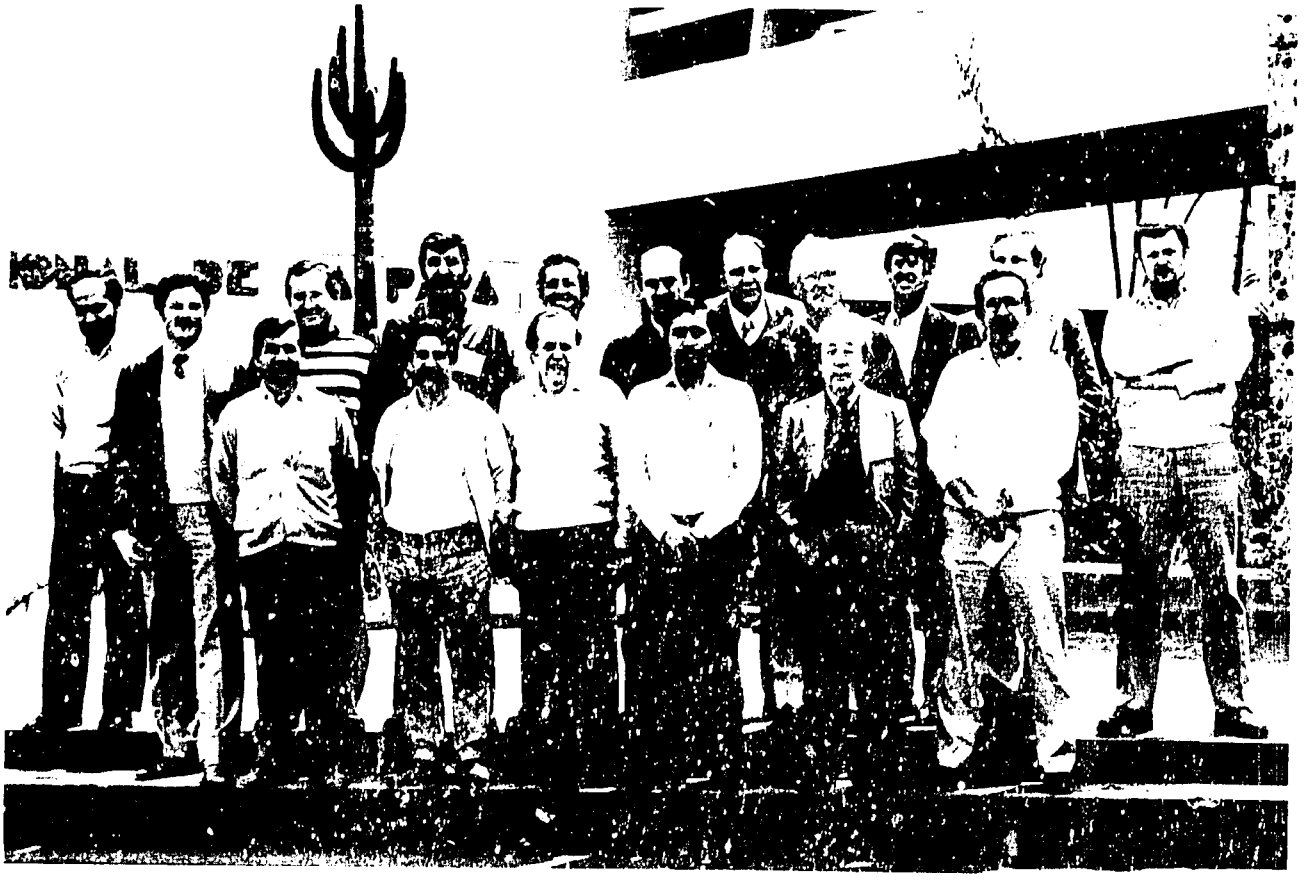
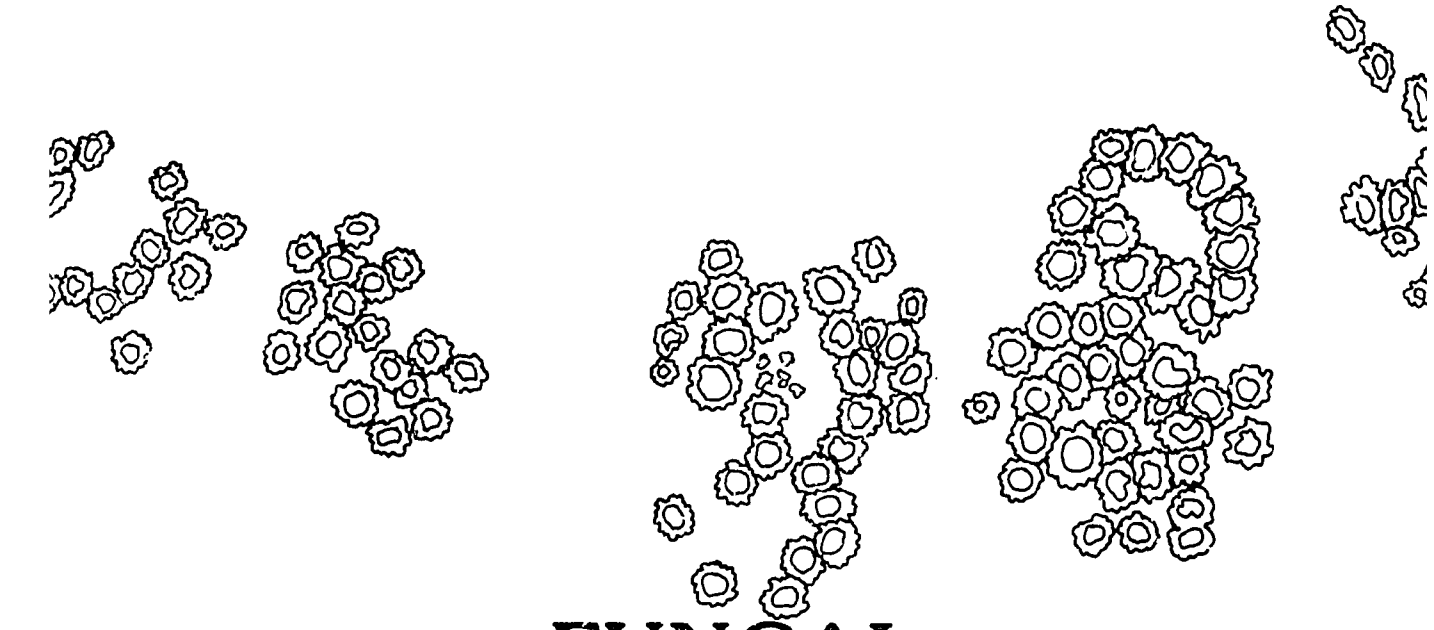

INTERNATIONAL POTATO CENTER (CIP)

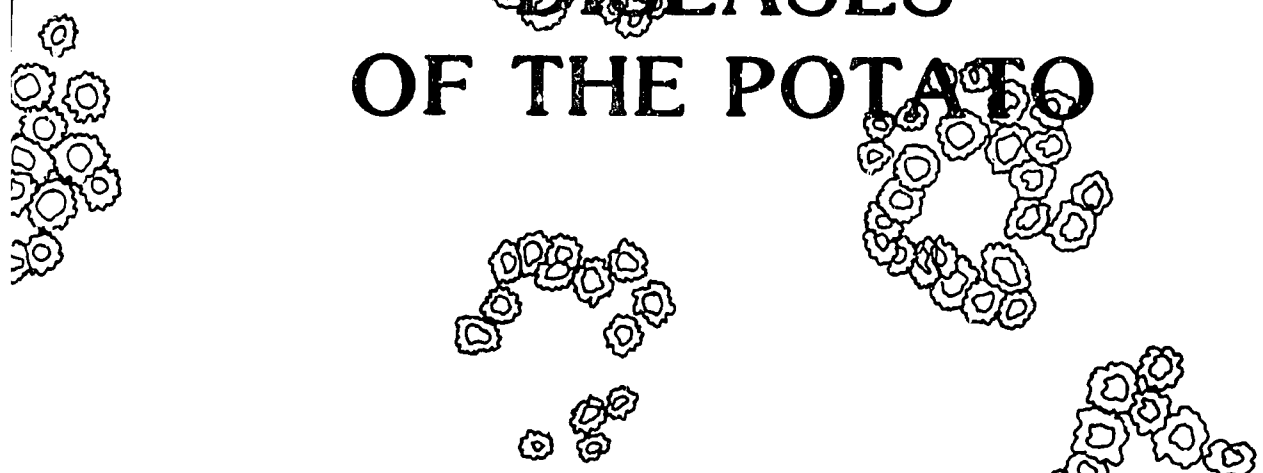


Left to right: First line: Carlos Castro, Herbert Torres, José Luis Zapata, Oscar Malamud, Hans Pinedo, Kohei Tomiyama, Oscar Hidalgo. Second line: Juan Landeo, Humberto Mendoza, Manuel Villareal, Víctor Otazú, Carlos Martin, Lod Turkensteen, Primo Accatino, William Fry, Paul Tooley, Peter Gregory.

Cover:
Underside of potato leaflet showing crowded groups of sacs of *Verticillium cantensis* Arthur, causal organism of Determining Rust of Potato also known as the Peruvian Potato Rust

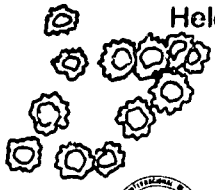


FUNGAL DISEASES OF THE POTATO



Report of the Planning Conference on Fungal Diseases of the Potato

Held at CIP, Lima, September 21-25, 1987



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CONTENT

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RECOMMENDATIONS

EARLY BLIGHT

1) Next to late blight, early blight (Alternaria solani) was considered by the previous Planning Conference, to be the second most important foliar disease in terms of crop loss in the subtropics. We propose to endorse this statement and to amplify it to warm areas in the tropics where potatoes are being introduced and are meeting this problem. However, the Committee feels that other biotic and abiotic factors may complicate disease assessment. Therefore, it is recommended that whenever possible a careful regional evaluation be made when the disease is reported as being a serious problem.

2) The Committee recognizes that since the last Planning Conference, a substantial amount of research has been conducted both on the pathogen as well as the disease. The Committee, however, recommends that this research be continued to further clarify aspects related to A. solani, physiological specialization, ecology and epidemiology.

3) It was previously recommended that efforts be made to determine whether usable levels of resistance to A. solani existed in potato germplasm. In the last five years valuable levels of resistance have been found. The Committee recommends that in this newly assembled gene pool, breeding and screening for resistance to A. solani should continue.

4) Andigena germplasm has been screened as recommended. Good levels of resistance have been found by using seedling screening techniques. The Committee recommends that further field evaluation should be done to confirm resistance.

5) The appearance of early blight epiphytotics in San Ramon complemented with artificial inoculations have resulted in a dependable field testing site for early blight research. So far all selection for resistance has been made in this location. Seedling screening is a valuable tool for selection, but the relationship between seedling and adult plant reaction is unclear. The Committee recommends that more research should be performed to establish correlation between seedling tests and adult plant reaction field tests.

6) The bulk of the selection work has been carried out in San Ramon, but certain genotypes have reacted differently when evaluated in other locations. This may suggest either a differential environmental effect or the presence of different Alternaria species or races. The Committee, therefore, recommends that an inter-regional network to evaluate the stability of resistance in both segregating and clonal materials be established.

7) The Committee encourages regional efforts involving national programs to evaluate fungicides and other control measures.

Other Foliar Diseases

Although the Committee recognizes the importance of several other foliar pathogens (Phoma spp., Septoria spp., Ulocladium spp., Didymella bryonae, Rhizopus spp., Puccinia pittieriana, Aecidium cantensis and others), we feel it is not recommendable for CIP to initiate specific research projects for diseases caused by these pathogens. Whenever necessary CIP should explore avenues for research on these diseases through local institutions in the regions where they are important.

Soil-Borne Diseases

1) The Committee reaffirms the primary importance of the wilt diseases caused by Verticillium spp. and Fusarium spp. as well as diseases caused by Rhizoctonia solani.

2) Increased attention should be given to both powdery scab caused by Spongospora subterranea and common scab caused by the bacterium Streptomyces scabies.

3) Efforts should be pursued to adapt and improve if necessary, the available methodology to evaluate the advanced clonal materials for their reaction to the above mentioned pathogens.

4) The Committee encourages research on chemical, cultural, and biological control measures for important soil-borne pathogens in regions where any of these diseases are a serious problem.

5) The Committee also feels that CIP should not initiate any program of breeding for resistance to the above mentioned diseases until more information on their relative importance is available.

Verticillium Wilt

1) The Committee recommends that CIP should continue surveys to determine geographic distribution and importance of Verticillium spp. and that proper methods for detection and identification be disseminated to regional potato workers.

2) The Committee encourages research in CIP's regions to estimate the relative importance of tuber-borne versus soil-borne inoculum for both V. albo-atrum and V. dahliae.

Fusarium

The Committee recognizes the importance of Fusarium spp. as causal agents of wilt and tuber dry rot, and recommends that surveys should be conducted in the regions to determine the relative importance of the two diseases and the interaction of Fusarium wilt and rot and Verticillium wilt. Studies on dry rot conducted at CIP to evaluate advanced clones for resistance and to determine the effect of storage conditions on disease severity should be continued.

Rhizoctonia

The Committee considers that Rhizoctonia is an important soil fungal pathogen of potatoes grown from tubers, but is a more serious disease either alone or in association with other soil-borne disease in TPS crops, especially when transplanted.

We recommend that research be conducted to evaluate specific factors related to losses after transplant of seedlings.

The Committee encourages collaboration with universities and research programs to determine which anastomosis groups of Rhizoctonia solani are affecting potatoes in different regions.

LATE BLIGHT

Late Blight and bacterial wilt were repeatedly identified in this conference as the most important disease problems of potato on a worldwide basis. Thus we endorse CIP's strategy of focusing personnel and resources on late blight. A series of recommendations on different topics are presented for consideration by CIP administrators and scientists.

1. Breeding

a) We endorse the current use of breeding population A (with R-genes) and breeding population B (without R-genes), with the idea that population A will eventually be phased out and that population B will be emphasized.

b) We encourage the use of a wider array of sources of R-gene-free resistances.

c) Late blight resistance should be incorporated into breeding populations with wide ranges of maturities under different day length conditions.

d) We encourage the integration of appropriate characteristics (other resistances or quality characteristics) from other breeding programs at CIP.

2. Screening

a) The validity of the current seedling screening method should be tested. If the correlation with appropriate field tests is poor then the method of seedling screening should be modified. The continuity of the seedling screen should be based on knowledge of its validity.

b) The seedling screen should use the appropriate complex, aggressive isolate of Phytophthora infestans. An appropriate complex race is one that is compatible with as many R-genes in parental lines as possible. Immune progeny should continue to be discarded.

c) We endorse the use of liquid nitrogen storage of P. infestans. Virulence of isolates should be maintained via intermittent culture on suitable (R-gene-containing) hosts. Virulence of isolates needs to be ascertained on an appropriate set of R-gene differentials before and at use in a screen.

d) Field screens should be conducted simultaneously on the same clones in Rionegro and in Toluca. Both sites are essential. In each location screens should be done via artificial inoculation with the most complex races occurring naturally

in each location. These steps are needed to assure that R-genes do not confound assessments. Sprinkler irrigation is required to assure good infection by the complex race during dry spells. It is important that the complex race used for inoculation contains at least the same set of virulences as the complex race used in the seedling screen.

e) The Committee endorses current CIP policy of evaluating the blight resistance in Rionegro and/or Toluca of all R-genes-containing clones which are subsequently listed as resistant to late blight.

f) The Committee expresses concern about the masking effect of R-genes in clones selected from segregating populations by national programs, and recommends efforts be made to help them manage this problem.

3) Epidemiology

The recent discovery of A2 mating types makes the occurrence of oospores possible in many parts of the world. We recommend that CIP encourage and/or support investigation on:

a) the importance of oospores as a source of inoculum;

b) the distribution and spread of the A2 mating type;

c) the survival and ecology of oospores.

4) Integrated Control

a) We recommend that CIP encourage and/or support research on how resistant clones might fit into integrated control programs (i.e. using also fungicides, sanitation, cultural factors, etc.), but we expect that the majority effort should be done by national programs.

b) We recommend that CIP encourage and/or support investigations on the role of mixtures of clones and mixed cropping as aids to resistance in late blight control.

5) Genetics

Widespread occurrence of both A2 and A1 mating types of P. infestans will increase the rate of evolution of P. infestans in several aspects, and the significance of this development needs to be investigated.

a) CIP should encourage and/or support investigations on the genetics of P. infestans.

b) CIP should encourage and/or support ecological and genetic studies on factors affecting the level of field resistance and on the durability of specific resistance.

c) CIP should develop a stand d R-gene-free set of clones to be used as references in the study and selection of field resistant materials. The reaction of the standard set in different locations may identify the appropriateness of specific test locations as predictors of field performance of clones in various regions around the world.

d) In order to avoid the risk of negative effects resulting from R-genes in breeding programs, CIP should continue studies on R-gene identification in existing and especially in new sources of field resistance.

e) CIP should encourage or support studies which identify the genetics and role of resistance in populations of Mexican wild potato species.

f) CIP scientists and administrators should remain aware of progress in areas of biochemistry, biotechnology, and genetic engineering of P. infestans, potatoes, and tomatoes. Discoveries in some of these areas may be especially useful in the near future.

6. Tuber Blight

Field resistance on foliage should be accompanied by tuber resistance in those locations where tuber blight is known to be a factor.

7. TPS

We recommend that CIP investigate the epidemiology of late blight in R-gene containing TPS families: The epidemiology will be influenced by climatic conditions and the manner in which TPS is used by recipients.

8. Farmer Acceptance

It is recommended that socioeconomic studies be conducted in locations where farmer adoption of late blight resistant clones is slow.

9. Other Factors

We observe a discrepancy between the apparent high priority given to late blight at CIP and the accomplishment in late blight breeding. We recognize that the discontinuity in late blight research is largely responsible, and we recognize that no one at CIP is at fault. Therefore, we endorse the present search for a new scientist in late blight pathology. However, we feel that this scientist should be located in Lima where she/he can interact with leaders of other programs. Location of the position in Lima should enhance the possibility of attracting a first class Ph.D. scientist.

The new late blight pathologist needs to be supported adequately. The screening facilities in Huancayo need to be upgraded. Personnel support is required in Rionegro and in Toluca. We feel that a full-time MS-level scientist is needed in each location for the next several years.

National Programs and regional scientists would benefit from training programs dealing with late blight. Workshops teaching methodology, biology of late blight and integrated control should enhance resistance terminology, late blight screening and selection by national programs and would contribute to a better overall understanding late blight.

CONTINUITY

Finally, the participants of the Planning Conference express their concern for the continuity of the research on fungal diseases and encourages CIP to take the necessary steps so that this continuity is assured.

WELCOME AND OPENING REMARKS

Richard L. Sawyer

Thank the participants for taking time from their busy schedules to help us with our work.

These Planning Conferences are a part of CIP's strategy for building bridges of collaboration to other institutions around the world.

The success of CIP is very dependent on the bridges of collaboration established, not only through Planning Conferences but also through contracts, and collaborative projects.

When CIP started operations, late blight was by far the most important disease with potatoes around the world. In the past 15 years the areas growing potatoes have changed considerably. Today, over half of the potatoes in the developing world are being grown in warm to hot tropical areas, in lowland tropical climates. Here Pseudomonas solanacearum is a major disease of potatoes and is rapidly out distancing late blight as the major potato disease in the developing world. And we have not got very far to solving the late blight problem.

As the potato has continued to extend into new areas additional fungal diseases have become important.

REVIEW OF RECOMMENDATIONS OF PREVIOUS PLANNING CONFERENCES

Peter Gregory

In the June 1978 Planning Conference on "Control of Important Fungal Diseases of Potatoes" recommendations were made in the areas of late blight, early blight, Verticillium and Fusarium wilt, Rhizoctonia and several other diseases.

In 1982 recommendations were on made late blight research during CIP's Tenth Anniversary Post Congress Workshop.

The purpose of this paper is to summarize the recommendations from both the Planning Conference and the Post Congress Workshop and to describe the actions CIP has taken as a result of them.

THE 1978 PLANNING CONFERENCE RECOMMENDATIONS

FOLIAR DISEASES

Late blight

This disease was considered by the Planning Conference's Panel to be the single most destructive potato disease in the world. As chemical control was becoming increasingly expensive and difficult the Panel strongly endorsed CIP's strategy of breeding for general resistance to Phytophthora infestans, a special advantage of this resistance being its stability over long periods. The Panel also endorsed CIP's policy of having a wide range of sources from which to obtain resistance to P. infestans. In addition to these general recommendations several specific areas of late blight research were covered:

The Panel pointed out that the simply inherited R-gene resistance that exists in CIP's materials should be used in combination with general resistance. However, future CIP breeding programs should not add R-genes, and in fact R-genes should gradually be eliminated from CIP's breeding lines. In response to this recommendation CIP has been developing resistant populations without R-genes, particularly during the past 5 years.

While recognizing the importance of the late blight resistance tests in Toluca, Mexico, the Panel said that it would be highly desirable to add an additional testing site in Costa Rica or other appropriate location and that material selected with resistance should not be maintained in new testing sites. Work was done in Costa Rica for three years where blight was more severe than in Toluca.

It was recommended that R-gene differentials be grown in the field at the sites used to test for late blight resistance. It was thought that this would monitor the development of the race complex present to assure that the resistance seen was general rather than specific. This was done to the extent possible but some materials were so poorly adapted that they died out.

It was suggested that race identification should be carried out to the extent that it helps to assure the gradual elimination of R-genes from CIP's breeding material. This was not implemented until the development of Population B in 1986.

It was recommended that CIP (perhaps through contracts) should assure that R-gene differentials were maintained and increased free of viruses or viroids so they would be readily available for use in the field both by CIP and by other breeding and testing programs. This has been done in Scotland and The Netherlands.

It was recommended that there should be continued international testing of advanced selections in CIP's Regions. This has been pursued vigorously.

The Panel recommended that CIP encourage the use in all late blight testing sites of at least three general resistance types, with high, low and intermediate resistance, to provide standards with which to compare new clones. This is now standard practice in sites to which these materials are adapted.

In order to find out whether or not general resistance could gradually be overcome, the Panel recommended that CIP should employ post-doctorals or graduate students or use other contractual arrangements, to initiate studies on whether isolates differing in relative aggressive ability exist or can be produced by P. infestans. This was not done.

It was recommended that CIP encourage basic studies on the physiology of parasitism of P. infestans as there may be a tremendous potential for rapid and simple biochemical tests for identification of stable resistance. This recommendation was acted upon by means of contract research but little practical progress was made.

Considering that the sexual stage might be distributed to other parts of the world it was recommended that CIP, perhaps through contract research with national scientists, might initiate studies on the presence of both mating types and the role of oospores in the epidemiology of P. infestans.

Type A_2 is all over Europe, and has been since 1984. Studies in this area are underway.

Early Blight

In its introduction to this section, the Panel made the following points: Next to late blight, early blight (Alternaria solani) was considered to be the second most important foliar disease in terms of crop loss in the sub-tropics. Although the disease can be effectively controlled by fungicide in developed areas it is more difficult to control than late blight and almost impossible to control in those areas where dust storms are prevalent. The Panel also stated that many subsistence farmers are unable to afford the necessary fungicides or they lack the essential knowledge for their proper use. Predicting the timing of application is often difficult and frequently leads to overspraying, or to inadequate coverage and protection.

For these reasons it was strongly recommended that efforts be made to determine whether usable levels of resistance to A. solani existed in potato germplasm. This was acted upon and, during the past five years, useful levels of resistance have been found.

Due to the very low incidence of early blight in Peru it was recommended that screening for resistance be carried out at an appropriate location where the disease occurs regularly. It was also recommended that andigena material be included in a screening program for early blight. Screening for early blight has been achieved in the screenhouse in Lima, in covered nurseries in San Ramon and also in field tests. In addition, a contract was established in Brasil.

It was recommended that new fungal-host interactions might be encountered as potato moved into lowland tropics and this should be closely monitored and their importance evaluated. This has been done.

SOIL-BORNE DISEASES

Verticillium and Fusarium Wilts

It was noted that wilt diseases are commonly found in the regions but there was confusion as to whether the main cause was Fusarium or Verticillium. Fusarium species are ubiquitous and readily isolated, whereas Verticillium is difficult to detect without the appropriate techniques. Because Verticillium is a threat to a wide range of agricultural crops and because the potato is often blamed for its introduction into an area the Panel recommended the following:

- CIP should initiate a survey to determine the geographic distribution and importance of Verticillium. This has been done in Peru and, in Central Africa, Pakistan and Turkey.

- CIP should acquire the simple known methods for detection and positive identification of Verticillium albo-atrum and V. dahliae and should make them available to regional potato workers. This has been done.

- CIP should not initiate an extensive breeding program with Solanum andigenum and other Solanum species for Verticillium resistance until the results of the survey were available, but should take advantage of existing resistant or tolerant materials in other breeding or screening programs. CIP concurred with this.

Rhizoctonia

This was considered to be the second most important soil-borne fungal pathogen of potatoes. In addition, it was known to be the cause of the unacceptable level of "damping off" of transplanted seedlings at La Molina, and possibly the cause of losses in the regional program. It thereby posed a threat to the distribution by CIP of true potato seed (TPS) breeding materials as well as a potential threat to the TPS research program. Thus the Panel recommended that an effort be made to develop specific procedures for controlling "damping off" of seedlings by Rhizoctonia and other potential pathogens. This was done in the context of TPS work.

It was considered that attention be paid to the development of chemical and cultural practices to limit the establishment of Rhizoctonia in the soil caused by planting contaminated tubers. Therefore, the Panel strongly recommended that initiative be taken to control Rhizoctonia through crop management practices. In order to achieve this they further recommended the setting up of an active program of technology transfer under the auspices of Regional Research and Training. This was not done.

OTHER DISEASES

The Panel recognized that there were many potentially important soil-borne pathogens and that their range and importance was poorly understood. They recommended that their significance be assessed and biology investigated as a basis for developing control measures.

Where control measures were already known, the information should be made available to farmers. Synchytrium endobioticum was considered to warrant high priority attention, as the disease could become even more important, especially considering the planned introdution of frost resistant cultivars into the higher Andes where the disease is endemic. In this respect, CIP supplied materials to the Peruvian program and also initiated a contract in Ayacucho which encountered serious difficulties.

The Panel also recommended that attention be given to the development of measures for controlling Fusarium on stored potato tubers both for existing stores and for those being developed through CIP for small farmers. Progress in development of resistance was made during 1986-87. The Panel pointed out that excellent chemical treatments were available for control of Fusarium in storage.

QUARANTINE

With respect to possible dissemination of pathogens in CIP's worldwide distribution of germplasm the Panel recommended that attention should continue to be given not only to viruses and bacteria but also to fungal pathogens. This continues to be an important aspect of our work.

RECOMMENDATIONS OF CIP'S 1982 POST-CONGRESS WORKSHOP RELATED TO LATE BLIGHT RESEARCH

It was recommended that breeding, testing and selection for late blight resistance should be located in suitable sites besides Peru. Breeding continues in Peru, but screening has been conducted on a much greater field scale in Colombia and Mexico than in Peru.

The Panel recommended that the seedling screening test should be evaluated rigorously and should be discontinued until its use could be justified. This recommendation was not acted upon since no alternative seemed feasible. The seedling screening method was shown to be useful in Britain, and its end product in CIP is resistance in field tests.

It was recommended that techniques be developed to evaluate tuber blight resistance and incorporate it into the breeding program. Techniques have been developed and some germplasm evaluated, but no specific breeding has been done.

Development of a standard set of differentials for global evaluation of general resistance was suggested so as to evaluate the stability of clones over several locations and years. This was not done on a wide scale because suitable differential materials able to grow well in diverse environments do not appear to exist.

It was recommended that parental clones with high levels of late blight resistance be produced for further breeding by national programs and that this resistance be combined with resistance to other diseases and to nematodes followed by distribution as TPS or tuber families. Although parental clones with high levels of resistance have not yet been produced population breeding at CIP has resulted in combining of some resistances e.g. with bacterial wilt, early blight, PLRV and PVY.

It was recommended that CIP begin the evaluation of alternative sources of resistance using primitive cultivars and other species. A breeding population for general field resistance based on primitive andigena cultivars and devoid of major genes has been established.

It was recommended that CIP should stimulate research into methods of evaluating late blight resistances in environments which were not optimal for screening. It turned out that the Colombian site had been non-optimal. Also, Huanuco in Peru was utilized.

It was recommended that CIP should stimulate research designed to study variability of P. infestans in situations where both sexual and/or asexual stages exist. Some work was done on this in Toluca, Mexico.

It was considered unlikely that the problem of late blight would be solved entirely by breeding. Thus, it was recommended that CIP should take a lead in developing integrated control systems for late blight, including the use of fungicides and cultural techniques as well as breeding. No action was taken on this as CIP considered that such work should be done by national institutions.

It was recommended that the R-gene differential set should continue to be maintained at an appropriate site such as the Scottish Crops Research Institute. As mentioned earlier, maintenance has been achieved in Scotland and also in The Netherlands.

CONCLUSION

We have seen that the recommendations of the 1978 Planning Conference and of CIP's Tenth Anniversary Post Congress Workshop in 1982 were carefully considered by CIP's pathologists and in most cases the recommended actions were taken.

The goals of this present Planning Conference are to review the work done since the recommendations were made and plan optimal future research to help CIP achieve its practical goals. I am optimistic that with the collective experience and expertise present here this Planning Conference has all the ingredients for success.

THE IMPORTANCE OF THE PERFECT STAGE OF *Phytophthora infestans* FROM THE STANDPOINT OF EPIDEMIOLOGY AND ADAPTATION

W. E. Fry, P. W. Tooley and L. J. Spielman

TRANSITION PERIOD

The early 1980's have been a transition period in the biology and epidemiology of *Phytophthora infestans*. Prior to the early 1980's, the sexual stage of *Phytophthora infestans*, and the occurrence of both A1 and A2 mating types, were limited to Central Mexico. This region had been considered the center of origin of this fungus, and both mating types occurred in approximately equal frequency (2, 15). Sexual recombination in Mexico occurred sufficiently often to maintain a Hardy-Weinberg equilibrium in the *P. infestans* population (15), whereas, populations from other regions were not in Hardy-Weinberg equilibrium.

During the early 1980's, the A2 mating type was discovered in locations other than Central Mexico, demonstrating the possibility that the sexual stage may occur in these locations. Thus, there may be a major change in the biology and epidemiology of *P. infestans* in areas outside of Mexico.

There are at least two major implications from the possibility of sexual reproduction in a region. First, sexual reproduction provides an avenue for creating enhanced variation within a population of *P. infestans*. Second, the products of sexual recombination, oospores, survive environmental conditions not survived by other life stages. Oospore survival in soil during adverse conditions is a distinct possibility, and the soil may then serve as a source of initial inoculum. This paper addresses some of the important implications of the potential for enhanced variation via recombination, and the increased possibility that soil will become a source of initial inoculum. However, we will first present background information pertaining to recent discoveries of the A2 mating type, studies of oospore survival, and studies of "adaptation" in *P. infestans*.

BACKGROUND

Recent discoveries of the A2 mating type. The first reports of A2 isolates outside of Mexico were from Switzerland in 1984 (3), and from the United Kingdom in 1985 (9,12). However, it appears that isolates of A2 mating type have been present in the United Kingdom since the early 1980's. In a study of isolates collected prior to 1984, Tantius, et al.(13) found A2 mating types collected as early as 1981

in North Wales. None of the isolates collected prior to 1981 were A2. Early and widespread distribution of the A2 mating type apparently is not restricted to the United Kingdom. In conjunction with our ongoing studies of the biology of P. infestans, we receive isolates from around the world. Some isolates received in 1985 from the Netherlands, and from Israel, turned out to be A2. Persons from whom we had received these isolates had assumed them to be A1. Although the A2 mating type has not yet been reported from the United States or Canada, the widespread occurrence of A2 mating types from many locations throughout the world increases the possibility that A2 mating types may be imported into the United States and/or Canada.

Survival studies. Oospores have been shown to survive freezing and thawing, long term storage, and passage through the digestive system of snails (11). Thus, survival of oospores in soil not associated with potato tubers is now a distinct possibility in the United Kingdom, Western Europe, and the Middle East.

To date, almost all of the studies on the role of oospores in epidemiology have been conducted in Mexico. Most of these were conducted by Jorge Galindo during the late 1950's and 1960's (Niederhauser, J. S., personal communication). Galindo observed oospores of Phytophthora infestans in soils which had supported a crop of potatoes in the previous season. These oospores germinated and led to infections on potatoes. The importance of oospores in the soil as a source of initial inoculum relative to sporangia dispersed on air currents is not known. We are unaware of studies identifying the longevity of oospores in soil. However, if the oospores of P. infestans are similar to those of other Phytophthora species, the oospores might survive for ten years or more.

Adaptation in P. infestans. The question of adaptation in P. infestans is of tremendous concern to potato breeders around the world. The possibility that sexual recombination may increase the potential of adaptation raises our concern about adaptation. Because different authors define adaptation somewhat differently, we provide our own explicit definition. Adaptation is the specific ability of some genotypes of P. infestans to cause greater amounts of disease on certain potato genotypes, exclusive of R-gene interactions. Thus, the isolates x host interaction is significant even in the absence of R-genes. Older uses of the term adaptation regarding R-gene virulences now are generally considered to be examples of mutation and selection. The hope has been that potato resistances to P. infestans not based on R-genes, would be stable (or "horizontal" Sensu Vanderplank, [17]). Adaptation in P. infestans is one explanation for apparent "erosion" of resistance in certain potato genotypes.

Adaptation and the erosion of resistance are controversial topics which have not been resolved satisfactorily during the past thirty years of debate. Because these issues are still debated, one must conclude that the magnitude of erosion and the magnitude of adaptation can not be large or that these phenomena are inconsistent. If the magnitude of each of these phenomena were large, they would be experimentally verifiable and no longer controversial. It seems most logical to assume that adaptation is controlled by many genes in the fungus and that specific adaptation to one host genotype would involve a unique set of genes. Thus, if these assumptions are accurate, one would expect the evolution of specific adaptation to be a very slow process.

The controversy concerning adaptation in P. infestans has involved scientists throughout the world (1, 4, 5, 8, 10, 18). Claims of adaptation in P. infestans have been controversial for a variety of reasons: some experiments were done in the laboratory on potato tubers rather than on foliage in the field; isolate x cultivar interactions were confused with isolate x cultivar x environment interactions; and the history of isolates used in laboratory studies has been unknown.

Unfortunately, the controversy surrounding adaptation remains despite more carefully controlled experiments with other host:pathogen systems. Work by K. J. Leonard and co-workers at North Carolina State University has been particularly carefully done. They have investigated two pathogens of maize: Cochliobolus heterostrophus and Colletotrichum graminicola. In one of their first studies, they demonstrated that significant cultivar x isolate interactions in one replicated trial were due to environment effects: if these trials were repeated, the cultivar x isolate interaction terms became non-significant. On the other hand, they demonstrated experimentally that C. heterostrophus responded to selection for length of lesion on a specific maize genotype during sexual generations of C. heterostrophus (Fig. 1) (7).

Thus, they confirmed that some aspects of the host:pathogen interaction could be altered via selection during sexual generations. It is not yet clear that this type of selection would lead to increased adaptation in the field. The concern for us, however, is that the occurrence of sexual recombination may increase the possibility of adaptation.

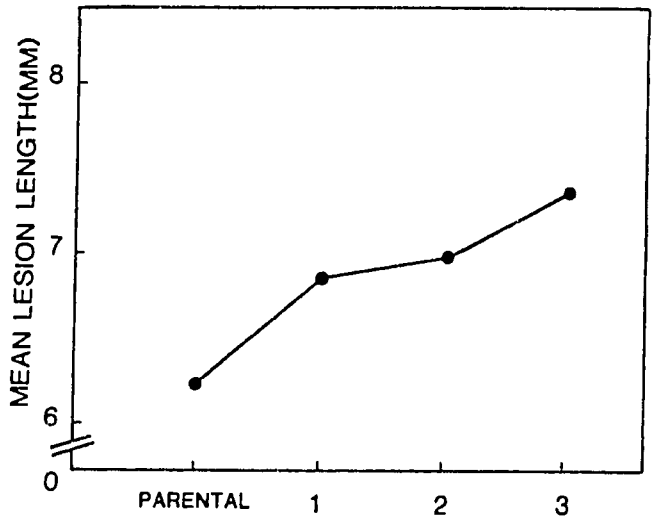


Fig. 1. Mean length of lesions produced by Cochliobolus heterostrophus in parental and selection generations on one maize inbred. Figure is from Kolmer and Leonard (7).

ROLE OF PERFECT STAGE ON EPIDEMIOLOGY

One of the very important impacts of sexual recombination will be the occurrence of oospores in soil. Although very little is known about the longevity of P. infestans oospores in soil, their occurrence will most certainly make the suppression of potato late blight more difficult by creating another source of initial inoculum. The importance of this source needs to be quantified. Thus, new investigations on the ecology of oospores and their subsequent role in late blight epidemiology need to be initiated. In locations such as Northeastern part of the United States, effective sanitation, and destruction of piles of cull potatoes have resulted in low levels of initial inoculum during the past several years. As a result, potato late blight has been a rare disease. If oospores become part of the epidemiology of late blight in this location, the disease may become more consistently serious.

Studies are needed to identify the following aspects of the ecology of oospores.

..characterization of factors affecting oospore survival in soil.
..quantification of the manner in which environmental factors affect infections resulting from oospores.
..identification of potential cultural, biological, and chemical techniques to suppress oospore populations.
..investigation of variation in host response to infections resulting from oospores.

ROLE OF PERFECT STAGE ON ADAPTATION

This topic has been the subject of investigation by a group of us at Cornell University for the past several years. Some preliminary studies have been concluded, and more extensive studies are in progress.

Aggressiveness of Isolates from Sexual and Asexual Populations. One of our first preliminary studies compared isolates from asexual populations with isolates from the Mexican sexual population in terms of components of aggressiveness. (Aggressiveness is the capacity of a pathogen to increase rapidly in population and, therefore, disease.) The sexual population was derived from Central Mexico, and the asexual population was composed of individuals from diverse locations in the United States, Canada, and Western Europe. Experiments were conducted in growth chambers during the winter months under containment conditions at Cornell University in Ithaca, New York. Individual isolate genotypes were inoculated onto potato leaflets. Subsequently, the infection frequency, lesion area, and sporulation capacity of each isolate on a susceptible potato cultivar were measured. These components were combined in a "composite fitness index" (=infection frequency X lesion area X sporulation capacity). Both populations contained individuals of dramatically different composite fitness index (CFI)(Fig. 2).

Although there were differences between the two populations (in opposite directions) for some of the components, there was no difference between the two populations for CFI (16). Thus, we conclude from this preliminary growth chamber experiment that sexual reproduction alone does not necessarily create isolates with enhanced aggressiveness.

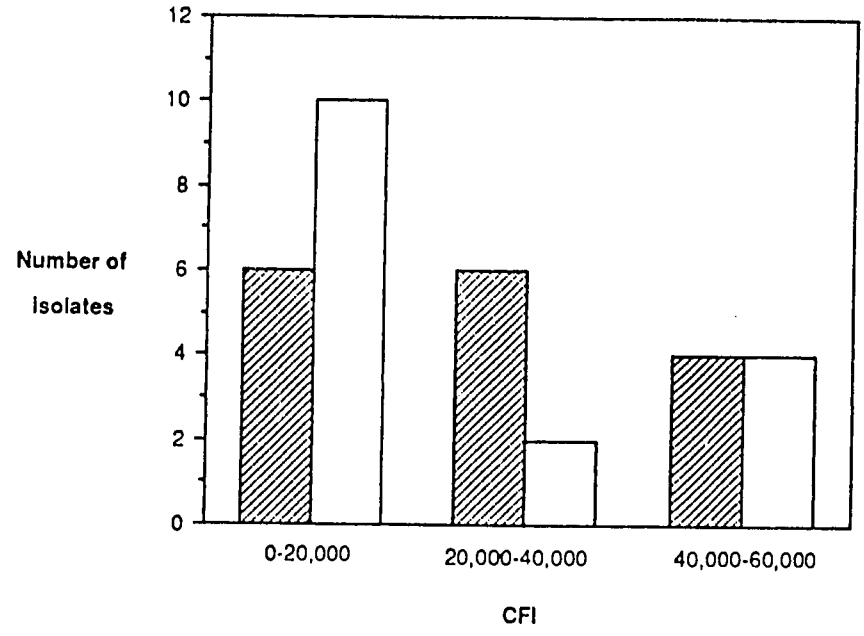


Fig. 2. Relative aggressiveness of Phytophthora infestans from Mexico (sexual population, hatched bars) or areas other than Mexico (asexual population, open bars) as determined in growth chamber studies involving infection frequency, lesion area and sporulation capacity (=Composite Fitness Index=CFI) on one potato cultivar. Data are from Tooley et al. (16).

Growth chamber studies of pathogen aggressiveness may not reflect aggressiveness differences accurately if the environment interacts with aggressiveness. Consequently, we have been especially concerned about the relationship between growth chamber assessments of aggressiveness and field assessments of aggressiveness. We and others have demonstrated previously that environmental changes can cause small differences in the expression of pathogen aggressiveness. Consequently, we have compared growth chamber studies with field studies on aggressiveness for the past several years. In general, there is a strong correlation between growth chamber studies and field studies (L. J. Spielman, unpublished) (Fig. 3). Field assessments were done by inoculating a single isolate onto a few leaflets on a small plot of potatoes, and then observing the disease increase over the next several weeks. Aggressiveness is reflected by disease severity (area under the disease progress curve)(14). These studies demonstrate that environment influences the expression of aggressiveness. Additionally, these studies document a phenomenon observed by late blight workers for years--that isolates tend to become less aggressive with length of time in culture (Fig. 4).

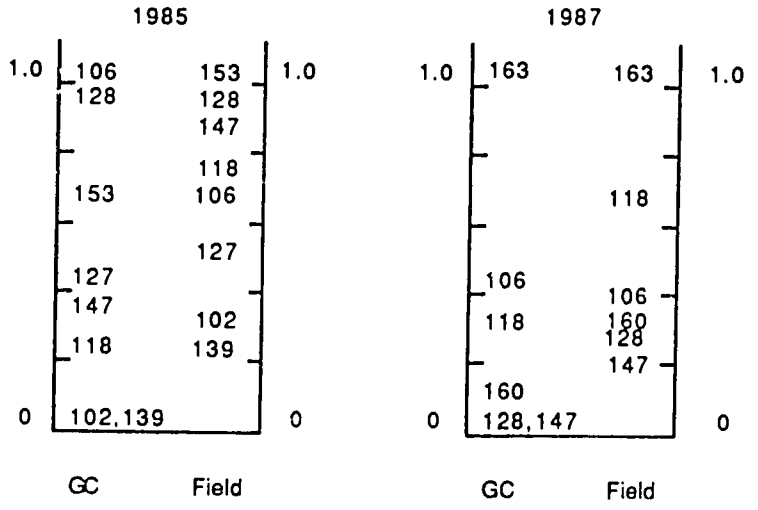


Fig. 3. Relative aggressiveness of isolates of Phytophthora infestans as determined in growth chamber (GC) or field tests. Growth chamber tests involved lesion area, sporulation capacity and (1985 only) infection frequency. Rankings of isolates in field tests are based on area under the disease progress curve.

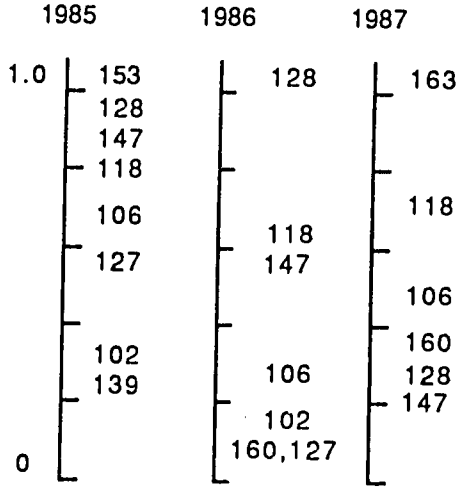
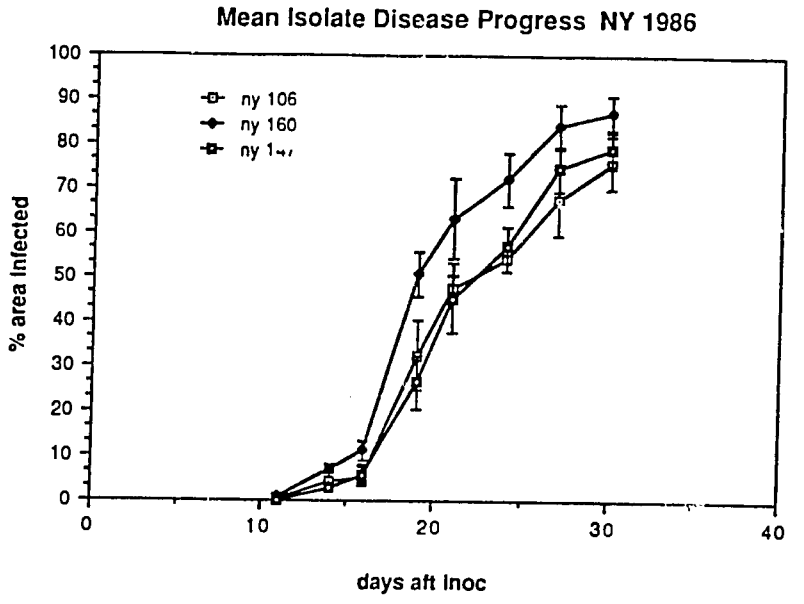


Fig. 4. Relative aggressiveness of isolates of Phytophthora infestans measured in three successive years in field tests. Inoculations were made with isolates maintained on artificial medium at 18C.

Because our preliminary results supported the hypothesis that changes in environment could influence the expression of aggressiveness, a series of comparisons was conducted between aggressiveness measurements in Toluca, Mexico with those in Ithaca, New York. The same set of isolates was used in each location. One experiment was conducted in Toluca during May and June, and the companion experiment in Ithaca in July and August. These experiments were conducted by J. M. Parker, a graduate student working with H. D. Thurston at Cornell University. Parker used three U. S. potato cultivars (Hudson, Sebago, and Superior) and Alpha. Among the three U.S. cultivars, Superior was the most susceptible cultivar in both locations, and Sebago was the most resistant cultivar in both locations, thus the different locations did not cause a differential expression of resistance. The isolates had similar aggressiveness but, in one experiment, one isolate appeared slightly more aggressive than the others (Fig.5). We conclude tentatively that aggressiveness appears fairly consistent. However, the sample size supporting this conclusion is small and needs to be broadened before important decisions are based on it. In neither location was there an isolate x cultivar interaction.

Changes in host resistance may be caused by differences in environment in different locations and can be confused with pathogen adaptation or erosion of resistance. For example environmental effects may explain why potato cultivar Alpha is viewed as resistant in some locations (Sweden, Ithaca, NY) but susceptible in others (Toluca, Mexico)(Fig. 6). In an effort to address this possibility, Parker investigated the effect of photoperiod on the expression of resistance by various potato genotypes. Although shorter photoperiods appeared to increase susceptibility, the effects were not differential. The relative resistance ranking of most potato genotypes was consistent over photoperiod. Unfortunately, cultivar Alpha has not yet been included in the photoperiod studies.



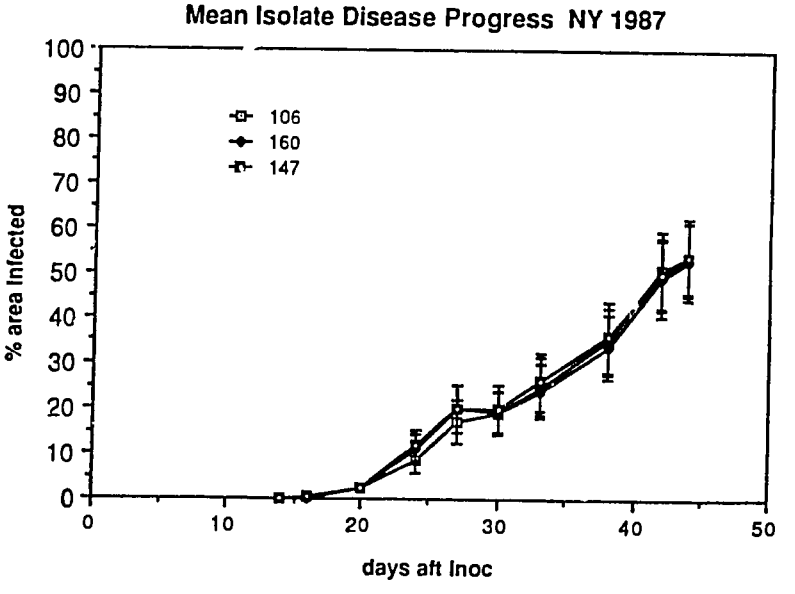
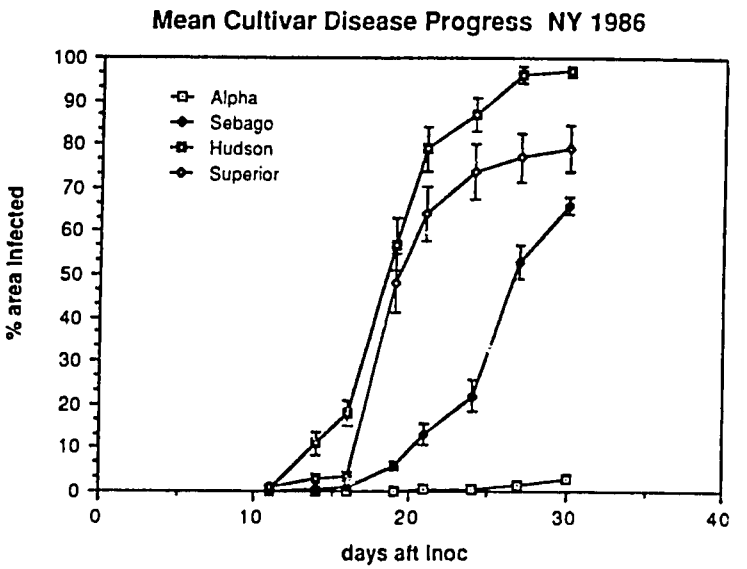


Fig. 5. Severity of epidemics induced by three different isolates of Phytophtora infestans - data are from J. M. Parker (unpublished).



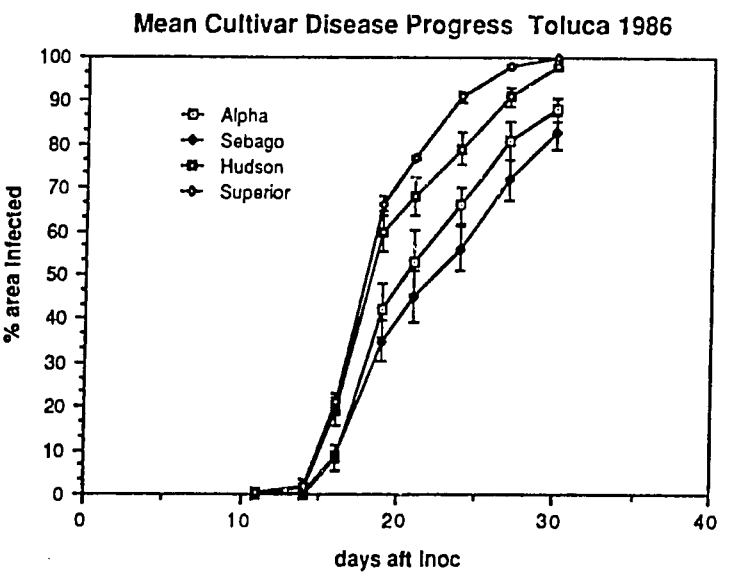


Fig. 6. Severity of potato late blight epidemics in four different cultivars in Ithaca, New York and Toluca, Mexico. Data are from J. M. Parker (unpublished).

Until the mechanisms of field resistance are known, the only way to determine whether the resistance in a cultivar is widely adapted, will be to grow that cultivar in different environments. It seems possible that environmental effects on cultivar resistance could be confused easily with adaptation in the pathogen population.

Resolution of the controversy regarding adaptation in P. infestans? Whether or not adaptation is a significant concern for potato late blight is still hotly debated. However, the sexual stage can be used to resolve the debate and provide guidance to potato breeding programs. Progeny of P. infestans from crosses should be selected for aggressiveness on specific host genotypes. Especially aggressive genotypes in the F1 should then be crossed among themselves to create an F2. Again, the especially aggressive genotypes should be used as parents to create the F3 generation. Comparison of adaptation in progeny after several sexual cycles of selection should be made with adaptation in unselected progeny. In this comparison, aggressiveness should be broadly defined to include survival characteristics, as well as pathogenicity characteristics. Presumably, the selection should be done within a reasonably narrowly defined environment. If intensive selection produces a population which is especially aggressive, or is specifically adapted to a given cultivar, we would have a basis for estimating the impact of adaptation on disease management and epidemiology.

The proposed selection experiment is technically possible, but extremely difficult. Comprehensive definitions of aggressiveness require estimates of pathogenicity and survival. Large numbers of progeny should be assessed and selected, but laboratory and field plot space are limiting factors. Finally, Phytophthora infestans is characterized by inbreeding depression (L. J. Spielman, unpublished data). Thus, these experiments would have to be conducted with several different families in parallel. Individuals from one F1 progeny could be crossed with individuals from a distinct F1 progeny. Perhaps as many as four interbreeding families should be used.

CONCLUDING THOUGHTS

The role of the perfect stage of Phytophthora infestans in the epidemiology and adaptation of this fungus, is not yet known. Contradictory hypotheses are plausible from our present basis of understanding : sexual recombination might prevent adaptation; or sexual recombination might enhance adaptation. Adaptation would be prevented if sexual recombination served to destroy combinations of genes that result in high levels of specific aggressiveness on a specific host genotype. This scenario is particularly plausible if oospores are the primary method of overwintering survival. Adaptation

would be enhanced if sexual recombination were to provide a greater population diversity from which adapted genotypes might be selected. This scenario is plausible if the fungus survives between seasons primarily as mycelium.

The type of agroecosystem is likely to influence selection for specific adaptation. For agroecosystems with vast acreages of homogeneous host genotypes, selection for a specific pathogen genotype is likely to be very consistent and strong. In contrast, for agroecosystems in which there are smaller fields each containing a unique host genotype, selection for a specific pathogen genotype will be much less intense. Not only will there be less of any one host genotype for selection, but there will be a greater degree of interchange of pathogen genotypes among fields. Therefore, there is likely to be selection in diverse directions. The result could be a diverse pathogen population composed of dissimilar individuals each more-or-less adapted to a specific host genotype. Alternatively, the result could be a pathogen population composed of individuals none of which is specifically adapted to any single host genotype.

If pressed to make predictions, we would suggest that specific adaptation in Phytophthora infestans is unlikely to have a dramatic effect on the "field" resistances of most commercial potato cultivars. We are reluctant to predict whether the occurrence of sexual recombination will enhance or retard selection for specifically adapted pathogen genotypes. However, we are fearful that widespread occurrence of both mating types of P. infestans will elevate the importance of this fungus. Survival of oospores in soil will provide yet another source of initial inoculum which will be unfortunately persistent and consistent. Thus it appears to us that the occurrence of the perfect stage will pose an immediate threat in the epidemiology of P. infestans.

REFERENCES

1. Caten, C. E. 1974. Intra-racial variation in Phytophtora infestans and adaptation to field resistance for potato blight. Ann. of Appl. Biol. 77:259-270.

2. Gallegly, M. E. and Galindo, J. 1958. Mating types and oospores of Phytophthora infestans in nature in Mexico. Phytopathology 48:274-277.

3. Hohl, H. R. and Iselin, K. 1984. Strains of Phytophthora infestans from Switzerland with A2 mating type behavior. Trans. Brit. Mycol. Soc. 83:529-593.

4. James, R. V., and Fry, W. E. 1983. Potential for Phytophthora infestans populations to adapt to potato cultivars with rate-reducing resistance. Phytopathology 73:984-988.

5. Jeffrey, S.I.B., Jinks, J. L., and Grindle, M. 1962. Intra-racial variation in Phytophthora infestans and field resistance to potato blight. Genetica 32:323-338.

6. Jenns, A.E., Leonard, K.J., and Moll, R.H. 1982. Variation in the expression of specificity in two maize diseases. Euphytica 31:269-279.

7. Kolmer, J. A. and Leonard, K.J. 1986. Genetic selection and adaptation of Cochliobolus heterostrophus to corn hosts with partial resistance. Phytopathology 76:774-777.

8. Latin, R. X., MacKenzie, D.R. and Cole, H. Jr. 1981. The influence of host and pathogen genotypes on the apparent infection rates of potato late blight epidemics. Phytopathology 71:82-85.

9. Malcolmson, J. F. 1985. Phytopthora infestans A2 compatibility type recorded in Great Britain. Trans. Brit. Mycol. Soc. 85:531.

10. Paxman, G.I. 1963. Variation in Phytopthora infestans. Eur. Potato J. 6:14-23.

11. Shaw, D. S. 1967. A method of obtaining single-oospore cultures of Phytophthora cactorum using live water snails. Phytopathology 57:454.

12. Shaw, D.S., Fyfe, A.M., Hibberb, P.G., and Abdel-Sattar, M.A. 1985. Occurrence of the rare A2 mating type of Phytophthora infestans on imported Egyptian potato and production of sexual progeny with A1 mating types with the U.K. Plant Pathology 34:552-556.

13. Tantius, P.H., Fyfe, A.M., Shaw, D.S., and Shattock, R.C. 1986. Occurrence of the A2 mating type and self-fertile isolates of Phytophthora infestans in England and Wales. Plant Pathology 35:

14. Tooley, P. W. and Fry, W. E. 1985. Field assessment of fitness of isolates of Phytophthora infestans. Phytopathology 75:982-988.

15. Tooley, P. W., Fry, W. E., and Villarreal-Gonzalez, M.J. 1985. Isozyme characterization of sexual and asexual Phytophthora infestans populations. J. Heredity 76:431-435.

16. Tooley, P. W., Sweigard, J. A., and Fry, W.E. 1986. Fitness and virulence of Phytophthora infestans isolates from sexual and asexual populations. Phytopathology 76:1209-1212.

17. Vanderplank, J. E. 1963. Plant diseases: epidemics and control. Academic Press, New York.

18. Vanderplank, J. E. 1971. Stability of resistance to Phytophthora infestans cultivars without R genes. Potato Res. 14:263-270.

GENETICS OF *Phytophthora infestans*

P. W. Tooley and W. E. Fry

Recent advances in the genetics of *Phytophthora infestans* have paved the way for the first in-depth analyses of virulence, mating type, and other characters of interest to plant pathologists and breeders. However, many aspects related to the basic biology, breeding behavior, and pathogenicity of the fungus still remain to be explored and characterized through genetic analysis.

Historically, progress in *P. infestans* genetics has been very slow in spite of the pathogen's worldwide importance. In 1875, Smith (24) described oospore-like bodies in leaves infected with *P. infestans*. Others, including deBary, disputed this claim believing them to be resting spores of a different fungus. In 1911, Clinton (4) reported oogonia with amphigynous antheridia and oospores in pure cultures of *P. infestans*. Similarly, in 1913, Pethybridge and Murphy (14) observed oospores in aged *P. infestans* cultures. It was not until 1956, however, that the naturally-occurring sexual stage of the fungus was discovered in Mexico (8,12), where two mating types of the fungus (designated A1 and A2) were found in approximately equal frequency (6). It is thus generally held that the fungus originated in Mexico, since this is also the region where many genes for blight resistance have been identified in wild *Solanum* species.

Due to the occurrence of only a single mating type (A1) of *P. infestans* in the U.S. and Europe until very recently (9,23,28), genetic studies were limited in the period from 1956 to 1985. Cultures of A2 mating type necessary for genetic studies were present only in Mexico, and quarantine restrictions in the U.S. and Europe prevented their distribution. Most studies during this period were directed at exploring mechanisms of asexual variation (5,11,18) or characterizing the cytogenetics of the fungus (3,17). In one study, however, sexual crosses were performed, and segregation for virulence and mating type was observed in the F1 generation (15).

During 1983 and 1984, Tooley et al. in collaboration with the Mexican National Potato Program collected *P. infestans* isolates of both mating types from Toluca and Chapingo, Mexico. These isolates provided the foundation for the most recent advances in *P. infestans* genetics research, and will continue to serve as valuable genetic materials for future studies. Although the collection of novel strains of both mating types was an important prerequisite for genetic studies, two other problems needed to be overcome before genetic analyses were possible.

First, an improved set of genetic markers was needed to analyze genetic crosses and evaluate segregation and recombination for genetic traits. Previous workers had used only virulence, mating type, and drug resistance as markers. Virulence and drug resistance are often variable in expression, and mating type is now known to show a complex mode of inheritance. Tooley et al. (29) investigated the use of isozymes for comparing levels of population diversity in P. infestans, and found that they made ideal genetic markers. Different P. infestans isolates were found to have distinctly different isozyme banding patterns at several genetic loci and these could be used as markers in genetic crosses.

The second problem which needed to be overcome for genetic studies was the difficulty in germinating the thick-walled oospores which resulted from sexual crosses. Previous workers had encountered very low oospore germination rates, making it difficult to obtain adequate numbers of progeny for genetic analyses. Shattock et al (19,20), adapting a technique used earlier by Shaw (22) passed oospores through live water snails to concentrate them and remove attached hyphae. These oospores showed high percentages of germination, allowing the establishment of numerous single-oospore progeny from specific crosses.

Genetic analysis of P. infestans using isozymes

The newly discovered isozyme markers and improved methods for germinating oospores allowed Shattock et al. (19,20) to study selfing, segregation, and recombination in the F1 generation of crosses between Mexican P. infestans isolates of opposite mating type. Since isozyme alleles show codominance, homozygous individuals can easily be distinguished from heterozygotes at specific loci. These workers found that crosses between parent isolates which were homozygous for different isozyme alleles yielded F1 progeny which were heterozygous (Table 1). Crosses between heterozygous parents yielded expected 1:2:1 ratios of homozygous:heterozygous:homozygous genotypes (Table 1). Selfing was found to occur at very low frequencies. Analysis of mating type ratios was complicated by the occurrence of homothallic strains among the progeny (Tables 1 and 2).

Further results (21,26) which followed the isozyme markers through F2 and backcross generations indicated that in some cases segregation ratios did not follow Mendelian expectation (Table 2). The causes of these disturbed segregations are not known, but could include inbreeding effects, gene interaction, or chromosome translocation. Additional genetic markers and data are needed to help determine the causes of disturbed ratios, so that the inheritance of more complex characters such as virulence, mating type, and fungicide resistance may be studied.

Genetic analysis of virulence in P. infestans

In spite of the recent advances in methodology, only limited progress has been made in analyzing the genetics of virulence. Early workers (13,25) observed genetic recombination among single-oospores derived from crosses between isolates of different race. However, too few progeny were obtained to determine the mode of inheritance. Later workers who obtained higher numbers of progeny (10,15) confirmed that segregation for virulence occurred, but did not formulate a specific hypothesis for genetic control of virulence.

Sweigard et al (27) using isozymes as independent genetic markers, analyzed 58 F1 progeny from a cross between two Mexican isolates of P. infestans which differed in virulence against potato resistance genes R2 and R4. Segregation in approximate 1:1 ratios was observed for virulence/avirulence to both host genes, and the two virulence factors appeared to be linked. These results indicated simple genetic control of the virulence factors, but dominance relationships could not be ascertained from the F1 data alone. More recent data obtained from F2 and backcross generations (Spielman, personal communication), revealed distorted segregation ratios for some of the isozyme markers. Because P. infestans is an obligate outcrosser, skewed segregation ratios were probably caused by inbreeding effects, indicating that inbred generations may be unsuitable for analysis of virulence. Progeny strains must be selected or produced that are free of deleterious inbreeding effects. These will be used as parents in additional backcrosses and sib crosses so that further analysis of virulence may proceed.

Genetic studies of pathogen virulence and host resistance will be enhanced by research utilizing other P. infestans hosts, notably tomato. To date, the entire tomato genome has been mapped using isozymes and DNA restriction fragment length polymorphisms (1). The PH1 gene for resistance to P. infestans has been mapped to chromosome 7, and future studies may allow characterization of the resistance gene product (Fry and Tanksley, personal communication). Such advances will allow for more detailed studies on the interaction of pathogen virulence genes and host resistance genes.

Ploidy variation and double-stranded RNA in P. infestans

Two additional developments have recently come to light which make the P. infestans genetics picture more interesting, and more complex.

First, Sansome and Brasier (16,17) viewed chromosomes of Mexican and British P. infestans isolates and found that British isolates appeared to contain twice the chromosome number of Mexican isolates.

They hypothesized that Mexican isolates are diploid, while isolates from the temperate regions may be tetraploid or of mixed ploidy. Tooley and Therrien (31) analyzed the nuclear DNA content of Mexican and non-Mexican P. infestans isolates and found that while Mexican isolates were all apparent diploids, the non-Mexican isolates appeared to consist of diploids, triploids, tetraploids, and aneuploids (Table 3).

Crosses performed using P. infestans isolates of recent Mexican origin may have been successful in part because isolates used as parents were of the same ploidy level. However, crosses attempted between Mexican and European isolates sometimes failed resulting in high levels of oospore abortion (Spielman, personal communication). These results indicate that heteroploidy should be considered an important factor contributing to variation in P. infestans. In future work, care must be taken to cross isolates of similar ploidy if genetic studies are to be successful.

A second factor which bears on future genetic studies with P. infestans is the discovery of large amounts of double-stranded RNA (dsRNA) in about 40 percent of all Mexican strains examined (30). The presence of dsRNA is often the result of infection with fungal viruses (mycoviruses). DSRNA has not been found in any non-Mexican P. infestans isolates including those from the U.S., Europe, and Peru. Gene products coded for by the dsRNA may interact with host gene products to yield novel phenotypes. Thus, the degree to which dsRNA affects virulence mating type, and other characteristics needs to be assessed. Since dsRNA appears to be inherited as a cytoplasmic character (Tooley, unpublished) it should prove useful as a cytoplasmic marker in genetic studies.

Potential applications to breeding for resistance to potato late blight

There are several ways in which increased knowledge of the pathogen's genetics could enhance efforts to breed for durable resistance to late blight.

First, specific pathogen races could be developed through genetic manipulation of the fungus. These could prove useful to potato breeders who wish to screen their material against specific races of P. infestans. Simple races such as race 1 or race 4 seem to occur rarely in nature, perhaps due to linkages between virulence genes. These linkages could perhaps be broken, and any desired race or combination of races could be provided to breeders. The availability of such races could allow more screening to be performed in the greenhouse, since field testing would not be necessary to assure that breeding material was exposed to a wide array of P. infestans races.

Secondly, P. infestans populations developed through genetic manipulation could be used to provide a rigorous screening test for horizontal resistance to late blight. Crosses between isolates heterozygous at virulence loci should generate an F1 population containing a broad virulence spectrum, since many recessive virulence genes would be expressed as homozygotes. A proportion of the individuals in such populations would likely contain virulence factors which could overcome any unidentified R genes present in the host, including those of intermediate effect which often interfere with screening for horizontal resistance.

Finally, by understanding the genetic basis for virulence and the gene products coded for by virulence loci, breeders will be better able to develop and deploy types of resistance and resistance gene combinations which are not so easily overcome by the pathogen.

Conclusions

The above studies have shown that detailed genetic analyses are now possible with P. infestans. Improved genetic markers and techniques for oospore germination and establishment of progeny have resulted in rapid progress toward understanding this important pathogen. However, much work remains to be done in identifying additional genetic markers and further probing the genetics of virulence, mating type, fungicide resistance, and other characters. The genetic studies now in progress and future studies will provide basic knowledge about the pathogen, leading to improved strategies for long-term control of potato late blight.

Literature Cited

1. Bernatzky, R. and Tanksley, S. D. 1986. Toward a saturated linkage map in tomato based on isozymes and random cDNA sequences. Genetics 112:887-898.

2. Bourke, P. M. A. 1964. Emergence of potato blight, 1843-46. Nature 203:805-808.

3. Brasier, C. M. and Sansome, E. 1975. Diploidy and gametangial meiosis in Phytophthora cinnamomi, P. infestans, and P. drechsleri. Trans. Brit. Mycol. Soc. 65:49-65.

4. Clinton, G. P. 1911. Oospores of potato blight. Science 33:744-747.

5. Denward, T. 1970. Differentiation in Phytophthora infestans. II. Somatic recombination in vegetative mycelium. Hereditas 66:35-48.

6. Galindo, J. and Gallegly, M. E. 1960. The nature of sexuality in Phytophthora infestans. Phytopathology 50:123-128.

7. Gallegly, M. E. 1968. Genetics of pathogenicity of Phytophthora infestans. Annu. Rev. Phytopathol. 6:375-396.

8. Gallegly, M. E. and Galindo, J. 1958. Mating types and oospores of Phytophthora infestans in nature in Mexico. Phytopathology 48:274-277.

9. Hohl, H. R. and Iselin, K. 1984. Strains of Phytophthora infestans with A2 mating type behaviour. Trans. Brit. Mycol. Soc. 83:529-531.

10. Laviola, C. and Gallegly, M. E. 1983. Genetic recombination and mode of inheritance of pathogenic characters by Phytophthora infestans through sexual reproduction. Pages 339-345 in: Durable Resistance in Crops, ed. by F. Lamberti, J. M. Waller, and N. A. VanderGraff. Plenum Press, NY.

11. Malcolmson, J. F. 1970. Vegetative hybridity in Phytophthora infestans. Nature 225:971-972.

12. Niederhauser, J. S. 1956. The blight, the blighter, and the blighted. Trans. NY Acad. Sci. 19:55-63.

13. Niederhauser, J. S. 1961. Genetic studies of Phytophthora infestans and Solanum species in relation to late-blight resistance in the potato. Pages 491-497 in: Recent Advances in Botany. Univ. of Toronto Press, Toronto.

14. Pethybridge, G. H. and Murphy, P. A. 1913. On pure cultures of Phytophthora infestans deBary, and the development of oospores. Sci. Proc. Roy. Dublin Soc. 13:566-588.

15. Romero, S. and Erwin, D. C. 1969. Variation in pathogenicity among single-oospore cultures of Phytophthora infestans. Phytopathology 59:1310-1317.

16. Sansome, E. 1977. Polyploidy and induced gametangial formation in British isolates of Phytophthora infestans. J. Gen. Microbiol. 99:311-316.

17. Sansome, E. and Brasier, C. M. 1973. Diploidy and chromosomal structural hybridity in Phytophthora infestans. Nature 241:344-345.

18. Shattock, R. C. and Shaw, D. S. 1976. Novel phenotypes of Phytophthora infestans from mixed culture of antibiotic resistant mutants. Trans. Brit, Mycol. Soc. 67:201-206.

19. Shattock, R. C., Tooley, P. W., and Fry, W. E. 1986a. Genetics of Phytophthora infestans: characterization of single-oospore cultures from A1 isolates induced to self by intra-specific stimulation. Phytopathology 76:407-410.

20. Shattock, R. C., Tooley, P. W., and Fry, W. E. 1986b. Genetics of Phytophthora infestans: determination of recombination, segregation, and selfing by isozyme analysis. Phytopathology 76:410-413.

21. Shattock, R. C., Tooley, P. W., Sweigard, J. A., and Fry, W. E. 1987. Genetic studies of Phytophthora infestans. Pages 175-185 in: Day, P. R. and Jellis, G. J., eds. Genetics and Plant Pathogenesis. Blackwell Sci. Publs., Oxford. 352 pp.

22. Shaw, D. S. 1967. A method of obtaining single-oospore cultures of Phytophthora cactorum using live water snails. Phytopathology 57:454.

23. Shaw, D. S., Fyfe, A. M., Hibberd, P. G., and Abdel-Sattar, M. A. 1985. Occurrence of the rare A2 mating type of Phytophthora infestans on imported Egyptian potatoes and production of sexual progeny with A1 types from the U.K. Plant Pathol. 34:551-556.

24. Smith, W. G. 1875. The resting spores of the potato fungus. Gardeners' Chronicle 4:68-70.

25. Smoot, J. J., Gough, F. J., Lamey, H. A., Eichenmuller, J. J., and Gallegly, M. E. 1958. Production and germination of oospores of Phytophthora infestans. Phytopathology 48:165-171.

26. Spielman, L. J., Sweigard, J. A., Shattock, R. C., and Fry, W. E. 1986. Use of isozymes for the analysis of unexpected segregation ratios in F2 and backcross progeny of Phytophthora infestans. Phytopathology 76:1102 (Abstr.).

27. Sweigard, J. A., Spielman, L. J., Tooley, P. W., Shattock, R. C., and Fry, W. E. 1987. The genetic control of virulence in Phytophthora infestans. Phytopathology 77:122 (Abstr.).

28. Tantius, P. H., Fyfe, A. M., Shaw, D. S., and Shattock, R. C. 1986. Occurrence of the A2 mating type and self-fertile isolates of Phytophthora infestans in England and Wales. Plant Pathol. 35:578-581.

29. Tooley, P. W., Fry, W. E., and Villarreal Gonzalez, M. J. 1985. Isozyme characterization of sexual and asexual Phytophthora infestans populations. J. Hered. 76:431-435.

30. Tooley, P. W., Hewings, A., and Falkenstein, K. F. 1986. Detection of double-stranded RNA in Phytophthora infestans. Phytopathology 76:1102 (Abstr.).

31. Tooley, P. W. and Therrien, C. D. 1987. Cytophotometric determination of the nuclear DNA content of 23 Mexican and 18 non-Mexican isolates of Phytophthora infestans. Exper. Mycol. 11:19-26.

Table 1. Mating type and genotype at the Gpi-1 locus of F1 progeny from matings between Mexican isolates of Phytophthora infestans[a/]

Parents	Progeny	
	Mating type[b/]	Gpi-1 genotype[c/]
533 (A1, 122/122)	150 (A1)	0 (86/86)
X	140 (A2)	329 (86/122)
550 (A2, 86/86)	71 (H)	3 (122/122)
533 (A1, 122/122)	63 (A1)	0 (100/100)
X	33 (A2)	158 (100/122)
525 (A2, 100/100)	38 (H)	0 (122/122)
529 (A1, 86/100)	26 (A1)	13 (100/100)
X	22 (A2)	23 (86/100)
519 (A2, 86/100)	7 (H)	15 (86/86)

a/ Data from Shattock et al. (20).

b/ The number of progeny in each class is followed by the mating type. 'H' indicates homothallic isolate.

c/ The number of progeny in each class is followed by the genotypic class at the Gpi-1 locus. Alleles are designated by numbers (86, 100, 122) corresponding to the relative mobility of the isozyme bands on starch gels (29).

Table 2. Mating type and genotype at the Gpi-1 locus of F2 and backcross progeny from matings between Mexican isolates of Phytophthora infestans[a/]

Parents	Progeny	
	Mating type[b/]	Gpi-1 genotype[c/]
533 (A1, 122/122)	30 (A1)	0 (86/86)
X	7 (A2)	27 (86/122)
1-104 (A2, 86/122)	19 (H)	30 (122/122)
1-327 (A1, 86/122)	92 (A1)	0 (86/86)
X	21 (A2)	116 (86/122)
550 (A2, 86/86)	3 (H)	0 (122/122)
1-246 (A1, 86/122)	95 (A1)	20 (86/86)
X	99 (A2)	105 (86/122)
1-104 (A2, 86/122)	20 (H)	96 (122/122)

a/ Data from Shattock et al. (21).

b/ The number of progeny in each class is followed by the mating type. 'H' indicates homothallic isolate.

c/ The number of progeny in each class is followed by the genotypic class at the Gpi-1 locus. Alleles are designated by numbers (86, 100, 122) corresponding to the relative mobility of the isozyme bands on starch gels (29).

Table 3. Nuclear Feulgen-DNA values in Mexican and non-Mexican isolates of *Phytophthora infestans*[a/]

Mexican isolates		non-Mexican isolates	
Isolate no.	mean DNA[b/]	Isolate no.	mean DNA[b/]
501	0.54	111	0.81
502	0.63	127	1.11
503	0.57	128	0.76
506	0.54	134	0.84
508	0.54	135	1.09
512	0.58	136	0.94
514	0.66	137	0.95
517	0.58	140	1.03
518	0.58	141	0.88
519	0.69	142	0.66

a/ Data from Tooley and Therrien (31).

b/ Standard errors were 0.01 for all Mexican isolates, and ranged from 0.01 to 0.04 for non-Mexican isolates. Data are means for 50 zoospore nuclei.

BIOCHEMICAL ASPECTS OF POTATO LATE BLIGHT WITH RESPECT TO COMPATIBILITY AND INCOMPATIBILITY REACTIONS

Kohei Tomiyama

ABSTRACT

Main well known phenomena in disease resistance of plants; hypersensitive cell death, activation of oxidative enzymes, browning, and phytoalexin production have been, often, investigated as independent phenomenon respectively. Moreover, main phytoalexins of potato, rishitin, lubimin and phytuberin were often investigated as an independent substance respectively. This often let researcher underestimate the roles of rishitin or other compounds in disease resistance, since it depends on experimental materials and conditions employed which compounds accumulate in great amount. However, it is becoming clear by research on potato late blight that all these factors are closely related together and are in the relation of cause and effect. These factors work together and constitute the defense response of potato plant.

Evidence for the close relation among these different phases of defense response are discussed. Recent status of research on the mechanism of host-parasite specificity in potato late blight is also discussed.

INTRODUCTION

In 1950s and in the first half of 1960s, the physiological studies on the disease resistance of plants centered around the physiology related to oxidation of phenolic compounds. This prevalence in research came of the following facts; (a) generally, disease resistance is accompanied with quicker and severer browning as compared with the case of susceptible host-parasite relationship, (b) browning is caused by oxidation of phenolic compounds, (c) phenolic compounds are often toxic and intermediary oxidation products are generally more toxic to fungi than original compounds.

However, extensive work could not demonstrate exclusively the presumption that phenolic compounds play a decisive role in disease resistance, since the toxicities of phenolic compounds are insufficient to explain the inhibition of hyphal growth of the parasite, even if their concentration increase in the infection site. Thus, as far as the inhibitory action on the hyphal growth are concerned, decisive conclusion could not be drawn as to their role in disease resistance, and the research on the phenolic compounds was brought to a stalement.

In 1961, pisatin was isolated as a kind of phytoalexin (PA) from pea pod by Cruickshank and Perrin. Since this finding, the PA-theory which was presented by K. O. Muller to explain the disease resistance of potato late blight, has been spotlighted. PA had reason to make researcher convince that it could explain disease resistance of plants, since (a) chemically or biologically detectable amount of PA appears after the infection by microbes and accumulate in sufficient amount to inhibit the hyphal growth, (b) a parasite is generally tolerant of PA which is produced by its host plant.

Thus, the research on PA took the place of research on phenolic compounds, oxidative enzymes and so on, and became main current of research on disease resistance, and the research on the latter was rather neglected.

Another distinct phenomenon was hypersensitive death of the plant cells infected by an incompatible parasites. Reports have been published in which the significance or role of the hypersensitive death was denied or doubted. However, many evidence presented by studies on potato late blight supported the conclusion that the hypersensitive cell death plays a key role in defense response. Data, which accumulated by research on potato late blight, strongly suggest that these apparently different phases of response accompanied with disease resistance not only cooperate together to perform the actual defense response to pathogens, but also each phase of defense factors is in the relation of cause and effect.

ROLE OF OXIDATION IN MAKING CELL WALL RESISTANT

When the cut surface cells of aged slices of tuber of potato cultivar Rishiri(R_1) were infected by an incompatible race of Phytophthora infestans, in about 40% of the infected cells, only one cell was browned, and no further invasion of hypha into second cell occurred. In about 50%, infected cell and next one were browned and further development was not observed. In the rest, the hyphae invaded into 3 or more cells (33). In the case of this sample 50% of the infected cells died within about 3 hr after the inoculation by the parasite. Microscopic observation on the infection process of the cut surface cells of aged leaf petiole slice by the incompatible race showed that hypha continued to grow in the same rate after the hypersensitive death of the host cell (Fig. 1). Intracellular hyphal growth began to be slowed down about 7 hr after inoculation when reshitin began to accumulate. The hypha was almost entirely inhibited about 11-15 hr after inoculation when the concentration of rishitin reached about that of ED_{50} for the hypha in vitro (33, 34). The incompatible hypha in the dead cell grew in the same rate as that of total length of the compatible hypha (length of main axis plus those of branches) but did not branch out. The unbranched hypha wound inside of the cell, and

could not go out of the cell. When infected by the incompatible race, no cell death and therefore no browning occurred for at least 2-3 days after inoculation. The hyphae branched soon after infection, and invasion into next cell was already observable 7 hr after inoculation. In the case of these samples, penetration by both races was achieved within about 2 hr after inoculation in average. These results indicated that the hyphal invasion into next cell or intercellular space had been inhibited before the growth of the intracellular hypha began to be slowed down.

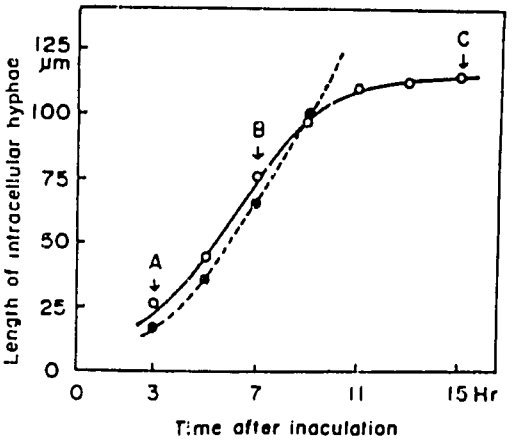


Fig. 1. Intracellular hyphal growth of P. infestans and rishitin content in infected potato cell.

A: Time when about 50% of cells infected by race 0 have died,

B: the time when rishitin began to accumulate,

C: the time when rishitin reached about the concentration of ED_{50} for hyphal growth (33, 34).

O----O: Infected by incompatible race.
●----●: Infected by compatible race.

Another noteworthy phenomenon was observed (39). When hypha of the incompatible race of P. infestans invaded into a corner of a cell and stick to host cell wall, the hyphal wall facing towards inside of the host cell turned brown, but that sticking to host cell wall did not turn brown. Continuous observation on the same cell showed that the hypha grew from the colorless hyphal wall side, but did not grow from the brown hyphal wall side. These results suggest that oxidation of phenolic compounds may make the host cell wall resistant against the penetration by the parasite. It may not be absurd to suppose that oxidized products of polyphenolic compounds alter the hyphal wall and inhibit branching but does not inhibit hyphal growth.

Following experiments (31) presented evidence showing that phenolic compounds play roles in sealing off the hyphae of the incompatible race into the infection site. When thin fresh slices 0.5 mm thick of potato cultivar Kennebec (R_1 gene) were inoculated with dense zoospore suspension of the incompatible race 0 of P. infestans, spores were produced abundantly a few days after inoculation. However, if the inoculated thin slices were treated with 0.01, 0.02, 0.03 and 0.05 M chlorogenic acid solution including ascorbic acid before inoculation

or 8 or 24 hr after inoculation. The treatment induced brown small flecks which could not be observed in untreated infected slice. Spore production on the slice was greatly reduced irrespective of the time of the treatment. In the case of compatible host-parasite combination, on the contrary, the inhibitory effect was far less than that in the case of incompatible combination (Fig. 2). These results support the presumption that oxidation of phenolic compounds caused by the hypersensitive death of the infected cells play an important role in inhibition of lesion development.

All these results indicated that oxidation of phenolic compounds play a role in sealing off the hypha into the infected cell which hypersensitively died.

SESQUITERPENOIDS (PHYTOALEXIN) IN POTATO

Phytoalexin of potato which had been predicted by K. O. Muller (1940) was first isolated by our group and named rishitin (45). In a short time, lubimin (25) and phytuberin (51) were found in diseased potato as phytoalexins (2). Since that time, joint work by organic chemist group (N. Murai, N. Katsui and T. Masamune) and biologist group (the author's group) has been carried out on the phytoalexin of potato. The results so far obtained by our group showed that rishitin and related sesquiterpenoids exclusively accumulated in dead cells caused by infection or chemical treatments and possibly in their just adjacent space (Fig. 3 and 4).

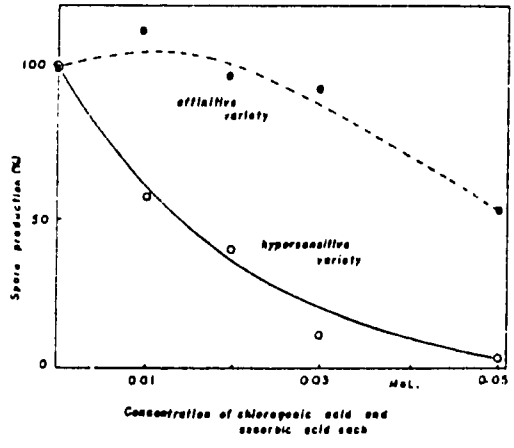


Fig. 2. Effect of mixture of chlorogenic and ascorbic acid on the spore production on thin disks of susceptible and hypersensitive cultivars infected by race 0 of P. infestans. (31).

It was demonstrated by using acetate-2-^{14}C that rishitin was synthesized in the healthy tissue neighbouring the dead tissue (28). In the adjacent living cells, where rishitin was actively synthesized, the synthesized rishitin was soon metabolized to rishitin-M-1 and to more hydrophylic compounds (17, 18). Rishitin-M-1 is far less toxic to both host and parasite. However, rishitin and related compounds which were transported to adjacent necrotic cells, accumulated, since they could not be metabolized further in the host cells. Here, a

question is paused as to whether or not synthetic pathway of rishitin operates in uninfected intact tissue. Experiments were carried out to answer this question. By treating for 10 min the fresh slices excised from intact buds of potato tuber with acetate-2-^{14}C soon after slicing, slow but evident synthesis of rishitin was demonstrated (29). This indicates that synthetic pathway operates in healthy intact tissue, although it is slow. Incorporation of radioactivity from acetate-2-^{14}C into rishitin began to increase rapidly soon after slicing (29).

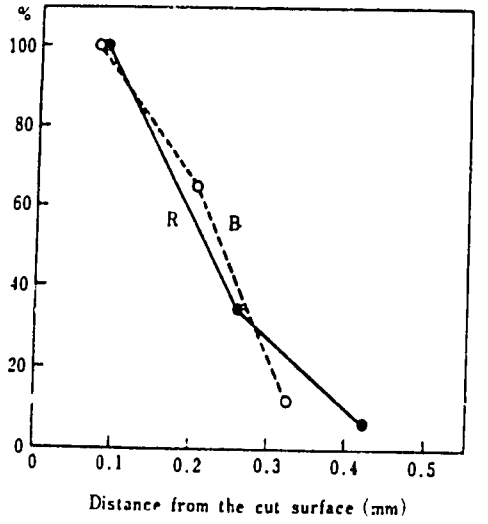


Fig. 3. Relation between rishitin content and percentage of browned infected cells in the superficial tissue of aged potato slice infected by the race 0. R: percentage of rishitin content when the content of rishitin in the superficial infected tissue was taken as 100%. B: percentage of brown cell per total cells in each zone (46).

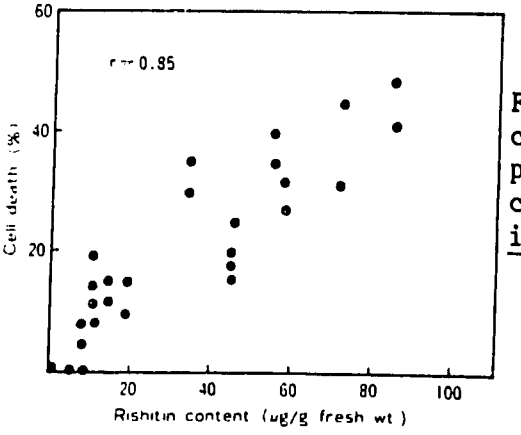


Fig. 4. Relation between rishitin content and percentage of cell death in potato disks treated with hyphal wall components of mycelia of Phytophthora infestans 18 hr after cutting (30).

About 20 compounds were isolated from diseased potato (cultivar Rishiri) (26, 27). All of these were sesquiterpenoids and found only in diseased lesion but, as far as the roles in disease resistance are concerned, rishitin, lubimin and phytuberin are main phytoalexins of potato. Experiments on the biosynthesis of rishitin and related compounds using ^{14}C labeled compounds and doubly deuterated compounds, biosynthetic pathways of the three main phytoalexins became clear (27). Elucidated biosynthetic pathways of main compounds of potato PA are shown in Fig. 5 (35). The results showed that solavetivon is a key metabolic intermediate of rishitin, lubimin and phytuberin. In some papers published, contradictory or negative results were often

reported as to the role of rishitin or other PA in disease resistance. These apparent negative results came of the fact that rishitin, lubimin and phytuberin are derived from the same origin, solavetivon. It depends on the circumstance of infection or difference in host-parasite combination which of these compounds accumulate in greater amount. Solavetivon, which is in a state of low degree of oxidation, is oxidized in succession and transformed to the main phytoalexins (27). This suggests that the activation of oxydative enzymes in the cells adjacent the infected one may afford physiological condition favourable for synthesis of rishitin and so on.

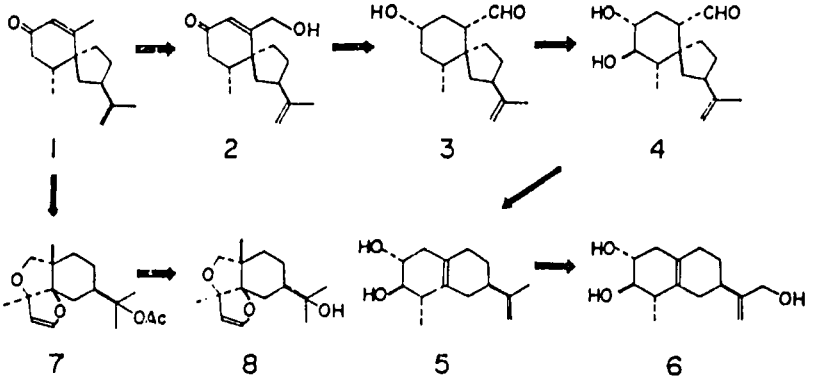


Fig.5. Elucidated biosynthetic pathways of main compounds of potato Phytoalexins. 1, solavetivone; 2, oxysolavetivone; 3, lubimin; 4, oxylubimin; 5, rishitin; 6, rishitin-M_1; 7, phytuberin; 8, phytuberol.

"Sato et al.(35)"

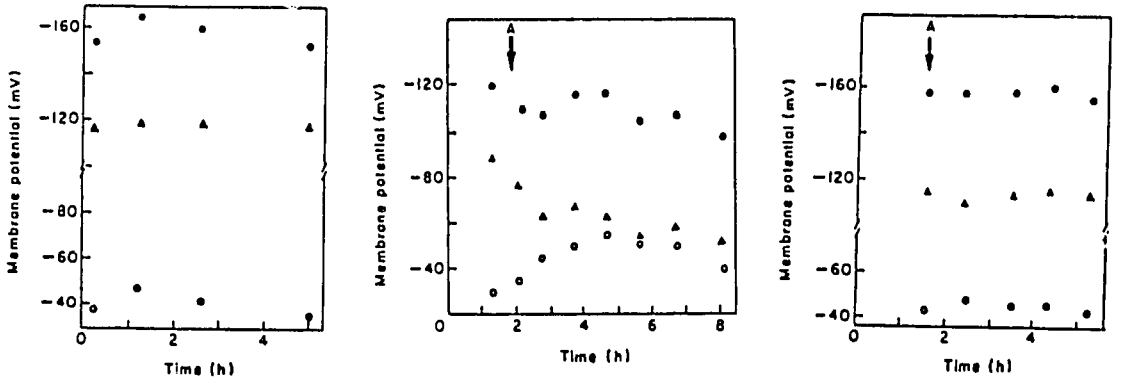
It has been known that most of the chemical compounds, which induced rishitin accumulation in potato tissue, caused a part of tissue necrosis at their effective concentration. It may be said that chemicals or other treatment, which cause a part of tissue necrosis, always induce accumulation of rishitin and related compounds. Although, as described formerly (29) cutting of potato tissue accelerated incorporation of radioactivity from acetate-2-^{14}C into rishitin, no accumulation of a great amount of PA occurred without necrosis in part of the tissue. The wounding or necrosis are known always to induce acceleration of oxidative enzymes. It seems probable that oxidation of lipids and intermediately produced superoxide relate to acceleration of synthesis of PA in potato tuber. Murai et al. (27, lecture in Nagoya University 1987) presented some evidence showing that H_2O_2 accumulated in the infected tissue may relate the PA synthesis. However, decisive conclusion cannot be drawn until it is confirmed that H_2O_2 at the concentration which does not induce tissue necrosis, accelerate synthesis of rishitin. Enzymatic study is necessary to make clear the mechanism of acceleration of PA synthesis.

HYPERSENSITIVE CELL DEATH

It has been known that the potato cells hypersensitively die rapidly by infection by incompatible race of P. infestans. With compatible race, on the contrary, no cell death occurs at least for 2 days or more. It is generally accepted that in many cases rapid browning accompany disease resistance. However, counteraugments were also presented as to the roles of hypersensitive death of the infected cells in disease resistance. Before argueing the problem cut, process of the hypersensitive cell death should be more precisely investigated. Light (e.g. 38, 47) and electron microscopic (36, 37) observations on the process of hypersensitive response were made.

Transmembrane electropotential of potato cell depolarized very soon after penetration by the incompatible race of P. infestans, but no depolarization occurred when infected by the compatible race (Fig. 6, 7, and 8). The depolarization of membrane potential caused by the incompatible race was due to depolarization in diffusion potential component (49, 50).

These results suggest that the alteration of the site of cell membrane bearing the function for the ion diffusion may be very early event which occurs after recognition by the potato cell of the attack by the incompatible race (49, 50).



It has been reported that the rapid occurrence of the hypersensitive death of the infected cell need metabolic energy, since the death was greatly prolonged by inhibitors of respiratory enzymes, SH-binding compounds at concentration which had little effect on the intracellular growth of the incompatible race (4, 40, 42). Pre-

infectional heat treatment inhibited greatly the hypersensitive cell death, although the treatment had no effect on the growth of the intracellular hyphae (Fig. 8 and 9) (41). The pre-infectional heat treatment inhibited also the depolarization of diffusion potential component of host cell membrane which was otherwise to be caused by the infection by the incompatible race (published elsewhere). It has been well known that heat treatment inhibit metabolic activity. Relation between metabolic energy and hypersensitive death of the cell infected by the incompatible race was also found in aging process. The relation between the rise in metabolic activity in aging process and rapidity in occurrence of the hypersensitive death of the cells infected by the incompatible race was almost linear, that is, the more active the metabolic activity, the shorter the time for the hypersensitive cell death (12, 42, 44).

Evidence, showing that superoxide produced intermediately by the infection by the incompatible race of P. infestans play an important role in the hypersensitive death of the infected cells, was presented (9, 10). The potato disks were infiltrated with solution containing superoxide dismutase (SOD) which catalyze the conversion of the superoxide anion (O_2) to hydrogen peroxide and O_2. The treatment significantly delayed the occurrence of the hypersensitive cell death caused by infection by the incompatible race (9, 10). Roles of superoxide in phytopathological phenomena were reviewed (11). It has been known that superoxide generation by leucocytes plays an indispensable role in defense of animals against invaders.

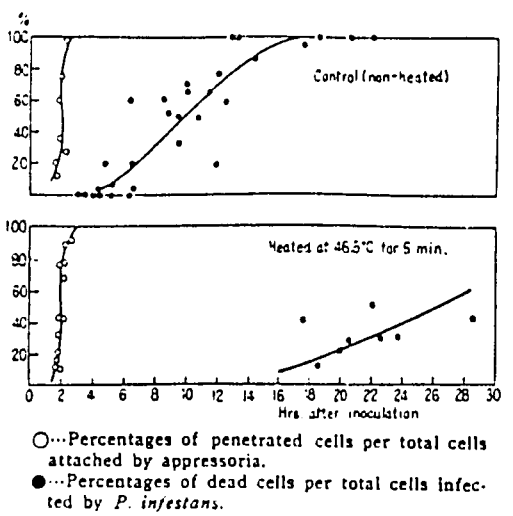


Fig.9. Effect of pre-infectional heat treatment (46.5°C, 5 min) on the penetration by the incompatible race and the time needed for the death of the infected cells. Open circle: penetration, closed circle : hypersensitive cell death. (41).

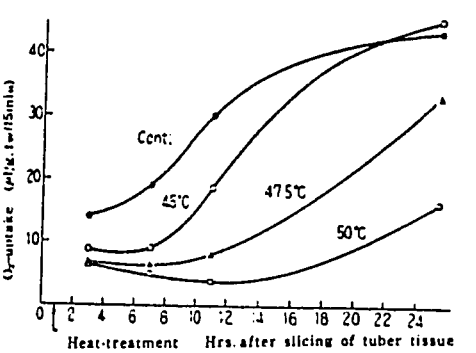


Fig.10 Effect of treatment with hot water for 5 min on respiration of potato slice (41).

OXIDATION OF LIPIDS BY WOUNDING OR INFECTION

Several reviews (14, 23, 52, 53) dealt with the relation between slicing of storage tissue and lipids destruction. Extensive damage occurs in the membrane system of neighbouring cells immediately after slicing and unusually large amount of lipids were released in cytoplasmic phase. It was reported that slicing of potato tuber resulted in the loss of 20% of endogenous membrane lipids within seconds and 40% loss within minutes after cutting (15, 16). The released fatty acids may be attacked by oxidative enzymes producing activated oxygen (11). It was reported that the oxygen activating reaction was observed also in cell wall (11, 54). Accordingly, oxygen activation may occur in both cytoplasm and cell wall after slicing. These compounds are potentially destructive to membrane structure and to enzyme processes.

Roles of the fatty acids and activated oxygen in plant pathological problems have been reviewed (11). Arachidonic and eicosapentaenoic acid were isolated from P. infestans as an elicitor of PA accumulation in potato tissue (1). Keppler and Novacky (21) presumed that the depolarization of membrane potential in the hypersensitive response of cucumber to pathogens may be caused by destruction of membrane lipids. Enzymes such as superoxide dismutase, catalase and peroxidase and small molecules having reducing activity were reported to play roles in protection of aerobic cells from the deleterious effect arising from oxygen activation (11).

BREAK OF OXIDATION-REDUCTION SYSTEM

When epidermal cell of midrib of potato (R-genes) leaf was infected by the incompatible race, a number of small granules of rod shaped or spherical in brownian movement appeared around the hypha and gradually increased in number. Then the half-moon shaped nucleus which had been sticking to the side cell wall, suddenly shrank, became round and was detached from the side wall and, immediately, turned pale brown (38). This process of morphological change of nucleus was almost momentary. The cinephotomicrographic observation (38) showed that the transition from the living state to the irreversibly destroyed state (death) seemed to occur momentarily, and instantaneously pale browning took place (38, 47), and then the color was gradually deepen. This indicate that break of the oxidation-reduction system may occur just at the moment of the transition from living state to dead state, and result in oxidation of various compounds. This oxidation, especially oxidation of phenolic compounds cause various phases of defense response described in the former sections. Thus, oxidation of lipids, oxidation of phenolic compounds and PA accumulation each are not independent phenomenon, but in the relation of cause and effect and constitute a complex defense reaction.

MECHANISM OF SPECIFICITY

Hyphal wall component (HWC) isolated from ground mycelia of P. infestans (24) was found to cause necrosis of potato tissue. HWCs isolated from different races had no specific effect on the tissues of potato cultivars having different R-genes. HWC, sometimes, was looked upon as elicitor of the hypersensitive response to the infection by the incompatible parasite. However, there is no evidence that the necrosis caused by the HWC is induced by the same mechanism as that of the hypersensitive cell death.

A blocker of the hypersensitive response was isolated (3, 5, 6 and 7). Later, the blocker was shown to be water soluble -1,3-glucan (38). The blocker appeared to be race specific and to inhibit specifically the hypersensitive cell death of the tissue of potato cultivars having R-genes.

However, recent study by Furuichi and Suzuki (13), using enzyme-linked immunosorbent assay technique, showed that both compatible race 1, 2, 3, 4 and incompatible race 0 contained the same blocker as far as the employed technique was concerned. They showed that the blockers obtained from both compatible race 1, 2, 3, 4 and incompatible race 0 inhibited in about the same rate the hypersensitive death of the cells infected by the incompatible race.

Thus, it must be stated that the role of the blocker : water soluble 1, 3-glucan and HWC in establishment of the host-parasite specificity in potato late blight still remains uncertain.

However, there is a possibility that the blocker plays a role in the loss of the hypersensitive reactivity which occurs gradually in the potato cells infected by the compatible (43). This process of losing hypersensitive reactivity is known to be time dependent. About 15 hr seemed to be necessary to lose fully the reactivity (43). A possibility that the blocker plays some role in this phenomena should be investigated.

DISCUSSION

The investigation on the defense response described above were mainly focussed on making clear the response of a potato cell to an incompatible race of P. infestans using a model system which is extremely hypersensitive to the incompatible race. The late blight resistance of the tissue is based on these responses of a cell although the defense phenomena of the commercial cultivars are apparently more complex in tissue level.

The two groups of compounds, polyphenolic and sesquiterpenoid compounds cooperate to protect the tissue from the hyphal development

of P. infestans. In the case of the highly hypersensitive potato cells which were used as an experimental model system by our group, the relation between both groups compounds are as follows. If the hyphal development were not inhibited by the altered host cell wall which is considered to be caused by intermediately produced oxidation product caused by the hypersensitive cell death, the accumulation of PA in efficient concentration may be too late to inhibit the hyphal development, because it takes several hr after cell-death for rishitin to accumulate in efficient concentration. In this case of highly hypersensitive type of potato cells, which were used as an experimental model system, PA may not be important to restric the hyphae within the infected site, and alteration of cell wall caused by oxidation of phenolics may be enough to inhibit the hyphal development.

However, the cells in the tissue of commercial cultivars usually not so highly sensitive, and also the tissue often consists of the cells having different sensitivity to an incompatible race. In these cases, even the hyphae of the incompatible race also often invade into the intercellular space. Originally, the hyphae of P. infestans spread through intercellular space after they proliferate in the cell, into which it invades in the first place, or in next cells. To inhibit the hyphal growth in the intercellular space, the most possible substance may be PA which accumulate in the space surrounding the dead cells.

Periderm may finally separate the disease lesion and the healthy tissue. However, it was shown that inhibition of hyphal development took place before the periderm formation (20). In the periderm, the cells divided a few or several times, but growth of their daughter cells was strongly retarded. Phenolic compounds (e.g. coumalin) are known as an inhibitor of IAA. Reshitin also was shown to be an inhibitor of IAA (19). It is supposed that there may be relation between these compounds and the periderm. Thus, in the tissue level, the defense responses are not only in the relation of cause and effect, but also cooperate in a very complex manner to seal off the parasite into the infection site.

REFERENCE

1. Bostock, R. M., and J. A. Laine (1981). Science 212:67.

2. Clark, D. D. (1983). In "Biochemical Plant Pathology", (J. A. Callow, ed.) John Wiley and Sons. Chichester, New York, Brisbane, Toronto, Singapore.

3. Doke, N. (1975). Physiological Plant Pathology, 7:1-7.

4. Doke, N. and K. Tomiyama (1978). Physiological Plant Pathology, 12:133-139.

5. Doke, N., N. A. Garas and J. Kuc (1979). Physiological Plant Pathology, 21:89-95.

6. Doke, N. and K. Tomiyama (1979). Physiological Plant Pathology, 16:169-176.

7. Doke, N. and N. Furuichi (1982). Physiological Plant Pathology, 21:23-30.

8. Doke, N. (1982). Physiological Plant Pathology, 21:85-95.

9. Doke, N. (1985). Physiological Plant Pathology, 23:345-357.

10. Doke, N. (1985). Physiological Plant Pathology, 23:359-367.

11. Elstner, E. F. (1982). Annual Review of Plant Physiology, 33:73-96.

12. Furuichi, N., K. Tomiyama, N. Doke and M. Nozue (1979). Ann. Phytopath. Soc. Japan, 45:215-220.

13. Furuichi, N. and J. Suzuki (1987). Ann. Phytopath. Soc. Japan, 53:391 and Ann. Phytopath. Soc. Japan (in press).

14. Galliard, T. (1978), in "Biochemistry of Wounded Plant Tissues" (Kahl, G., ed.). Walter de Gruiter, Berlin, New York, pp. 155-201.

15. Hasson, E. P. and G. G. Laties (1976). Plant Physiology, 57:142-147.

16. Hasson, E. P. and G. G. Laties (1976). Plant Physiology, 57:148-152.

17. Horikawa, T., K. Tomiyama and N. Doke (1976). Phytopathology, 66:1186-1191.

18. Ishiguri, Y., K. Tomiyama, N. Doke, A. Murai, N. Katsui, F. Yagihashi and T. Masamune (1978). Phytopathology, 68:720-725.

19. Ishizaka, N., K. Tomiyama, N. Katsui, A. Murai and T. Masamune (1969). Plant and Cell Physiol., 10:183-192.

20. Ishizaka, N. and K. Tomiyama (1970). Ann. Phytopath. Soc. Japan, 36:243-249.

21. Keppler, L. D. and A. Novacky (1985). Phytopathology, 76:104-108.

22. Kitazawa, K., H. Inagaki and K. Tomiyama (1973). Phytopathol. Zeitschr., 76:80-86.

23. Laties, G. G. (1978). In "Biochemistry of Wounded Plant Tissue" (Kahl, G., ed). Walter de Gruiter, Berlin, New York. pp, 421-466.

24. Lisker, N. and J. Kuc (1977). Phytopathology, 67:1356-1359.

25. Metlitsky, L. V. and O. L. Ozertskovaya (1970). Mikol. Fitopatol., 4:146.

26. Nasamune, T., A. Murai and N. Katsui (1978). Kagaku to Seibutsu, 16:646.

27. Murai, A. (1987). In "Pepticide Science and Biotechnology". (R. Greenhalgh and T. R. Roberts), pp. 81-88. Blackwell Scientific Publications.

28. Nakajima, T., K. Tomiyama and M. K. Kinukawa. Ann. Phytopath. Soc. Japan, 41:49-55.

29. Sakai, S., K. Tomiyama and N. Doke (1979). Ann. Phytopath. Soc. Japan, 45:705-711.

30. Sakai, S., N. Doke and K. Tomiyama (1982). Ann. Phytopath. Soc. Japan, 48:238-240.

31. Sakuma, T. and K. Tomiyama (1967). Ann. Phytopath. Soc. Japan, 33:48-58.

32. Sato, N. and K. Tomiyama (1969). Ann. Phytopath. Soc. Japan, 35:207-217.

33. Sato, N., K. Kitazawa and K. Tomiyama (1971). Physiological Plant Pathology, 1:289-295.

34. Sato, N. and K. Tomiyama (1976). Ann. Phytopath. Soc. Japan, 43:598-600.

35. Sato, N., Y. Yoshizawa, H. Miyazaki and A. Murai (1985). Ann. Phytopath. Soc. Japan, 51:494-497.

36. Shimony, C. and J. Friend (1975). New Phytol., 74:59-65.

37. Shimony, C. and J. Friend (1977). Physiological Plant Pathology, 11:243-249.

38. Tomiyama, K. (1954). Research Bulletin Hokkaido, National Agr. Exp. Sta., 67:28-38.

39. Tomiyama, K. (1955). Ann. Phytopath. Soc. Japan, 19:149-154.

40. Tomiyama, K. (1957). Ann. Phytopath. Soc. Japan, 22:75-78.

41. Tomiyama, K. (1957). Ann. Phytopath. Soc. Japan, 22:237-242.

42. Tomiyama, K. (1960). Phytopathol. Zeitschr. 39:134-148.

43. Tomiyama, K. (1966). Ann. Phytopath. Soc. Japan, 32:181-185.

44. Tomiyama, K. (1967). Phytopathol. Zeitshr. 58:115-116.

45. Tomiyama, K., T. Sakuma, N. Ishizaka, N. Sato, N. Katsui, M. Takasugi and T. Masamune (1968). Phytopathology, 58:115-116.

46. Tomiyama, K. (1970). Shokubutsu no Kagaku Chosetsu, 5:105-115.

47. Tomiyama, K. (1982). In "Plant Infection" (Y. Asada et. al. ed.) Japan Scientific Soc. Press, Springer-Verlag, Berlin. pp. 329-344.

48. Tomiyama, K. (1983). Ann. Rev. of Phytopathology, 21:1-12.

49. Tomiyama, K., H. Okamoto and K. Katou (1983). Physiological Plant Pathology, 22:233-243.

50. Tomiyama, K., H. Okamoto and K. Katou (1987). Ann. Phytopath. Soc. Japan, 53:310-322.

51. Varns, J. L., J. Kuc and E. B. Williams (1971). Phytopathology, 61:174-177.

52. Van Steveninck, R. F. M. (1975). Ann. Rev. Plant Physiol. 26:237-258.

53. Van Steveninck, R. F. M. (1978). In "Biochemistry of Wounded Plant Tissues" (Kahl, G, ed.). Walter de Gruiter, Berlin, New York, pp. 421-466.

54. Yamazaki, I. and L. H. Piette, (1963). Biochem. Biophys. Acta. 77:47-64.

LATE BLIGHT BREEDING STRATEGY AT CIP

J. Landeo

1. INTRODUCTION

Breeding for resistance to late blight (Phytophthora infestans), in potato, since the early years at CIP has focused on non-specific type of resistance (also called horizontal resistance, general resistance, field resistance, minor-gene resistance, polygenic resistance, etc.). This type of resistance as opposed to specific type of resistance (also called R-gene resistance, major-gene resistance, monogenic resistance, etc.), was thought to be the most meaningful resistance to breed for, since it was shown to be more effective against all physiological races of the pathogen, and more stable over years environments (3, 9, 10).

Despite, considerable effort of most breeding programs to select for non-specific resistance, little progress has been made on its combination with early maturity along with agronomic attributes well-suited for the growers and the consumers (12). All evidence from past experiences, indicated that there was a positive correlation between non-specific resistance and late maturity in potato (12). However, it can be safely said, that effort has not been fully exhausted. The successful growth of susceptible early maturing varieties fully protected against late blight by the use of fungicides, slowed the effort of breeders, to develop varieties with non-specific resistance in combination with early maturity.

The International Potato Center, as a research institute where most relevant research is addressed to help developing countries, in the light of raising costs of the chemical control, undertook the responsibility of developing genetic resistance as the most practical and meaningful means to control late blight. To this end, non-specific resistance to late blight in combination with early maturity and gradual introduction of other major disease resistances were the primary objectives of the late blight breeding program.

2. SOURCES OF RESISTANCE

The overall sources of genetic resistance to late blight, that was used at CIP in the development of a breeding population and advanced clones involved the following germplasm:

a. Solanum tuberosum Gp. Tuberosum clones, made available by several potato breeding programs of some temperate countries working on late blight resistance. Resistance to late blight is inherited from early introduction of S. demissum.

b. Solanum tuberosum Gp. Andigena and Gp. Phureja clones from the germplasm bank maintained at CIP.

c. Neo-tuberosum (Gp. Andigena adapted to long days), clones made available by Cornell University, USA.

d. ABPT (Acaule-Bulbocastanum-Phureja-Tuberosum), clones from a special project with the Agricultural University in Wageningen, The Netherlands.

3. BREEDING AND TESTING STRATEGIES DURING THE FIRST PERIOD (1973-1980)

Two main approaches were followed during this period:

a. The development of a breeding population with enhanced non-specific resistance to late blight, and

b. The selection of late blight resistant clones in combination with desirable agronomic characters, directly from already existing advanced breeding materials in some potato breeding programs.

3.1. Breeding population at CIP

An extensive clonal screening for late blight resistance of the native germplasm during the early years at CIP, helped identify a number of clones with different levels of non-specific resistance to late blight. Since all clones belonged to Gp. Andigena and Gp. Phureja, the type of resistance that was present in these clones was of a non-specific nature as no R genes have been reported in the past in these groups.

In an attempt to improve some of the unfavorable agronomic characters of the native germplasm, crosses to select Tuberosum resistant clones were done, resulting in a mixture of the two types of resistance in the population, the specific resistance inherited from S. demissum in the Tuberosum germplasm and the non-specific resistance as provided by native Andigena and Phureja germplasm. At this point this newly created population was unfortunately not different than any other available advanced resistance germplasm except for the added Andigena contribution.

Seedling screening and field testing was done during following years against local isolates of the pathogen. This helped advance the population during the first period. Selected clones although highly resistant to local pathogenic isolates and good yielding ability, were late in maturity, therefore, limited in their wider acceptability. Besides, it was uncertain whether the resistance of these clones would hold in different environments with different spectrum of the physiological races of the pathogen.

3.2. Selection of clones from advanced breeding material

This second approach was the most fruitful in selecting resistant clones for potential variety releases. it involved the testing and selection of advanced breeding lines from leading potato programs working on late blight resistance.

For this approach to take place, it was necessary to establish an International Late Blight Trial in a close collaboration with the Mexican Potato Program. This international testing scheme was established at Toluca Valley in Mexico, a location where the largest variability of the pathogen exists, thanks to the sexual recombination of the two mating types that have been reported there.

The large number of physiological races of the pathogen has made this location the most suitable environment to select for non-specific resistance in the presence of R genes. It was assumed that most R genes for resistance to late blight would be matched by their corresponding compatible races, therefore, allowing for selection of non-specific resistance (9, 10).

As a result of this international trial, a number of clones were selected and distributed to CIP regions and country programs for further testing and selection of potential cultivars.

Some years later, after the distribution of these clones, the resistance of some of them appeared to break down and some were not well adapted to some environments, although some were successfully named as varieties.

Apparently, it seemed that selection in Toluca, despite of being the best approach, is not complete insurance of the stability of this resistance worldwide nor of the adaptability to most environments. However, from the practical point of view, it may still remain as the best location where useful levels of non-specific resistance can be selected. Even low levels of resistance may be useful in combination with minimum use of chemicals to achieve adequate control.

To date, most of these clones are kept as pathogen tested material at CIP headquarters and in some CIP regions. From here they are available for further distribution to requesting countries. Some of these clones have become varieties in certain countries, some are used as parental lines in their breeding programs, and some are still under evaluation--particularly in those countries that recently received this material.

4. BREEDING AND TESTING STRATEGIES DURING THE SECOND PERIOD (1980 - 1986)

A position paper on the improvement of potato resistance to late blight as developed at CIP during 1981, brought about several changes in the breeding and testing strategies.

The International Late Blight Trial that was established during the early period in Toluca, Mexico, was not longer operating as a cooperative effort between the Mexican Program and CIP. Therefore, selection of new clones from advanced material provided by temperate breeding programs was not possible anymore.

The new genetic material for testing and selection for late blight resistance from there on, would have to depend completely on CIP's breeding population under development at headquarters. It was immediately recognized the need to improve earliness in the population, and pertinent steps were taken accordingly. Likewise, a new testing scheme was outlined as an alternative test to the International Late Blight Trial (Fig. 1).

The important features of this testing scheme included a mass seedling screening under quarantine greenhouse, field testing and selection of a decreasing number of selected clones in two different environments, and the multiplication of clean duplicates of selected clones throughout the test, stored at CIP.

The locations chosen for technical reasons, included Huancayo (Peru), for the seedling screening Rio Negro (Colombia), for preliminary field testing and selection under natural late blight infection in the field and Toluca (Mexico), for the final test of a reduced number of selected clones also under natural blight infection in the field.

It is also evident in this scheme that Mexico is still needed for the ultimate test of the material to select for non-specific resistance, since R genes are known to be present in the population under development.

4.1. Results

A total of 266,800 seedling have been screened through the new testing scheme from 1981 to 1986 (Table 1). From these, 14,122 selected survivors were tested in Colombia as clones, resulting in an average over the years of 1,036 selected clones during the first test in the field, and 322 clones during the second testing. Subsequent field tests in Mexico, yielded 100 selections during the first year, and 28 out of 58 the second year.

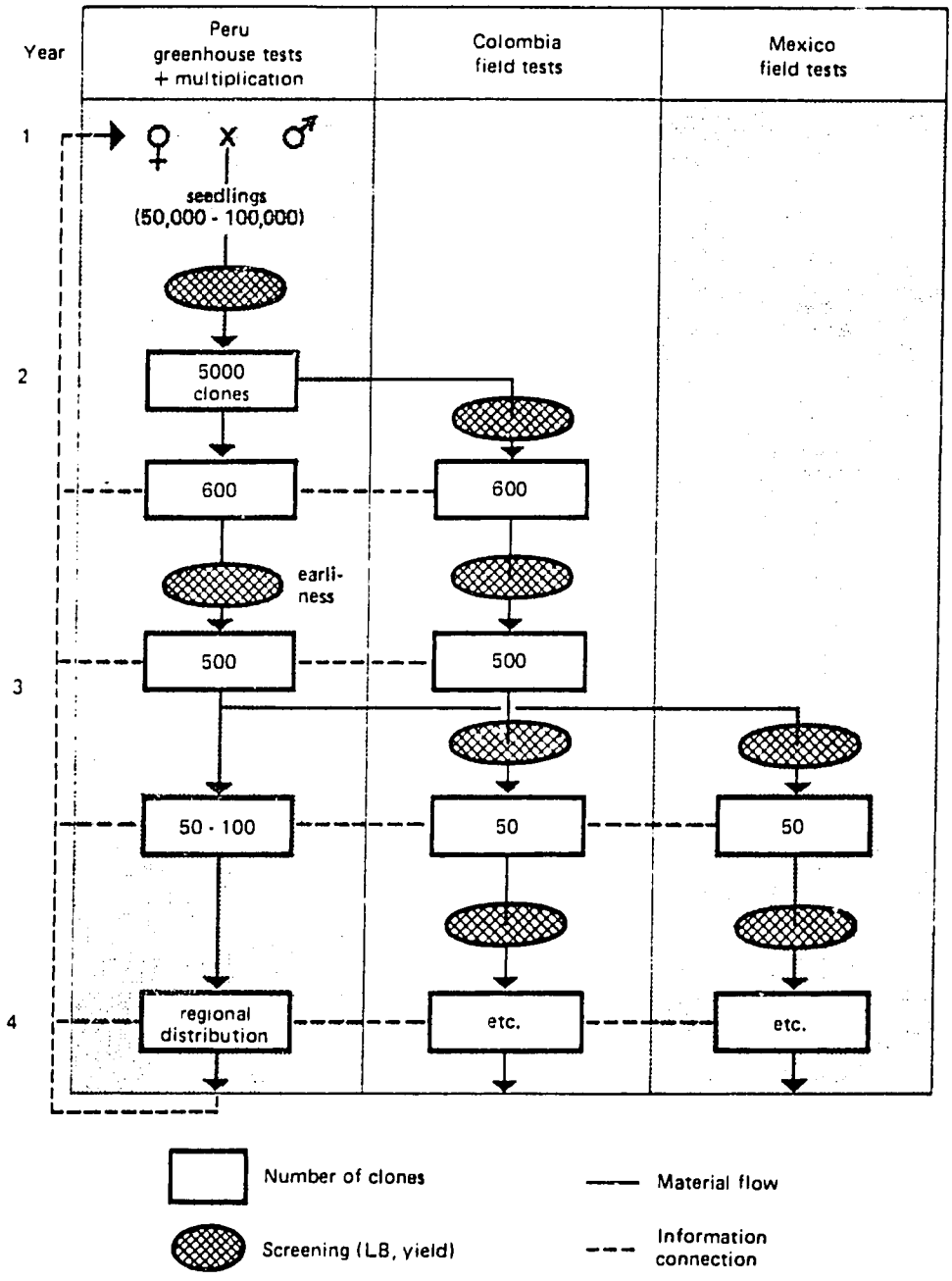


Fig. 1. Testing scheme for late blight resistance at three locations.

Table 1. Progress in developing non-specific resistance to late blight

		Peru	Colombia			Mexico	
Year	Gp.	Seedlings	Tested	1st Sel.	2nd Sel.	1st Sel.	2nd Sel.
1981	I	41,000	3,049	183	63		
1982	II	34,000	3,320	111	73		
1983	III	46,600	3,331	376	109	100	28
1984	IV	55,000	1,541	100	45		
1985	V	42,200	1,681	116	32		
1986	VI	48,000	1,200	150			
Total		266,800	14,922	1,036	322	100	28

Parallel to the selection of new clones (which were immediately made available to requesting countries) tuber families were also distributed to a number of countries after seedling screening. Table 2, shows an overall distribution account of genetic material with resistance to late blight from CIP headquarters during 1979 to 1987. A total of 108 shipments to 34 different countries were made during this period. They included 2,554 clones and 2,153 tuber families containing 48,997 individual clones.

Table 2. Distribution of genetic material resistant to late blight from CIP Headquarters

Year	Shipments to countries[1]	Number of clones[2]	No. of tuber families[2]	No. of clones in families[2]
1979	10	40	129	6,508
1980	14	114	73	4,307
1981	17	107	161	8,728
1982	18	253	71	1,989
1983	4	60	272	4,028
1984	8	48	404	3,843
1985	11	1,070	316	4,759
1986	16	686	518	11,323
1987	10	176	209	3,512
Total	108	2,554	2,153	45,997

1) From overall shipments, 34 different countries are covered
2) Same clones and same tubers families could have been sent several times

4.2. Weaknesses of the testing scheme

Some of the most important limitations of the testing scheme were recognized in the last two years and they were pointed out as follows:

a. The seedling screening as it is being used until now, tends to accumulate R genes at the expense of eliminating genes for non-specific resistance (Turkensteen personal communication).

b. The field testing of clones, particularly in Colombia, under an unknown complexity of the pathogen, usually results in a high proportion of resistant genotypes. This could indicates that the complexity of the races of the pathogen is not large enough or if it is developed at the end of the growing season, it may not be as effective as they would, if they had appeared earlier. This last statement may also be true for Toluca.

c. The most important limitation of all perhaps lies in the effect of certain R genes present in our current breeding population that interfere with the assessment of truly non-specific resistance. These genes (R11, R10, and in a lesser extent R2 and R4) in the presence of incompatible races, show partial incompatibility simulating the expression of non-specific resistance (Turkensteen personal communication). In other words, selected clones can easily be confused as having non-specific resistance. Another important limitation is the presence of undefined R genes in different sources of germplasm. This is especially important where compatible races are not yet developed in the field, nor isolations available to break down this resistance. These genes may also encourage the selection of clones regarded as having very high levels of non-specific resistance.

4.3. Correcting measures

In regard to the seedling screening, the use of a single complex-compatible race was recommended during the last review of the late blight strategy in late 1984, as the most effective way to improve seedling screening. During the screening, the highly susceptible individuals and those without visible blight symptoms should be eliminated and the remaining survivors kept as potential genotypes with different degrees of non-specific resistance for further testing and selection in the field.

With respect to the field testing identification of the complexity of races, collection of inoculum in the field at the end of the growing season, and inoculation of the experimental plots early the following season will eventually provide the most reliable approach.

As far as the interference of genes that show partial incompatibility and new undefined R genes are concerned, a new breeding population exempt of R genes, stands as the best alternative to circumvent such a problem. This population should in turn replace the current R-gene containing population in use at this moment.

The main advantages of this new population (exempt of R genes) would be the following:

a. The nature of resistance to late blight would be entirely non-specific, therefore, effective against all races of the pathogen.

b. The absence of R genes in this population would simplify the seedling screening and field testing of the genetic material as the need for complexity of races for both tests would no longer be required.

c. The combination of non-specific resistance to late blight with other disease resistances, could be done more effectively since testing for late blight resistance can be done under the presence of any undefined isolate of the pathogen.

d. The selected clones with high levels of non-specific resistance can be safely use as parental lines in other breeding programs for further selection and improvement of local important characters. This is perhaps the most important feature in this population since, it will allow breeding programs of developing countries the use of this type of resistance without being hindered by the variability of the pathogen.

5. ADJUSTMENTS IN THE CURRENT BREEDING STRATEGIES

Starting in 1986, two main approaches in breeding and selection for late blight resistance were considered and they are presently in process of being implemented. First is the continuation in the use of the current population that contains R genes to improve useful levels of non-specific resistance (Population A); and second is the development of a new population with non-specific resistance to late blight, free of R genes (Population B).

5.1. Population A

This population contains non-specific resistance to late blight in the presence of R genes, and will still be under use once correcting measures are in place. However, it will be gradually phased out as population B develops and replaces it. It will be strictly directed to the development of resistant clones in combination with earliness and desirable agronomic characters by using the current testing scheme. Parental lines will be selected with full knowledge of their R genes.

5.2. Population B

The development of population B involves two lines of strategy: first, the extraction of R-gene-free genotypes with some level of non-specific resistance from population

A, and second, the development of a new population free of R genes with non-specific resistance derived mainly from Andigena and Phureja native germplasm.

5.2.1. Extraction of R-gene free genotypes from population A

The extraction of R-gene free genotypes with non-specific resistance to late blight from population A, will involve the systematic seedling screening of segregating progenies against the race "0" first, and then the single complex race (1.2.3.4.5.6.7.10.11), respectively. The race "0" would allow the selection of genotypes without R genes and the complex race would eliminate the possibility of the presence of genes for partial incompatibility in the selected clones after race "0" (Fig. 2).

This approach, although time consuming, may turn out as the best means to eliminate R genes from our current late blight population and rescue useful levels of non-specific resistance. The advanced level of agronomic improvement in population A is expected to speed up the progress in the development of suitable genetic material with non-specific resistance. However, it is also possible that the level of non-specific resistance may not be high enough, therefore, it may need some recombination cycles to improve this character.

5.2.2. Development of a new population from sources exempt of R genes

This population is now based mainly on Andigena and Phureja sources of non-specific resistance. Initially two routes will be followed and eventually they will merge into one. It is intended to be a mid-to-long term strategy, since the improvement of agronomic characters along with high levels of non-specific resistance may require several cycles of recombination and selection.

Two parallel sub-populations will be initiated from of a number of native clones previously identified as having some levels of resistance to late blight during the early years at CIP (Fig. 3).

a. The first one is based completely on intercrosses among Andigena clones, followed by screening and selection for non-specific resistance to late blight and desirable agronomic traits.

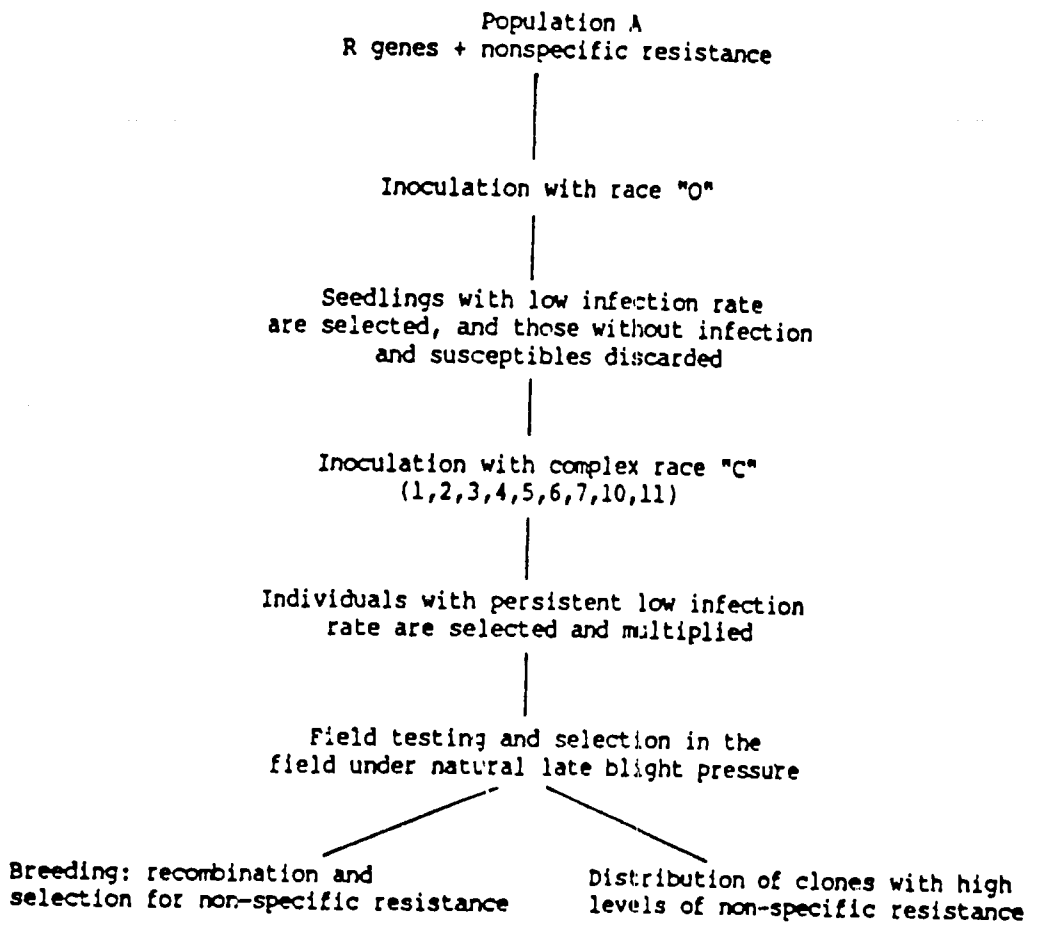


Fig. 2. Extraction of R-gene free genotypes with non-specific resistance from Population A

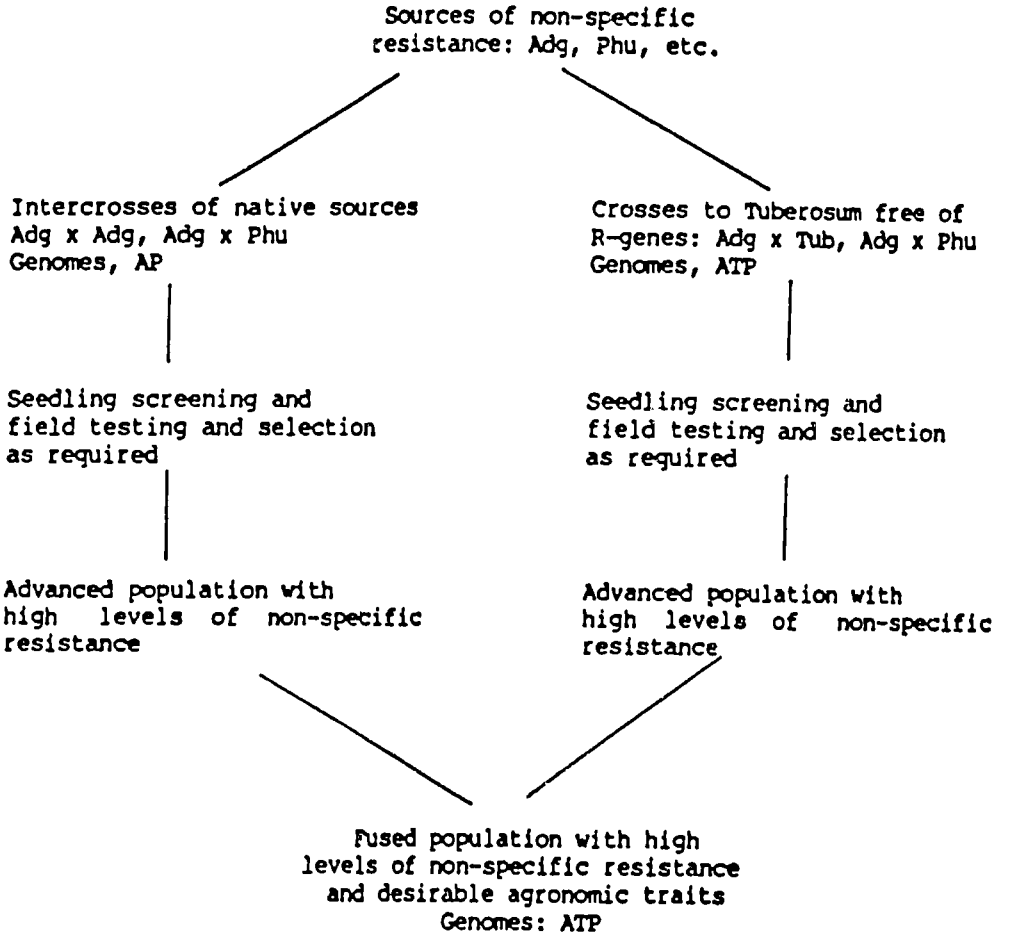


Fig. 3. Development of a new population with non-specific resistance to late blight from native sources of resistance.

b. The second one is based on crosses of Andigena clones to Tuberosum varieties free of R genes. This will be done at the start as required to improve both agronomic characters and non-specific resistance to late blight at an early stage.

Once both, agronomic characters and frequency of non-specific resistance to late blight have been improved through several cycles of recombination and selection, the two sub-populations will be fused into one for further use and improvement.

An additional third route is also under consideration and it may initiated as soon as physical facilities become available. This third approach will be worked out at the diploid level making use of Andigena haploids from resistant sources, Tuberosum haploids, Tuberosum haploid-diploid hybrids, Phureja and other diploid sources of non-specific resistance to late blight (Fig. 4). Once high levels of non-specific resistance together with desirable agronomic traits have been achieved, then transfer to the tetraploid state will be done through the 2n gamete approach described elsewhere (6, 8).

Several theoretical advantages may result form the application of this third approach:

a. A rapid progress in increasing gene frequency for non-specific resistance at the diploid level, as compared to a tetraploid level can be achieved.

b. Heterosis in resulting progenies as a consequence of uniting gametes from relatively distant parents will be expressed.

c. The introduction of other disease resistances to the population will be easier and faster.

d. Selected diploid hybrids with high levels of non-specific resistance to late blight, agronomic characters, and 2n gamete producers by first division restitution mechanism (FDR), can be distributed to CIP regions and country programs for their use in crosses to selected tetraploid parental lines. The absence of R genes will facilitate selection for non-specific resistance in the resulting progenies and on-site selection will undoubtly ensure the adaptability to local environments and farmer's acceptability.

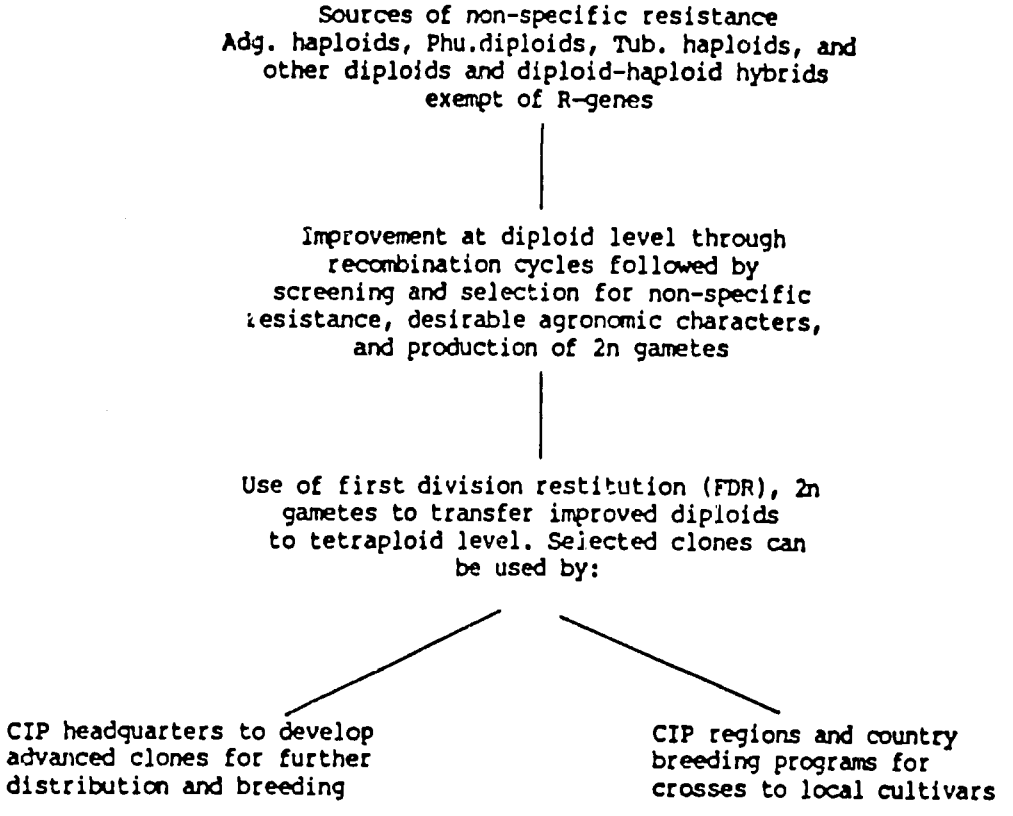


Fig. 1. Development of a population with non-specific resistance to late blight at diploid level, by using haploids and diploid sources of resistance.

6. CONCLUDING REMARKS

There is no doubt that the overall outcome of the late blight program at CIP has been more than effective. The late blight resistant clones selected during the first period through the International Late Blight Trial, still represent the largest portion of the pathogen tested material for distribution. The new clones that are coming out the new testing scheme during the second period are also being widely distributed. A large number of tuber families after seedling screening within the testing scheme has also been distributed to a number of countries for testing and selection under their own local environments. Despite the limitations of the seedling screening, there is a good possibility after careful testing and selection of clones for late blight resistance to select valuable material as potential variety releases.

In addition, if we take into account the new adjustments of the breeding strategies and the correcting measures of the seedling screening and field testing, the success of developing durable resistance to late blight in combination with other favorable traits looks promising.

REFERENCES

1. Black, W. 1954. Late blight resistance work in Scotland. Am. Potato J. 31:93-100.

2. Black, W. and M.E. Gallegly. 1957. Screening of Solanum species for resistance to physiological races of Phytophthora infestans 34:273-281.

3. CIP. 1973. Late blight strategy. Report of the Late Blight Planning Conference, 1973. International Potato Center (CIP). Lima, Peru. pp. 43.

4. CIP. 1978. Control of important fungal diseases of potatoes. Report of the Planning Conference, 1978. International Potato Center (CIP). Lima, Peru. pp. 184.

5. Hodgson, W.A. 1962. Studies on the nature of partial resistance in the potato to Phytophthora infestans. Am. Potato J. 39:8-13.

6. Landeo, J.A. and R.E. Hanneman, Jr. 1979. Crossability of Solanum tuberosum Gp. Andigena haploids. Am. Potato J. 56:427-434.

7. Mastenbroek, C. 1966. Some major points from 22 years of experience in breeding potatoes for resistance to late blight. (P. infestans). Am. Potato J. 43:261-267.

8. Mendiburo, A.O., S.J. Peloquin, and D.W.S. Mok. 1974. Potato breeding with haploids and 2n gametes. p. 249-258. In K.J. Kasha (ed.). Haploids in higher plants. University of Guelph, Guelph.

9. Niederhauser, J.S. 1962. Evaluation of multigenic "field resistance" of the potato to Phytophthora infestans in 10 years of trials at Toluca, Mexico. 52 (746 abst.).

10 Niederhauser, J.S. and W.R. Mills. 1953. Resistance of Solanum species to Phythophthora infestans in Mexico. Phytopathology 43 (456-457).

11. Thurston, H.D. 1971. Relationship of general resistance of potato. Phytopathology 61 (620-626).

12. Vander Zaag, D.E. 1959. Some observations on breeding for resistance to Phytophthora infestans. European Potato J. 2:278-286.

13. Van der Plank, J.E. 1968. Disease resistance in plants. academic Press New York and London. pp. 206.

14. Van der Plank, J.E. 1971. Stability of resistance to Phytophthora infestans in cultivars without R genes. Potato Res. 14:263-270.

SCREENING FOR LATE BLIGHT RESISTANCE

V. Otazu

Many methods are used to screen potatoes for late blight resistance. Most of them use seedlings, tuber or field plants (3,4,5). After the last late blight strategy Conference in 1984, (2) the CIP late blight screening process was analyzed reviewed and new conclusions and recommendations were drawn. Major enphasis was placed on the development of material with general resistance. As a result especially the seedling screening needed modification. Most of this report will deal with aspects related to the seedling screening.

EFFICACY OF THE SEEDLING SCREENING

A breeding program directed towards obtaining of late blight resistance usually handles a considerable number of seedling progenies at the early stage. At CIP, usually over 100,000 seedlings per year are screened for late blight resistance. The seedling screen eliminates over 80% of the populations because of susceptibility, unwanted resistance or undesirable agronomic characteristics. This test is, therefore, highly valuable since the population is sufficiently reduced to allow immediate field testing. The reliability of the seedling test has been a controversial subject, although recent evidence suggests a correlation between disease incidence in artificially inoculated seedlings and naturally attacked plants in the field (1,7). Field evaluations in Colombia indicate that seedling screening is succesful, however, further research is required to establish correlations between results from seedling testing and field evaluations of CIP germplasm.

SEEDLING SCREENING FOR VERTICAL RESISTANCE

Initially this method was employed to distinguish resistance in populations in which R genes were incorporated from *S. demissum*. The method to differentiate this resistance is simple. The inoculum usually consists of a complex race or a mixture of virulent isolates of *P. infestans* in a highly concentrated zoospore suspension. Susceptible plants are self eliminated, whereas, healthy or hipersensitive plants are selected.

SEEDLING SCREENING FOR HORIZONTAL RESISTANCE

Screening methods often use either race "o" or a complex race in order to overcome any R genes that may be present in a population (7). Since complex races are difficult to obtain, the use of race "o" is usually preferred. A low spore concentration is normally used and only plants which show slight or low levels of infection are selected.

SEEDLING SCREENING AT CIP

Presently there are two populations that are screened at Huancayo. Population "A" consists of advanced material nevertheless "contaminated" with R genes. These seedlings are screened twice. Initially, they are inoculated with race "o" and susceptibles are eliminated. Seedlings with only slight infection (fewer and smaller lessions) are transplanted, whereas, the remaining comprising symptomless and highly susceptible seedlings are eliminated. Inmunes probably have R genes incompatible with the genes of the race used. By selecting slightly attacked plants, hopefully vertical resistance was broken down, thus remaining some field resistance. Seedlings affected by race "o" are re-checked later for the presence of the so called "weak R- genes". This tests consists of inoculations of one half of a leaf with race "o" and the other half with a race which is compatible to these "weak" genes. It is known that plants having these genes (2, 4, 10, 11) will show some reaction with any race of the pathogen. Hence, horizontal resistance may be falsely diagnosed. If equal reaction in both halves is observed, it is assumed that the plant has no R genes. If the reaction is less in the half inoculated with race "o", then the plant must have R genes and is eliminated. Material selected with a complex race (1, 2, 3, 4, 7, 10) is sent to Colombia and later to Mexico for field tests.

Population "B" started with andigena progenitors selected in previous years on the basis of field resistance. Since they were andigena, the absence of R genes was expected. However, race "o" was still used for inoculation and selected material is rechecked later for presence of weak genes. Since this material has initially a low degree of resistance, it is screened with a low spore concentration (1000-2000 zoospores/ml). Also, subsequent field screening is done in a place with a mild disease pressure (San Ramon). Results of screening during the last 3 years are shown in Table 1. Population B is in the initial phase.

IMPROVEMENT OF THE TEST

Humidity and temperature are essential factors for the success of the seedling screening. Plastic bags and an incubation chamber with temperatures fluctuating between 15-20 C provided satisfactory results. There were other factors, however that needed to be tested.

(1) The question whether to use zoospore or sporangial suspension for inoculum was considered. Fig. 1 shows results of tests performed with different concentrations of both inocula. It is obvious that zoospore suspensions always caused significantly more disease, proving to be better inoculum.

(2) Substrates for growing the inoculum were also evaluated. Comparisons between V-8 agar medium (1 and 3 week old cultures)and seedling and tuber plant leaves indicated that inoculum grown in live plant material caused significantly more infection than inoculum grown on agar media (Fig.2). The use of tuber slices is recommended for its practicality.

(3) Inoculators were also tested for efficacy. Three General Electric vacuum-pressure pump inoculators calibrated at a pressure of 0.2Kg/cm2 and one knapsack sprayer were compared. Known spore concentrations were placed in each inoculator and the outflow was measured. Results indicated that there was considerable variation among inoculators (Table 2). The knapsack sprayer was better because it allowed the passage of almost 100% of spores, while the pump inoculators yielded about only 85% of the initial spore concentration (Table 2,3). It was also evident that all inoculators delivered over 80% non motile zoospores (Table 2). Later tests based on zoospore dilution techniques and infectivity curves as well as zoospore germination revealed that a significant proportion of these non-motile zoospores were dead. Thus, it was demonstrated that inoculum viability was increasingly lower during various steps in the inoculation procedure (Fig.3). It was estimated that with an initial inoculum concentration of 1000 zoospores/ml, only about 400 viable zoospore /ml reach the leaf surface.

Still more factors should be considered to refine the screening test. Light quality during incubation may be important. A smaller standard error in last year's screening (Table 1) may be an indication that the test has improved compared to previous years. However correlation between seedling and field screening have yet to be established.

LITERATURE CITED

1. CALIGARI, P. D.S., G. R. MACKAY, H. E. STEWART and R. L. WASTIE. 1985 confirmatory evidence for the efficacy of a seedling progeny test for resistance to potato foliage blight (Phytophthora infestans). Potato Research 28:439-442.

2. CIP. 1984 Internal. Late blight strategy Conference. Conclusions. Lima-Peru, 4 pp.

3. CIP. 1986. Field screening procedures to evaluate resistance to late blight. Technology Evaluation Series 5. International Potato Center, Lima, Peru. 17 pp.

4. GARCIA, V. S., H. D. THRUSTON and A. T. TSCHENZ. 1977. A greenhouse method for large scale testing of potatoes for general resistance to P. infestans Pl. Dis. Rep. 61:820-822.

5. LAPWOOD, D. H. 1965. Laboratory assessments of the susceptibility of potato tuber tissue to blight (P. infestans). European Potato Journal 8:215-229.

6. MALCOMSON, J. F. 1986. Assessment of field resistance to blight (P. infestans) in potatoes. Trans. Br. Mycol. Soc. 67:321-325.

7. STEWART, H. E., P. H. FLAVELLE, D. C. Mc CALMONT and R. L. WASTIE 1983 Correlation between glasshouse and field tests for resistance tp. foliage blight caused by Phytophthora infestans. Potato Research 26:41-48.

TABLE 1. RESULTS OF LATE BLIGHT SEEDLING SCREENING AT CIP DURING THE LAST 3 YEAR

Year	Families	Total Seedlings	% Resistance "A"	% Resistance "B"	Standard Error
1985	130	38,267	11	N.T.	7.06
1986	129	41,450	29	8.0	7.74
1987	130	52,720	19	8.3	1.73

* Resistance "A" inoculation with complex race
  Resistance "B" inoculation with race "o"

Table 2. Performance of inoculators in CIP's late blight seedling screening using sporangia

Inoculator	Output (ml/min)	% sporangial passage
Motor 1	26.2	88.6
Motor 2	30.6	79.9
Motor 3	31.7	86.1
Knapsack sprayer	628.0	96.6
LSD (P = .05)		9.1

Table 3. Performance of inoculators in CIP's late blight seedling screening using zoospores

		Zoospore passage (%)		
Inoculator	Output	Total	Non motile	Motile
Motor 1	25.5	79.5	90.2	9.8
Motor 2	23.8	100.3	79.6	20.4
Motor 3	26.7	73.6	94.4	5.7
Knapsack sprayer	660.0	82.0	82.0	18.0
LSD (P = .05)		24.7		

Figure 1. Infectivity curves of zoospore and sporangial inoculum on DTO-33 seedlings. Bars represent standard deviation of means.

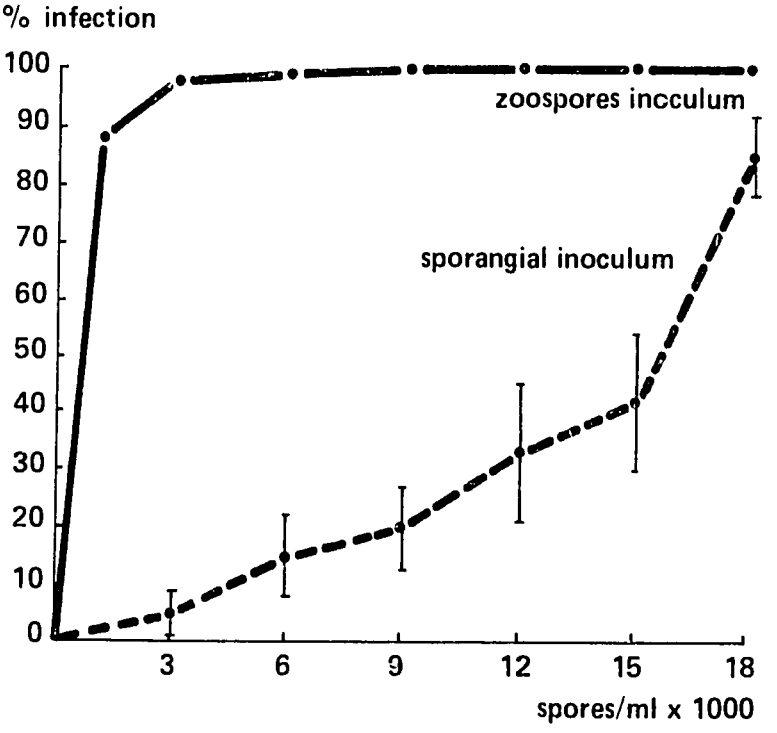


Figure 2. Infectivity of inocula grown on various substrates. Different letters represent statistical differences (P = .05).

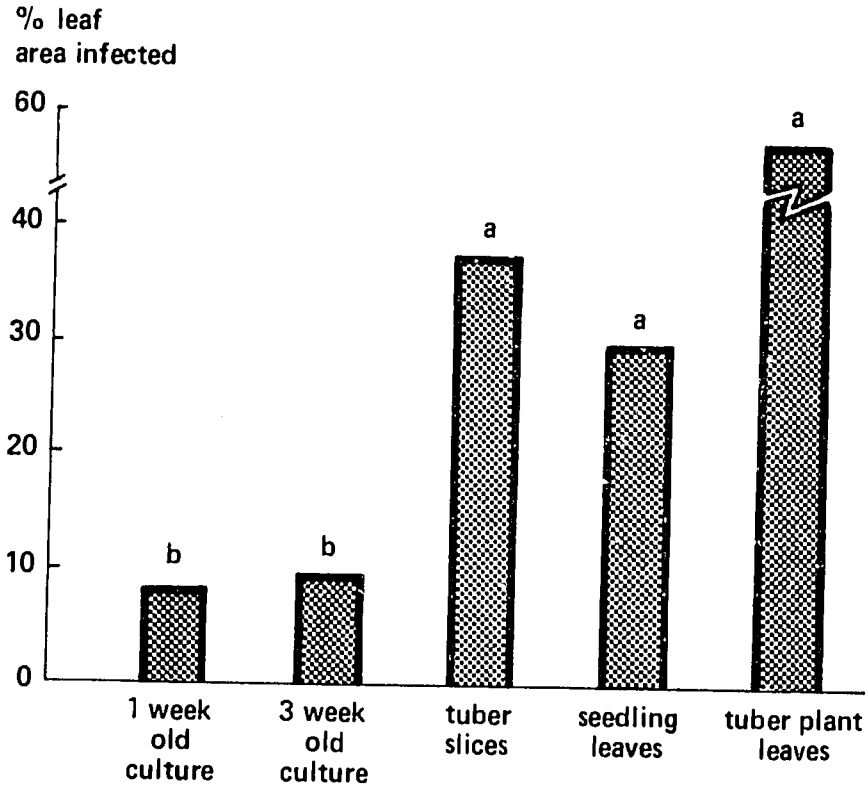
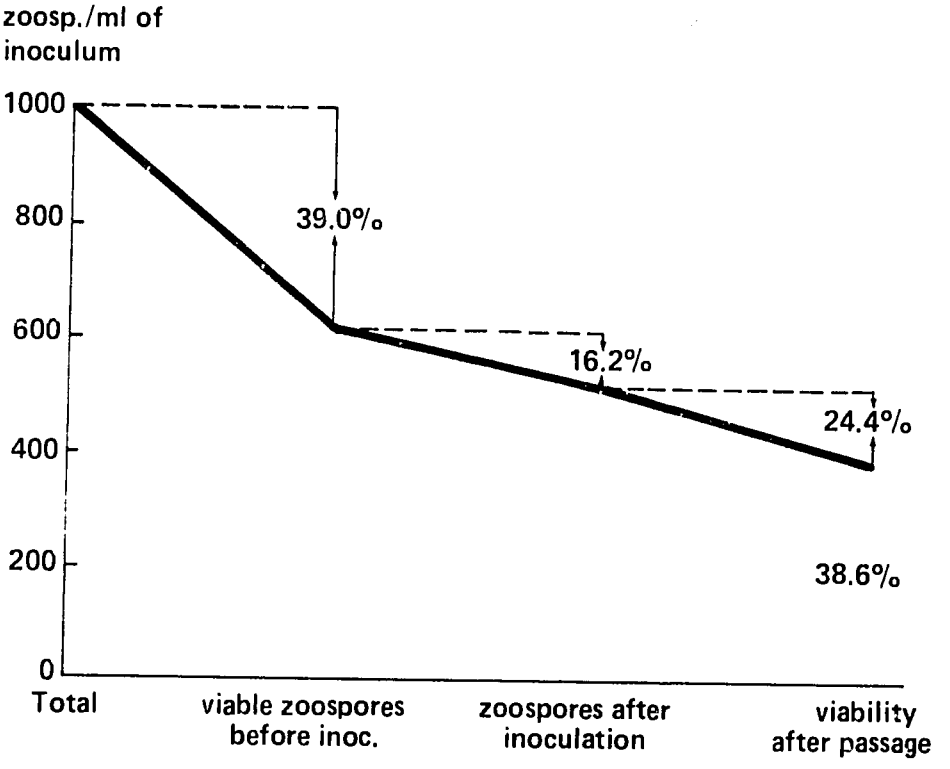


Figure 3. Decreasing gradient of inoculum viability of *P. infestans*. CIP seedling screening.



INTERACTION OF R-GENES IN BREEDING FOR RESISTANCE OF POTATOES AGAINST *Phytophthora infestans*

L. J. Turkensteen

1. Introduction

An integral part of breeding for resistance against late blight is testing for resistance. Seedlings, clones and varieties are selected because of the level of resistance they present. Resistance may be assessed in the field and/or laboratory and greenhouse. Resistance may be assessed with the help of especially designed screening tests or may be assessed in an opportune way whenever late blight appears in the field. The level of resistance for a particular genotype may vary from one test or one observation to another. Resistance of host tissues, plant parts, a single plant or a crop as expressed under specified test conditions may be considered as intrinsic resistance for those conditions. Under fixed environmental conditions, the level of resistance expressed by a specified genotype depends only on the pathogen population. With various pathogen populations, it may vary from one population to another. To distinguish intrinsic resistance from resistance as expressed in tests or as observed in the field, the latter may be named apparent resistance. Unfortunately to the breeder and the farmer, apparent resistance may deviate considerably from intrinsic resistance.
A role played by R-genes is to cause deviation of the apparent resistance from intrinsic resistance, and the various types of interaction of these genes with breeding and selection for resistance are elucidated in this paper.

2. Compatibility versus incompatibility

In testing for resistance the conceptions, compatibility and incompatibility, are often used in relation to the R-genotype of the host and the virulence factors of the pathogen.

A compatible isolate (race) is an isolate (race) that is not restricted in its pathogenicity to a host genotype by the action of R-genes. It refers to host genotypes free of R-genes and to those host genotypes with R-genes, the action of which is overcome by specific virulence factors of the specified isolate (race).

Incompatibility is due to the effect of one or more R-genes through which an isolate (race) of the pathogen is restricted in its pathogenicity with respect to the host genotype concerned as compared to similar host genotypes void of those R-genes.

3 Interaction of R-genes

3.1 Masking of horizontal resistance

There are several modes of interaction of R-genes in breeding programs. A well known feature is the masking effect of R-genes with respect to horizontal resistance if no compatible race is available for testing or is not present in the field at the time of introduction of a R-gene protected clone. There is no possibility to select for horizontal resistance, and as a consequence, there is no selection pressure to maintain horizontal resistance in the breeding material concerned. At the appearance of a compatible race, the level of horizontal resistance was in general low. This feature is well known and is called the Vertifolia effect (14). However a few other interactive processes may go on in breeding programs with respect to selection for resistance. They can be described as inoculum dilution, epidemic retardment and mimesis of horizontal resistance.

3.2 Inoculum dilution

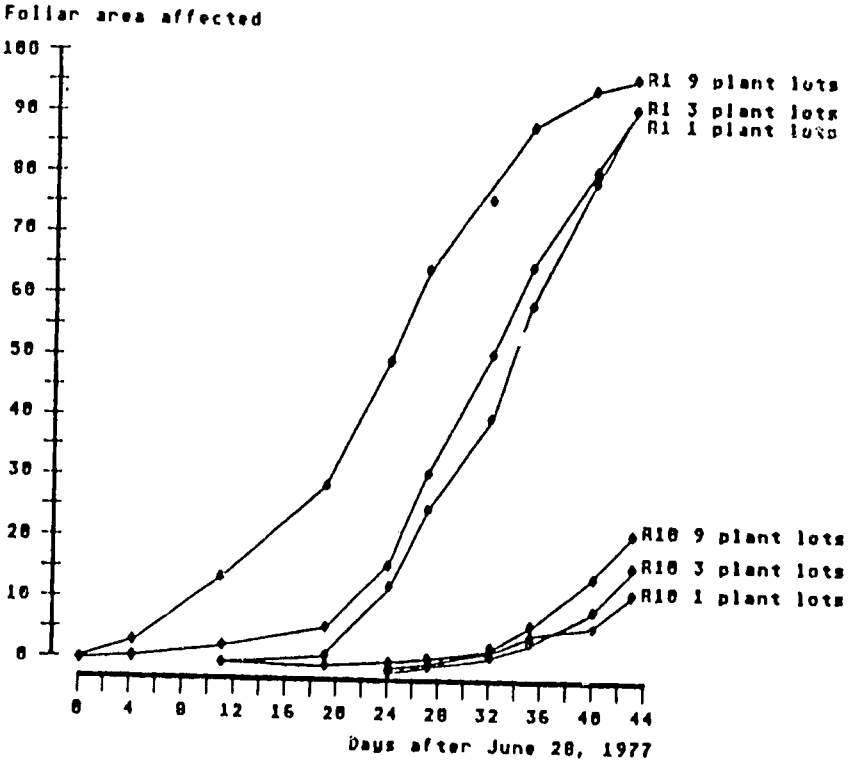
Inoculum dilution is due to incompatibility between a part of the host and pathogen population submitted to testing. It plays a role if within a host population genotypes are present with one or more R-genes or R-gene combinations, which are incompatible to one or more of the isolates (races) of the pathogen population to which they are exposed. Genotypes containing these R-genes are affected less than genotypes without these genes. These R-genotypes are preferentially selected at the cost of those host genotypes, which do not carry those R-genes. This mechanism leads to containment and accumulation of R-genes in breeding and breeding populations.

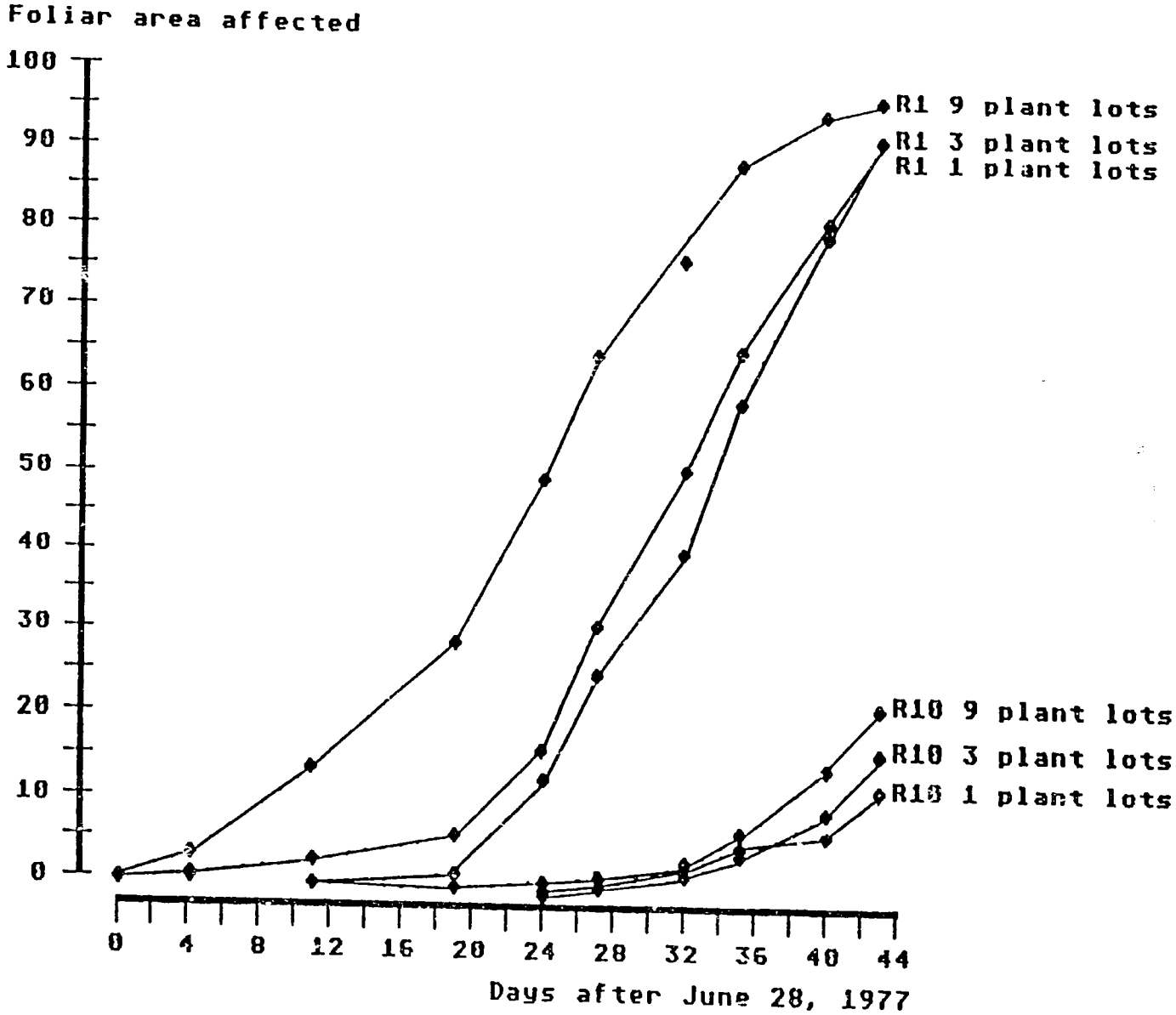
It is easily understood that this mechanism is effective in monocyclic seedling screening tests. In this type of test the effective spore-load to a host genotype is directly related to the proportion of spores compatible to this genotype. Genotypes free of R-genes are affected by the total spore-load, whereas genotypes containing R-genes are affected by the compatible proportion of the spore-load only. Pending the proportion of spores of incompatible races, the effective spore-load and, consequently, disease incidence is accordingly less. Effects of spore dilution are easily calculated (Table 1).

Table 1. Spore-load in relation to inoculum dilution. Frequency of each race is 0.25.

	R-genotypes				
Races:	r	R1	R2	R1R2	R3
Race 0	0.25	0	0	0	0
Race 1	0.25	0.25	0	0	0
Race 2	0.25	0	0.25	0	0
Race 1.2	0.25	0.25	0.25	0.25	0
Spore-load	1.00	0.50	0.50	0.25	0

Graph 1. Epidemic retardment in relation to plant number (respectively 1, 3 and 9 plants per experimental plot) and a common R-genotype (R1) and a rare one (R10) at Atizapan, Toluca Valley, Mexico, 1977.




Foliar area affected
100
90
80
70
60
50
40
30
20
10
0
R1 9 plant lots
R1 3 plant lots
R1 1 plant lots
R10 9 plant lots
R10 3 plant lots
R10 1 plant lots
0
4
8
12
16
20
24
28
32
36
40
44
Days after June 28, 1977

With respect to polycyclic field tests there may also exist effects of spore dilution on disease incidence of R-genotypes. This will especially be the case whenever R-genotypes concerned are growing in a low frequency and in small groups (Graph 1) (1,2). However, effects of spore dilution in field trials are entangled with others, especially those of epidemic retardment, and are difficult to calculate.

3.3 Epidemic retardment

Epidemic retardment is associated with the relative rareness of compatible races of the pathogen and the according host R-genotype. It is found with polycyclic screening trials which, in general, are field trials, and with common epidemics. The phenomenon may be explained in the following way. A genotype free of R-genes is affected by any race of the pathogen. A genotype containing R-genes is affected by compatible races only. The lower the proportion of compatible races in the pathogen population and the lower the proportion of the R-genotype concerned, the smaller will be the chance that a compatible spore lands on a plant of the specified R-genotype. The consequence is that at the average epidemics on these genotypes will start later than on genotypes

Table 2. Results of a race identification trial at Atizapan, Toluca Valley, Mexico, in 1976. Virulence is expressed as the number of R-genes which were overcome per isolate in relation to sampling date. It concerned the following R-genes: R1, R2, R3, R4, R5, R7, R10, R11.

Sampling dates	Number of R-genes matched per isolate	Mean number of R-genes matched
July 5	0,1,5,2,5,0,0	1.9
July 14/16/18	2,2,4,2,4,2,5,1,2,2,1,2	2.4
July 25/26	5,2,2,2,1,4,2,1,4,2	2.5
August 14/17	8,8,7,7,6,8,6,8,7,8,2,3 4,3,3,8,8,8,6,7,6,5,8,4	5.2
August 21	8,8,5,8,4,4,5,6,2,6,2,5 4,2,4,4,4,4,8,8	5.0
August 25	5,3,5,4,6,1,2,3,2,5,5,3 5,1,5,4,5,6,1,5	3.8

void of R-genes and as a consequence less disease symptoms may develop. Consequently the genotype involved will be considered as being more resistant than it should be as based on the level of its intrinsic resistance. This phenomenon is well known from varieties protected by R-genes at the time of their introduction (5,15). However it was also demonstrated in field trials in 1976 and 1977 when testing clones for resistance in Toluca Valley in Mexico (Graph 1) (2). It was found associated with a tendency for races compatible to the relatively rare genotype R10, which races happened to be the most complex ones, to appear later than races compatible to the more common genotype R1 (Table 2).

Like inoculum dilution, epidemic retardment will lead to containment and accumulation of R-genes in breeding populations and resulting cultivars.

3.4 Mimesis of horizontal resistance

The two ways of interaction described herefore could also be seen as mimesis of horizontal resistance. However, in this context mimesis of horizontal resistance is associated with the intrinsic partial resistance of a R-genotype as expressed in case of an incompatible reaction. Apart from typical hypersensitivity lesions, a number of slowly expanding lesions may also be formed. Especially genotypes with R2, R4, R10 and R11 (and their combinations) express in decreasing order partial resistance with incompatible races. The problem for the breeder is that apart from their level of partial resistance, these genes give similar reactions with race 0 and single and complex races as long as these are not compatible to the R-gene involved (Table 3).

A very notorious case of the effect of R-genes mimicking horizontal resistance is the presence of R10 in many modern cultivars. In the Dutch potato variety list there are 34 cultivars known to carry R-genes of which 21 cultivars possess the gene R10 single or in combination with other R-genes. Two clones Multa (R1R4R10) and Hertha (R1R3R10) were considered to be field resistant at the time of their introduction. At that time the common races were race 0, race 1.3 and race 1.4. At present these cultivars are affected by the common race 1.3.4.7.10. Also in the germplasm for late blight resistance R10 is present (Table 4). In most cases it seemed to be combined with R3 and R1R3.

There appears to be a tendency to combine R-genes mimicking horizontal resistance. New Dutch introductions tested possessed the genes R2, R4 single or together combined with R10. The presence of these R-genes may lead to partial or total masking of horizontal resistance. Consequently selection for horizontal resistance is less effective or totally impossible.

The result of the three mechanism described here is that R-genes tend to contain themselves in breeding populations and to accumulate in advanced breeding materials. If in breeding populations single R-genes are present or combinations of R-genes can be formed for which no compatible races are available, then the corresponding R-genotypes will be preferentially selected.

Table 3. Spore-load in relation to mimesis of resistance and inoculum dilution. Frequency of each race is 0.25

	R-genotypes					
	r	R1	R2	R4	R10	R11
Relative susceptibility:	1	1/0	1/.1	1/.05	1/.2	1/.5 *
Race 0	0.25	0	0.025	0.013	0.05	0.125
Race 1	0.25	0.25	0.025	0.013	0.05	0.125
Race 2	0.25	0	0.25	0.013	0.05	0.125
Race 1.2	0.25	0.25	0.25	0.013	0.05	0.125
Spore-load	1.00	0.50	0.55	0.05	0.20	0.50

* first figure: compatible combination
second figure: incompatible combination

4. Combined vertical and horizontal resistance

4.1 Introduction

One of the approaches towards resistance breeding might be the deliberate combination of horizontal resistance with R-genes in single varieties to use the beneficial effect of both types of resistance. After releasing such varieties there might be a beneficial effect of R-genes as long as compatible races are relatively rare. For this type of breeding compatible races should be available to be able to screen for horizontal resistance. At present race 1.2.3.4.5.7.10.11 is available at CIP. This race is compatible to most of the clones of the Germplasm collection. Technically it is possible for CIP to breed for the two dimensional type of resistance. There are however a few drawbacks elucidated in paragraphs 4.2 to 4.3, some of which interfere with CIP's breeding goals.

4.2 Environment and horizontal resistance

A first drawback is that horizontal resistance may vary very much in various environments. There exist strong interactions with day length, temperature and light intensity. The consequence is that a cultivar may show an acceptable level of horizontal resistance in one environment and a non-acceptable one in another. In case no compatible race is present at the introduction of a cultivar, there is no way to test its level of horizontal resistance for the environment concerned. At the appearance of a compatible race, the level of horizontal resistance may not answer so well as was expected. As CIP develops and spreads advanced breeding materials for world-wide use, it experienced this phenomenon more than once.

4.3 Continued breeding in other regions

A second drawback is related to continued breeding with advanced CIP materials supplied to breeding programs in the regions. In the regions compatible races may not be present at testing. As people suppose that the reaction exposed in the field is due to horizontal resistance, the ultimate result of their breeding efforts might well be materials without horizontal resistance temporarily protected against the local pathogen population by one or more R-genes.

4.4 Testing facilities

A third drawback is that for breeding and consequently testing for combined horizontal and vertical resistance, there must be facilities present. It means facilities to maintain and to test races, knowledge on the presence and the nature of R-genes in breeding materials and advanced clones as well as knowledge on race spectra for the regions involved. One of the consequences will be a voluminous administration system. All together this is a costly operation as well as being labour intensive and time consuming.

4.5 R-genes in germplasm for late blight resistance

Most of the clones of the germplasm collection for late blight resistance contain one or more of the R-genes R1, R2, R3 , R4 and R10 (Table 4). At testing the combinations R1R3 and R3R10 are the most frequently encountered ones. It should, however, be noted that since not all races needed for testing are available, some of the genotypes indicated as R1R3 may contain R7 and those indicated as R3R10 may contain one or more of the genes R1, R2, and R4 as well. The effect of these identified R-genes are overcome by race 1.2.3.4.5.7.10.11, which

is used in selection at present at CIP. There are, however, a few genotypes which are incompatible with this complex race. It concerns clones of Indian origin obtained from germplasm developed by Dr. Black in the Scottish Breeding Station at Pentlandfield and in Kenya. There are indications that they may have one or more unidentified R-genes. The genetic background of this resistance is being studied at IPO at present.

To escape from these drawbacks, CIP has chosen to breed for germplasm and advanced breeding materials with resistance to late blight free of R-genes.

Table 4. R-genotypes identified in 49 clones of the germplasm collection with late blight resistance.

Genotype	Number of times identified	Genotype	Number of times identified
R1	8	R1R4	2
R1R2	1	R3	3
R1R2R3 1)	2	R3R4	1
R1R2R3R4	1	R3R10	10
R1R3	7	R4	2
R1R3R4	3	R10	3
R1R3R10 2)	3	R1R4	2

1) Genotypes containing R3 may also contain R7.
2) Genotypes containing R3 and R10 may also possess one or more of the genes R1, R2 and R4.

5. The race spectrum and R-genes in Mexico

In the past material with resistance was collected in Mexico (3,7,9,10) Resistance transferred from this material to Solanum tuberosum showed to be based on R-genes. Apparently, these R-genes did not protect these materials when tested in Toluca Valley in Mexico. It is very likely that not all resistance was transferred from the wild species to S. tuberosum.

There is, however, the phenomenon of accumulation of R-genes in wild potato species. In this respect, it is of interest to note that six out of the eleven identified R-genes were found to be present in the single S. demissum accession CPC 2127 (6), which has been widely used in breeding. These six genes are R1, R2, R3, R4, R7 and R8. In addition there is evidence that the genes R1, R2, R3 and R4 were present twofold

in that same clone (3). Since R-genes were not successful in breeding, two questions arise. Why were these genes nevertheless so commonly found in wild species and why they accumulated to such an extent as in accession CPC 2127? Simple accumulation of these six R-genes does not explain why the original material would have been resistant in Mexico. Races compatible to the combined effect of these R-genes were found to be common in Mexico (Table 5). Similar experiences were obtained by other researchers (4,11,13). There was no evidence that these races were noticeably less aggressive than more simple ones.

Table 5. Results of a race identification trial of 98 isolates of Phytophthora infestans obtained from the International Late Blight Trial at Atizapan, Toluca, Mexico in 1976. Tested on genes r0, R1, R2, R3, R4, R5, R7, R10 and R11.

Race	Times found	Race	Times found	Race	Times found
0	3	2.7.11	1	2.3.5.7.11	1
1	2	3.4.7	1	2.4.7.10.11	1
2	4	1.2.3.4	2	3.4.5.7.11	1
3	1	1.2.3.5	1	1.2.3.4.5.7	1
4	1	1.2.4.11	2	1.2.4.5.7.11	2
7	2	1.3.10.11	1	1.3.4.7.10.11	1
1.2	1	1.4.10.11	4	2.3.4.5.7.10	1
2.3	4	2.3.4.7	2	2.3.4.5.10.11	1
2.4	10	2.4.7.11	2	1.2.3.4.5.7.10	1
2.7	3	1.2.3.4.7	6	1.2.3.4.5.7.11	1
4.5	1	1.2.3.4.11	2	1.2.3.4.7.10.11	1
4.7	1	1.2.4.7.11	1	1.2.3.5.7.10.11	1
1.2.4	2	1.2.4.10.11	1	1.2.3.4.5.7.10.11	9 1)
2.4.7	6	1.3.4.5.11	1		
2.5.10	1	2.3.4.7.11	1		

1) Five of these isolates were also tested against R8 and R9 and showed to be race 1.2.3.4.5.7.8.9.10.11.

The presence of R-genes accumulated in single genotypes of wild potato species and the occurrence of a broad variability in virulence within the pathogen population is unexpected in a system where R-genes do not have a lasting protective effect. In this respect there is ample room for speculation. However, it is apparent that R-genes must play a role

in nature. If they were not of any importance, they would either get lost or become fixated within the host populations. The same holds true with respect to virulence genes.

The mechanisms by which this genetic variability is maintained within the populations of the pathogen and the different host species are not known. The same holds for the nature of the resistance as expressed by wild species in Mexico and possible interaction with R-genes. One thing is obvious. The procedure of taking resistant materials out of Mexico to breed for resistance in the way as has been done in the past, has failed to yield the wanted type of resistance.

In all breeding programs for late blight resistance, the greatest shortcoming has been, that no proper study was made on the nature and the basics of the resistance involved. It is obvious that more knowledge is needed on the nature and genetic make up of resistance to late blight as present in wild potato species in Mexico. It is recommended to investigate thoroughly the genetic make up of resistance in populations of wild potato species and of pathogenicity in the pathogen population. Obviously, Mexico is the best place for it.

6 Present and future use of R-genes

At present there is an increasing interest in R-genes. They are used as marker genes in studies on genetic mapping of potato species. They are also used in genetical studies of the late blight fungus. The latter type of research is stimulated very much because of the recent spread of the A2-mating type of P. infestans.

6.1 R-genes and genetic manipulation

There exist interesting possibilities for genetic manipulation with R-genes. Of course, the direct impact on breeding because of the introduction of R-genes in potato clones is as limited concerning durable resistance as common breeding with R-genes. It offers, however, the tools to make series of R-gene differentials with the same genetic background. These series may be very useful for the identification of races of the fungus. At present, it is not known which is the specific chemical compound associated with a specified R-gene leading to incompatibility. The use of the same genetic background for R-gene free and R-genotypes may offer a help to unravel the specific biochemical impact on the cellular level.

Last but not least, there is the view that R-gene resistance might be manipulated in such a way that this type of resistance may not be overcome by a compatible race. In most cases, compatibility to a R-gene is based on recessive virulence genes in the pathogen. A compatible race has to be homozygous for recessive gene(s) involved. An incompat-

ible race carries the dominant analogue of the virulence gene and may be heterozygous with respect to virulence gene. If the dominant analogue of a virulence gene can be introduced into the host genotype in such a way that this gene expresses itself effectively, then it might lead to an incompatible reaction with all races including those which should be compatible with the R-gene involved. The future will tell if this view is more than mere science fiction.

Literature

1 Anonymous, 1976. Thrust III. Control of important fungal diseases of potatoes. Annual report CIP 1976: 21-27
2 Anonymous, 1977. Thrust III. Control of important fungal diseases of potatoes. Annual report CIP 1977: 33-44.
3 Black, W., 1952. Inheritance of resistance to blight (Phytophthora infestans) in potatoes: Inter-relationships of genes and strains. Proc. Roy. Soc. Edinb. B64: 312-352.
4 Huerta Miranda, E., 1977. Aparicion cronologica de razas fisiologicas de Phytophthora infestans (Mont.) de Bary, causante de tizon tardio de la papa y del tomate. Thesis. Instituto Polytecnico de Ciencias Biologicas. Mexico, DF.: pp 65.
5 Kirste, 1958. Ergebnisse von Krautfäule-Spritzversuchen. Kartoffelbau 9: 114-115.
6 Malcolmson, J.F. & Black, W., 1966. New R genes in Solanum demissum lindl. and their complementary races of Phytophthora infestans (Mont.) de Bary. Euphytica 15: 199-203.
7 Müller, K.O., 1925. Neue Wegen on Zielen in der Kartoffelzüchtung. Beitr. Pflanzenzücht 8: 45-72.
8 Müller, K.O., 1932. Bemerkungen zur Frage der biologischen Spezialisierung von Phytophthora infestans. Angew. Bot. 15:84-96.
9 Niederhauser, J.S. & Mills, R.W., 1953. Resistance of Solanum species to Phytophthora infestans in Mexico. Phytopat. 43: 456-457.
10 Reddick, D., 1934. Elimination of potato late blight from North America. Phytopath 24: 555-557.
11 Rivera Pena, A., 1987. Wild tuber bearing Solanum species and Phytophthora infestans (Mont) de Bary on the slopes of the "Nevado de Toluca" Volcano, Mexico. Abst. of conference papers and posters. 10th Triannial Conf. European Assoc. Potato Res., Aalborg Denmark, 26 July-31 July, 1987: 383-386.
12 Salaman, R.N., 1932. Recent progress in the breeding of potato varieties resistant to blight (Phytophthora infestans). Deuxième Congr. Int. Pathol. Comp. 1932: 436-437
13 Turkensteen, L.J., 1976. Observations on the race spectrum of Phytophthora infestans at Toluca, Mexico. CIP circular. International Potato Center, Lima, Peru. Vol. V, 2:2.
14 Van der Plank, J.E., 1963. Plant disease, epidemics and control. Academic Press, New York: 349 pp.
15 Van der Plank, J.E., 1968. Disease resistance in plants. Academic Press, New York and London: 266 pp.

ACCEPTANCE OF NEW VARIETIES WITH RESISTANCE TO LATE BLIGHT WHEN CHEMICAL CONTROL IS NOT AVAILABLE: THE CASE OF RWANDAN FARMERS

Pierre Tegera

Introduction

Land scarcity in Rwanda is pronounced, with an average of about one hectare of land per household (Scott, 1986). Households use fragmented parcels of land across different ecological zones, across local administrative units, and even across national boundaries. There is a low degree of specialization; each family produces a range of essential foods for the household and minimizes reliance on markets and exchange. Cropping systems are complex and a wide diversity of crops and cultivars are grown. Farmers can afford few cash inputs, and have a low capacity for risk because they operate close to food subsistence margins. Opportunities for formal sector salaried employment are limited, therefore, more than 90 percent of the population resides in rural areas and practices agriculture.

Potatoes are a relatively recent introduction in Rwanda. They arrived early this century with explorers, traders, soldiers and missionaries but local taboos at first limited their adoption (Poats, 1981). While it took europeans two centuries to accept potatoes, in Rwanda they were widely produced and consumed within a few decades of their introduction (Poats, 1988). In areas over 2000 meters above sea level, mainly in the northern volcanic soils zones, potatoes have became both a primary food staple and a cash crop. Potato rank among major food crops in terms of planned increases. Average annual growth rates achieved for the period 1982-1986 were 8.0 and 5.0 percent for production (360,000 t/year) and yield (7, 5 t/ha) respectively. Yield increases resulted from the use of improved seed and from potato extension into fertile marshlands and newly cleared forests (Scott, 1986). Plant breeding and selection of new disease resistant, higher yielding and better adapted potato varieties, are the foundation of PNAP's research work since it was created in 1979.

Improving crop cultivars is one of the least expensive and most readily adopted means of improving the productivity of small scale agriculture (Riley, 1983). Potential yield improvements are achieved, however, only if plant breeding and selection efforts are suited to farmers' needs and constraints. This paper reports on the acceptance of new potato varieties with resistance to late blight in the absence of chemical control. The discussion is based on the results of PNAP studies and surveys in Rwanda.

Interaction Between Late Blight Resistance and Planting Dates

The Rwandan farmer can produce two to three potato crops per year (Durr, 1980) with two rainy seasons and in the marshy valleys (July-October).
Yield of potatoes may be increased by early planting of cultivars if they are resistant to late blight. Early planting of non-resistant cultivars, however, lowers yields since late blight pressure increases with the rainy season. Farmers who do not have access to improved cultivars or fungicides follow a strategy of intentionally planting potatoes near the end of the rainy season (May or November) in order to diminish the risk of late blight attacks. The delayed planting reduces the period of tuber growth because the onset of dry weather occurs earlier in the crop's development.

Important Trade off Between Yield, Earliness and Late Blight Resistance

In land-scarce farming systems with bimodal rainfall, late blight resistant cultivars that have short dormancies are advantageous so that at least two crops can be planted annually. Alternatively, if dormancies are long, farmers must be willing and able to store seed potatoes for six months or more, and to plant a harvested crop after another growing season has elapsed. Some Rwandan farmers have difficulty storing seed for such long periods because of the pressures of food and cash needs or of social demands to aid neighbours. Therefore, some farmers who have access only to cultivars with long dormancies plant seed before it is well sprouted, a practice which diminishes yields (Haugerud, 1986).

The adoption of faster maturing potato cultivars is often preferred since more of the agricultural calendar may be devoted to the production of other food staples on the same land. If labor is scarce, work time required for a longer maturing potato cultivar may be a constraint if it concurs with existing bottlenecks at critical points in the production cycles of other crops. A later cultivar may not be accepted if its longer period in the field coincides with a period of non availability of starch substitutes (Franzel, 1983), or if delayed harvest prolongs a period of cash shortage.

New Varieties and Farmer Cropping System

Cropping patterns influence suitability of cultivars with long and short dormancy and vegetative cycles. It is important that the cycle of the potato cultivar fit well with that of other major crops in rotation with it or that the growth habit of the potato cultivar fit well with other crops intercropped with it. Intercropping is a widespread practice by which the Rwandan farmer recognizes the advantages of yield stability and better use of scarce land. Nearly half of the potatoes cultivated are associated with up to three or more crops (Durr, 1980). When intercropped fields contain potatoes,

farmers preferences are oriented to varieties characterized by short stolons and non extensive leaf coverage to decrease competition with associated crops (Haugerud, 1986). In the pure potato stands, farmers strongly prefer to maintain diversity and grow both long and short cycle cultivars in order to increase the number of months when potatoes are available for sale and consumption, as well as to reduce risks due to environmental hazards such as uncertain rainfall, diseases and pests (Hagumimana, 1987).

New Varieties and Varied Production Goals

Farmers produce potatoes for both home consumption and for sale, and many use different cultivars for the two purposes. Farmers distinguish among cultivars according to their suitability for production goals. For example, many farmers prefer to keep potatoes with high dry matter and starch content (for home consumption or for long storage). Those with high water content, which weigh more but store poorly, are sold directly after harvest. Because there are production deficits in neighbouring countries and in some regions of Rwanda, large tubers with high water content are marketable (Haugerud, 1988). Nonetheless, since 1985 there has been success in increasing potato production which has led to price decreases and increased demand for cultivars with better consumer qualities such as high dry matter content.

Potato markets and consumer preferences are not specialized with respect to skin color in Rwanda. Preferences for a particular skin color may arise by association with a successful cultivar. Popular varieties in Rwanda include those with purple, red and white skins.

In Rwanda, potatoes are currently cropped, stored, marketed and cooked as color variegated mixtures without distinction among cultivars or skin color, except that russet skins are mostly not acceptable. Tuber shape is also relatively unimportant; even a sweet potato type of shape is accepted in some regions.

Discussion and Conclusion

Because breeding progress slows as the number of cultivar selection criteria increases, it is important to give priority to those criteria when there are trade offs between characters; breeding scientists have to determine which traits should be more emphasized to encounter the farmers' needs. Ranking of cultivar attributes may usefully serve a breeding program. In the Rwanda case, not surprisingly, the most important traits are yield, resistance to late blight and to bacterial wilt, medium earliness with short dormancy, storability, taste (high dry matter content) and suitability to the existing cropping systems. Of course, no set of preferred cultivar attributes is immmutable. Farmers may, for example, accept an undesirable trait if another

important attribute such as yield or disease resistance is a striking improvement over existing alternatives. It is therefore important to understand how farmers use existing cultivars and which characters they value for which purposes (Devaux and Tegera, 1987; Rhoades and Booth, 1982).

REFERENCES

1. Devaux, A. and Tegera, P. (1981). Les parcelles d'evaluation: Une Solution au Problème de Transmission Technologies. In Bulletin Agricole du Rwanda, N° 14: 165-167.

2. Durr, G. (1980). Potato Production and Utilization in Rwanda. Social Science Department Working Paper 1983 - 1. International Potato Center, Lima.

3. Franzel, S. (1983). Planning an Adaptive Production Research Program for Small Farmers: A Case of Farming Systems Research in Kirinyaga District, Kenya. Doctoral Dissertation, Dept. of Agricultural Economics, Michigan State University.

4. Haugerud, A. (1986). Biological and Social Scientists in Inter-disciplinary Agricultural Research - What Can Anthropologists Gain and Contribute?

5. Hagumimana, L. (1987). Contribution a' l'ètude de l'influence du Mélange de Variétés de Pomme de Terre sur le Développement Epidemiologique du Mildiou. Travail de fin d'Etudes, Faculté d'Agronomié, Université Nationale du Rwanda, 69 pp.

6. Poats, S. (1981). La Pomme de Terre au Rwanda: Les Resultats Préliminaires d'une Enquête de Consommation. In Boulletin Agricole du Rwanda, N°14:82-91

7. Rhoades, R. and Booth, R. (1982). Farmer-back-to-Farmer. A Model for Generating Acceptable Agricultural Technology. Agricultural Administration 11:127-137.

8. Riley, R. (1983). Plant Breeding - An Integrating Technology. In Plant Breeding for Low Input Conditions. Proceedings of a Workshop on Plant Breeding for "Lousy" Conditions. Makule Central Research Station, Chilanga, Zambia.

9. Scott, G.J. (1986). Potatoes in Central Africa. A Survey of Burundi, Rwanda and Zaire. International Potato Center, Lima, Peru. 202 p.

FACTORS AFFECTING RESISTANCE TO *Alternaria solani* AND PROGRESS IN EARLY BLIGHT RESEARCH AT CIP

Carlos Martin and H. David Thurston

Early blight, caused by *Alternaria solani* (Ell & Mart) Jones and Grout, is one of the most important fungal diseases of potatoes, especially under conditions of high temperature and humidity. In several countries the disease does not usually reach epiphytotic proportions and it is generally considered a disease of senescent tissue. However, early severe infections normally occur in countries such as Brazil, Uruguay, Caribbean Islands, India, and others (1,3,6,15,22). In most cases early blight affects potato foliage but in some places, as in Colorado (USA), tuber infection may also induce severe losses (12,15).

As stated during the Planning Conference on the control of important fungal diseases held at CIP in June 1978, early blight was rated immediately next to late blight as the second most important foliar disease of potatoes (15). During that Conference, Dr. M.D. Harrison presented an extensive literature review on the symptomatology, importance and control of early blight, covering almost every report on this disease published up to 1978.

During this presentation we will concentrate on a few topics in which substantial progress has been achieved in the past few years and which are directly or indirectly related to breeding for resistance to early blight. We will also report on research advances in some areas of an early blight project initiated in late 1984 at CIP.

IMPORTANCE. The expansion of potato cultivation throughout the world and the increase of irrigation systems (mostly sprinkler overhead) has increased the severity of early blight disease in recent years. Harrison in his report to the Planning Conference of 1978 (15) discussed numerous examples of yield reductions due to early blight, losses that ranged from 10 to more than 50% from widespread locations in the world. More recently, reports on yield reductions due to early blight also agreed with those reported by Harrison in 1978 (3,6,17,18,20,22,24). Most of these yield losses are normally estimated by comparing fungicide treated and non-treated plots. In several occassions, although fungicides reduced early blight infection, this did not result in an increase in yield (8,9,10,11). This lack of yield increase has been attributed

to a late development of the disease in certain areas, and to the difficulty in measuring yield effects due to early blight in the presence of other diseases (11).

Before concentrating on the effect of certain factors on disease development and expression, it might be instructive to outline how researchers have measured infection level. This will also be helpfull in understanding early blight evaluation data. Commonly used in early blight work is the Horsfall-Barratt grading scale (13) which goes from 1= 0% to 12= 100% diseased tissue, having 6= 25-50% as the midpoint. Douglas and Pavek (7) modified the Horsfall-Barratt scale extensively adding lesion size, percent infected leaves, percent defoliation, severity, presence of stem lesions and plant maturity as parameters for measuring infection levels. Rowell (21) used a scale of 0 to 4 based on percent leaf area diseased, combining Horsfall-Barratt grades 1-5 (0-25%) and 8-12 (75-100%). Arzuaga, in Cuba (4), compared several methods of evaluating resistance to early blight and concluded that assest of defoliation on a scale of 1 (healthy) to 9 (dead) was the most recommended method, and that spot diameter was not well correlated with resistance. These results agreed with those reported by Nunes et al. (19) at Brazil who found that lesion size and number of lesions per area of tissue were not reliable parameters to estimate resistance. Finally, Reifschneider et al. (20) used lesion size and lesion number per leaf to compute, as a non-subjective alternative, an index of relative disease (they used a Li-cor electronic leaf-area meter, Li-3000), and developed a scale of 1=0% to 5=50% leaf area infected. Here at CIP, at the initiation of the early blight research, we used this scale but later the decision was made to use the international late blight scale of 1= 0% to 9=100% of the foliage infected, having 4=25-50% as the midpoint and using also half of a point for readings that do not exactly coincide with one of the categories, e.g. 3.5, 4.5, etc.

PLANT NUTRIENT STATUS

As has been mentioned before, early blight is primarily a disease of senescent tissue, and the highest sporulation has been measured as highest on older leaves. In general,older leaves are more susceptible than younger leaves to disease (2,7,11). These observations have suggested the assignation for early blight as a low sugar disease since older leaves contain less sugar than younger leaves (14). Horsfall and Dimond (14) after several studies concluded that any factor reducing the sugar content of leaves would increase early blight infection.

In 1983, Barclay et al. (5) after a two year study at Maine concluded that both high nitrogen and low phosphorus

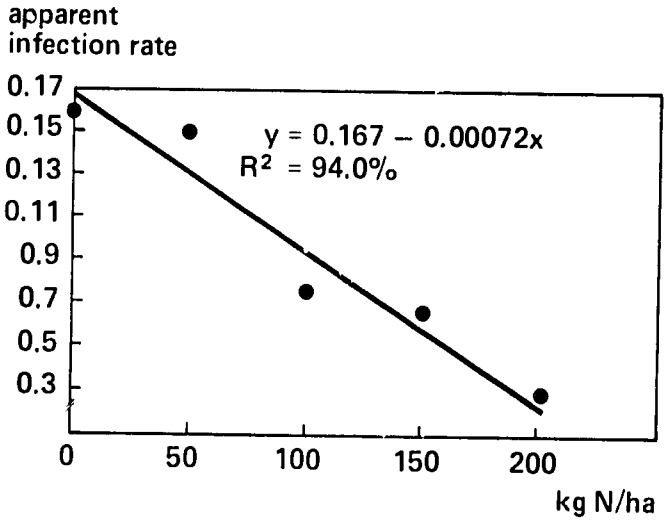


Figure 1. Effect of nitrogen fertilizer application on the apparent infection rate of potato cultivar Kennebec by *Alternaria solani*. Mackenzie, D. 1981.

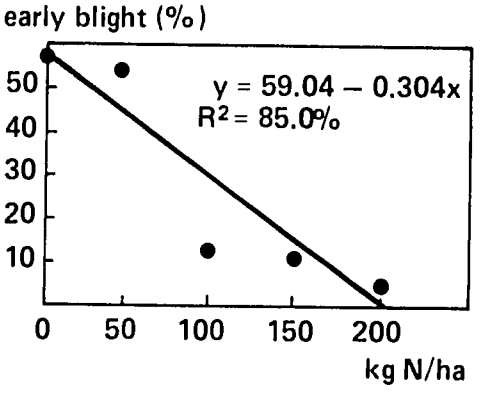


Figure 2. Effect of nitrogen fertilizer on the final severity of early blight disease of potato cultivar Kennebec. Mackenzie, D. 1981.

treatments significantly reduced the incidence of early blight. Results suggested that this combination of nutrients may be related to early blight resistance in the plant by extending the period of meristematic activity, permitting the plant to wall off infection. Later, Soltanpour and Harrison (23) confirmed the above results and concluded also that higher nitrogen levels produce a larger plant and delay senescence which would decrease early blight severity. Similar results were also obtained by MacKenzie (18) in 1981 at Pennsylvania, who concluded that apparent infection rates and the final amount of early blight decreased with increased rate of applied nitrogen (Figs. 1 and 2). Finally, a recent publication by Johanson (17) at Cornell University confirmed the effect of high nitrogen levels in reducing the susceptibility of potato plants to early blight. Both resistant and susceptible genotypes were affected in the same manner (Figs. 3 and 4).

RESISTANCE AND PLANT MATURITY

Another very important characteristic in selecting resistant genotypes which is poorly reported in the literature is the relationship between plant maturity and resistance to early blight infection. Douglas and Pavek (7) in 1972 concluded, after evaluating several clones under field conditions, that resistance to foliar infection was generally associated with plant maturity. Late maturing selections were generally quite resistant as compared to early maturing selections that were usually extremely susceptible. Several other researchers have noted this type of relationship (personal communications) and recently a detailed study of this subject confirmed that earlier varieties are more susceptible than later varieties. In this study, during 1985 and 1986 Johanson (17) rated potato cultivars for maturity and resistance to early blight under field conditions. Statistical analysis revealed a strong correlation between late maturing cultivars and early blight resistance (Figs. 5 and 6).

RESEARCH PROGRESS AT CIP-PERU.

Due to increasing demands during the past years for early blight resistant germplam from different CIP regions, and the increasing appearance of this disease at San Ramon's CIP experimental fields, a research project was initiated in late 1984 with the following objectives:

1.- Identification of the Alternaria species affecting potatoes in Central Peru.

2.- To determine the possibilities for early blight field screening at San Ramon.

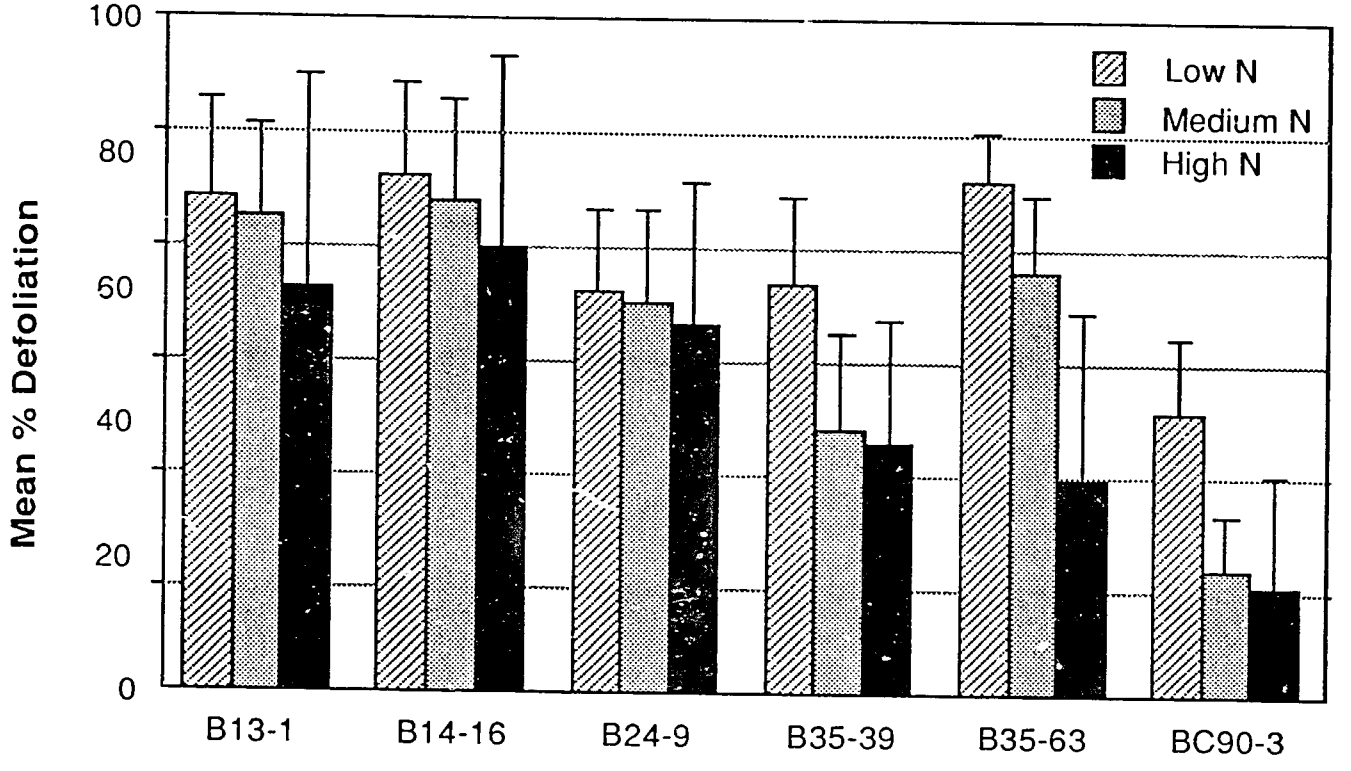


Figure 3 Percentage defoliation due to early blight, of the B - clones in the Cornell breeding program, at three different levels of nitrogen fertilizer. Error bars show standard errors of the mean of eight replications. Johanson, A. 1987.

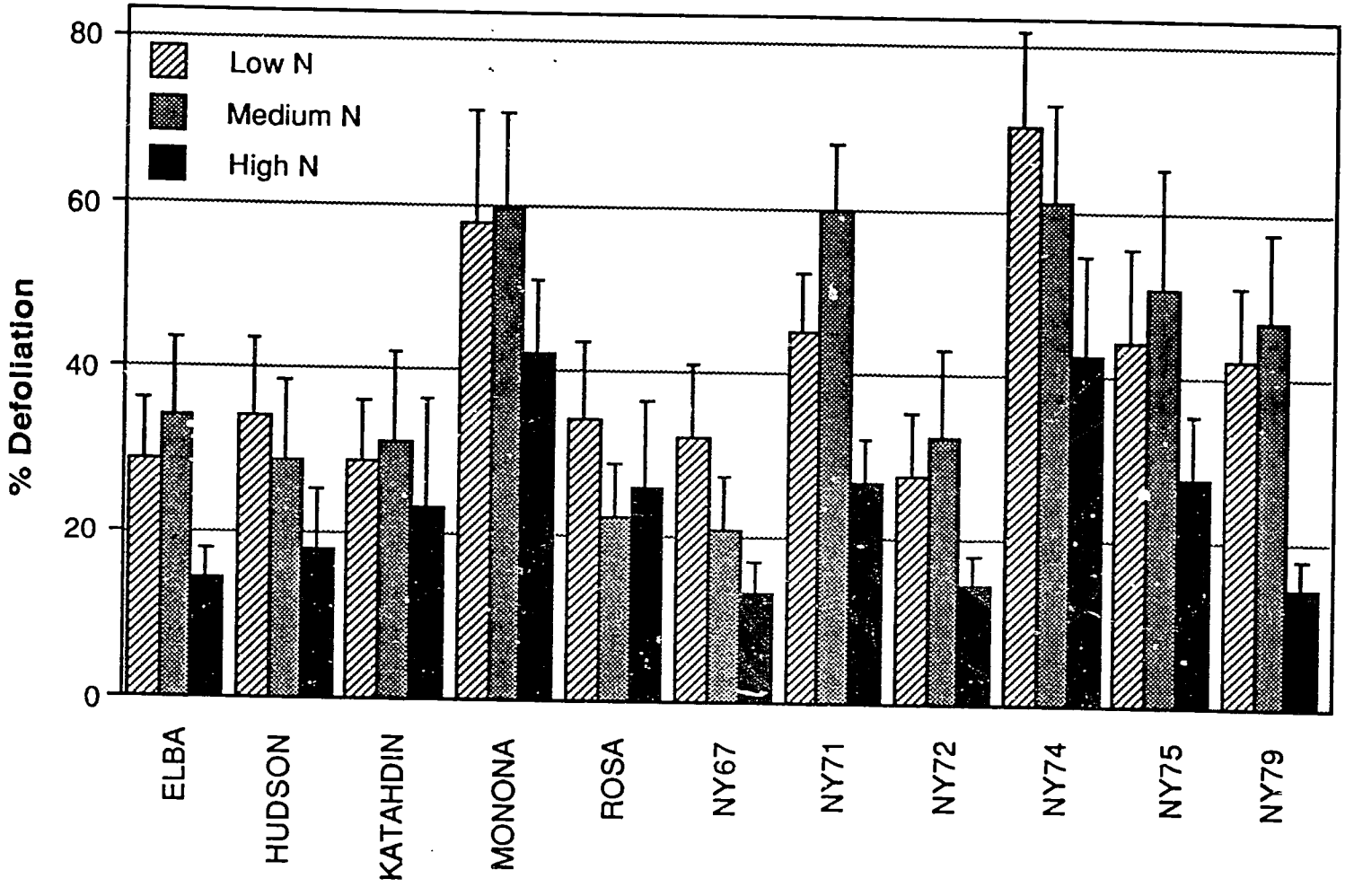


Figure 4 Percentage defoliation due to early blight of potato clones at three different levels of nitrogen fertilizer. Error bars show standard errors of the mean of eight replications. Johanson, A. 1987.

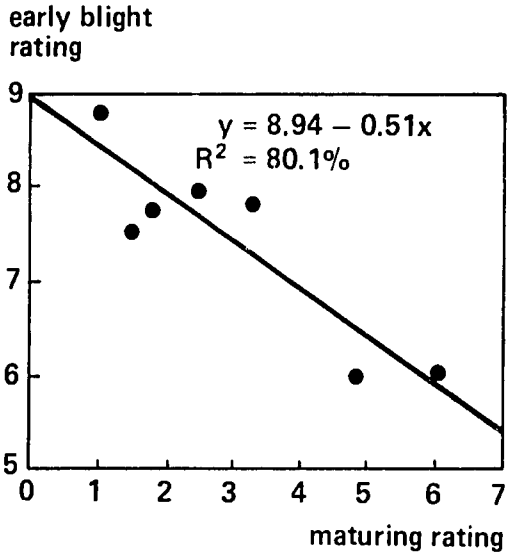


Figure 5. Simple, linear regression of early blight rating on maturity rating, for potato cultivars classified as early-maturing in 1985. Johanson, A. 1987.

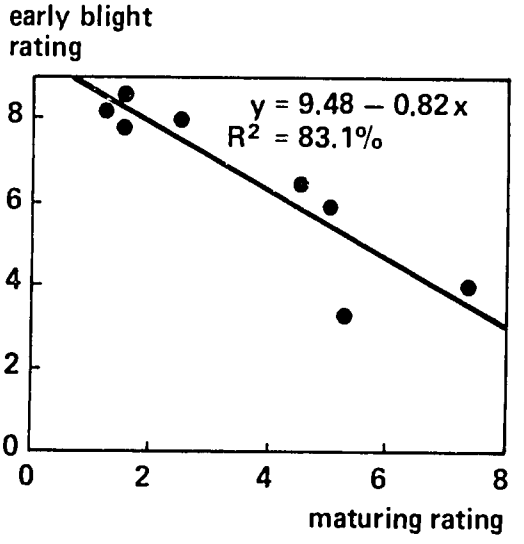


Figure 6. Simple, linear regression of early blight rating on maturity rating, for potato cultivars classified as late-maturing in 1985. Johanson, A. 1987.

3.- To develop a seedling screening test to select resistant genotypes at early stages of plant development, and therefore to speed up the selection process for resistant families.

During this presentation only the first two topics mentioned above will be briefly discussed; the third topic will be discussed during the later presentation of "Breeding for Early Blight Resistance."

Identification of Alternaria species. Studies conducted at CIP-Lima during 1984-86 with Alternaria isolates obtained from potato plants with typical early blight symptoms at San Ramon (Chanchamayo Valley,middle elevation) and The Mantaro Valley (highlands) indicated the possibility of the presence of more than one species of Alternaria causing early blight in potatoes.
Morphological studies of four distinct isolates obtained in this study indicated the possibility of the presence of three Alternaria species (Table 1). Conidial length, width, and number of septa agreed with the description of Alternaria solani (isolates SR from San Ramon, and LV from Mantaro Valley), A. brassicae (isolate LVa from Mantaro Valley), and A.porri (isolates SIC from Mantaro Valley). Cultural characteristics of these isolates confirmed the above observations: isolates SR and LV developed much faster on PDA medium than LVa and SIC isolates, although none of them sporulated on this medium at room temperature. All isolates sporulated under constant light at 18 C (SR and LV isolates) or 22 C (LVa and SIC isolates) but spore production differed significantly depending on the medium (Figure 7). Spore production for SR and LV isolates was much higher on PDA, V-8 agar and corn meal agar as compared to LVa and SIC isolates that sporulated better, although in less quantity in corn meal agar only. Pathogenicity tests of the four isolates on seven plant species further confirmed the previous observations on the presence of different Alternaria species causing early blight of potatoes in Peru (Table 2).

Field screening at San Ramon. Studies on early blight infection and severity were conducted during 1984-85 at San Ramon by using natural as well as artificial inoculations. To enhance disease development overhead sprinkler irrigation was used constantly for 5 days following inoculation. Forty day old plants were inoculated with a suspension of 3,000 to 3,500 spores/ml at the rate of 15 l of inoculum per 350 m-linear row. Results indicated that high levels of infection were obtained with artificial inoculation, specially during

Table 1.

Principal conidia characteristics of four Alternaria isolates causing Early Blight on potatoes in Central Peru.

Isolate*	Conidia (μ)		Septa (No.)		Named Specie
	Length	Width	Transv.	Longit.	
SR**	143-288	13-20	8-12	0-5	A. solani
LV	141-255	12-20	8-12	0-4	A. solani
LVa	92-226	19-24	8-16	0-7	A. brassicae
SIC	115-211	12-20	8-12	0-4	A. porri

* Average of three isolates and 50 measures per isolate.

** SR = San Ramon, Chanchamayo Valley (mid elevation)

LV = La Victoria, Mantaro Valley
LVa= La Victoria, Mantaro Valley } Highlands
SIC= Sicaya, Mantaro Valley

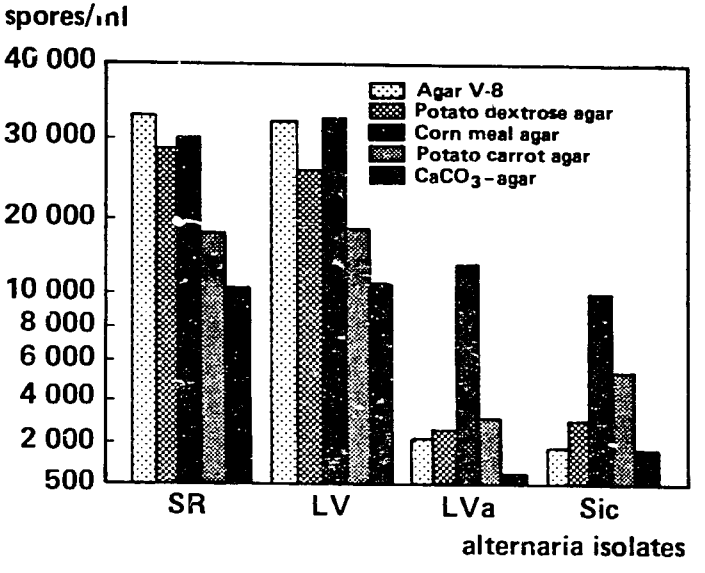


Figure 7. Spore production of four Alternaria isolates (SR = Chanchamayo Valley; LV, LVa and SIC = Mantaryo Valley) on five different media at 18-22°C.

Table 2.

Pathogenicity of *Alternaria solani* (SR and LV), *A. brassicae* (LVa) and *A. porri* (SIC) on seven plant species.

Host Plant	Alternaria isolates			
	SR	LV	LVa	SIC
Solanum tuberosum	TS*	TS	TS	TS
Lycopersicum esculentum	TS	TS	TS	TS
Brassica oleraceae	TP	TP	TP	AT
Brassica campestris	AT	AT	TP	NL
Allium cepa	AT	AT	AT	TP
Allium sativum	NL	NL	NL	NL
Datura sp.	NL	NL	NL	NL

* TS = Typical symptoms; TP = typical but relatively small lesions; AT = small lesions not typical of early blight; NL = no lesions.

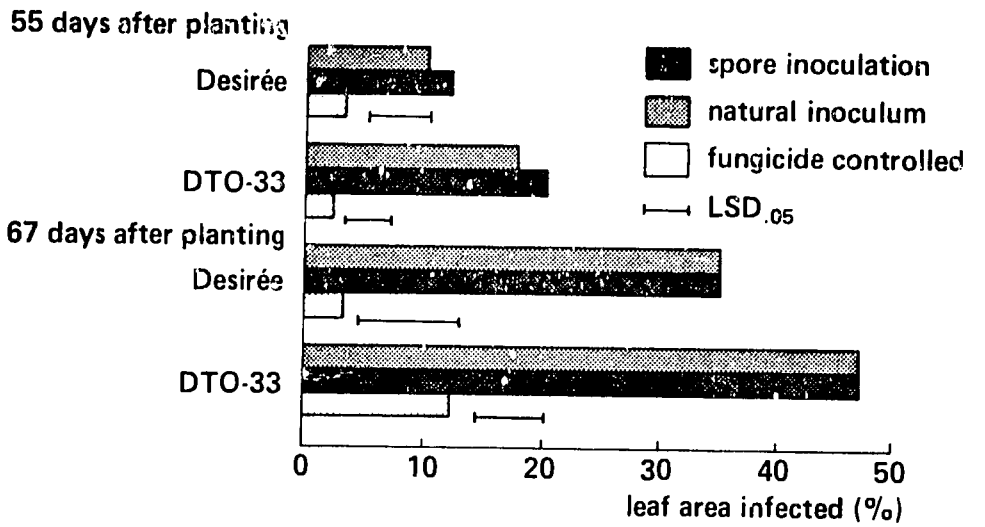


Figure 8. Percent leaf area infected by Alternaria solani on two potato cultivars under three treatments, at two dates after planting. San Ramon, Peru - 1984 dry season.

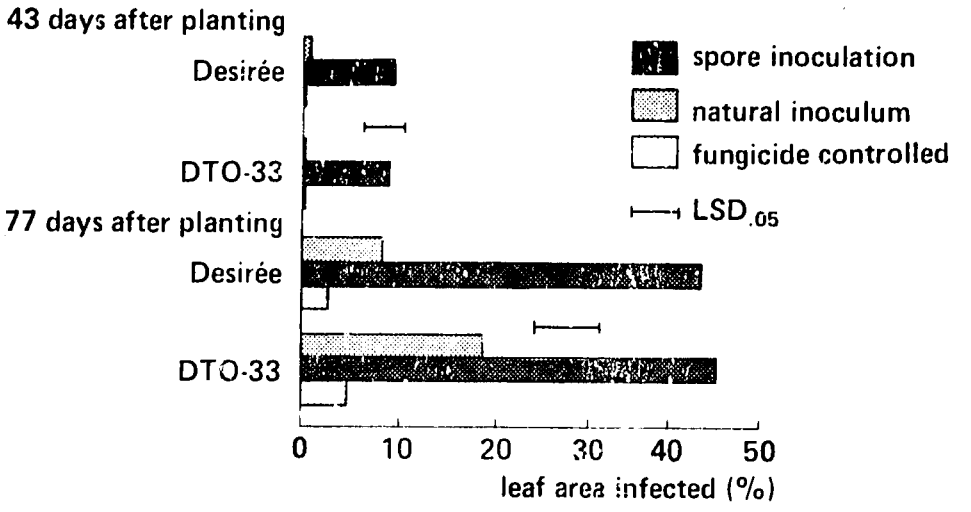


Figure 9. Percent leaf area infected by Alternaria solani on two potato cultivars under three treatments, at two dates after planting. San Ramon, Peru - 1984/85 rainy season.

Table 3.

Early blight evaluations (scale of 1 = 0% to 9 = 100% infection) and yield of clone DTO-33 (susceptible, early maturing) treated with different fungicides under San Ramon conditions, rainy season (Jan-Apr) 1987. Plots harvested at 105 days.

Fungicide (5 applications)	Early blight evaluations			Yield	
	1st[x]	2nd	3rd	Plot (Kg/6.3 m^2)	Ton/ha
Dyrene	2.3	3.0 b[z]	6.8 b	15.3 a	25.5 a
Euparem	2.1	3.1 b	7.3 b	14.7 ab	24.5 ab
Dithane/Cupravit/ Polyram Combi[y]	2.2	3.0 b	7.3 b	14.7 ab	24.6 ab
Dyrene + Dithane	1.5	1.6 a	5.2 a	15.9 a	26.5 a
Control	3.2	5.4 c	8.8 c	12.6 b	21.0 b

[x] First, second and third evaluation conducted at 20, 40 and 57 days after first field inoculation, respectively (first inoculation conducted 50 days after planting and second inoculation 10 days later).

[y] Three fungicides applied separately and rotated.

[z] Means followed by a common letter are not significantly different ($p = 0.05$). Early blight (= non-parametric Friedman test); Yield (= LSD).

Table 4.

Early blight evaluations (scale 1 = 0% to 9 = 100% infection) and yield of cultivar Revolucion (susceptible, late maturing) treated with different fungicides under San Ramon conditions, rainy season (Jan-Apr) 1987. Plots harvested at 105 days.

Fungicide (5 applications)	Early blight evaluation			Yield	
	1st[x/]	2nd	3rd	Plot ($Kg/6\ m^2$)	Ton/ha
Dyrene	1.5 a	1.8 a	3.1 a	10.0 b	16.7 b
Euparem	1.9 a	2.2 b	4.5 b	11.0 b	18.3 b
Dithane/Cupravit/ Polyram combi[y/]	1.9 a	2.2 b	4.3 b	9.7 b	16.3 b
Dyrene + Dithane	1.5 a	1.5 a	2.8 a	12.6 a	21.0 a
Control	2.4 b	3.1 c	5.2 c	10.2 ab	17.2 ab

x/ First, second and third evaluation conducted at 20, 30 and 47 days after first inoculation, respectively (first inoculation conducted 50 days after planting and second inoculation 10 days later).

y/ Three fungicides applied separately and rotated.

the rainy season (Figures 8 and 9)(16). During the dry season, infection levels of both natural and artificially inoculated plots were similar. This has not been the normal situation for the dry season during the last two seasons however; thus artificial inoculations have been used to enhance disease development in the field.

Chemical control and yield losses. Studies carried out at the San Ramon Station on the control of early infection during the past two years (4 seasons) have permitted a good level of chemical control of the disease as reported in the literature for other places. Control has been effective in early as well as late maturing cultivars. As shown in Table 3, significant differences were obtained in early blight incidence between control and chemically treated plots during the last rainy season on DTO-33, an early maturing clone. All the fungicides significantly controlled early blight although the application of Dyrene (Anilazine) + Dithane M-45 (Mancozeb) was the best treatment. Yields from plots treated with Dyrene also yielded significantly more than the untreated control. An extra yield of 5.5 Ton/ha was obtained when plots were treated with Dyrene + Dithane M-45 as compared to the control plots. Similar results were also obtained with Revolucion, a late maturing cultivar, although in this case early blight infection was lower (Table 4). Again, the best control of the disease and the highest yield was obtained from plots treated with Dyrene + Dithane M-45. An extra yield of 3.8 Ton/ha was obtained in plots treated with this mixture of fungicides as compared to the control plots.

LITERATURE CITED

1.- AGUILAR, J.A.E., and REIFSCHNEIDER,F.J.B. 1984. Efficiency of fungicides on the control of Alternaria solani in potatoes (Abstr.). Fitopatologia Brasileira 9:363.

2.- AMERICAN PHYTOPATHOLOGICAL SOCIETY. 1981. Compendium of potato diseases. (Ed.) W.J. Hooker. St. Paul, Minnesota, USA. 125 pp.

3.- ARZUAGA,J. 1983. Estimacion de las pérdidas causadas por Alternaria solani (Ell & Mart) Jones and Grout en seis variedades de papa (Solanum tuberosum). Cultivos Tropicales (Cuba) 5:255-264.

4.- ARZUAGA,J. 1985. Comparación de diferentes métodos para evaluar la resistencia a Alternaria solani (Ell & Mart) Jones and Grout en variedades de papa. Cultivos Tripicales (Cuba) 7: 141-146.

5.- BARCLAY, G.M., MURPHY, H.J., MANZER, F.E., and HUTCHINSON,F.E. 1973. Effects of differential rates of nitrogen and phosphorus on early blight in potatoes. Am. Potato J. 50: 42-49.

6.- CRUZ, DA, J. and NACIMIENTO, E.J. DA. 1984. Effect of fungicides on the control of Alternaria solani in potato (Abstr.). Fitopatologia Brasileira 9:362.

7.- DOUGLAS, D.R.and PAVEK,J.J. 1972. Screening potatoes for field resistance to early blight. Am. Potato J. 149: 1-6.

8.- EASTON, G.D., NAGLE,N,E, and BAILEY, D.L. 1975 Lack of foliar protection from early blight by aircrfat-applied fungicides on sprinkler irrigated potatoes. Plant Dis. Reptr. 59: 910-914.

9.- EASTON, G.D.,and NAGLE, N.E. 1985. Lack of economic benefits by fungicides applied through center-pivot irrigation systems for control of Alternaria solani on potato. Plant Disease 69: 152-153.

10.-FRANC, G.D., NNODU, E.C., HARRISON, M.D., and SADLER,A.J. 1983. evaluation of sprinkler application of fungicides for control of potato early blight in Colorado. Am. Potato J. 60:631-643.

11.-HARRISON, M.D., LIVINGSTON, C.L., and OSHIMA.N. 1965. Control of potato early blight in Colorado. I. Fungicidal spray schedule in relation to the epidemiology of the disease. A. Potato J. 68: 5-8.

12.-HARRISON, M.D.,and VENNETTE, J.R. 1970. Chemical control of potato early blight and its effect on potato yield. Am. Potato J. 47: 81-86.

13.-HORSFALL, J.G., and BARRATT, R.W. 1945. An improved grading system for measuring plant diseases(Abstr.). Phytopathology 35:655.

14.-HORSFALL, J.G., and A.E. DIMOND. 1957. Interaction of tissue sugars, growth substances and disease susceptibility. Z. Pflanzenkund Pflanzenhutz 64:416-421.

15.-INTERNATIONAL POTATO CENTER. 1978. Report of the Planning Conference on the control of important fungal diseases of potatoes. Lima, Peru.184 pp.

16.-INTERNATIONAL POTATO CENTER. 1986. Annual Report CIP 1985. Lima, Peru. 176 pp.

17.-JOHANSON, A. 1987. Factors affecting the resistance of potatoes to early blight, caused by Alternaria solani. Master of Science Thesis, Cornell University. 120 pp.

18.-MAC KENZIE, D.R. 1981. Association of potato early blight, nitrogen fertilizer rate, and potato yield. Plant Disease 65: 575-577.

19.-NUNES, M.A.L., ZAMBOLIN, L., MIZUBUTI, A. e CHAVEZ, G.M. 1983. Parameters that express the resistance of the potato plant at early blight (Abstr.). Fitopatologia Brasileira 8: 545.

20.-REIFSCHNEIDER, F.J., FURUMOTO, O.,and FILGUEIRA,F.A.R. 1984. Illustrated key for the evaluation of early blight of potatoes. FAO Plant Protection Bull. 32:91-94.

21.-ROWELL, J.B. 1953. Leaf blight on tomato and potato plants. Rhode Island Agr. Exp. Sta. Bull. 320. 29 pp.

22.-SAAD, N.H., and STINO, M.N. 1986. Effect of early blight infection on potato tuber yield in A.R.E. Potato Abstracts 11:159.

23.-SOLTANPOUR, P.N., and HARRISON, M.D. 1974. Interrelations between nitrogen and phosphorus fertilization, and early blight control of potatoes. Am. Potato J. 51:1-7.

24.-TENG, P.S., and BISSONETTE, H.L. 1985. Potato yield losses due to early blight in Minnesota fields, 1981 and 1982. Am. Potato J. 62: 619-628.

BREEDING FOR RESISTANCE TO EARLY BLIGHT (*Alternaria solani*)

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I. Introduction

During the last Planning Conference on the control of important fungal diseases, held at CIP-Lima in 1978, the three major recommendations made for early blight research were the following: first, efforts should be made to determine whether usable levels of resistance to *A. solani* exist in potato germplasm; second, due to the low incidence of early blight in Peru, it was recommended that screening for resistance be carried out at an appropiate location elsewhere, and, third, that *Solanum tuberosum* sp. *andigena* materials should be included in any screening program for early blight.

A research project on early blight was initiated in late 1984, as a response to increasing demands for *A. solani* resistant germplasm from different CIP Regions. The appearance and increasing incidence of early blight at CIP experimental station in San Ramon was also one of the factors that facilitated the initiation of several studies on this disease. Good levels of infection were obtained under field conditions by using artificial inoculations. The obtention of good levels of field infection and the finding of acceptable levels of resistance in CIP materials permitted to take further steps in CIP's early blight research. The principal goals were: 1) to evaluate the field resistance of advanced CIP clones 2) to develop a seedling screening test, and 3) to develop an strategy and a research project on breeding for early blight resistance.

II. CIP's Early Blight Research

1. Early blight field resistance of CIP advanced clones

Advanced clones from CIP's pathogen tested collection were field evaluated during three seasons at the San Ramon experiment station.

A great deal of variability for resistance was found during these evaluation trials indicating that breeding could be a feasible task. Table 1 presents information on the results of the

evaluation of a sample of clones at San Ramon where clones with the disease rating of 4 (25% attack) or less were considered resistant. It is noticeable that there are several clones which maintained an acceptable level of resistance throughout seasons, i.e. Cruza 148, F-4, ARX 69.1, Mex 21, and Yungay. Other clones exhibited a variable rating from moderately susceptible, up to 50% of foliar attack, i.e. CFC 69.1, ASN 69.1, and Aracy to susceptible, i.e. LT-1, Shuang Feng, Serrana, B71.240.2, and Atlantic (foliar damage of 75% or more).

The resistant clones with the exception of Mex 21, were late maturing and their yield of immature peeling tubers was from low to medium. In the group of moderately susceptible to susceptible clones, some produced a good yield which may indicate that they escaped the effect of the disease, i.e. when the foliage was seriously damaged the tuberization phase was already well advanced.

Table 1. Disease rating of a sample of CIP's clones in a field exposure to A. solani at 75 days of planting. San Ramon 1985-87.*

	S E A S O N			
	Rainy (85/86)	Dry 86	Rainy (86/87)	
Clone	75d	75d	75d	85d***
Cruza 148	3.4	3.0 (718)**	3.0	4.1 (320)
F-4	3.2	2.7 (150)	3.3	4.0 (395)
ARX-69.1	3.0	2.8 (611)	3.1	4.2 (528)
Yungay	3.2	2.0 (455)	2.7	3.6 (610)
CFC69.1	3.6	2.3 (521)	3.5	4.6 (732)
Serrana	4.5	--- (---)	3.0	5.8 (783)
Kinigi	6.0	4.2 (582)	3.3	4.3 (283)
LT-1	7.0	5.3 (---)	4.7	6.6 (600)
Aracy	4.5	4.5 (916)	3.7	5.7 (567)
Mex 21	3.3	3.0 (944)	3.3	4.3 (600)
ASN69.1	---	5.8 (---)	3.4	5.0 (779)
Shuang Feng	---	4.8 (---)	3.4	5.2 (428)
B71.240.2	---	4.0 (665)	4.6	6.2 (520)
Atlantic	---	--- (---)	7.3	8.5 (467)

* Disease rating scale 1:no disease 9:100% attack.
** Numbers in parenthesis are yield in gr/plant.
*** In the rainy season 86/87 a second rating was made 85-days after planting.

2. Seedling screening

The availability of efficient seedling screening techniques, provided that the behavior at both the seedling and adult plant stages are highly correlated, are a valuable tool in resistance breeding (8). These techniques permit to save time, space, and labor and can be applied with either of the two following purposes or both:

a) To determine resistance at the progeny mean basis permitting the identification of the resistant progenies. In this way one can test for resistance, in a reduced space and time, large numbers of progenies and rapidly identify without a need of further testing, a smaller number of progenies that carry adequate levels of the trait under selection. This is very important for selection of parental lines in particular and in population breeding in general.

b) To identify within progenies the individual resistant genotypes that will be later tested for other attributes. This is very important in variety selection.

Seedling screening for Alternaria resistance has given good results in tomatoes and musk melons (1,3). A potato seedling test for resistance to A. solani has been developed at the Cornell University. The test seems to work well although the correlation with adult plant behavior under field conditions has not been definitively established (5). Recently, Bussey and Stevenson (2) reported a correlation value of r=.75 between the resistance at the seedling and adult plant stages. The test might have certain limitations to screen large populations since it is performed on leaf discs maintained under controlled conditions.

Within CIP's research to develop an efficient seedling screening technique two aspects were considered: a) to develop a reliable an efficient inoculation and selection method, and b) that a high correlation must exist between the seedling test and the field evaluation.

For inoculation and selection purposes two alternatives were considered: a) Seedlings inoculated in a screenhouse under La Molina conditions, and b) Seedlings inoculated in large seedbeds under San Ramon conditions. For both alternatives, preliminary results indicated that seedlings must be inoculated when they are between 35-40 days of age with an inoculum suspension of 3,500 spores/ml at the approximate rate of 1 liter/1 m^2. After inoculation the seedlings were covered with a plastic sheet for 4 days to keep high relative humidity. The temperature at La Molina ranged from 12 to 35°C and at San Ramon from 18-36°C. After four days an average disease evaluation was made for the entire progeny and not for individuals within the progenies.

Under field conditions at San Ramon the plants were inoculated 30-35 days after transplanting with a suspension of 3,500 A. solani spores/ml at the rate of 15 L/350-400 m-row. Sprinkler irrigation was provided four times during the day for the first 4 days, and later at least 2-3 times a week for 30 min. Early blight readings were made 60 and 75 days after transplanting.

Although in the seedling test, infection and disease development occurred, disease severity and consistency was better under La Molina than under San Ramon conditions. So far, evaluations of two sets of TPS progenies at seedling as well as adult plants under field conditions have indicated a low correlation value for progeny means for most cases (Tables 2 and 3). For the first evaluation (Table 2), only the seedling test at La Molina and the field test were positively correlated and significant at the .05 probability level (6). In the second test, however, there were no significant correlation values (Table 3). This lack of association between seedling and adult plant to inoculation could be due to the impossibility of keeping constant, during the seedling inoculation, certain environmental parameters, i.e. temperature, humidity, and solar radiation. Another reason could be that the factors responsible for resistance at the seedling stage may be different from those conferring resistance at the adult plant stage.

Table 2. Spearman's correlation coefficients between 26 progeny means at different evaluations for early blight resistance at seedling and adult plant stages under field and semi-controlled conditions at two locations.

	Lima screenhouse	San Ramon field-55 days	San Ramon field-75 days
San Ramon seedbeds	- 0.062	- 0.374*	- 0.204
Lima screenhouse		0.180	0.445*
San Ramon field-55 days			0.335

Table 3. Spearman's correlation coefficients between 23 progeny means at different evaluations for early blight resistance at seedling and adult plant stages under field and semi-controlled conditions at two locations.

	Lima Screenhouse	San Ramon Seedbed	San Ramon Field	
	winter	4 days	55d	75d
Lima screenhouse summer	- 0.091	0.002	- 0.228	- 0.304
Lima screenhouse winter		0.228	0.213	0.115
San Ramon seedbed 4 days			0.322	0.318
San Ramon field 55 days				0.147

In spite of the modest positive correlations, between the seedling and adult plant stages, on a progeny mean basis, it seems that a higher level of concomitance exists between individual genotypes at both plant stages as indicates by the following experimental results. After a seedling screening of 23 progenies, carried out at La Molina 326 resistant seedlings were transplanted to pots to be grown to maturity. Tubers of the resistant seedlings were planted in the field at San Ramon where the plants were inoculated 40 days after planting and evaluated 25 and 35 days after inoculation. At the first evaluation, 235 out of the 326 clones were resistant (rating of 4 or less) indicating a coincidence of 72%. At the second evaluation, 124 out of the 326 clones were still resistant with a coincidence of 38% which is acceptable considering that: 75 day old plants growing at San Ramon, where many stresses affect the plant growth, start to show some symptoms of senescence and other foliar damage which may bias upwards the early blight damage overestimating it.

It seems necessary to continue the research to upgrade the efficiency of the seedling screening as well as to gain more information about the response of individual genotypes at both seedling and adult plant stages.

To comply with one of the priority recommendations of the 1978 Planning Conference, S. tuberosum sp. andigena germplasm was subjected to the previously described seedling screening for A. solani resistance. In the period 1986-87, Mihovilovich and Martin, 1987, unpublished results; evaluated the resistance of 425 open pollinated progenies from an equal number of clonal accessions from CIP's World Potato Collection. The work was carried out at La Molina and a sample of 100 seedlings per accession, grown in plastic trays, was utilized. The results showed that 96 progenies (22.6%) were resistant but the length of the growing period of their clonal accessions was in the late side. Only 23 resistant progenies (5.4%) corresponded to clones of medium early maturity. Disease ratings in the range of 2.3 to 4.0 were found among these 23 progenies. These results indicate that S. tuberosum sp. andigena is an important source of resistance to early blight.

3. Breeding research for early blight resistance

The breeding research work for A. solani resistance started in late 1984 and it was developed in a stepwise fashion including: a) to assemble a collection of resistant clones with a wide genetic base, b) to make genetic studies about the nature of the genetic variability for resistance and correlation between resistance and important agronomical traits, c) to select the most suitable progenitors on the basis of progeny testing, d) to determine the most adequate breeding methodology, and e) performance of the selected clones coming from the breeding work.

a. Genetic resources

i. Clonal materials as well as progenies from resistant progenitors were made available by CIP's research contracts at Cornell University (neo-tuberosum), and North Carolina State University (S. phureja diploid clones and 4x-2x S. tuberosum sp. tuberosum x S. phureja hybrids). In addition, selected S. tuberosum sp. tuberosum clones from Dr. A. Reeves (University of Maine), and Dr. J. Pavek (Idaho) were obtained.

ii. A group of CIP clones selected for their resistance to early blight (see Table 1), and some of them also carrying other resistances.

iii. A group of highly selected progenitors from CIP's lowland tropic population which had been progeny tested for yield, adaptation to heat and carrying resistance to several diseases, i.e., late blight, bacterial wilt, and immunity to PVY and PVX. These clones had not been selected before for early blight resistance.

These genetic resources of a broad genetic base and formed by highly selected materials formed the initial gene pool.

b. Genetic research

i. Three samples of 7, 6, and 6 clones were taken from the previously mentioned collection and crossed following a diallel mating design without reciprocals. Diallel A was a 7 x 7 with the following progenitors: LT-7, 378015.16, 575049,

Katahdin, 377892.7, LT-9, and Maine-28. Diallels B and C were 6 x 6 including the clones NDD277.2, 378676.6, C83.119, Atlantic, 7XY.1, and BR63.65 for diallel B, and 378676.6, 377964.5, WNC 521.12, 378015.16, Atlantic, and India-1035 for diallel C.

From all these progenitors it was known that Katahdin, Atlantic, Maine-28, NDD277.2, and WNC521.12 had an adequate level of resistance to A. solani when tested under the long day growing conditions of the USA where they originated. The level of resistance to early blight of all the rest of the clones was unknown. However, 575049 and India-1035 were resistant to late blight, BR63.65 resistant to late blight, bacterial wilt and viruses. LT-9 and 7XY.1 were immune to both PVY and PVX.

ii. A larger sample of clones were crossed following a North Carolina Design I formed by 12 males mated each to a sample of 6 females, involving a total of 84 progenitors.

All these genetic designs were evaluated with seedlings transplanted to the field. Three replications of 40 seedlings/rep were utilized in the experimental layout. The plot size and number of replications was found to be the optimum to evaluate segregating progenies at San Ramon (10). The seedling were field inoculated 45 days after transplanting. Only one disease evaluation was made in the diallel experiments 30 days after inoculation and 2 disease ratings in the Design I, the first 10 days and the second, 30 days after inoculation, respectively. Harvesting in all experiments was made 90 days after transplanting.

Tables 4, 5, and 6 present the average performance of the 10 best progenies in the diallel experiments A, B, and C, respectively. The three tables show that the best progenies were from semi-late to late with very high yields and the average progeny resistance was relatively low (50% or more foliar damage). However, since the progenies were of a wide genetic basis there was a great deal of variability for all the traits and several clones were selected for high yield, medium earliness, and resistance rating of 4 (25% foliar damaged) or less.

Table 4. Average performance of top progenies in the diallel A (7x7). San Ramon, winter, 1985

Progeny	Yield/ plant (gr.)	Earli- ness	Early blight resistance	Number selected clones/rep.
Maine-28 x LT-7	1340 a	3 a	5 abc	2
377892.7 x 575049	1261 ab	2 abc	5 abc	4
Katahdin x LT-7	1244 ao	4 a	5 abc	3
Maine-28 x 378015.16	1207 ab	3 ab	6 bcd	1
Maine-28 x 379706.34	1206 ab	4 a	5 abc	0
377892.7 x Katahdin	1150 ab	3 ab	5 abc	0
379706.34 x 377892.7	1140 ab	2 abc	5 abc	1
379706.34 x 378015.16	1135 ab	4 a	6 bcd	2
379706.34 x 575049	1124 ab	4 a	5 abc	5
379706.34 x LT-7	1102 ab	2 abc	5 abc	1

Earliness: 1:very late 9:very early
Early blight resistance: 1:no damage 5:50% damage 9:destroyed

Table 5. Average performance of top progenies in the diallel B (6x6). San Ramon, winter, 1985

Progeny	Yield/ plant (gr.)	Earli- ness	Early blight resistance	Number selected clones/rep.
Atlantic x NDD277.2	1303 a	4 ab	6 b	0
BR63.65 x NDD277.2	1289 a	4 ab	6 b	0
7XY.1 x Atlantic	1221 a	4 ab	5 ab	1
C83.119 x NDD277.2	1163 a	3 abc	6 b	5
7XY.1 x NDD277.2	1163 a	2 bc	5 ab	1
Atlantic x 378676.6	1158 a	4 ab	6 b	6
7XY.1 x 378676.6	1072 a	1 c	5 ab	0
C83.119 x 378676.6	1035 a	2 bc	5 ab	0
BR63.65 x 7XY.1	992 a	4 ab	6 b	0
7XY.1 x C83.119	978 a	2 bc	6 b	3

Earliness: 1:very late 9:very early
Early blight resistance: 1:no damage 5:50% damage 9:destroyed

Table 6. Average performance of top progenies in the diallel C (6x6). San Ramon, winter, 1985

Progeny	Yield/ plant (gr.)	Earli- ness	Early blight resistance	Number selected clones/rep.
378015.16 x 378676.6	1372 a	1 c	5 a	6
378015.16 x 377964.5	1365 ab	4 ab	7 b	2
Atlantic x 378676.6	1145 ab	5 a	6 ab	5
378015.16 x WNC521.12	1137 ab	4 ab	6 ab	4
WNC521.12 x 377964.5	1128 ab	4 ab	6 ab	1
India 1035 x 377964.5	1125 ab	4 ab	6 ab	2
377964.5 x 378676.6	1096 ab	4 ab	5 a	6
WNC521.12 x 378676.6	1050 ab	2 bc	5 a	4
India 1035 x WNC521.12	1050 ab	5 a	7 b	3
India 1035 x 378686.6	1044 ab	2 bc	6 ab	0

Earliness: 1:very late 9:very early
Early blight resistance: 1:no damage 5:50% damage 9:destroyed

The phenotypic correlations between early blight resistance and other traits followed the same pattern in the three diallel experiment and hence only results of the diallel A are presented in Table 7. More vigorous plants have less early blight damage, flower more, are later maturing and have higher yield. It is interesting to note that there is a highly significant correlation between early blight and earliness. The earliest the genotypes, the highest the attack.

Table 7. Phenotypic correlation matrix. Diallel A (7x7). San Ramon, winter, 1985

	Early blight	Flower- ing	Earli- ness	Yield/ plant
Vigor	-0.231	.463**	-.349**	.114
Early blight		-.395	.563**	-.042
Flowering			-.322**	.030
Earliness				.093

Table 8 presents the average progeny performance (with 6 females) for each of the male progenitors of the N.C. Design I experiment. One can notice that the clone 378676.6 gave the most resistant progenies followed by BL2.9 but both were medium late. The US clones NDD277.2, WNC521.12 and the variety Katahdin which are resistant under long day conditions had a rating of 6 suggesting that growing under short days they loose their resistance.

Table 8. Average performance of the 12 males utilized in the N.C. Design I experiment. San Ramon, winter, 1985

Male clones	Yield/ plant (gr.)		Earli- ness		Early blight (1st. Eval.)		Early blight (2nd. Eval.)	
378015.16	789	a	5	a	4	b	6	c
Murca	769	a	6	b	4	b	6	c
BL-2.9	766	a	6	b	3	a	5	b
DTO-28	744	a	6	b	4	b	6	c
NDD277.2	723	a	6	b	4	b	5	b
377892.7	719	a	6	b	4	b	5	bc
WNC521.12	714	a	6	b	4	b	6	c
378676.6	714	a	6	b	3	a	4	a
Katahdin	713	a	6	b	4	b	6	c
LT-7	711	a	5	a	4	b	5	b
377964.5	711	a	6	b	4	b	6	c
377873.9	686	a	6	b	4	b	5	b

Earliness: 1: very late 9: very early
Early blight resistance: 1:no damage 5:50% damage 9:destroyed

The performance of the top 12 progenies of the N.C. Design I is showing that in this larger experiment better levels of resistance and more earliness was obtained with respect to the diallels. The progeny 65-ZA-5 x 378676.6 was highly resistant but extremely late. On the other hand, the progeny Maine-47 x 378015.16 had an acceptable yield, medium to semi-early and it had unusually high number of selected clones.

Table 9. Sample of top performing progenies from the N.C. Design I experiment. San Ramon, winter, 1985

Progeny	Yield/ plant (gr.)	Earli- ness	Early blight (1st. Eval.)	Early blight (2nd. Eval.)	Number of selected clones
MS42.3 x BL2.	1078	4	3	5	1
A503.42 x DTO-28	1067	4	4	6	2
78.4.5F2 x 377873.9	945	4	3	5	2
CCN69.1 x NDD277.2	902	4	3	6	0
A66107.5 x 378676.6	871	1	2	4	4
65-ZA.5 x 378676.6	871	1	2	2	3
XY121.1 x Katahdin	866	3	3	5	11
Maine-35 x 377892.7	830	4	4	5	1
B71-240.2 x Murca	829	2	3	5	3
B78-1168.26 x 377892.7	807	3	3	4	3
Maine-47 x 378015.16	768	6	2	5	15
7XY.1 x BL-2.9	751	3	2	5	0

Table 10 shows the heritability estimates for the diallel experiments. The average narrow sense heritability estimate (h^2=.7) for early blight resistance is high (7). These estimates agree with an estimate of h^2 = .81 obtained with diploid potatoes (4). These estimates suggest that the control of resistance to A. solani might be dependant of a reduced number of loci. It also indicates that a rapid increase in resistance could be achieved at the population level by applying mass selection or recurrent phenotypic selection. However, this aspect will be discussed later. The h^2 estimates for the other traits are within the range of estimates previously obtained in CIP's genetic research (9).

Table 10. Narrow sense heritability estimates (h^2) in three diallel experiments in San Ramon, winter, 1985

	Yield/ plant	Earli- ness	Plant type	Early blight resistance
Diallel A	.45	.49	.70	.61
Diallel B	.15	.79	.83	.85
Diallel C	.56	.67	.50	.67

c. Selection of progenitors

Estimates of general combining ability for yield, earliness, and early blight resistance for the progenitors involved in the three diallel experiments were obtained (Table 11). These estimates indicate that the GCA for early blight resistance for the clones NDD277.2, WNC521.12, and Maine-28 and the varieties Katahdin and Atlantic are either 0 or negative, but have positive GCA estimates for earliness. On the other hand, the clones 378676.6, LT-7, 377892.7, and 7XY.1 have shown a good parental value for early blight resistance (positive g_i estimates). Also from Table 8, BL2.9 showed an acceptable parental value.

From some line x tester analysis made recently it has been found that the three clones 575049, Maine-47, and, at a smaller extent, Atzimba are also good general combiners for early blight resistance. Maine-47 deserves a special mention because it transmits not only resistance but also earliness. At present the breeding program counts on at least 10 good progenitors for A. solani resistance. It is, however, indispensable to continue the search for more parental materials.

Table 11. Estimates of GCA effects ($\hat{g}_i$) for various traits in the Diallels A, B, and C, respectively. San Ramon, winter, 1985

Clone	Yield/ plant	Earliness	Early blight resistance
Maine-28	+ 68.06	+ 0.20	- 0.20
379706.34	+ 34.66	+ 0.40	0.00
LT-7	+ 17.25	- 0.40	+ 0.40
377892.7	+ 8.85	- 1.20	+ 0.60
575049	- 4.94	0.00	0.00
378015.16	- 47.54	+ 0.20	- 0.40
Katahdin	- 76.34	+ 0.80	- 0.40
s.e. ($\hat{g}i$)	70.37	0.30	0.16
s.e. ($\hat{g}i-\hat{g}i$)	107.50	0.46	0.25
NDD277.2	- 141.16	+ 0.33	- 0.25
7XY.1	- 40.66	- 0.66	+ 0.25
Atlantic	- 33.42	+ 1.08	- 0.50
378676.6	+ 35.58	- 0.92	+ 0.50
C83.119	+ 77.33	- 0.16	0.00
BR63.65	+ 102.33	+ 0.33	0.00
s.e. ($\hat{g}i$)	114.12	0.31	0.19
s.e. ($\hat{g}i-\hat{g}i$)	176.80	0.48	0.30
378015.16	+ 298.16	- 0.83	- 0.17
378676.6	+ 258.66	- 1.08	+ 0.83
377964.5	- 33.08	+ 0.67	- 0.16
India 1035	- 121.58	+ 0.16	- 0.16
WNC521.12	- 136.08	+ 0.16	+ 0.08
Atlantic	- 266.08	+ 0.92	- 0.42
s.e. ($\hat{g}i$)	175.33	0.38	0.17
s.e. ($\hat{g}i-\hat{g}i$)	271.62	0.58	0.26

d. Breeding methodology

Within CIP's population breeding strategy the purposes of the genetic research are basically: i) to maintain a wide genetic diversity, ii) to increase the frequency of genes controlling desirable attributes, and iii) to recombine the genes controlling these attributes. From the populations developed in this manner the national programs of the developing countries are selecting new potato cultivars carrying the attributes of adaptation, yield, and tolerance to pests, diseases and stresses that they need for their growing conditions.

In CIP's genetic research on A. solani resistance, the studies on quantitative genetic variation, estimation of heritabilities (Table 10), and estimation of general combining ability effects for a group of progenitors (Table 11), permit to establish a breeding methodology. The high narrow sense heritability estimate for early blight resistance, i.e., $h^2=.7$ suggests that repeated cycles of mass selection or phenotypic recurrent selection could produce an adequate gain on resistance to the disease. However, some undesirable correlations between resistance and earliness, and resistance and yield, and earliness and yield, (Table 7) place a warning about the breeding methodology.

Since the population development focus the important characteristics under selection, the progenitors which transmit well a certain trait have also to be studied on their ability to transit to their progenies the other important attributes. One does not want to utilize as progenitors clones that either are very resistant to early blight but extremely late or very early and extremely susceptible to the disease. A balance has to be maintained to improve the entire population. Moreover, considering that the various attributes have a range of heritability values, an efficient breeding methodology to cope with these differences is the recurrent selection with progeny testing. This methodology has been applied with success for several years and now the early blight resistance has been incorporated an additional character for selection.

All the genetic research previously reported in this paper has been based on evaluation of transplanted seedlings that were inoculated under field conditions at San Ramon. By comparing the resistance of each genotype recorded in the seedling generation with that observed in the first clonal evaluation it appears that the percent of escape is relatively low i.e. no more than 10%. Therefore, this method of screening seems to be dependable and would permit a significant increase in the frequency of genes controlling resistance to A. solani.

In spite of this, the development of a seedling screening for early blight resistance that would be highly correlated with the behaviour of the plants in the field would considerably improve the effectiveness of the recurrent selection scheme. Moreover, such an efficient scheme would facilitate the selection at an early stage for combined immunity to PVY and PVX and resistance to early blight. This combination is highly needed in many developing countries.

e. Performance of clones selected for resistance to A. solani

Since 1985 when the breeding for resistance to A. solani was started several clones have been selected for further testing. Most of the results shown in this paper indicate that there is a correlation between resistance and lateness. Since this association is undesirable, during the evaluation of the selected clones, special emphasis was placed to look for materials which would not carry this correlation.

Tables 12 and 13 present the earliness and disease resistance of the best second and third generation clones, respectively, evaluated at San Ramon. It is noticeable that the clones LT-7, 575049, and India 1035 have produced a high frequency of clones with good levels of resistance accompanied by a medium or semi early maturity. The agronomical characteristics of most of these clones are very good since they are the progenies of clones highly selected for other attributes besides of their resistance to A. solani.

Table 12. Selected second generation resistant clones tested at San Ramon, summer, 1987

Clone	Earli-ness	Early blight 50d	Early blight 63d	Early blight 75d
C86.117 (Maine-28 x 377888.8)$_{2}$3	5	2.5	5	5
C86.153 (BR63.65 x 575049)$_{32}$	3	1.5	3.5	4
C86.118 (India 1035 x 377888.8)$_{11}$	5	2.5	5.0	5
C86.057 (Tollocan x LT-7)$_{22}$	5	2.0	3.5	4.5
C86.151 (BR63.65 x 575049)$_{15}$	5	2.0	3.5	5.0
C86.154 (BR63.65 x 575049)$_{34}$	3	1.0	2.5	4.0
C86.055 (377887.25 x LT-7)$_{33}$	5	2.0	5.0	6.0
C86.054 (377887.25 x LT-7)$_{32}$	3	1.0	2.5	4.0
C86.152 (BR63.65 x 575049)$_{23}$	5	2.0	3.5	5.0
C86.156 (India 1035 x 575049)$_{31}$	7	1.5	2.5	4.0

Table 13. Selected third generation resistant clones tested at San Ramon, summer, 1987

Clone	Earli-ness	Early blight 50d	Early blight 63d	Early blight 75d
C85.012 (Beauvais x LT-7)41	5	2.5	3	4
C85.010 (India 1035 x LT-7)62	7	2	3.5	4
C85.031 (377887.25 x 377964.5)41	5	1.5	2.5	3.5
C85.054 (Maine-47 x 378015.16)66	7	2	3.5	5
C85.002 (Desiree x LT-7)42	5	2.5	3.0	3.5
C85.009 (India 1035 x LT-7)54	7	1.5	2.5	3.0
C85.102 (377880.23 x Bulk PSR)51	5	1.5	3	3.5
C85.051 (Maine-47 x 378015.16)51	5	2	3	3.5
C85.036 (377843.3 x 377964.5)51	5	2	3	3.5

Summarizing the research aspects discussed in this paper a few points can be stressed:

i. The urgent need of a sound seedling screening technique for resistance to early blight.
ii. The availability of acceptable level of resistance in advanced genetic materials.
iii. S. tuberosum spp. andigena contains a wide range of variability for A. solani resistance and can be, whenever necessary, a valuable available extra source.
iv. The heritability for resistance to early blight is high (h^2=.70) which can permit a rapid increase in the frequency of genes controlling this trait.
v. The identification of several progenitors with a high general combining ability for this trait could facilitate the combination of resistance with other traits of importance.
vi. In spite that a significant correlation between lateness and resistance exists, medium to medium early clones with an acceptable resistance have been identified.
vii. After the results of this research carried out in a stepwise manner it is clear that genetic resistance is a viable route for the control of this disease.

REFERENCES

1. Barksdale, T.H. 1968. A method of screening for resistance to early blight on tomato seedlings. Phytopathology 58:883. (Abstr.).

2. Bussey, M.J., and W.R. Stevenson. 1987. Development of a disease screen for early blight resistance in Solanum spp. (Abstr.). Paper presented at the APS Annual Meeting, Cincinnati, Ohio, USA. August 2-6,1987.

3. Carmody, B.E., M.E. Miller, and M.P. Grisham. 1985. A technique to screen muskmelons for resistance to Alternaria leaf blight. Plant Disease 69:426-428.

4. Herriott, A.B., and F.L. Haynes. 1984. The heritability of resistance to early blight disease in diploid potatoes (S. tuberosum subspp. phureja and stenotomum). Am. Potato J. 61:524. (Abstr.).

5. Hoopes, R.W., R. Plaisted, and H.D. Thurston. 1986. Seedling screening for early blight resistance. Am. Potato J. 63:433. (Abstr.).

6. Martin, C., H. Torres, and H. Mendoza. 1986. Development of an early blight seedling screening test in potatoes. Am. Potato J. 63:444. (Abstr.).

7. Mendoza, H.A., C. Martin, R. Vallejo, and J. Espinoza. 1986. Breeding for resistance to early blight (Alternaria solani). Am. Potato J. 63:444-445. (Abstr.).

8. Mendoza, H.A. 1987. Progress in resistance breeding in potatoes as a function of efficiency in screening procedures. In. Report of the Planning Conference on Bacterial Diseases of the Potato. CIP-Lima (In Press).

9. Thompson, P.G., and H.A. Mendoza. 1984. Genetic variance estimates in a heterogenous potato population propagated from true seed (TPS). Am. Potato J. 61:697-702.

10. Vallejo, R.L. and H.A. Mendoza. 1987. Determination of optimum plot size and adequate number of replications for yield trials using potato seedling populations. Am. Potato J. (Manuscript sent for publication).

RESEARCH PROGRESS ON *Verticillium dahliae* Kleb.

Oscar S. Malamud

The first CIP's Planning Conference on Control of Important Fungal Dieseases, in addition to "Late Blight Strategy", was held at CIP headquarters in 1978. Then an initial survey of *Verticillium* wilt, its importance and control was presented by Krikun and Orion (14). The conference participants recommended that CIP undertake the following research:

"Wilt diseases are commonly found in the regions but there is confusion as to whether the main cause is *Fusarium* or *Verticillium*. *Fusarium* spp. are ubiquitous and readily isolated, whereas *Verticillium* is difficult to detect without the apropriate techniques. Because *Verticillium* is a threat to a wide range of agricultural crops and because the potato is often blamed for its introduction into an area we recommend that CIP:

1. Should initiate a definite survey to determine the geographic distribution and importance of *Verticillium* by a CIP pathologist and/or regional workers.

2. Should acquire the simple known methods for detection and positive identification of *Verticillium albo-atrum* and *V. dahliae* and should make them available to regional potato workers.

3. Should not initiate an extensive breeding programme with *Solanum andigenum* and other *Solanum* spp. for *Verticillium* resistance until the results of such a survey are available, but rather, advantage should be taken of existing resistant or tolerant material in other breeding or screening programs."

This paper intends to summarize and only partially review the present knowledge in main topics pertinent to the former conference recommendations based on new information published recently or obtained through personal communications.

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An in-depth review of the disease including incidence in various developed and developing countries, its potential spread, insect and pest interaction, bacterial and other fungi synergistic effect, detection and possible control was conducted at a special Early Dying Symposium of the Potato Association of America in 1985 (31).

Incidence and importance

Although mainly recognized as affecting warmer potato growing regions of the USA and Canada, and drier and desertic areas in the Middle East and Asia (11, 29), Verticillium dahliae Kleb. has spread to Northern regions of the North American continent (15) and was detected also in the Highlands of the Andean Region (19, 32). The disease is of serious importance in areas of long-term or intensive potato production and is largely uncontrolled (29). Wilt incidences are as high as 60% for susceptible cultivars resulting in yield losses of 25% or more, and up to 46%.

Other basic facts of the disease could be pointed out:

- Symptoms are difficult to distinguish from normal senescence.
- Infection occurs through the roots, followed by colonization of the vascular system.
- Contamination of uninfected fields can occur by wind or mechanical movement of soil-borne propagules, or by planting infected seed stocks.
- Interaction with other organisms is very common, resulting in a disease complex, making it difficult to properly identify its casual agent.
- The fungus may exist naturally on roots of native vegetation.

Research performed at CIP in 1986 has shown that many plant species in potato growing areas of Peru are infected with the organism under natural field conditions in the highlands. A total of 18 species, including the most common weeds from the Compositaceae and Cruciferaceae families were contaminated, some symptomless. Further testing of those weeds under controlled environment and through artificial inoculations yielded results which agreed with those found under field conditions (34).

Infection and colonization

It is clearly known that infection occurs through the roots, but it has not been conclusively demonstrated that the tubers become infected by internal vascular spread through the stolons. Regardless of foliar incidence, tubers are likely being infected through the eyes or the skin (15, 18).

There is no correlation between foliar symptoms and tuber infection or yield (24) (Table 1). Year to year fluctuations of tuber infection is large (Table 2). In most countries were applied, certification of *Verticillium*-free tubers is based on visible symptoms of the disease. This procedure does not accurately measure latent tuber infection (15). Therefore, it is proposed that potato seed certification be based on serological tuber examination and not on the foliar symptoms iniciated by the pathogen (15).

Although believed that potato seed is the basic transmission agent, many opposite results are available. When tubers heavily inoculated with the fungus were planted in virgin soils in Idaho (USA), no disease problem was observed the first year. Conversely, in the same type soils using clean seed lots, inoculum build up was detected, regardless of seed source (10, 16). Soil inoculum build up however, could be heavy after several succesive potato crops, and short rotations are not useful (7). Even with high inoculum levels, not always yield reduction possitively correlates with the incidence of wilt symptom expression and organism stem colonization.

In recent studies in Israel it was found that all the fields that were planted with contaminated seeds were infested with the disease. The correlation between the seed contamination level and the crop infection was relatively good. Usually the crop was infected in a higher rate than the seed contamination level. Additionaly, seed contamination occurred mainly in the spring season and fungus invaded the tuber also after the killing of the foliage. Seed contamination was very low during the fall and when harvesting in short days. Irrigation with saline water increased susceptibility to the fungus (25).

Root biomass is reported to decrease rapidly after stems are inoculated with conidial suspensions. Root deterioration was reported associated with premature senescence of foliar

Table 1. The occurrence of symptom expression of Verticillium dahliae on potato, isolation from haulms, percent tuber infection, and the effect of tuber infection on percent infected plants in the subsequent crop. 1/

Year	Cultivar	Infected plants[a]		Stems infested (%)	Daughter tubers[b] (%)	Infection of the subsequent crop[c] (%)
		Sample size	Symptoms present (%)			
1980	Blanka	880	50	37.0	0.25	0
1981	Desiree	540	2	3.0	11.0	8.0
1981	Desiree	370	80	30.0	1.4	0.5
1981	Alpha	500	15	7.5	5.0	5.0
1981	Spunta	500	12	1.0	7.0	5.0
1982	Spunta	120	0	3.0	11.6	10.0
1982	Pentland Crown	120	0	1.5	21.6	12.0
1982	Desiree	480	100	95.0	36.0	17.1

[a] Infected plants in the spring crop, determined 100 days after planting.

[b] Infection in the spring crop daughter tubers that were used as seed tubers.

[c] From 1.000 hills grown during the autum season.

1/ Data by Nachmias and Krikun. (24)

Table 2. Percentage of infected tubers in 11 potato cultivars during a 5-yr test period at Gilat, Israel. 1/

Cultivar	1978	1979	1980	1981	1982
Desiree	12	44	19	36	29
Spunta	16	100	29	14	38
Blanka	24	36	60	20	19
Up-to-Date	35	90	29	16	66
Kondor			7	0	30
Alpha			26	54	35
Patroness			43	72	60
VK 505			9	8	20
Croft			60	60	4
Maris Bard			38	16	28
Cardinal			41	20	6

1/ Data by Nachmias and Krikun. (24)

tissue, ocurring independently of root colonization by the pathogen (13).

Interactions

The incidence of the disease is increased and yields are considerable lowered by often synergestic effects of interactions with other organisms.

Fungi

A complex of organisms including *V. dahliae*, *Fusarium* spp, *Colletotrichum atramentarium*, *Erwinia carotovora* and *Rhizoctonia* spp have been associated with "Early Dying" disease syndrome in North Dakota (11).

In other somewhat similar associations plus the nematode *Pratylenchus penetrans*, singly and in all possible combinations, stem colonization by *V.dahliae* was a more sensitive measure of soil infestation than root colonization. Symptom severity was significantly greater in treatments with *V. dahliae* than in those without, influencing yield reduction (13).

Nematodes

The most prevalent and damaging interactions with the fungus is considered to include species of the genus *Pratylenchus*, *Globodera* (10, 28) and *Meloidogyne* (14).

Synergism was demonstrated by the occurrence of disease when both pathogens were present at populations which individually had little or no effect. Interaction with nematode lession *per se* was not considered the cause. Rather it was the nematode altering the host physiology so that the tissues were made more attractive or less tolerant to *Verticillium*.

Bacteria

Erwinia carotovora ssp. *carotovora* (Ecc) and *Erwinia carotovora* ssp. *atroseptica* (Eca) have shown synergistic effects, inducing more severe symptoms by *V. dahliae* (26).

When tested simultaneously, growth is reduced and foliar chlorosis and wilting is increased. Additionally, stem soft- rot, characteristic of Ecc, develop in stems of more plants inoculated with both pathogens than in plants were only Ecc is present. Similar trend can be detected with Eca showing an increased colonization of V. dahliae.

Inoculation methods

The need for a reliable soil inoculation method to assure homogenity and degree of severity in field trials to conduct clone selection for resistance, is a major concern for researchers. The main variations include root- dipping in spore suspensions, the addition of grains contaminated with fungal structures and the inoculation of the stem- base with pocking instruments carrying propagules (4, 14, 17).

For field trials CIP's researchers utilize a base "perlite" media on which fungal propagules are spread, supplemented with agar and nutrients and uniformly distributed at given rates over the tubers in the row at planting time (12). Nachmias et al. (21) obtain repeated and reliable soil contamination by mixing fixed rates of highly infected, naturally dried-up potato stem debries with microsclerotia, obtained from plots of susceptible varieties (24).

Detection

Away from visual symptom evaluation, a very difficult problem is to measure disease incidence in the tubers and predict future season's prevalence. The irregularity of disease outburst complicates research on control or breeding for resistance.

In one season, percent contamination of tubers harvested from a heavily infected area may be extremely low. (Tables 1 and 2). As a consequence, the number of tuber samples to be disected and cultured for a reliable determination is so large that it becomes extremely difficult to handle. Also in many cases, all tubers fron a sample are contaminated and in others the fungus can be detected only in as low as 1% of the tubers (15).

To date, there are no practical and completely reliable immuno-diagnosis techniques in place to detect latent infections of tubers.

The main problem seems to be the lack of specificity of the serum produced from toxins and fungal extracts or structures. Present technology is not sensitive and does not differenciate between the fungal species. The lack of specificity is seemingly related to heterogeneous, high molecular weight compounds (lipopolysaccharides) which contaminates the serum even after fraction separation (15). The subject will be expanded when discussing biossays.

Several researchers stressed the need to evaluate plant tolerance by the level of Verticillium colonization in potato tissue, isolating its propagules at different heights of the stem. (Figure 1) (5, 17).

Prediction

With the advances in computer technology, numerous potato growth and yield prediction models were developed (8), a few of them including Verticillium incidence and yield reduction as a major factor in prediction parameters (1, 9).

In most of them, the model prediction values are compared against plant growth data from field experiments on-site. Due to the complex background information needed, and the difficulty to handle additional traits or characteristics, the models to-date are highly specific for site, variety and pathogen interaction.

Biassays

One of the main questions on methods for screening for resistance is to determine how to measure it. We have seen that research is well developed in symptom expression, fungal isolation from stems and roots, testing methods in greenhouse and field conditions and inoculation procedures.

We have also discussed the fact that selection based solely in symptom expression is not reliable and cited a few research papers emphasizing the need to base the screening methods on the levels of Verticillium colonization in plant tissues.

As a result of potato breeders and pathologists concern to select stable resistant varieties, several bioassays were developped in the last years to study compounds produced by the pathogen or the plant. Once their characteristics are

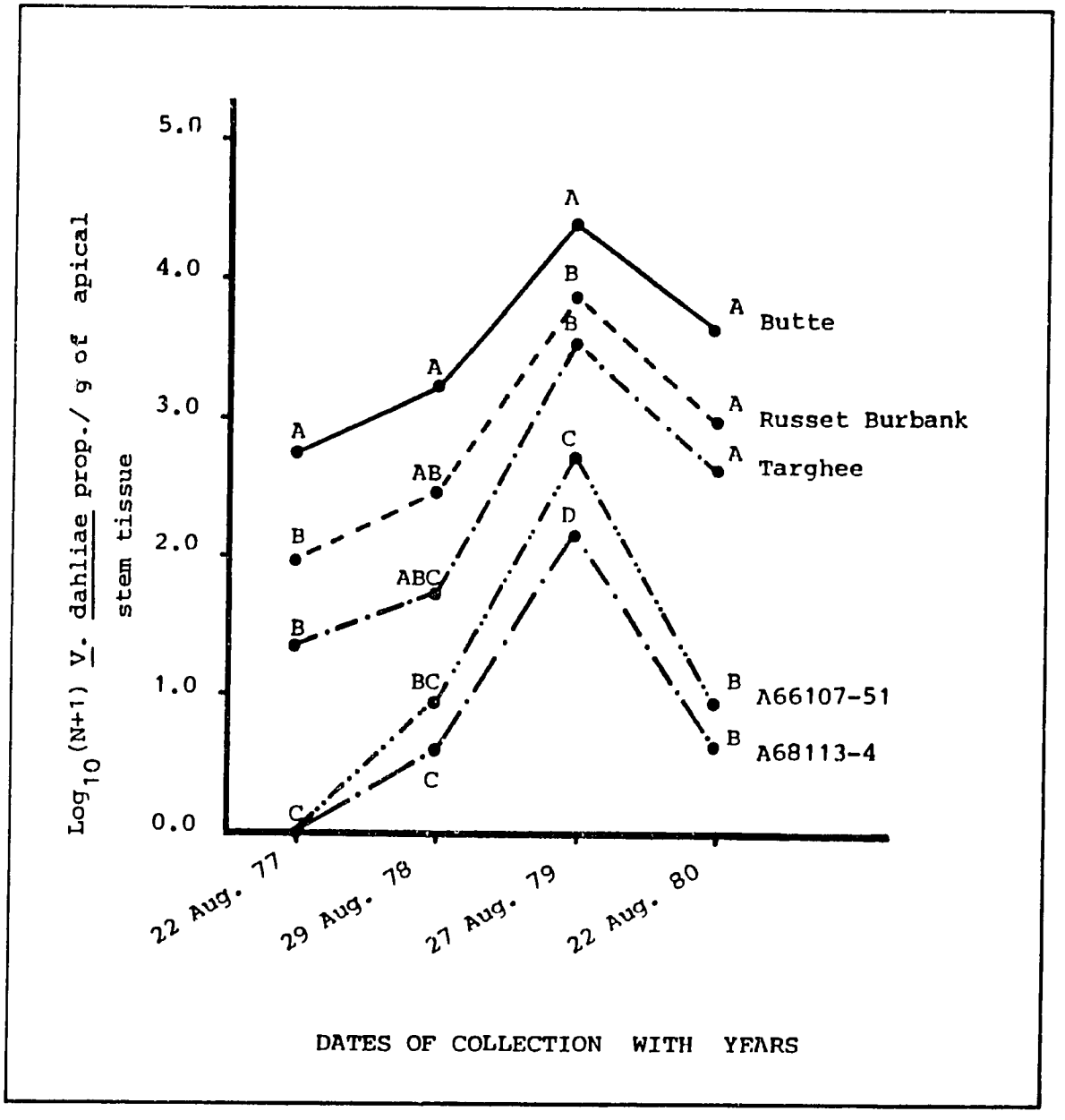

Figure 1. Relative Verticillium dahliae colonization of apical stem tissue among potato cultivars with continuous cropping. After Davis, Pavek and Corsini (1983).

known, these could be utilized to design immuno-serology type techniques.

The most extensive research on the subject was done at the Neguev region in Israel (20, 22, 23, 25) and in Canada (15). A Verticillium dahliae toxin in the form of a heat stable, high molecular weight protein-lipopolysaccharide complex was isolated, extracted and purified from infected tissue. Such toxin or toxins causes damage to potato tissue and is identical to the toxin that is secreted by the fungus in liquid culture. The toxin was tested on leaflets, cali, roots and protoplasts of resistant and susceptible cultivars. A differential effect using the toxin was obtained in leaflets, roots and protoplasts but not in the cali of the cultivars, corresponding to their resistance or susceptibility. The resistant or tolerant do not show damage to the compound. Furthermore, researchers of the same group compared three immunoassay techniques with the standard isolation method for determining the presence and percent of V. dahliae in seed potato tubers. They are still using the immunofluorescent method characterized by stained vascular xylem bundles, following a reaction using antisera produced against highly purified toxin (25).

These xylem staining techniques, in conjunction with specialized immunodifussion procedures improved the potential detection of antigen-antibody reaction of infected tubers, stems and leaves. The procedure is still laborious and not clear cut.

Using dot-Elisa system for detection of fungal antigens with sera prepared from washed spores Lazarovich (15) concluded that Verticillium produces a compound containing a high molecular weight polysaccharide which behaves as a dominant antigen and supresses the elicitation of antibodies against other components of the fungus. The antigen is prevalent in nature and therefore it is not possible to eliminate it from antigen preparations by the filtration procedures utilized so far. Both V. dahliae and V. albo-atrum produce this product. As a consequence, the methodology for antibody production has to be refined before a species specific antibody could be obtained.

Moderate high correlation (r=0.60) was found in Holland between toxin infection of V.dahliae and wilt and yellowing symptoms seen in pots on 15 genotypes. Reactions of tests agreed closely for cultivars like Alpha, Cardinal and Estima but disagreed for others (30).

A bioassay could also be applied to detect inoculum potential of V. dahliae in the soil by a baiting technique, using eggplant or flax as susceptible hosts on soil samples sistematically taken from a given plot (33). Complementary data on nematode population and infection rates of seed lots could help reach decisions on utilization of fields, specially for seed production.

Control

Chemical

The most prevalent means of control of Verticillium species is soil fumigation with chemicals. The continous development of new products and further reinfection of the soil by the fungus' resistant propagules or new strain-races appearances makes it very difficult to rely only on this procedure. The current status on chemical control was reviewed in 1985 (31) and is the subject of almost yearly trials elsewhere (7).

Physical

A mean of naturally controlling the fungus is by the use of solarization. Repeated studies involving polyethylene mulching at moderate temperatures demonstrated disease supression and significant improvements in yield and quality of tubers of Russet Burbank cv. and breeding clones (6).

Biochemical

Studies are being conducted in Canada trying to look at long term control procedures; specially the effect of melanine inhibitors on survival of microsclerotia and on changes in the chemical balance of the parasite (15).

Cultural practices:

Easton et. al. (7) showed that short cropping rotation (1-2 years) with immune hosts to V. dahliae had no effect on soil propagules of the fungus. Subsequent fumigation with methylbromide on part of the field and replanting of potatoes showed again that no cropping or fumigation treatment significantly affected soil propagules. Nevertheless, it reduced wilt and number of propagules in potato stems, and increased yield of marketable tubers.

Breeding for resistance

Strain-race concept

Recently, more evidence is being found about strain differentiation and possible existence of races which differ in their pathogenicity and biological characteristics. Puhalla and Hummel (26) tested a worldwide collection demonstrating that *V. dahliae* populations in the northwestern USA were uniform (P-4), being similar to a non-defoliating *V. dahliae* strain of cotton, but different fron European isolates. Nachmias and Malamud (unpublished) were able to compare isolates from Israel and Peru, showing that the latter induced foliar symptoms in the cultivar Blanca (susceptible) and also in the highly tolerant Alpha cv., in which the Israeli strain does not produce symptoms. Alpha is used as a tolerant standard to select resistant clones in southern Israel, a region of severe and endemic soil and foliage infestation by *V. dahliae*.

Clone Selection

Among all the methods to control *Verticillium* wilt the greatest success was achieved by soil fumigants and clonal tolerance (3). Costs and environmental dangers limited the use of fumigants. Lack of grower acceptance of newly developed clones with tolerance, limited their use (29). Ample reservoir of resistance is available and is still not fully exploited in cultivated and wild species over a wide range of ploidy (17).

Considerable evidence of stability of selection under different environmental conditions and fungus variability was presented and discussed in the last 5 years (2, 4). Yield and quality were improved and were correlated with wilt reduction and decreasing fungal stem colonization. With resistant clones, soil-borne propagules of *V.dahliae* dimished significantly while the less pathogenic species *V. tricorpus* was increased in the soil in plots where these clones were grown.

In the latest years CIP has also iniciated a screening program utilizing its pathogen-tested reservoir of worldwide cultivars and new introductions of tolerant clones from the USA (7). In 1985, five clones were found highly tolerant in greenhouse tests. In 1986, a broader population was tested

also in the field but the results were interfered by the interaction of other ecological and pathological problems. Also in 1986 in greenhouse tests, seven of 41 clones were rated as resistant, based on the lack of microsclerotia formation or mycelia detection in stems, and a very slight chlorosis with no defoliation. Furthermore in 1986, fifteen of 65 clones were rated as resistant in a trial at the irrigated Coastal Region of Peru.

The percent yield reduction in each of the rated resistant, intermediate and susceptible clones is shown in Figure 2.

Breeding clones with resistance to V.dahliae were produced in Idaho (2). Clone A66107-51 showed no wilt when tested against several strains from North America, Eurasia and Australia while Russet Burbank cv. showed variable response depending on strain.

Conclusions

The topics described above represent a brief summary of current research developments on the disease, specially in areas which I consider important for the planning of future research at CIP and its cooperators in different regions of the world.

As it becomes apparent, in the main areas of concern for potato growers (i.e.) yield reduction, seed contamination and soil infection, the assessment of the relative importance of the disease worldwide is complicated by:

a) The lack of reliable and easy methods for early detection of the disease and for measuring yield losses.
b) The lack of fast reliable methods to determine seed tuber contamination.
c) The difficulty of measuring soil contamination and disease expression in a given region and year.

These facts, among others, could account for the apparent lack of progress in our own research in some aspects of the proposed recommendations of the previous CIP's Planning Conference, specially in regard to the geographic distribution and importance of Verticillium.

Since there is a growing concern among potato researchers on the advance of the disease in may areas of potato production both traditional and newly developed, it seems pertinent to

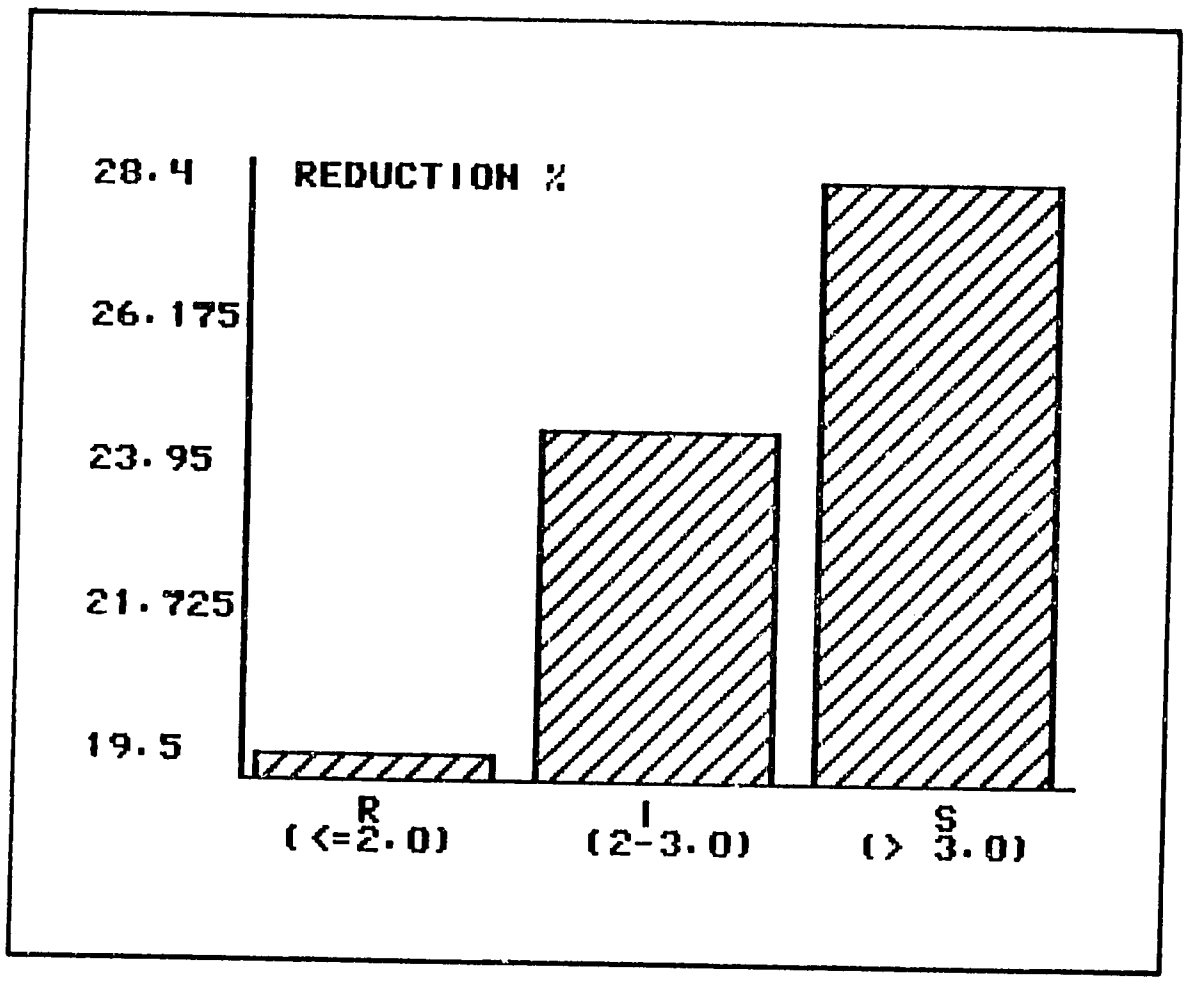

Figure 2.- Percent yield reduction due to Verticillium wilt in Cañete, Peru, in a group of resistant (R), intermedium (I) and susceptible (S) clones. After Martin (19).

bring to the attention of this Conference the following recomendations:

CIP, in the next few years should:

1. Initiate the evaluation of the relative importance of the disease in differente major potato growing areas of CIP's Regional Program.
2. Develop a screening test for Verticillium wilt for breeding purposes, using small plots (beds) in the field.
3. Initiate research to improve existing bioassays or develop new biological techniques to detect V. dahliae infection in tubers.
4. Increase its input in germplasm evaluation and selection for wilt resistance.
5. Study the potential for biological control of soil-borne fungal pathogens.
6. Continue its limited evaluation of new chemical products to control soil-borne fungal pathogens.

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LITERATURE CITED

1. Adams, S.S., D.I. Rouse and R.L. Bowden. 1987. Performance of alternative versions of Potwill: A computer model that simulates the seasonal growth of Verticillium-infected potato crops. Abstracts, Potato Association of America 71st. Annual Meeting: 67 (abs.)

2. Corsini, D.L., J.R.Davis and J.J. Pavek. 1985. Stability of resistance of Potato to strains of Verticillium dahliae from different vegetative compatibility groups. Plant Disease 69 (11):980-982.

3. Davis, J.R. 1985. Approaches to control of potato early dying caused by Verticillium dahliae. Am. Potato J. 62(4):177-186.

4. Davis, J.R., J.J. Pavek, D.L. Corsini, L.H. Sorensen and S.L. Hafez. 1983. Evaluation of field resistance to Verticillium dahliae among potato clones and relationships of potato clones to the soil environment. Abstracts of EAPR-84 Conference papers: 327-328. Interlaken, Switzerland.

5. Davis, J.R., J.J. Pavek and D.L. Corsini. 1983. Development of potato cultivars with Verticillium resistance and increased yield potential by the year 2000. In Proceedings International Congress "Research for the potato in the year 2000":71. International Potato Center. Lima, Peru.

6. Davis, J.R. and L.H. Sorensen. 1986. Influence of soil solarization at moderate temperatures on potato genotypes with different resistance to Verticillium dahliae. Phytopathology 76(10):1021-1026.

7. Easton, G.D., M. Nagle and M.C. Seymour. 1987. One and two year cropping to immune hosts on Verticillium dahliae control and potato production of Russet Burbank potato. Abstracts Potato Association of America 71st. Annual Meeting: 44.

8. Ewing, E., W.D. Heim, J.Shustek. R.G. Snyder and A.G. Nicholson. 1987. Evaluation of plant growth models for use in a system analysis approach to potato IPM. Potato Association of America 71st. Annual Meeting: 89. Abstract.

9. Francl, L.J., L.V. Madden, R.C. Rowe and M.R. Riedel. 1987. Validation of a model that discriminates yield reductions due to Potato Early Dying. Potato Association of America 71st Annual Meeting: 91. Abstract.

10. Franco, J. and E. Bendezu. 1985. Estudio del complejo Verticillium dahliae Kleb. y Globodera pallida Stone y su efecto en el comportamiento de algunos cultivares peruanos de papa. Fitopatologia 20(1):21-27.

11. Gutmestad, N.C., T.T. Zink and V. Otazu. 1977. North Dakota Potato wilt survey. N.D. Farm Res. 35:26-31.

12. International Potato Center Annual Report. 1985. pp 17.

13. Kotkon, J.B. and D.I. Rouse. 1984. Root deterioration in the potato early dying syndrome: Causes and effects of root biomass reductions associated with colonization by Verticillium dahliae. Am. Potato J. 61:557-568.

14. Krikun, J. and D. Orion. 1978. In Report of the Planning Conference: Control of Important fungal Diseases of Potatoes. Centro Internacional de la Papa. Lima, Peru: 110-123.

15. Lazarovich, G. 1987. Agriculture Canada. London, Ontario. Canada (personal communication).

16. Mace, M.E., A.H. Bell and C.H. Beckman (eds.). 1981. Fungal wilt diseases of plants. Academic Press, N.Y. 640 pp.

17. Malamud, O.S. 1975. Species and families of tuber-bearing Solanum with resistant or tolerant response to infection with Fusarium solani, F.eumartii and Verticillium dahliae. Univ. Nebraska. Lincoln, Nebraska. PhD. Thesis.

18. Malamud, O.S. 1986. Potato tuber infection by Verticillium dahliae. ARO-Guilat Experiment Station, The Volcani Center, Israel. Unpublished.

19. Martin, C. 1985. Verticillium wilt of potato in Central Peru. Am. Potato J. 62(4):195-199.

20. Nachmias, A., M.Tal and J. Krikun. 1983. Screening for Verticillium tolerance by the use of toxic metabolites produced by the pathogen. Proceedings International Congress "Research for the potato in the year 2000".:138. International Potato Center. Lima, Peru.

21. Nachmias, A., L. Livescu, J. Krikun,G.R. Mackay, P.D.S. Caligari and D.E. Van Der Zaag, 1984.. Screening for resistance in potato breeding lines to Verticillium dahliae and Alternaria solani under field conditions. Abstracts EAPR-84 Conference papers: 329. Interlaken, Switzerland.

22. Nachmias, A. and J. Krikun. 1984. Diagnosis of Verticillium dahliae in potato seed tubers with immunoassay techniques. Potato Research 27:423-426.

23. Nachmias, A., J. Orenstein and M.Tal. 1984. Biological activity of a Verticillium dahliae toxin on potato tissues. Abstracts EAPR-84 Conference papers:81 Interlaken, Switzerland.

24. Nachmias, A. and J. Krikun. 1984. Transmission of Verticillium dahliae in Potato tubers. Phytopathology 74:535-537.

25. Nachmias, A. et. al. 1987. Potato seed contamination with Verticilium dahliae (unpublished).

26. Puhalla, J.E. and M. Hummel. 1983. Vegetative compatibility groups within Verticillium dahliae. Phytopathology 73:1305-1308.

27. Rahiman, M.K. and J.E. Mitchell. 1984. Relationship of Verticillium dahliae and Erwinia carotovora pv. carotovora in the early dying disease of potatoes. Phytopathology 74:327-332.

28. Riedel, R.M. and R.C. Rowe. 1985. Lesion nematode involvement in potato early dying disease. Am. Potato J. 62(4):163-172.

29. Rowe, C.R. 1985. Potato Early Dying - A serious threat to the potato industry. Am. Potato J. 62(4):157-161.

30. Stichting voor Plantenveredeling SVP. Netherlands. 1986. Jaarverslag 1985. 29, 71. Abstract.

31. The Potato Association of America. 1985. Potato Early Dying Symposium. Am. Potato J. 62(4):157-205.

32. Turkensteen, L. and L. Nieto. 1984 and 1986. Reports on a survey on potato diseases in Colombia. Research Institute for Plant Protection IPO, Wageningen, The Netherlands and ICA-Instituto Colombiano Agropecuario, Tibaitata, Colombia (unpublished).

33. Tzror (Lahkim), L., A. Nachmias and J. Krikun. 1985. Improvement of potato profitability through field mapping of Verticillium dahliae inoculum in the soil. Am, Potato J. 62:445-446 (Abstract).

34. Vargas Machuca, R. 1986. Host range of Verticillium dahliae in the Mantaro Valley. The International Potato Center. Lima, Peru. Thesis research report: 4pp.

WIDESPREAD SOIL-BORNE FUNGAL DISEASES OF POTATO

L. J. Turkensteen

Abstract

From 1979 on surveys on bacterial and fungal diseases of potatoes were made in several countries in Africa, Asia and Latin America. These surveys were made to obtain a better understanding of the importance of these diseases on a worldwide scale. There was a tendency of increasing importance for Verticillium albo-atrum (wilt), Fusarium solani (wilt) and Spongospora subterranea (powdery scab). The increase in importance of these problems was found associated with lack of rotation and lack of a proper seed inspection scheme. In hot climates Macrophomina phaseoli (charcoal rot) was a principal problem.

1. Introduction

An important issue to the International Potato Center (CIP) is control of major potato diseases. However, as there are many diseases, choices must be made concerning those ones to work on at present and/or in future. To set the right priorities, knowledge on the importance of diseases is necessary. During the last six years surveys have been made in Bhutan, Burundi, Colombia, Pakistan, Rwanda, Tunisia, Turkey and Zaire (5,6,7,8,9,10,11,12,13). These countries were selected because they present a broad range of environments in which potatoes are grown. These surveys were made in the frame of a linkage contract between CIP and the Research Institute for Plant Protection (IPO). In this paper an overview of the results of these surveys is given concerning world-wide spread of major soil-borne fungal diseases.

It is somewhat difficult to confine soil-borne diseases as such. Most potato diseases have a more or less pronounced soil-borne phase. In the case of diseases such as early blight (Alternaria solani) and gray mold (Botrytis cinerea), epidemics may be started because of soil-borne inoculum. However, most of the inoculum for epidemic development is generated within the same crop during that same growing season. With the world-wide spread of the mating types A1 and A2 of Phytophthora infestans at present, this pathogen may also be counted in that same

group. In this presentation soil-borne diseases will be restricted to those fungi causing wilts, tuber blemishes and tuber rots. The soil-borne inoculum is largely responsible for epidemic disease development during the growing season. Inoculum generated during the growing season does not contribute much to epidemic development but may serve as initial inoculum for a next crop.

2. Results

2.1 Tuber rots

2.1.1 Dry rot

Dry rot caused mainly by Fusarium solani and Fusarium oxysporum was a common problem in all regions surveyed (See also Fusarium wilt, 1.2.1). Lifting immature tubers was more the rule than the exception. As a consequence tubers were often damaged. In addition, conditions for curing were often improper as either temperatures were too high or too low or the relative humidity was too low. As a consequence, these tubers were very prone to dry rot infection. Potatoes had to be consumed within two months after harvesting to avoid inacceptable losses. Under these conditions storage losses were estimated to fluctuate between 3 to 10%. In Rwanda and North Kivu gangrene caused by the weakly pathogenic fungus Phoma exigua var. exigua was common.

The main mechanisms of control are improved harvesting and storage methods including proper curing. Varieties, which are too susceptible, should be replaced.

2.1.2 Charcoal rot

Charcoal rot caused by Macrophomina phaseoli was a serious problem at the harvest of the spring crop in Tunisia in June, 1981, when hot summer weather started earlier than normal. Losses of between 2 and 30% were recorded. This disease is common in regions where soil temperature exceeds 32°C yearly, and starts when soil temperature exceeds 28°C. It was also observed to cause problems in the summer of 1982 in the plains of Turkey. For many regions where the growing season usually ends with a hot period, it is charcoal rot which dictates the harvest time. This is a marked problem of potato growing areas in the Mediterranean area and the Indian subcontinent. The main mechanism of control is to avoid having potatoes in the soil when soil temperatures are too high. Resistance to charcoal rot may prolong the growing season as well as increase the potato growing area in hot climates.

2.2 Wilt

2.2.1 Fusarium wilt

In all regions surveyed Fusarium oxysporum and Fusarium solani were often found associated with wilt. In most cases Fusarium wilt was found associated with excessively wet field conditions. However, as these organisms are omnipresent and behave also as secondary invaders, they are problematic to diagnose them either as primary or secondary wilt causing organisms. Also in association with Verticillium wilt, Fusarium spp. are commonly found to be present. They may not be the primary invaders but, nevertheless, may increase disease incidence. It is difficult to estimate the importance of Fusarium wilt on a world-wide scale. In many regions Verticillium wilt is erroneously identified as Fusarium wilt. This is mainly because the two Verticillium spp. involved develop very slowly and are outgrown on culture media by accompanying Fusarium spp. In any case, during the surveys Fusarium wilt did not show up as such an important disease as local people believed. Evidence was found of the presence of a wilt causing strain of F. solani in Tunisia (5). This strain affected the vascular system of plants and tubers but did not develop tuber rot. It was found to be associated with fields on which an annual potato crop was produced.

Control of wilt causing Fusarium spp. is mainly by the use of disease free seed and proper rotation. On soils with problems, the use of less susceptible cultivars may be a help.

2.2.2 Verticillium wilt

Wilt caused by Verticillium spp. was found very commonly during the surveys. It should be noted that the two main species involved, Verticillium albo-atrum and V. dahliae are distinguished according to the descriptions made by Smith, 1965.

2.2.2.1 Verticillium dahliae

V. dahliae was found in cool and warm growing areas. It is known as an important disease problem in a rather large part of the potato growing areas of the world. It concerns regions with a high evapo-transpiration rate during the growing season. The pathogen causes early maturing of the crop whenever the crop is stressed. When stress occurs, the crop becomes damaged by the fungus in an irreversible way. As a consequence the crop does not recover when stress is over. The pathogen destabilizes the production capacity of the crop. Stress may be have several causes, related or not, such as heat, drought, excess of water and unbalanced fertilization. Especially the last two conditions were

encountered too often during the surveys. Many farmers tend to over-irrigate either because of ignorance or in order to counter salt accumulation. In the plains of Pakistan, excessive irrigations were an adapted practice to avoid damage by light night frosts. Zinc deficiency was common in many alcalic soils and aluminum and manganese toxicity was common in acid soils. These problems tend to increase problems with Verticillium species.

As crops may mature two to six weeks before their time, considerable losses may occur in warm climates (2). For susceptible varieties losses up to 90% are experienced; for resistant varieties losses are 30% at the average and may go as high as 50%. An additional problem is the increased susceptibility of affected plants to early blight caused by Alternaria solani. Very often, early blight is found associated with Verticillium wilt, which is not recognized as such by the local farmer or extension specialist. In cool climates, the presence of V. dahliae is in general not a problem as far as yield is involved but may be a problem, whenever healthy seed is to be produced. V. dahliae is longlived in soils and control is mainly through the cultivation of resistant and tolerant cultivars and the avoidance of too short rotation with potatoes and other susceptible hosts. Generally, varieties adapted to grow under warm conditions show some level of resistance or tolerance to this pathogen. In spite of heavy infestation, farmers obtained good results with the application of high amounts of stable manure.

2.2.2.2 Verticillium albo-atrum

Wilt caused by Verticillium albo-atrum was found in the cool growing areas in most of the countries surveyed (7,8,11,12,13). There were no earlier reports on its presence and importance to the crop, which may point to a recent development. In all cases it was found with lack of rotation. Losses in affected crops were estimated to be between 10 to 70%. In cool areas of Colombia and Pakistan, where viral seed degeneration was low, V. albo-atrum seemed to be a main cause of seed degeneration. In fact losses due to Verticillium wilt were more pronounced than those caused by late blight. This was especially the case when late blight was chemically controlled.

Build up of the disease in seed stocks is through the use of infested seed and lack of rotation (4). A vicious circle is the consequence. Clean seed becomes infested through cultivation in infested soils and clean soils become infested through infested seed. At the end all seed stocks become infested. In cool humid climates the fungus was found to sporulate densely on affected dying stems. It is supposed that this sporulation plays a role in in epidemic spread. There are indications that improved late blight control may increase problems with V. albo-

atrum. Crops and stems stay green longer giving more opportunities to the fungus to infect the crop and to colonize infected stems. Infected stems again are a source of inoculum because of sporulation developed upon them as well as because these stems are incorporated together with the fungus into the field soil.

Because of its devastating effects on the crop, it is recommended for seed programs in cool areas in developing countries to consider V. albo-atrum as a seed degenerating agent and to take proper action for its eradication from seed stocks. Fortunately, with the introduction of healthy seed and rotation periods with non-host crops of more than one year, infestation can be brought back to acceptable levels.

In certain areas, the use of resistance to this disease may offer a way out. Cultivar Seseni, which locally is permanently grown in the same field in Zaire, has been found to be very resistant to Verticillium (Spek, J. van der, pers. comm.). Unfortunately, its yield and quality are low. The introduction of new varieties failed as they could not withstand the high level of soil infestation with V. albo-atrum. In Colombia there are regions where potatoes are continuously grown during a number of years in fields opened in grassland. In fact they are grown in these fields as long as the level of infection with V. albo-atrum permits. As a major part of the cost of potato production are the costs to open these fields, it is of importance to keep those fields free of disease as long as possible. Therefore, prevention of infestation should be by using healthy seed. Resistance to slow down disease build up in the crop and field infestation might be useful as well. However, in general, control of disease problems lay more in the field of prevention and sanitation than breeding for resistance.

2.3 Rosellinia

Rosellinia necatrix occurrs in restricted cool and humid areas. It was found to cause considerable losses locally in Zaire, Rwanda, Central America and the Andes. It was associated with growing potatoes in rotation with fallowing. In Tunisia, Rosellinia was very rarely found in association with hedgerows of cypresses. On a world-wide scale, Rosellinia is of limited importance.

2.4 Powdery scab

A problem of a somewhat different dimension is the spread of powdery scab caused by Spongospora subterranea. This disease showed a strong tendency to spread and to grow in importance in new non traditional potato growing areas, which was especially the case in Turkey (11) and Pakistan (8,9,10). Recently, there were also reports from other regions

in the world on increased problems with this disease. The fungus, which is longlived in soils, seemed to survive very humid and very dry hot summers as well as bitter cold winters in the regions visited.

To prevent build up of the pathogen population to inacceptable high levels, rotation periods of three to four years are recommended (14). To reduce the level of heavy soil infestation to acceptable levels rotation periods of five years and more are necessary.

In general physical yield is not affected but occasionally roots may become attacked to such an extent that young plants wither away. However, as tubers are attacked, quality and consequently marketable yield is affected. In Pakistan, farmers could not sell heavily affected potatoes and received only half the price for lightly to moderately affected ones. As a consequence of skin attack, storability is poor because of increased evaporation losses and because of the effects of secondary invaders leading to dry and soft rots. Under very humid growing conditions deep craterlike lesions may develop which was the case in Pakistan and Turkey. This deep pitted type of powdery scab is very often confounded with deep pitted common scab.

In Turkey materials were screened for resistance to powdery scab. Most clones were susceptible but a few were much less affected. S. subterranea is reported as a vector and source of potato mob top virus (PMTV) (1). This virus affects yield and tuber quality. Due to the spread of the vector also the spread of this virus is to be expected. In Tunisia powdery scab was observed in 1980 on plants with typical PMTV symptoms. In Africa, powdery scab was observed in all areas surveyed and was rather widespread in Rwanda and Burundi. In some areas attack was very severe. Due to lack of rotation and the use of infested seed, it is expected that powdery scab will spread in many areas.

2.5 Wart

During the surveys no attention was paid to wart. However, in some regions problems with wart caused by Synchytrium endobioticum are developing. The disease is confined to the cool potato growing regions of the Andes and the southern slopes of the Himalayas. So far the disease is controlled by the use of resistant cultivars. However, as there is no prohibition to grow susceptible cultivars on infested land, this resistance will be overcome by races of the fungus. Plant health services and regulations in the countries concerned should be upgraded to such an extent that effective control of wart can be achieved.

2.6 Rhizoctonia

In the areas surveyed, many people complained about the effects of Rhizoctonia. However, during the surveys not much damage due to Rhizoctonia solani was observed. It is very likely that symptoms due to other diseases such as leafroll, V. albo-atrum and mycoplasms (stolbur) were taken for Rhizoctonia. In the Punjab of Pakistan severe stem attack associated with very humid conditions and with deep planting was a general problem. Black scurf was rather commonly observed. In general, the impression was given that problems due to Rhizoctonia were over-estimated.

2.7 Other soil-borne diseases

Stalk break caused by Sclerotinia sclerotiorum and S. minor were found to affect the crop in a few occasions. As attack occurred generally in aging crops, losses were very limited. The disease did not appear as a major disease. Basal stem rot caused by Sclerotium rolfsii is a problem in humid tropical and subtropical climates. Losses can be considerable. However, during the surveys it was rarely observed. Another typical soil-borne disease, pink rot caused by Phytophthora erythroseptica and other Phytophthora spp., was very rarely found during the surveys. Consultation of local specialists did not reveal any evidence on its occurrence as a considerable disease with the exception of the basic seed production farm at Phubjika in Bhutan.

3. Conclusions

Based on the results and experiences obtained with surveys the three most important fungal potato pathogens after late blight seem to be V. albo-atrum, V. dahliae and S. subterranea. In the course of these surveys it became clear that both Verticillium spp. cause more damage than was suspected. The importance of these two pathogens is probably underestimated. Contrarily, problems with Fusarium wilt and Rhizoctonia were found to be over-estimated in most places.

It is recommended to pay more attention to the two Verticillium species involved. Work to be done on the control of V. albo-atrum lies mainly in the field of extension. Farmers should become aware of the importance of disease free seed and proper rotation. Work to be done on control of V. dahliae lies in the field of breeding for resistance or tolerance and of agronomic measurements to prevent stress to the crop.

Powdery scab is becoming a serious problem in areas with a relatively short tradition of potato growing.

References

1 Jones, R.A.C. and Harrison, B.D., 1969. The behaviour of potato mob top virus in soil, and evidence for its transmission by Spongospora subterranea (Wallr.) Lagerh. Ann. Appl. Biol. 63: 1-17.

2 Krikun, J. and Orion, D., 1978. Verticillium wilt: Importance and control: Report of the Planning Conference: Control of Important Fungal Diseases of Potatoes. Cip-Lima, Peru, June 19-23, 1978: 110-123.

3 Smith, H.C., 1965. The morphology of Verticillium albo-atrum, V. dahliae, and V. tricorpus. N.Z.J. Agric. Res. 8: 450-487.

4 Thanassoulopoulos, C.C. and Hooker, W.J., 1970. Leaf and sprout infection of potato by Verticillium albo-atrum. Phytopath. 60: 196-203.

5 Turkensteen, L.J., 1981. Report of surveys on fungal and bacterial potato diseases in Tunisia made in 1980 and 1981. Research Institute for Plant Protection, Wageningen: 9 pp.

6 Turkensteen, L.J., 1982. Rapport d'une visite aux régions productrices de pommes de terre du Burundi, Rwanda et Zaïre pour inventorier les maladies bactériennes et fongiques de la culture de pomme de terre - Juillet 1982. Institute pour la Recherche Phytopathologique (IPO), Wageningen: 14 pp.

7 Turkensteen, L.J., 1984. Report Survey Potato Diseases: Rwanda, Burundi, and N. &. S. Kivu, Zaire, 1984. Research Institute for Plant Protection (IPO), Wageningen: 18 pp.

8 Turkensteen, L.J., 1985. Survey on bacterial and fungal potato diseases in the hilly areas of Pakistan - August-September 1985. Pakistan-Swiss Potato Development Project - PARC - POB. 1031 Islamabad, Pakistan: 41 pp.

9 Turkensteen, L.J., 1987a. Survey on bacterial and fungal potato diseases in the plains of the North West Frontier Province and Punjab of Pakistan - December 1986. Pakistan-Swiss Potato Development Project - PARC - POB. 1031 Islamabad, Pakistan.

10 Turkensteen, L.J., 1987b. Survey on fungal and bacterial potato diseases in Baluchistan, Pakistan - August 1987. Pakistan-Swiss Potato Development Project - PARC - POB. 1031 Islamabad, Pakistan. (In press)

11 Turkensteen, L.J. and Eraslan, F., 1985. Surveys on fungal and bacterial potato diseases in Turkey, 1981 - 1983. Aegean Regional Agricultural Research Institute, Menemen, Izmir, Turkey. Bulletin No: 62: 20 pp. English summary.

12 Turkensteen, L.J. and Nieto, L.E., 1984. Report on a survey on potato diseases in Colombia - October 21 till November 8, 1984. Research Institute for Plant Protection, Wageningen/Instituto Colombiano Agropecuario, Bogota: 9 pp.

13 Turkensteen, L.J. and Nieto, L.E., 1987. Report on a survey on potato diseases in Colombia, November 10 - 29, 1986. Research Institute for Plant Protection, Wageningen/Instituto Colombiano Agropecuario, Bogota: 10 pp.
14 Wenzl, H., 1975. Die Bekämpfung des Kartoffelschorfes durch Kulturmassnahmen. Z. Planzenkr. Pflanzenschutz 82: 410-440.

SOIL-BORNE AND FOLIAR DISEASES IN THE HIGHLAND TROPICS

Hebert Torres

INTRODUCTION

The highland tropics, for the purpose of this presentation, are the cool highlands of the tropical belt where potatoes have been traditionally grown in altitudes ranging from 1800 m to slightly above 4000 meters above sea level (masl). Although potatoes were taken from South America to the rest of the tropical belt of the world, principally through Europe, only a few of the diseases from the center of origin of the potato were carried to the highland tropics outside the Americas. I will restrict this presentation to the highland diseases of the tropical South American Andean highlands. I will discuss the actual status of the potato diseases present in the highlands of South America, their distribution and results from projects carried out by CIP as a result of recomendations given in the last Planning conference in 1978.

The potato in these Andean countries is grown from 1000 to 4000 masl. Farmers cultivate commercial varieties of Solanum tuberosum ssp. andigena; but, native varieties of other species of excelent characteristics are also cultivated such as S. gonyocalix, S. stenotomum, S. chaucha, and S. phureja. They are grown in the highest parts of the Andean zone.

PRESENT STATUS OF SOIL-BORNE AND FOLIAR DISEASES

Soil-borne diseases are present in all Andean countries, the incidence of these diseases varying from country to country and within each country. In spite of the wide distribution of some of these diseases, there are still areas free of them. The incidence of a particular disease will depend on climatic factors, primary inoculum concentration, aggressiveness of strains and relative susceptibility or resistance of the cultivar.

In the Andean region potatoes are mostly planted during the rainy season. In very wet seasons the incidence of a disease such as Pink rot (Phytophthora erythroseptica) will increase significantly as compared to diseases such as

Verticillium wilt and Potato smut (Angiosorus solani) that will be more severe in dry seasons.

Another factor that influences the low or high level of soil infestation by soil-borne pathogens in the Andean zone is the size of the farm and the economic situation of farmers. Thus, small farms are generally under highly intensive cultivation and therefore rotation is not practiced. On the other hand big farmers have more access to credit, better seed, fertilizers, pesticides, etc.

Farmers in the Andean region, as in the case of Bolivia and Peru live every day with soil-borne diseases. In this way, the presence of diseases in the tubers such as Black wart or Powdery scab are in most cases overlooked. When tubers are totally infected, farmers discard them, but if the infection is partial they will cut the infected part out and the rest of the tuber is then sold or saved for food. This is one of the reasons why it is very common to find in the market diseased or low quality tubers.

Chemical control of soil-borne diseases is difficult, expensive and the majority of farmers in the highlands do not practice this type of control. However, control of soil-borne diseases is possible by using a combination of fungicides, soil fumigants, solarization, etc.

In the case of foliar diseases, late blight is considered as the most important potato disease in the highlands of the Andes. Septoria leaf spot (6,7,15) and Phoma leaf spot (28,29) have also been reported, but most of the andean farmers generally consider these diseases as late blight too. In most cases chemicals used by the farmers for controlling late blight, also control other foliar diseases. If environmental conditions are adequate for late blight development and the farmers are planting a susceptible variety, the number of fungicide applications may range from 15 to 25. On the contrary, if environmental conditions are not adequate, the number of applications may be up to three per growing season.

DISTRIBUTION AND INCIDENCE OF DISEASES IN THE HIGHLAND TROPICS

A) SOIL-BORNE DISEASES

Thirteen soil-borne diseases are present in the Andean highlands (Table 1). I will briefly discuss the distribution, incidence and importance of these diseases.

Table 1._ Soil-borne diseases in the Andean highlands

Powdery scab (Spongospora subterranea (Wallr.) Lagerh.)
Black wart (Synchytrium endobioticum (Shilb.) Perc.)
Verticillium wilt (Verticillium spp.)
Potato smut (Angiosorus solani (Barrus) Thirum & O'Brien)
Pink rot (Phytophthora erythroseptica Pethybr.)
Rhizoctonia canker (Rhizoctonia solani Kühn)
Dry rot (Fusarium spp.)
White mold (Sclerotinia sclerotiorum (Lib.) de Bary)
Rosellinia black rot (Rosellinia sp.)
Black dot (Colletotrichum atramentarium (Berk. & Br.) Taub)
Stem rot (Sclerotium rolfsii Sacc.)
Charcoal rot (Macrophomina phaseoli (Maubl.) Ashby)
Common scab (Streptomyces scabies (Thaxter) Waksman & Henr.)

Powdery scab (S. subterranea) is present in all Andean countries. It is more severe between 3200 to 3800 m elevation. In the central and southern highlands of Perú and in the altiplano of Bolivia, powdery scab is considered as the most important potato disease. In the southern part of the highlands of Peru (Cusco and Puno Departments), the losses are estimated at between 5 and 60% of the total yield. Commonly grown comercial varieties such as Yungay and native varieties such as Ccompis are very susceptible to the disease.

Black wart (S. endobioticum).- It is also present in the highlands of all the Andean zone, but it is more severe between 3500 to 3800 masl. In Bolivia this disease is considered as the most important, and most potato growing areas are infested. Losses are estimated between 5 to 20% of the total yield. When asociated with the false root-knot nematode (Nacobbus aberrans), losses are even as high as 20% (A. Gandarillas, personal comunication). It is also present in the potato growing areas of the highlands of Peru. In this country there are fields free or with little infestation, but, there are places seriously infested too. The losses in these infested places reach 20% of the total yield (24). In other countries the losses are not significant and in Venezuela the disease is found only occasionally.

Pink rot (P. erythroseptica).- This disease, in the cases of Perú and Bolivia, is present in areas with poor drainage, where there is a lot of rain or when heavy irrigation is applied. The losses in the highlands of Perú can reach 80% of the total yield (26).

Verticillium wilt (Verticillium spp.).- It is present in all the Andean zone from sea level to 4000 m elevation. The incidence is more severe in the dry season than wet season. When soil moisture is normal, Verticillium wilt is not a problem. Apparently V. dahliae is the more common species in the Andean zone, although V. albo-atrum has also been reported in Colombia (14) and in Peru (4). Losses are estimate range between 5 and 15%. The losses depend on the susceptibility of the cultivar, soil moisture and sometimes nematode association such as with the Potato Cyst nematode (Globodera pallida)(8).

Potato smut (A. solani).- This disease was reported for the first time by Barrus and Muller in 1943 in Venezuela (3); but, according to Eduardo Ortega (personal comunication), the disease has not been recently observed in potato growing areas of Venezuela. In Peru, the symptoms of this disease was mixed up by Abott (1) when he reported the Powdery scab symptoms in 1928. In this country the disease is present in potato growing areas located between sea level to 3500 m elevation, in different soil types and climates (22). Losses can reach up to 80% (5). The disease has also been reported in Bolivia, Colombia and Ecuador (12).

Rhizoctonia canker (R. solani).- The disease is present in all countries of the Andean zone. As reported by Anguiz (2), there are two groups of Rhizoctonia anastomosis (AG) in Peru. The AG3 is present in the highlands like Huancayo (3320 masl) and AG4 is present in the lowlands like San Ramón and La Molina (800 and 400 masl, respectively). Losses caused by Rhizoctonia canker depends of the AG. In places where AG4 is present, the losses will be more than in places where AG3 is present, because AG4 is more aggressive and it has a wider host range than AG3 (2).

Rosellinia black rot (Rosellinia sp.).- With exception of Ecuador where the disease affects the potato crop seriously (17), in other countries this disease is present only occasionally. In all cases it has been observed in warm areas where the soil has high organic matter content. Under favorable environmental conditions (high soil humidity and poorly drained), the losses in Ecuador can reach up to 100% (17).

White mold (S. sclerotiorum).- This disease is present in cold places of all Andean zones. In Bolivia, the disease has been observed at 3700 masl (18). In Peru it has been observed between 3000 and 3700 masl; but, in wet and cool seasons it is possible to find it in the coastal areas. In Cañete-Peru (sea level) 44% of potato plants showed wilting symptoms caused by Sclerotinia (9).

Charcoal rot (M. phaseoli), Black dot (C. atramentarium), Stem rot (S. rolfsii), Dry rot (Fusarium spp.) and Common scab (S. scabies) are mostly present in the lowlands; but sometimes they are also in the highlands but are not of economic importance. Dry rot is very important in storage, but not in the field. Losses will depend especially on managment during harvest and transit to storages. Common scab was reported in the lowlands like Santa Cruz (Bolivia) and Monagas (Venezuela), apparently as a consequence of the importation of the seed tubers from countries where the disease is present.

B) FOLIAR DISEASES

Eight foliar diseases are present in the Andean highlands (Table 2). Apart from late blight wich is very severe throughout the Andean zone, other foliar diseases such as phoma leaf spot (28,29) and septoria leaf spot (6,7,15) and early blight (Alternaria solani, A. brassicae and A. porri) can be present in this region (11). However, their economic importance is generally low. Apparently phoma leaf spot and septoria leaf spot commonly affect more late maturing cultivars as compared to early blight that affect more early maturing cultivars. Phoma leaf is present in dry and cool areas in altitudes between 2800 and 3500 m., septoria leaf spot is in wet and cold areas from 3500 to 4000 m elevation and early blight is in interandean valleys and in warmer places. There is no information on the losses caused by septoria leaf spot. Losses caused by phoma leaf spot has been reported to reach up to 80% (12).

Table 2.- Foliar diseases in the Andean highlands

Late blight (Phytophthora infestans (Mont) de Bary)
Early blight (Alternaria spp.)
Phoma leaf spot (Phoma andina Turkensteen)
Septoria leaf spot (Septoria lycopersici Speg.)
Common rust (Puccinia pittieriana P. Henn.)
Peruvian rust (Aecidium cantensis Arthur)
Powdery mildew (Erysiphe cichoracearum DC. ex Merat)
Ulocladium blight= Kasahui (Ulocladium spp.)

Common rust is present in Bolivia, Peru and Ecuador, but it is only important in Ecuador. Peruvian rust is present in Peru without economic importance. Powdery mildew is present in all Andean zone affecting senescent plants and Ulocladium leaf spot is important in altiplano zone of Perú and Bolivia (4000 masl).

CIP EXPERIMENTS TO CONTROL SOIL-BORNE DISEASES

In the last Planning Conference on fungal diseases carried out in 1978 in Lima, it was recomended to initiate research to control some of the soil-borne diseases. Verticillium wilt (Verticillium spp.) was considered as one the most important soil-borne disease in the world. Pink rot (P. erythroseptica), Powdery scab (S. subterranea), Potato smut (A. solani) and Black wart (S. endobioticum) were considered as local or regional diseases. These diseases are present in CIP's experimental station at Huancayo. They seriously threatened the maintenance of the Germplasm Collection and most experiments.

Verticillium wilt (Verticillium spp.)

Samples of wilting plants collected at Huancayo, had up to 40% infection with Verticillium sp. Further studies indicated that the morphological characteristics of the fungus corresponded to V. dahliae (23). Later, the fungus was detected in the highlands of the Mantaro Valley (16,19) and in all the Coastal areas of Peru where potatoes are planted (Torres and Tivoli 1986 unpublished). Based on these results, V. dahliae is probably more wide spread than V. albo-atrum, wich was also reported in Peru (4).

To study the host range and soil survival of V. dahliae in the Mantaro Valley, 23 fields used in rotation for potato production were sampled to collect weed plants. V. dahliae was recovered from eighteen out of 65 weed species under natural conditions. Ten of these weeds did not show Verticillium wilt symptoms (14).

Pink rot (P. erythroseptica)

Pink rot of potatoes was reported for the first time in Peru by Torres et al (20). At Huancayo where CIP maintains the Potato Germplasm Collection and where the disease has been extremely severe on some of the diploid clones, tuber losses exceeding 80% have been reported (21,26). To find an effective chemical control, various field trials were carried out from 1975 to 1981. These included evaluation of different chemical products and optimun dose levels, e.g. Basamid (Dazomet 98%), fumigants alone and in combination with Metalaxyl (Ridomil 5G), Basamid and Ridomil 5G alone and in combination, and different doses of Basamid + Ridomil 5G.

The fumigants Basamid, Methyl bromide, Sodium azide and Ditrapex increased production, but did not control the

disease. The most effective chemical control of pink rot was obtained with the use of Basamid (300 kg/ha) + Ridomil 5G (30 kg/ha) wich produced a 100% yield increase and reduced disease incidence from 31% to 1%. Basamid was applied one month before planting and Ridomil 5G at planting time accomplishing good results (26).

Potato smut (A. solani)

This disease also severely affected several accessicns of the CIP Potato Germplasm Collection at Huancayo (21,22). Hypertrophic symptoms in stems, stolons, and tubers were commonly observed at harvest. Symptoms like protuberances on the surface of the tuber were also observed (22). These protuberances after 2-3 months of storage became sunken lesions and subsequently hyperyhrophied tissues developed in or close to the new sprouts. Later the epidermal membrane of the hypertrophied tissues broke off and the spores spread.

Experiments to control this disease using fumigants and fungicides were conducted during 1979 to 1981 at Comas (3100 m), Peru. The fumigants Basamid and Methyl bromide controlled the disease and increased yields (25).

Potato clones and cultivars were also evaluated to determine resistance to A. solani in a naturally infested field at Comas, Peru (3100 m elevation). Among 179 accessions tested, seven were resistant: Cusco, Mariva, Mi Peru, Participación, Revolucion, 376181.5, 6959.52 (27).

Results on the chemical control have been effectively applied at CIP's Huancayo Station. Additionally, the resistant peruvian cultivars have been recommended to farmers as an inmediatly solution to control potato smut in places of Peru where the disease is a problem.

Black Wart (S. endobioticum)

Inicially, screening for resistance to black wart was done in naturally infested soil and in greenhouse conditions from 1968 to 1975. Out of 1500 accessions evaluated from the Potato Germplasm Collection, accessions G0075, G0116, G0382 and G0672, all of which belong to S. tuberosum ssp. andigena were resistant to the disease (24).

Later, crosses between resistant clones and with clones resistant to Globodera pallida were made by breeders Proudfoot and Soto. Using the Glynne-Lemershall method, 684 clones from 130 families were tested against Black wart under laboratory conditions. Using the Spieckerman method

the resistant clones were evaluated under greenhouse conditions. After 3 seasons of evaluation 24 resistant clones and 6 tolerant clones were selected (10).

Thirteen European differentials were all susceptible to 14 isolates of S. endobioticum from different places in Peru as determined by the Spieckerman method. Thus none of the Peruvian isolates were similar to any single European wart race (13).

Premature-death of Potato seedlings.

Premature-death of potato seedlings caused by a complex of pathogens, mainly Rhizoctonia, Fusarium, and Erwinia species is common in the coastal valleys of Peru. Four field trials were carried out in La Molina from 1984 to 1987 under irrigated desert conditions to study the control of this problem with chemicals. In the first two experiments, treatments included the aplication of fungicides Metalaxyl 5G, Tolclofos Methyl and PCNB + Benomyl (control) in combinations with or without a preplanting application of Basamid and the use of solarization. Plots treated with preplanting fumigant and plastic cover during one month + Tolclofos methyl applied at planting time yielded up to 150% more than the control.

Potato seedlings are also severely affected by this pathological complex at San Ramón. Pythium, Rhizoctonia, Fusarium, Macrophomina, Erwinia and Pseudomonas were identified affecting potato seedlings. The treatments Basamid + Tolclofos Methyl and Methyl bromide + Tolclofos Methyl were more efficient than the other treatments tested.

LITERATURE CITED

1. Abbott E.V. 1928. La Roña y La Rancha o Hielo de la Papa. Estación Experimental Agrícola de la Sociedad Nacional Agraria Lima-Perú. Circular No 7 11 pp.

2. Anguiz R. y C. Martin 1986. Identification of Anastomosis groups (AG) of Rhizoctonia solani isolated from potatoes in three ecological areas in Peru. Identificación de los grupos de Anastomosis(AG) de Rhizoctonia solani aislados de papa en tres zonas ecológicas del Peru. Fitopatología 21(1):3. Resumen.

3. Barrus, M.F. & A.S. Muller. 1943. An Andean disease of Potato tubers. Phytopathology 33:1086-1089.

4. Bazán de Segura Consuelo. 1965. Enfermedades de los cultivos tropicales y subtropicales. Edit. Jurídica. Lima, Peru. 439 pp.

5. Bazán de Segura Consuelo. 1960. The Gangrene disease of Potato in Peru. Plant. Dis. Rep. 44:257.

6. Carrera J. y H. Orellana. 1978. Estudio de la Mancha Foliar de la papa Septoria lycopersici Sub grupo A en el Ecuador. Fitopatología 13(1):51-57.

7. Ciccarone C. y G. Malagutti. 1968. Una interesante septoriosis de la papa en los Andes Venezolanos. Fitotecnia Latinoamericana 5(1):51-53.

8. Franco J. y Esther Bendezú. 1985. Estudio del Complejo Verticillium dahliae Kleb. y Globodera pallida Stone y su efecto en el comportamiento de algunos cultivares peruanos de papa. Fitopatología 20(1):21-27.

9. French E.R., H. Torres, et al 1972. Enfermedades de la Papa en el Peru. Ministerio de Agricultura. Boletín Técnico No 77, 36 pp.

10. Galindez W., F. Frey and G. Lopez. 1982. Laboratory and Greenhouse selection of Synchytrium endobioticum resistant clones. En Fitopatología 17(1):4 Resum.

11. García Evelyn L., C. Martin y H. Torres 1986 Isolation and identification of the causal agent of Early blight of Potatoes in San Ramón and Huancayo. Aislamiento e identificación del agente causal del Tizón temprano de la papa en San Ramón y Huancayo. En Fitopatología 21(1):6 Resumen.

12. Hooker W.J. 1981. Compendium of Potato Diseases. International Potato Center. 125 pp.

13. International Potato Center. 1980. CIP Annual Report. 133 pp.

14. _____ 1985. CIP Annual Report. 175 pp.

15. Jimenez A. y E.R. French. 1972. Mancha anular foliar Septoria lycopersici Sub-grupo A de la papa. Fitopatología 5-6:15-20

16. Martin C. 1985. Verticillium wilt of Potato in Central Peru. Am. Pot. Journ. 62:195-199.

17. Orellana H. 1978. Estudio de la enfermedad "Lanosa" de la papa en el Ecuador. Fitopatología 13(1):61-66.

18. Otazú V., W.M. Brown y Mery de Quitón. 1982. Enfermedades de las plantas en Bolivia. Ministerio de Asuntos Campesinos y Agropecuarios. Instituto Boliviano de Tecnología Agropecuaria. Consorcio Internacional para el Desarrollo. 30 pp.

19. Pinillos Olga, W. Galindez y C. Martin 1986. Occurrence and distribution of potato wilt caused by Verticillium dahliae in the Mantaro valley. Ocurrencia y distribución de la papa causada por Verticillium dahliae en el Valle del Mantaro. En Fitopatología 21(1):9. Resumen.

20. Torres H., E.R. French and L.W. Nielsen. 1970. Potato diseases in Peru 1965-1968. Plant. Dis. Rep. 54(4):315-318.

21. _____ 1978. Evaluación de enfemedades fungosas en el Banco de Germoplasma del CIP (1975-1978). Informe no publicado 142 pp.

22. _____ 1979. Carbón de la Papa (Thecaphora solani Bar.) en Banco de Germoplasma de Papa. Fitopatología 14(1):15-22.

23. _____ y H. Gutierrez 1981. Verticillium dahliae Kleb., Identificación y síntomas que produce en seis variedades comerciales de papas peruanas. Fitopatología 16(2):60-68.

24. _____ 1981. Búsqueda de Fuentes de Resistencia a la Verruga de la Papa (Synchytrium endobioticum (Shilb.) Perc.). Univ. Nac. Agraria La Molina. Tesis Magister Scientiae 56 pp.

25. _____ y J. Henfling. 1984. Control químico del carbon de la papa (Thecaphora solani). Fitopatología 19:1-7

26. _____, C. Martin and J. Henfling. 1985. Chemical control of pink rot of potato (Phytophthora erythroseptica Pethyb.). Am. Pot. Journ. 62:355-362.

27. _____ and C. Martin. 1986. Field Screening for resistance to Potato Smut in Peru. Am. Pot. Journ. 63:559-562

28. Turkensteen L.J. 1978. Tizón foliar de la apa en el Peru. I Especies de Phoma asociadas. Fitopatología 13(1):67-69.

29. _____ 1978. Tizón foliar de la apa en el Peru. II Patogenecidad de las especies de Phoma y otros hongos asociados y el organismo causal. Fitopatología 13(1):70-72.

ECOLOGY OF *Rhizoctonia solani* AND DISEASE DEVELOPMENT IN RELATION TO ANASTOMOSIS GROUPS

Carlos Castro

Rhizoctonia solani Kühn is worldwide in distribution in crop plants and even occurs in uncultivated areas. This distribution clearly indicates that the fungus has been present a long time. Although it is a well known soilborne pathogen of potato, data on total losses caused by *R. solani* are, without question, excessively low and quite variable.

Despite being endemic it is not considered an economically important pathogen in potato areas in Australia (16) and United States (21). Davis and Groskopp (19) found no yield reduction, but tuber size differences were evident, whereas yield decrease have ranged from 15 to 30% in England (51), in Canada (5) and in Germany (50).

During the past 37 years, considerable knowledge has become available on the ecology of *R. solani*. However, with a few exceptions, most of the ecological research on *R. solani* has been done in the laboratory and greenhouse. Although studies with models may sometimes be extrapolated to the field, such studies often do not reflet the natural conditions and stresses to which pathogens are subjected in the field.

This paper will focus primarily on recent researches concerning the parasitic, saprophytic and survival aspects of *R. solani*.

THE FUNGUS

R. solani is currently composed of several anastomosis groups (AG's) distinguished by hyphal anastomosis (41, 48). These groups may differ morphologically, physiologically and serologically (1, 36, 53) and host range, virulence, and distribution in nature (28, 39, 48). On a worldwide basis eight anastomosis groups have been reported (4, 30, 38, 48). With the exception of AG-BI (30), the anastomosis groups are genetically isolated (7, 31).

Anastomosis groups of *R. solani* are found all over the world, but their distribution and population depend on the crops that are cultivated there. In a small area or in a particular agricultural field, the anastomosis groups that occur are limited (25, 54). AG-2 and AG-3 have shown greater host specificity whereas AG-1 and AG-4 lack such specificity (9). Although AG-2 and AG-4 have been found associated to potato in Brazil and Peru (12, 14) the AG-3 is likely to be the most pathogenic group to potato (40, 42). This group has also induced stunt of barley and oats in England and Scotland (34, 37).

Thus the cultivated crop must exert a strong influence on the prevalence of a given group in certain areas. In the state of our knowledge, to have quarantine on groups of the fungus is still impossible, but this may be desirable in the future. Practical agriculturists have considered *R. solani* as a single entity and have

concluded that, since the species is widespread, the introduction of more of the fungus to a given soil is unimportant. It obviously ignores the significance of anastomosis group differences. The future emphasis on anastomosis groups of R. solani should be on recognition of their distribution, host range, ecology and economic importance.

However, the behaviour of this fungus cannot be completely understood only in terms of the anastomosis group.

THE DISEASE

R. solani spreads mainly by mycelium and sclerotia (0.1-10 mm) in soil, plant propagules and equipments. Sclerotia resist drying and may be viable for up to 5 years. When mycelia contact a host, they form infection cushions or appressoria and infection pegs. R. solani AG-3 penetrated roots of barley by infection pegs and penetrated coleoptile by cushions (34). Infection of potato is generally manifested in one or more of three ways:

a) infection of emerging sprouts, delaying or preventing emergence, or decreasing stem number. Sprout infection more commonly decreases the number of stems per hill, but the number of tubers set per stem then increases. Correlation between stem canker and yield has been controversial; while Cother (16) found no correlation, Weinhold (57) reported a negative correlation ($r = -0.25$). Experiments of Cother and Cullis (17) showed that stem canker did not significantly affect haulm fresh weight or tuber yield.

b) pruning of stolons resulting in fewer tuber forming sites, or possible initiation of other tubers that are unlikely to contribute to marketable yield. When the stolon pruning was below 18%, Cother and Cullis (17) did not detect yield reduction and for every 20% increase in pruning above this threshold yield was reduced only 6%. The general consequence of stolon pruning is fewer, larger tubers of the same total weight that has been produced as smaller tubers by more but uninfected stolons.

c) sclerotia on tubers which may be undesirable if market quality is downgraded or long term storage is required. Infection through lenticels of Streptomyces scabies lesions have resulted in deeper penetration (13).

The importance of each of these categories in a particular region needs to be assessed to establish the relative significance and economic impact of the disease before control measures are devised. In new growing areas (e.g. lowland tropics) or where R. solani is not present, tuber treatment to prevent introduction of this pathogen may be warranted. However, where R. solani is endemic, research has to demonstrate that this pathogen causes a consistent loss of yield.

R. solani is a primary pathogen of juvenile tissues and is unable to maintain aggressive pathogenicity as tissues mature (32). The

meristematic tips of tuber sprouts are very susceptible to R. solani, therefore, shallow planting or favorable growing conditions are likely to reduce the time of exposure and thus decrease the losses. As the plant stems mature and become more resistant, the lesions formed become smaller and less injurious. Stem cankers may restrict downward movement of photosynthates, which then accumulate in the tops. This accumulation may produce stunting and rosetting of the top, the resultant production of anthocianin may cause purple pigmentation, and aerial tubers may be formed in the axils of branches and petioles. Infection may result from mycelia either in the soil or produced from sclerotia on the seed piece. Stem canker incidence have been more closely related to the incidence of sclerotia on seed tubers than to the frequency of R. solani hyphae on growing tuber surfaces (2).

Among many environmental factors, temperature and moisture of soil have great effect on the disease development. Susceptibility of seedlings (22, 23) and plants (50) is strongly related to the soil temperature between 10 and 25 C (29). Damping-off due to R. solani increased with temperature above 20 C (22). The most disease is induced at relatively high osmotic potencial (-1 to -10 bars), where the soils are moist and plants are not stressed for lack of water (15).

METHODOLOGY

Accurated studies on the ecology and epidemiology of R. solani are dependent on sensitive and reliable methods for its detection and quantification. Several methods have been developed for estimating populations of R. solani, but their efficiency for recovering slow growing anastomosis groups, such as AG-3, usually is not tested. The soil-pelleting technique (27) was found to be more efficient than the wet-sieving technique (10) and, after improvement (11), its efficiency for recovering AG-3 isolates was 75%. It has also been demonstrated that storing soil at room temperature significantly reduce the number of viable propagules of R. solani (11), therefore, the native populations of this fungus in field soils may be best represented in freshly collected soil samples.

EPIDEMIOLOGY AND SURVIVAL

Populations of R. solani in natural soil, although highly variable (56), are relatively low (32), exist in the upper 15 cm of the soil (44), and the distribution of propagules is clumped or nonrandom (32, 35). Epidemiological studies indicate that inoculum levels in field soils depend on time of sampling, absence of susceptible host, weather conditions and kind of propagule (dormant mycelia or sclerotia). Soil populations were larger at the earlier stages of crop growth (34); and the highest inoculum levels were detected in the fall and the lowest in the spring (44). It is a well established

viewpoint that R. solani is able to make free and independent growth in natural and complex soil microenvironment. It has evolved rather complicated relationships with other microorganisms in its ability to survive and grow through the soil quite successfully under a wide range of conditions, despite the large variety of antagonists involved.

It is extremely difficult to determine which characteristics of R. solani are the most important for its survival or predominance in soil ecosystem. The degree of saprophytism and longevity is strongly interrelated. R. solani persists in soil as saprophyte in tissues infected during parasitism or by saprophytically colonizing dead plant tissues in which it can remain dormant or active for long periods of time. According to Sanford (52), parasitism is more important than saprophytism in the survival. He could not detect Rhizoctonia in a soil heavily infested after four months in the absence of a susceptible crop; however, he did recover the fungus up to 8 months under soils planted to susceptible crops.

Saprophytic survival of R. solani may be influenced by several soil factors:

a) carbon-to-nitrogen balance of the substrate. Findings of Papavizas et al.(46) showed that oat straw with C/N ratio of 30 and 85 was more effective in reducing saprophytic survival of R. solani in colonized substrate segments than oat straw with C/N ratio 10.

b) the soil environment. Depletion of certain nutrients in ephemeral food bases of the soil ecosystem led to a decline of parasitism and saprophytism of this fungus (43). A positive correlation was found between saprophytic activity of R. solani and total inorganic nitrogen and NH_4-N, but not NO_3-N (44). Dube (20) found that mycelium of R. solani grown under moist conditions died when exposed to sudden dessication; mycelium grown first under moderate drought and exposed gradually to the same degree of dessication survived. In soils which are moist for most of the year, R. solani remains more or less continuous vegetative growth; in soils which become extremely dry for several months (e.g. South Australia and South California) the fungus necessarily becomes dormant, either as thickwalled mycelia or sclerotia (3). These findings agree with Ploetz (49), who demonstrated that survival of R. solani was generally greater at intermediate water potentials of -2 to -15 bars than moister or drier soils. It is also known that the pathogenic phase of R. solani is more sensitive to high CO_2 concentrations than its active saprophyte phase (45).

What are the most important soil environmental factors to the incidence of R. solani on potatoes? The ability of certain anastomosis groups to survive different soil temperatures still needs to be studied more deeply. The predominance of AG-3 on potatoes in cold growing areas of the world may suggest this group is more adapted to low

temperatures. Such assumption remains to be investigated. It has been reported (6, 44) that R. solani may survive in soil with temperatures between - 5 and 30 C, but survives three times better at 15 than 26 C.

c) the innate constitution of isolates. Research carried out by van Bruggen and Arneson (55) demonstrated that the ability of large sclerotia to survive in soil and infect bean hypocotyl was greater than small sclerotia. Weak sclerotia (0.3-0.5 mm) needed an extraneous nutrient source to stimulate hyphal growth.

Significant contribution on population and survival of R. solani has recently been published by Naiki (35). He showed that the sclerotial number declined progressively with soil depth and increasing distance from diseased roots. There was also a progressive decline in sclerotial vigor and viability with increasing distance from an infected root and with increasing sampling depth.

The importance of the soilborne and tuberborne inocula has been related to disease development and survival of the fungus, respectively. Potato tubers commonly carry sclerotia of R. solani on their surface and the use of infected tubers for seed may result in infection of stems and new tubers. Tuberborne sclerotia are considered the most important source of inoculum in some areas, and seed treatment have resulted beneficial (8, 18). In other areas, because of the high inoculum potentials of the pathogen in soil, chemical treatments are no longer recommended.

CULTURAL PRACTICES AND BIOLOGICAL CONTROL

R. solani is an aggressive saprophyte that can increase its biomass and energy for attack of a crop when supplied with a fresh plant residue. However, R. solani may also be more subject than most soilborne plant pathogenic fungi to attack by several mycoparasites in soil. Biological control in less developed countries is accomplished mainly through agricultural practices because expensive equipments and large capital investiment are not required for some types of biological control practices. Reduced colonization of plant residues by R. solani was accomplished by plowing the field with a moldboard plow to 20-25 cm deep, rather than disking to 3-7 cm (47). Deep plowing buried the inoculum in soil where it could not survive or colonize organic substrates, decreased root rot, and reduced inoculum in the upper layers of soil. Gudmestad et al. (26) in a crop sequence of potato-wheat, plowing the wheat stuble 26 cm deep increased disease, but mixing the straw into the top 10 cm of soil suppressed disease. The disease suppression obtained with wheat straw resulted from the intensification of microbiological activity and antagonism. Management of the residue of potato plants infected with R. solani was a different matter, because such residue serves as an important source of inoculum for infection of the next potato crop. So, Gudmestad et al. (26) recommended disking as the best tillage method where potatoes

follow wheat, they recommended deep plowing when potatoes follow potatoes to bury the infested host residue beyond the reach of stolons and stems of the next potato crop. Undoubtedly, persistence of R. solani in crop residues is somewhat dependent on the crop species. In selecting cropping sequences to control R. solani we must consider the pathogen susceptibility and the persistence of the pathogen in crop residues. Very few researches report survival of R. solani on different crop residues in field soil under different environmental conditions. Different anastomosis groups of R. solani may differ significantly in their sensitivity to the antagonists. Very little is known about the effect of different cultural practices on certain antagonists. Soils differ markedly in their antagonistic effect. We also lack knowledge of the effect of agrichemicals on biological balance.

In summary, much more work will need to be done to determine the distribution and potential importance of anastomosis groups of R. solani as a cause of potato loss. The ultimate control of the Rhizoctonia disease will depend on our understanding on the ecology and epidemiology of this pathogen in the field and the influence of soil, climate, plant residues and antagonistic microorganisms. Lines for future research were suggested in this article in the belief that they may lead to further understanding of these aspects of the biology of this pathogen in order to develop rational and effective control.

LITERATURE CITED

1. Adams, G.C., Jr., and Butler, E.E. 1979. Serological relationships among anastomosis groups of Rhizoctonia solani. Phytopathology 69:629-33.
2. Adams, M.J., Hide, G.A., and Lapwood, D.R. 1980. Relationships between disease levels on seed tubers, on crops during growth and in stored potatoes. 1. Introduction and black scurf. Potato Res. 23:201-14.
3. Baker, K.F. 1970. Types of Rhizoctonia diseases and their occurrence. Pages 125-148 in Rhizoctonia solani: Biology and Pathology. University of California Press, Berkeley, CA. 255 pp.
4. Bandy, B.P., Zanziger, D.H., and Tavantzis, S.M. 1984. Isolation of anastomosis group 5 of Rhizoctonia solani from potato field soils in Maine. Phytopathology 74:1220-24.
5. Banville, G.J. 1978. Studies on the Rhizoctonia disease of potatoes. Amer. Potato Journal 55:56.
6. Benson, D.M., and Baker, R. 1974. Epidemiology of Rhizoctonia solani preemergence damping-off of radish: survival. Phytopathology 64:1163-68.
7. Bolkan, H.A. 1976. Attempts to bridge anastomosis groups of Rhizoctonia solani. Fitopatol. Bras. 1:14-17.
8. Bolkan, H.A. 1976. Seed tuber treatment for the control of black scurf disease of potatoes. N.Z.J.Exp. Agric. 4:357-61.
9. Bolkan, H.A., and Ribeiro, W.R.C. 1985. Anastomosis groups and pathogenicity of Rhizoctonia solani isolates from Brazil. Plant Disease 69:599-601.
10. Bruggen, A.H.C. van, Arneson, P.A. 1986. Quantitative recovery of Rhizoctonia solani from soil. Plant Disease 70:320-23.
11. Castro, C. 1983. Method for the quantitative estimation of Rhizoctonia solani in soil. Ph.D. Thesis, Univ. of Idaho, ID, U.S.A. 70pp.
12. Castro, C., and Tavares, F.N. 1986. Caracterização de isolados de Rhizoctonia spp. no Brasil. Summa Phytopathologica 12:26.
13. Chand, T., Logan, C. 1984. Postharvest development of Rhizoctonia solani and its penetration of potato tubers in Northern Irland. Trans. Br. Mycol.Soc. 82:615-19.
14. Centro Internacional de la Papa. 1986. Investigación en enfermedades bacterianas y fungosas. Pages 49-61 in Informe Anual del CIP, 1985. Lima, Peru. 184pp.
15. Cook, R.J., Baker, K.F. 1983. The pathogen in biological control. Pages 134-171 in The Nature and Practice of Biological Control of Plant Pathogens. The American Phytopathological Society, St. Paul, MN 539 pp.
16. Cother, E.J. 1983. Response of potato in a semi-arid environment to chemical control of Rhizoctonia solani. Potato Res. 26:31-40.

17. Cother, E.J., and Cullis, B.R. 1985. Tuber size distribution in cv. Sebago and quantitative effects of Rhizoctonia solani on yield. Potato Res. 28:1-14.
18. Davis, J.R. 1973. Seed and soil treatments for control of Rhizoctonia and black leg of potatoes. Plant Dis. Reptr. 57:803-06.
19. Davis, J.R., and Groskopp, M.D. 1979. Influences of the Rhizoctonia disease on production of the Russet Burbank potato. Am. Potato J. 56:253-64.
20. Dube, A.J. 1971. Studies on the growth and survival of Rhizoctonia solani. Ph.D. Thesis, Univ. Adelaide, Adelaide, Australia. 144 pp.
21. Easton, G.D. 1978. The Rhizoctonia disease of potato in Washington. Am. Potato J. 55:57-58.
22. Elango, F. 1982. A simple greenhouse inoculation technique for screening true potato seedlings for their tolerance to Rhizoctonia solani-induced damping-off. Am. Potato J. 59:466-7.
23. Elango, F. 1983. Strategy for controlling potato true seedlings damping-off caused by Rhizoctonia solani in the Peruvian lowland tropics. Pages 77-78 in Research for potato in the year 2000, Proceedings. International Potato Center, Lima, Peru. 199 pp.
24. Frank, J. A. 1975. The relative importance of potato tuber-borne Rhizoctonia inoculum in comparison to soil-borne inoculum. Am. Potato J. 52:244.
25. Grisham, M.P., Anderson, N.A. 1983. Pathogenicity and host specificity of Rhizoctonia solani isolated from carrots. Phytopathology 73:1564-69.
26. Gudmestad, N.C., Huguelet, J.E., and Zink, R.T. 1978. The effect of cultural practices and straw incorporation into the soil on Rhizoctonia disease of potato. Plant Dis. Reptr. 62:985-89.
27. Henis, Y., Gaffar, A., Baker, R., and Gillespie, S.L. 1978 A new pellet soil-sampler and its use for the study of population dynamics of Rhizoctonia solani in soil. Phytopathology 68:371-6.
28. Herr, L.J., and Roberts, D.L. 1980. Characterization of Rhizoctonia populations obtained from sugarbeet fields with differing textures. Phytopathology 70:476-80.
29. Hollins, T.W., Jellis, G.J., and Scott, P.R. 1983. Infection of potato and wheat by isolates of Rhizoctonia solani and R. cerealis. Plant Pathology 32:303-10.
30. Kuninaga, S., Yokosawa, R., and Ogoshi, A. 1978. Anastomosis groupings of Rhizoctonia solani Kühn isolated from noncultivated soils. Ann. Phytopathol. Soc. Jpn. 44:591-98.
31. Kuninaga, S., and Yokosawa, R. 1980. A comparison of DNA base composition among anastomosis groups in Rhizoctonia solani Kühn. Ann. Phytopath. Soc. Jpn. 46:150-58.

32. Martin, S.B., Campbell, C.L. and Lucas, L.T. 1983. Horizontal distribution and characterization of Rhizoctonia spp. in tall fescue from turf. Phytopathology 73:1064-68.
33. Moen, R., and Harris, J.R. 1985. The Rhizoctonia disease complex of wheat. Pages 48-50 in Ecology and management of soilborne plant pathogens. The American Phytopathological Society, St. Paul, MN. 358 pp.
34. Murray, D.I.L. 1981. Rhizoctonia solani causing barley stunt disorder. Trans. Br. Mycol. Soc. 76:383-95.
35. Naiki, T. 1985. Population and survival of sclerotia of Rhizoctonia solani in soil. Pages 51-51 in Ecology and management of soilborne plant pathogens. The American Phytopathological Society, St. Paul, MN. 358 pp.
36. Naiki, T., and Ui, T. 1978. Ecological and morphological characteristics of the sclerotia of Rhizoctonia solani Kühn produced in soil. Soil Biol. Biochem. 10:471-78.
37. Neate, S.M. 1985. Rhizoctonia in South Australian wheat fields. Pages 54-56 in Ecology and management of soilborne plant pathogens. The American Phytopathological Society, St. Paul, MN. 358 pp.
38. Ogoshi, A. 1975. Groupings of Rhizoctonia solani and their perfect stages. Rev. Plant Prot. Res. 8:93-103.
39. Ogoshi, A. 1976. Studies on the grouping of Rhizoctonia solani Kühn with hyphal anastomosis and on the perfect stages of groups. Bull. Natl. Inst. Agric. Sci., Ser. C., 30:1-63.
40. Ogoshi, A., and Ui. T. 1983. Diversity of clones within an anastomosis group of Rhizoctonia solani kühn in a field. Ann. Phytopathol. Soc. Jpn. 49:239-245
41. Ogoshi, A., and Ui, T. 1985. Anastomosis groups of Rhizoctonia solani and binucleate Rhizoctonia. Pages 57-58 in Ecology and management of soilborne plant pathogens. The American Phytopathological Society, St. Paul, MN. 358 pp.
42. Otrysko, B.E., Banville, G.J., and Asselin, A. 1985. Membership of anastomosis group AG-5 and pathogenicity of Rhizoctonia solani isolates obtained from sclerotia from the surface of potato tubers. Phytoprotection 66:17-21.
43. Papavizas, G.C., and Ayers, W.A. 1965. Virulence, host range, and pectolitic enzimes of single-basidiospore isolates of Rhizoctonia practicola and Rhizoctonia solani. Phytopathology 55:55:111-16.
44. Papavizas, G.C., Adams, P.B., Lumsden, R.D., Lewis, J.A., Dow, R.L., Ayers, W.A., and Kantzes, J.G. 1975. Ecology and epidemiology of Rhizoctonia solani in field soil. Phytopathology 65:871-7.
45. Papavizas, G.C., and Davey, C.B. 1962. Activity of Rhizoctonia in soil as affected by carbon dioxide. Phytopathology 52:759-66.

46. Papavizas, G.C., Davey, C.B., and Woodard, R.S. 1962. Comparative effectiveness of some organic amendments and fungicides in reducing activity and survival of Rhizoctonia solani in soil. Can. J. Microbiol. 8:915-22.
47. Papavizas, G.C., and Lewis, J.A. 1979. Integrated control of Rhizoctonia solani. Pages 415-424 in Soilborne Plant Pathogens. Academic Press, New York, NY. 686 pp.
48. Parmeter,J.C., Jr., Sherwood, R.T., and Platt, W.D. 1969. Anastomosis groups among isolates of Thanatephorus cucumeris. Phytopathology 59:1270-8.
49. Ploetz, R.C., and Mitchell, D.J. 1985. Influence of water potential on the survival and saprophytic activity of Rhizoctonia solani AG-4 in natural soil. Can.J. Botany 63:2364-68.
50. Roth, R. 1985. Occurrence and injurius effect of Rhizoctonia solani Kühn in field plot trials on soundy brown earth soil at Münchenberg. Archiv. für Phytopathologie und Pflanzenschutz 21: 465-69.
51. Rothamsted Experimental Station. 1981. Potato diseases. Harpenden, Herts., U.K. Report for 1983: 126-127.
52. Sanford, G.B. 1952. Persistence of Rhizoctonia solani kühn in soil. Can. J. Botany 30-652-64.
53. Sherwood, R.T. 1969. Morphology and physiology in four anastomosis groups of Thanatephorus cucumeris. Phytopathology 59:1924-9.
54. Sterne, R.E., and Jones, J.P. 1978. Sharp eyespot of wheat in Arkansas caused by Rhizoctonia solani. Plant Dis. Reptr. 62:56-60.
55. van Bruggen, A.H.C., and Arneson, P.A. 1985. A quantifiable type of inoculum Rhizoctonia solani. Plant Disease 69:966-69.
56. Weinhold, A.R. 1977. Population of Rhizoctonia solani in agricultural soils determined by a screening procedure. Phytopathology 67:566-9.
57. Weinhold, A.R., Bowman, T., and Hall, D.H. 1982. Rhizoctonia disease of potato: Effect on yield and control by seed tuber treatment. Plant Disease 66:815-18.

CONTROL OF RHIZOCTONIA AND OTHER SOIL-BORNE DISEASES OF TPS

Carlos Martin and Hebert Torres

Traditionally, farmers around the world have used seed tubers to produce potatoes for food as well as for planting. In developing countries, one of the most important factors limiting potato production is the lack of good quality and inexpensive seed tubers. These factors not only reduce potato production but also reduce the possibilities of expanding the potato into new areas of potential production. Seed tubers represent 40-70% of the crop production cost, and approximately two tons of costly, perishable seed tubers are needed to plant one hectare (1,13,14,22,23). These considerations have led the International Potato Center (CIP) along with other research institutions, to study the use of true potato seed (TPS) as an alternative method for potato production.

The advantages of using TPS have been extensively discussed by several potato scientists in the past years (1,13,14,22) and they can be summarized as follows:

1.- It is a low-cost planting material, and total production costs can be reduced drastically in those cases where hand labor is not expensive or potatoes are grown by subsistence farmers or in backyard gardens.
2.- There are several tuber-transmitted diseases which are not TPS transmitted.
3.- No seed tuber storage is involved, decreasing total production costs and storage losses.
4.- Finally, but no less important, TPS would make possible expanding potato cultivation to new areas previuosly unable to obtain good quality seed tubers.

Producing potatoes from TPS is not a new idea and scientists have used it for several decades in breeding research to develop new varieties (1).

Sowing TPS directly in the field or sowing in seedbeds for later transplant to the field are the two methods of growing potatoes from TPS that have been studied the most (1,13,14,22). Potatoes produced by either method can be used for food or as seed tubers (seedling tubers) depending on the advantages of each method at a given location.
Independently if TPS is sown for ware potato production or

for production of seedling tubers, there are a few diseases that can affect plant stand and therefore the final tuber production. Most of these diseases are caused by soil-borne pathogens or microorganisms that are on the TPS surface. Because potato diseases affecting adult well established plants are the same in plants coming from tubers or TPS, we will discuss during this presentation those diseases that affect seedlings during the first stage of plant emergence and development in the seedbeds as those affecting older seedlings after transplant to the field.

DISINFECTION OF TPS SURFACE

Seed surface contamination by saprophytes and pathogenic microorganisms have been reported several instances in the literature (4,16,20). Regulations for tomato seed certification in the USA states that a tolerance of 4 bacteria or 12 fungi per 200 seed will only be allowed (4). TPS does not escape to this situation of surface contamination, and disinfection has become a general practice at CIP.

Even when seedling damping-off is controlled by fungicides or soil sterilization, the quality of the TPS must still be considered as an important factor when establishing a seedbed. Normally, TPS inside the berry are free of pathogens, however, during the process of berry ripening or extraction TPS may become highly contaminated. TPS extracted aseptically from healthy looking berries collected at San Ramon and Huancayo were free of contaminants except for the presence of two yeasts that probably were involved in the fermentation process (C. Martin, unreported data).
Preliminary results obtained on TPS disinfection at CIP in 1983 recommended a treatment with 0.5-1% NaOCl for 10 minutes (13). Later, in order to study the presence of contaminants on the TPS surface Torres et al. (unreported data submitted for publication) assayed TPS from three different origens : San Ramon, Huancayo and La Molina. As shown in Table 1, 16 different fungi were isolated in large numbers from the seed surface. Pathogenicity tests carried out in PDA plates indicated that the mayority of these fungi were pathogenic to young seedlings, causing necrosis and also killing the hypocotyl. Within the same experiment, different chemical products were tested to control the contaminants. As shown in Table 2, TPS treated with any of the chemical products germinated significantly more than their respective untreated controls. The ANOVA also indicated that there was not a significant interaction between the products and the different types of seed treatment with gibberellic acid (GAc),indicating that

Table 1. Fungi isolated from TPS plated on PDA medium. TPS berries collected at La Molina (LM), San Ramon (SR) and Huancayo (Hyo).

Fungal sp.	Fungal sp.
Alternaria helianti (LM)	Curvularia lunata*(Hyo)
Alternaria alternata (LM, SR, Hyo)	Epicoccum sp. (LM, SR, Hyo)
Aspergillus niger* (LM, SR, Hyo)	Fusarium oxysporum* (LM, SR, Hyo)
Botrydiplodia sp (LM)	Monilia sp. (Hyo)
Botrytis cinerea* (Hyo)	Nigrospora sp. (LM)
Bipolaris sp* (LM)	Penicillium sp* (LM, SR, Hyo)
Cladosporium sp. (LM, SR, Hyo)	Trichoderma sp. (LM, SR)
Colletotrichum sp.* (Hyo)	Ulocladium sp.* (LM, Hyo)

* Pathogenic to TPS in laboratory assay.
Torres et al. (unreported data).

Table 2. Average percentage germinated TPS of clone DTO-33 disinfected with different chemical products with and without Gibberellic acid treatment.

Chemical Products	Gibberellic Acid (1000 ppm)			
	A[1/]	B	C	D
Hcl 1.8%	55	34	47	61
ClONa 0.5%	67	49	37	48
ClONa 1.0%	57	41	61	63
Dimanin 0.5%	82	73	64	79
Dimanin 1.0%	68	46	55	52
Alcide 1:10	46	0.0	7	59

Control - Gibb. Ac.	1.6
Control H_2O	0.0
Control - w/H_2O	0.0
LSD.05	5.8

[1/] A = 24 h before disinfection; B = 24 h after disinfection; C = together with disinfection; D = without Gib. Ac.

Torres et al. (unreported data).

disinfectans act independently of the GAc. Similar results were also obtained with regards to data collected on percent infected TPS and seedling height. As a consecuence of the studies it is a normal practice now at CIP to treat TPS with 0.5% NaOCl for 10 min. Disinfection with sodium hypochlorite has also proved to be effective on seed of other crops (4,16).

PRE AND POST EMERGENCE DAMPING-OFF.

At the nursery stage, when TPS is germinating and seedlings beginning to pop-out through the soil, several pathogens may affect seed germination and later plant development if non-sterilized soil or clean seed is used. Pre and post-emergence damping-off is the most common and prevalent disease affecting seed germination and seedling survival. Among the pathogens causing damping-off, Rhizoctonia solani and Pythium spp. has been frequently reported to cause severe reduction in seedling stands of a variety of plants, including bedding and non-bedding plants (3,6,7,12,13,14,22). These pathogens attack the growing, tender seedlings whose tissue is composed mostly of cells that have not yet developed thick secondary walls.

Pre and post-emergence damping-off of TPS due to R. solani became known to CIP scientists as soon as they started to develop the TPS technology, and several studies have been reported on different aspects of it, specially on control measurements (3,8,9,10,13,14). Results from these studies have shown that percentage of seedling damped-off increases with high temperatures, being maximun at 30-35 C (3,8,9)(Table 3). At CIP-Lima, two anastomosis groups (AG) of R. solani have been reported to cause damping-off in Peru, AG-3 and AG-4 (2,15). Independently from temperature effects, AG-4 was more pathogenic than AG-3 isolates, although pathogenicity of both AG increased with high temperatures (Figure 1). As for Peruvian conditions, AG-3 has only been isolated from potato plants at the highlands (2000-4500 m) as compared to AG-4 that has only been isolated from potato at lower elevations in the coast as well as from the jungle (200-1500 m)(2,15).

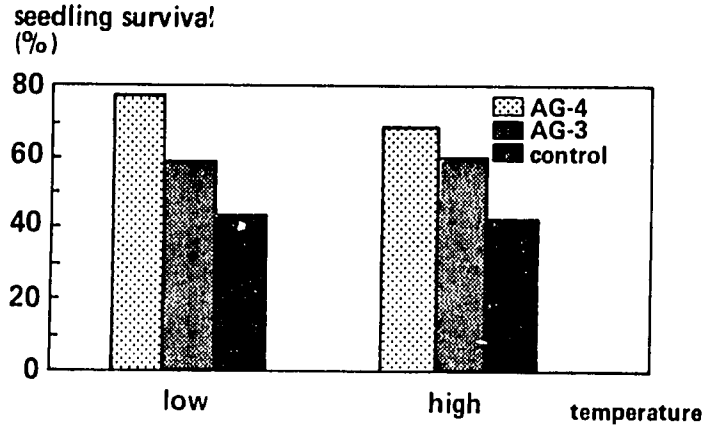

Figure 1. Percent seedling survival of clone DTO-33 sown in soil infested with Rhizoctonia solani AG-3 and AG-4 at low (16-18° C) and high (16-34° C) temperatures. Martin, unreported data.

Table 3. Seedling susceptibility to Rhizoctonia damping-off at three different temperatures.

Variety or clone of open-pollinated TPS	Percent damping-off		
	15° C	20° C	35° C
Yungay	1	20	90
Renacimiento	0.5	30	100
Molinera	1	40	100
Tomasa Condemayta	0	50	80
CFK-69-1	5	45	100
CEX-69-1	4	50	95
Caxamarca	4	35	75
OCH 11983	1	80	100
Ranrahirca	4	50	90
Participacion	3	20	95
DTO-33	4	70	100

Average of 2 experiments.

International Potato Center (1982)

Information on the effect of chemical products to control soil-borne diseases and damping-off in TPS is almost nil, and if TPS is to become widely used in the future by farmers from developing countries much more information on this subject is needed. As a direct consecuence of this demands, several studies on controlling damping-off of TPS have been conducted in CIP during the past years (8,9,10,13,14,21). Control of Rhizoctonia damping-off in seed nurseries for

production of transplants and for production of seedling tubers have been obtained by using several chemical products at a low dosage. In studies conducted by one of the authors during his sabbatic leave at The Netherlands, and later at CIP-Lima, products such as Furmecyclox, Benomyl, Tolclofos methyl, Chloroneb and Benodamyl significantly reduced Rhizoctonia damping-off in seedbeds (Table 4). Further studies conducted by Martin and Torres (14) on the control of Rhizoctonia damping-off in seedbeds for the production of seedling tubers indicated that a significant increase in yield and number of tubers per m2 were obtained in plots treated with some of the chemicals mentioned above (Table 5).

Table 4. Effect of nine fungicides on the control or Rhizoctonia solani damping-off of seedlings derived from true potato seed and sown in infested nurseries for production of transplants Lima, Peru.

TREATMENT	Dose/tray (gr/a.i.)	Dose/M^2 (gr/a.i.)	Seedling survival (%)
Benomyl	0.60	3.9	100.0 a
Chloroneb	0.75	5.0	99.3 a
Furmecyclox	0.45	3.1	99.0 a
Tolclofos methyl	0.55	3.0	98.7 a
Benodamyl	0.40	2.8	94.7 a
Vinclozoline	0.50	3.2	78.7 b
PCNB	0.45	3.3	77.7 b
Pencycuron	0.30	1.9	76.0 b
Carboxamide	0.10	0.7	53.7 c
Non-treated	0.0	0.0	22.3 d

Means followed by the same letter are not significantly different at the 5% level according to Duncan's Multiple Range Test.

Martin and Torres (1986)

Table 5. Effect of three fungicides on the control of Rhizoctonia solani damping-off of seedlings derived from true potato seed and on their plant height, yield and number of tubers>1 g produced in 1 m2 nurseries.

TREATMENT	Seedling survival at 30 days (%)	Plant height at 63 days(cm)	Yield (g/m2)	No. of tubers per 1 m2
Dazomet (control)[2]	100 a[1]	32.5 a	12,070 a	1,293 b
Dazomet + Inoculum[3]	28 b	19.6 c	7,685 c	693 c
Dazomet + Inoculum + Tolclofos Methyl (3.5 g a.i./m2)	96 a	32.5 a	11,381 ab	1,980 a
Dazomet + Inoculum[4] Benomyl (4 g a.i./m2)	99 a	33.3 a	11,652 ab	1,341 b
Dazomet + Inoculum + Furmecyclox (2.5 g a.i./m2)	97 a	28.4 b	9,803 b	937 b

[1] Means followed by the same letter in a column are not significantly different at the 5% level according to Duncan's Multiple Range Test (p = 0.5).

[2] Initially, soil mixture was treated with dazomet 35 days before planting.

[3] Four days before sowing colonized wheat grains was mixture soil substrate at the rate of 2 g kg of substrate.

Martin and Torres (1987)

Pythium sp. have also been reported to cause pre and post-emergence seedling damping-off in several different plants, being the most common causal species reported P. deliense, P. ultimun and P. splendens (5,12,17,18,19). Pythium spp. have been reported in several instances during the past few years to cause seedling damping-off at CIP experimental stations (8,9,10,11). Of six differnet isolates of Pythium sp. collected at San Ramon from damped-off seedlings, two of them caused about 80 % seedling mortality (Table 6, Gamarra et al., unreported data). Further studies conducted by the same authors (6) indicated that Pythium deliense and P. splendens are the two most common species causing pre ans post-emergence damping-off of TPS under tropical rainy conditions in Peru. Control of Pythium damping-off have been reported in the literature by using different fungicides, specially Metalaxyl (Ridomil) (12,17,18). At CIP,

significant seedling survival have been also observed with the application of Ridomil at sowing time (7). Other fungicides as Homai and PCNB have also been found to control Bfficiently Pythium damping-off (Table 7).

Table 6. Percent damping-off of seedlings from two potato cultivars inoculated with six different Pythium isolates from San Ramon, Peru. Soil artificially infested before sowing TPS.

Cultivar (OP - TPS)	Pythium isolate	Seedling damping-off (%)[1/]
Atzimba	1	45.4
	2	86.0
	3	37.7
	4	17.0
	5	25.0
	6	71.3
	Control	0.0
Participacion	1	41.7
	2	91.1
	3	52.0
	4	51.3
	5	23.7
	6	46.0
	Contr-l	0.0

1/ Average of 3 replications, 50 TPS/replication.
Gamarra et al. (unreported data).

Table 7. Fungicides used to control Pythium damping-off, percent seedling survival and fresh weight of seedlings grown from true potato seed(TPS) in artificially infested soil kept at 27-34º C.

Fungicides	Dose (ppm a. i.)	Seedling survival (%)1/	Seedling weight (g)2/
Ridomil	10	89.0	0.640
Homai	400	87.0	0.580
PCNB	7500	75.0	0.320
Benlate	5000	61.5	0.492
Rizolex.	5000	36.0	0.240
Demosan	6500	30.0	0.402
Control	---	22.0	0.230
LSD .05		14.10	0.341

1/ Average of four replications (100 TPS/replication) 30 days after sowing.
2/ Average of four replications (10 seedlings/replication) 30 days after sowing.

Gamarra et al. (1987).

SEEDLING DAMPING-OFF AFTER TRANSPLANT.

Seeedling losses after transplant to the field is probably one of the most important problems that TPS technology has faced at CIP. After transplant, damping-off and premature dying of plants have severely affected CIP's TPS research at La Molina and San Ramon experimental stations;plant losses up to 70% in some field trials have been recorded in some seasons. To control this important problem several studies have been conducted at CIP during the past years, and the following factors, conditions and combinations of the two have been taken in consideration (most of this information have not yet been published, however data has become available in internal reports, annual reports, seminars and other meetings):

Seedling vigor: potato seedlings at the moment of transplant to the field are generally between 30-40 days old and extremely fragil compared to sedlings of other plant species. Probably because potatoes has always been planted and multiplied as tubers, seedlings derived from TPS has not developed through evolution too much resistance to what is called"transplant shock". Seedling vigor has become a mayor aspect of TPS research at CIP and constant evaluations and selections are conducted on this aspect. More vigorous and strong seedlings will have more possibilities than weak and poor developed seedlings to survive after transplant, specially under high temperature conditions in the tropics where this technology can be readily applied.

Agronomic practices: It has also been found that special care must be taken on aspects such as irrigation, pest control, weed control, fertilization, etc. during and after transplant to permit seedlings survive the most. Hilling operations after transplant has become one of the important aspects in seedling survival at CIP. It has been constantly observed that the greatest seedling losses occur after hilling, specially when no fungicides are used to treat the soil that will cover the young, succulunt stems, or when non-experienced workers carry out this operation.

Complex pathological situation: R. solani, P. deliense, P. splendens, Fusarium sp., Erwinia sp., and other pathogens have been normally isolated from damped-off seedlings after transplant at CIP experimental stations. The presence of cut-worms, stem borers, bacterial wilt, excess/lack of water, high temperatures, etc. have made extremely difficult to point for the first and real causal agent of seedling damping-off in the field. Probably two or more of these factors act synergistically to cause severe plant losses. R. solani is probably the most common pathogen isolated from diseased seedlings throughout the year at La Molina, and at San Ramon during the dry season (Martin and Torres, unreported data). Good levels of control of this pathogen and other associated soil-borne diseases have been achieved by the application of different chemical products to the soil or to the seedlings prior to transplant. Products such as Rhizolex, Methyl bromide and Basamid have efficiently controlled R. solani and other soil microorganisms as shown in Table 8 (21). Preliminary results from trials using some of the fungicides reported to control damping-off in the seedbeds have also controlled damping-off. It is interesting to note at this moment that in most of our trials PCNB has not controlled soil-borne pathogens at high temperature conditions (8,9,10,14).

Table 8. Number of surviving plants (out of 60) and yield per 13.5 m^2 of plots treated with different chemical products to control damping-off after transplant.

Treatment	No. Surviving Plants (out of 60)	Yield (Kg/13.5 m^2)
Methyl Bromide + Rhizolex	47.0[1/]	23.86
Methyl Bromide	46.2	20.12
Rhizolex + Plastic cover	39.6	17.54
Rhizolex	37.2	16.76
Rhizolex + Basamid	44.0	16.62
Plastic Cover	35.8	15.16
Rhizolex + CaO Cl	25.4	10.40
Rhizolex + NaO Cl	25.4	9.04
PCNB + Benlate - control	19.2	7.94
LSD.05	10.63	6.51

[1/] Average of 5 replications.
Torres and Elphinstone (1987)

Pythium sp. has been isolated up to 22 % from seedlings showing symptoms of damping-off after transplant. These high values have been obtained normally during the rainy season at San Ramon (8,9,10). Studies on the control of Pythium damping-off after transplant have been successful by using Ridomil 5G (Table 9)(7,8,9,10). A normal procedure at San Ramon station during the rainy season in the soil application of Ridomil 5G or Homai at the moment of transplant.

Table 9. Fungicides used to control Pythium damping-off, percent seedling survival and fresh weight of seedlings grown from TPS and transplanted to artificially infected soil, and kept at 27-24°C.

Fungicides	Dose (ppm a. i.)	Seedling survival (%)1/	Seedling weight (g)2/
Ridomil	10	80.0	3.440
	50	58.3	0.850
Homai	400	66.8	3.32
	800	45.8	0.93
Benlate	5000	58.5	1.23
	10000	62.5	0.95
Demosan	6500	50.0	1.07
	13000	50.0	1.04
Rizolex	5000	37.5	1.29
	10000	62.5	1.23
PCNB	7500	45.0	1.29
	15000	41.5	1.04
Control	____	19.7	0.90
LSD .05		7.43	2.14

1/ Average of four replications (six seedlings/replication)

2/ Average of four replications (suvival seedlings/replication)

Gamarra et al. (1987)

Probably there are more pathogens that can affect TPS in the field but most our research has concentrated in those pathogens that normally produce damping-off in our conditions at CIP. Seedling damping-off in seedbeds can be controlled efficiently by using clean seed and by applying different fungicides to the soil at the moment of sowing. In small scale operations, such as for subsistent farmers in developing countries the cost of fungicides will be relatively small because of the reduced field size and

fungicide dosage.This will be particularly the case when seedling tubers are produced for planting. The control of damping-off after transplant is much more complicated because of the different factors involved. In this case, an integrated control approach must be considered in which besides chemical control other aspects such as insect control, hilling operations, fertilization, adecuate irrigation,etc. must be taken in consideration.

LITERATURE CITED

1.- ACCATINO,P., AND MALAGAMBA,P.1982. Potato production from true potato seed. International Potato Center, Lima, Peru. 20pp.

2.-ANGUIZ, R. y MARTIN,C. 1986. Identificacion de los grupos de anastomosis(AG) de Rhizoctonia solani, aislados de papa en tres zonas ecologicas del Peru.(Abstr.) Fitopatologia 21:3.

3.- ELANGO, F. 1986. The role of high soil temperatures in the damping-off of true potato seedlings in the lowland tropics. Trop. Agric. (Trinidad) 63: 66-67.

4.- EMMATTY, D.A., GEORGE, B.F., AND FISHER. M. 1977. Tomato seed treatment method to meet certification for production of Georgia transplants. (Abstr.) Proc. of the American Phtopathol. Soc. 4: 153.

5.- ENDO, R.M., and COLT, W.M. 1974. Anatomy, cytology, and physiology of infection by Pythium. Proc. Am. Phytopatol. Soc. 1:215-223.

6.- GAMARRA,D., TORRES H. and MARTIN, C. 1986. Pythium splendens y Pythium deliense causantes de damping-off en plantulas de papa en San Ramon, Peru.(Abstr.). Fitopatologia 21:5.

7.- GAMARRA, D., MARTIN, C. and TORRES, H. 1987. Control of Pythium damping-off in seedlings grown from true potato seed in the warm tropics. Submitted for publication to Tropical Agriculture (Trinidad).

8.- INTERNATIONAL POTATO CENTER. 1982. Annual Report CIP 1981. 130 pp.

9.- INTERNATIONAL POTATO CENTER. 1983. Annual Report CIP 1982. 144pp.

10.-INTERNATIONAL POTATO CENTER. 1984. Annual Report CIP 1983. 164pp.

11.-INTERNATIONAL POTATO CENTER. 1985. Annual Report CIP 1984. 168 pp.

12.-KAISER, W.J.,and HANNAN, R. 1983. Etiology and control of seed decay and preemergence damping-off of chickpea by Pythium ultimun. Plant Disease 67: 77-81.

13.-MARTIN, C. 1983. Control of Rhizoctonia damping-off on seedlings grown from true potato seed. CIP Circular, Vol.11(4): 1-4. International Potato Center, Lima, Peru.
14.-MARTIN,C. and TORRES, H. 1986. Fungicides for the control of Rhizoctonia solani damping-off in seedlings derived from true potato seed. Fitopatologia 21: 74-80.
15.-MARTIN, C., and ANGUIZ, R. 1987. Anastomosis groups and pathogenicity of Rhizoctonia solani from potatoes in Peru. (Abtsr.). Paper presented at the APS Annual Meeting, August 2-7, 1987, Cincinnati, CHIO.
16.- SAUER, D.B. AND BURROUGHS, R. 1986. Disinfection of seed surfaces with sodium hypochlorite. Phytopathology 76: 745-749.
17.-SONODA, R.M. 1972. Control of tomato transplant damping-off. Plant Dis Reptr. 56:840-842.
18.-STEPHENS,C.T., and STEBBINS, T.C. 1985. Control of damping-off pathogens in soilless container media. Plant Disease 69: 494-496.
19.-STEPHENS, C.T., and POWELL, C.C. 1982. Pythium species causing damping-off of seedlings bedding plants in Ohio greenhouses. Plant Disease 66: 731-733.
20.-TORRES, H. AND ROMAN, E. 1986. Control of contaminant microrganisms of true potato seed.(Abstr.). Fitopatologia 21: 11.
21.-TORRES, H. y ELPHINSTONE, J. 1987. Eficiencia del bromuro de metilo en el control de enfermedades de suelo. XII Reunion Asociacion Latinoamericana de la Papa (ALAP). Ciudad de Panama, Panama, Marzo 9-13, 1987. 556 pp.
22.-WHITE, J.W. and SADIK,S. 1983. Potatoes from true potato seed: A promising alternative. SPAN 26: 23-25.
23.-WIERSEMA, S.G. 1985. Production of seed potatoes derived from true potato seed. CIP Circular Vol 13 (1): 1-4. International Potato Center, Lima. Peru.

Planning Conference on

Fungal Diseases of the Potato

A G E N D A

September 21, Monday

Chairperson, morning session: Humberto Mendoza

8:30 - Inaugural comments.
Richard L. Sawyer, Director General

8:45 - Review of recommendations of previous planning conferences.
Peter Gregory, Director of Research

FIRST TOPIC: Late blight caused by *Phytophthora infestans*.

9:00 - The importance of the perfect stage of *P. infestans* from the standpoint of epidemiology and adaptation.
W. E. Fry

9:30 - Genetics of *P. infestans*
Paul Tooley

10:00- Break

10:30- Biochemical aspects of late blight with respect to compatibility and incompatibility reactions.
Kohei Tomiyama

11:00- Discussion

12:30- Lunch

14:00- Tour of CIP installations.

15:00- Break

Chairperson, afternoon session: L. J. Turkensteen

15:10- Late blight breeding strategy at CIP.
Juan Landeo

15:50- Screening for late blight resistance.
Víctor Otazú

16:10- Discussion

September 22, Tuesday

Chairperson, morning session: W. E. Fry

8:30 - Interaction of R-genes in breeding for resistance of potatoes against Phytophthora infestans.
L. J. Turkensteen

9:15 - Acceptance of new varieties with resistance to late blight when chemical control is not available.
Pierre Tegera

10:00- Break

10:30- Discussion on the strategy of breeding and screening for resistance to late blight.

12:30- Lunch

Chairperson, afternoon session: Primo Accatino

SECOND TOPIC: Early blight caused by Alternaria solani.

14:00- Factors affecting resistance to Alternaria solani and progress in Early blight research at CIP.
Carlos Martin and H. David Thurston

14:30- Breeding for resistance to Early blight (Alternaria solani).
Humberto Mendoza and Carlos Martin

15:00- Break

15:15- Discussion on the strategy of breeding and screening for resistance to early blight.

September 23, Wednesday

THIRD TOPIC: Importance of soil-borne diseases.

Chairperson, morning session: Víctor Otazú

8:30 - Research progress on Verticillium dahliae Kleb.
Oscar Malamud

9:00 - Widespread soil-borne diseases of the potato.
L. J. Turkensteen

9:30 - Soil-borne and foliar diseases in the highland tropics.
Hebert Torres.

10:00- Break

10:30- Discussion on the importance of soil-borne diseases of potatoes in the developing world and means to control them.

12:30- Lunch

Chairperson, afternoon session: Manuel Villarreal.

14:00- Ecology of Rhizoctonia solani and disease development in relation to anastomosis groups.
Carlos Castro.

14:30- Control of Rhizoctonia and other soil-borne disease of TPS.
Carlos Martin and Hebert Torres

15:00- Break

15:15- Discussion on CIP's role in studies of Rhizoctonia and its control.

September 24, Thursday

A small committee will prepare recommendations.

September 25, Friday

Discussion, modification and approval of recommendations.

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William Fry
Pierre Tegera
Kohei Tomiyama
Paul Tooley
Lodewijk Turkensteen
Manuel Villarreal

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Oscar Hidalgo
Juan Landeo
Oscar Malamud
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Hans Pinedo
Víctor Otazú
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