BACTERIAL DISEASES OF THE POTATO

Report of the Planning Conference on Bacterial Diseases of the Potato

1987

INTERNATIONAL POTATO CENTER (CIP)

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INTERNATIONAL POTATO CENTER (CIP)
Address Apartado 5969 - Lima Peru Cables CIPAPA Lima
Telex 25672 PE Telephones 366920 - 354354

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RECOMMENDATIONS

BACTERIAL WILT

1. SCREENING AND FIELD EVALUATION

Successful breeding for resistance to P. solanacearum is basically a function of adequate and effective screening procedures. The well-known complexity of the pathogen and genetic basis for resistance in the host accounts for the inherent extraordinary difficulties in developing adequate screening procedures. The conference recommends therefore that CIP consider allocating more resources for intensified research on screening at CIP and in the regions.

The conference recommends that basic screening for the identification of sources of resistance be conducted at CIP, Peru, but that regional sites - some of them as yet to be identified - be used for the final steps of CIP's breeding populations under specific environmental conditions.

1.1. Studies to determine the correlation between resistance screening of seedlings and plants from tubers should be done to determine the efficiency of the former to evaluate progenies for variety selection and/or TPS.

1.2 Screening of prospective progenitors for variety selection and/or TPS should be done in CIP Lima challenging the clonal materials by inoculating with the appropriate strains of P. solanacearum from the given geographical target area.

1.3 Preliminary screening should be done in Lima on materials to be sent to the regions for use in variety selection and/or TPS.

1.4 The regional evaluation and utilization of the genetic materials should proceed as follows:

a) Multiplication of materials in the most disease and pest free environment possible. At this stage individuals showing poor plant or tuber characteristics must be eliminated.

b) Preliminary clonal evaluation, still in a P. solanacearum free environment, to permit further multi-

plication as well as further selection for adaptation, agronomical attributes and other resistances.

c) In a third season enough tubers should be available from each clone to evaluate resistance to P. solanacearum.

d) In the following seasons trials should be designed to continue selection and confirmation of resistance.

1.5 a) Efforts should be made to improve standardized screening procedures, including use of uniform planting media (such as Jiffy mix) and inoculation procedures.

b) A standardized set of clones needs to be selected to serve as base line materials for evaluations under different environments.

c) Controlled environment facilities should be utilized to examine selected clones for influence of temperature on expression of wilt resistance.

d) A biological assessment of pathogens and other factors (water status, soil characteristics, etc.) present at the regional test locations needs to be completed in order to evaluate results obtained in field evaluations. In addition, the standard cultural practices need to be determined.

2. BREEDING

2.1 CIP should continue the development of P. solanacearum resistant progenitors. As they are identified they should be integrated into the populations for hot climates to develop P. solanacearum resistant clones combined with other attributes, i.e. resistances to late blight, root knot nematode, and viruses as well as earliness and heat tolerance. All these attributes are already present in the hot climate populations.

For short term use of the P. solanacearum resistant population present in the Regions, its incorporation through crosses with specific, adapted material present in a given Region is encouraged, in collaboration with National Programs.

3. GENE INSERTION/PROTOPLAST FUSION/RELATED TECHNIQUES

3.1 The conference recognizes that methods such as gene insertion, protoplast fusion and related techniques are not likely to become routine measures for solving problems of bacterial diseases of the potato in the immediate future. The conference, therefore, endorses CIP's present cautious approach to this type of research which, however, assures that CIP will be in a position to utilize immediately potential breakthroughs in this field. The conference further endorses CIP's use of contracts to conduct the basic molecular aspects of this research.

4. ADDITIONAL SOURCES OF RESISTANCE

4.1 The source of resistance from S. phureja has been successful in a variety of environments including the cool tropics and also the hot tropics when proper combination with adaption has been achieved. Therefore, accessions of S. phureja already available in CIP's world potato collection should be evaluated for resistance to P. solanacearum and further utilized in pre-breeding.

4.2 Cultivars of S. tuberosum, i.e. Achat, Prisca, AVRDC 1289.19 and others, which have been proven to have a certain level of resistance to P. solanacearum must be included in the breeding populations.

4.3 Additional sources of resistance from wild species should be considered in a long term germplasm enhancement effort.

5. NEMATODES

5.1 Both high level and high frequencies of resistance to root knot nematodes are present in the Phureja, stenotomum sparsipilum, chacoense and microdontum population. Since the same population contains resistance to P. solanacearum, a sequential screening for both resistances must be applied. First, screening for root knot nematode resistance followed by P. solanacearum. Selected individuals carrying both resistances will be rechecked for resistance and field evaluated for adaptation and agronomical performance.

5.2 Root lesion nematodes (Pratylenchus spp.) are widely distributed in the tropics as well as in the temperate regions. These nematodes are also known to interact with P. solanacearum. The above mentioned population also has a

certain degree of resistance to these nematodes. Therefore, screening for resistance should be carried out when appropriate.

6. LATENT

6.1 There is a need for more research work on methods for the detection of latent infections in potato stems, tubers, weed hosts and other crops.

6.2 In addition to the use of latex agglutination and Elisa kits, new serological methods employing monoclonal antibodies should be evaluated.

6.3 That more information be obtained on the relative importance and detection of latent infection of lenticels and vascular tissue in potato tubers.

6.4 New sources of resistance should be tested for their susceptibility to develop latent infection.

7. CHARACTERIZATION AND IDENTIFICATION OF P. SOLANACEARUM

P. solanacearum is a distinct, separate and easily recognized species. However, there is an urgent need for a natural classification which provides the kinds of information needed to screen for resistance or develop control methods based on an understanding of the distribution of strains. It is therefore recommended that:

7.1 CIP encourage and promote an international cooperative project to assemble the additional information needed to develop an improved classification system applicable for potatoes.

7.2 Because of limitations imposed by quarantine regulations, studies of cultures collected worldwide be conducted at laboratories that can receive them and that the means be provided or sought for scientists, from CIP or elsewhere, to conduct these studies. Initial work should be of an international collaborative nature on the conventional characterization of P. solanacearum.

7.3 A standard set of differentials be assembled, including resistance recognized from different Solanum species origin (i.e. Phureja, tuberosum, microdontum, sparsipilum, etc.) that may lead to the identification of conventional races

pathogenic on potatoes. The methodology to classify these differentials must be carefully defined (environment, inoculation procedures, etc.) so that comparable efforts can be conducted at different locations.

7.4 Efforts to assemble a representative worldwide collection be increased and that reliable conservation means for cultures (i.e. Lyophilization), be established at more than one location.

7.5 That modern classification tools such as production of monoclonal antibodies, cellular fatty acid analysis and application of DNA: DNA and DNA: rRNA hybridization be utilized for which purpose CIP must seek collaboration and/or funding.

8. INTEGRATED CONTROL

Information on agronomic practices that reduce P. solanacearum severity has been gathered at CIP, its regional centers and national programs. To make use of this information, the following complementary information is needed:

8.1 Seed of resistant/tolerant commercial cultivars needs to be produced so that the problem of contamination is reduced. Wherever possible each country should identify P. solanacearum free soils for seed production.

8.2 Early maturing varieties are needed to make use of optimum dates of planting for reducing bacterial wilt. Other agronomic practices should be investigated, including intercropping and its socio-economic aspects.

8.3 Suppressive soils for P. solanacearum have been observed. The mechanisms involved in pathogen suppression need to be investigated for practical application.

8.4 The Committee recommends that the individual elements of strategies for P. solanacearum control as listed in the proceedings of this conference receive careful attention in CIP's regional research in collaboration with national programs to assign research priorities with regard to each element under their specific environmental and socio-economic conditions. CIP is encouraged to consider the production of a document that contains the information at present available in order to assist regional researchers and personnel of national programs in this effort.

ERWINIA

Since the last Planning Conference on Bacterial Diseases in 1979 considerable progress has been made towards a better understanding of the biology, ecology and epidemiology of the *Erwinia* species and the diseases they cause in the temperate regions. This has led to the development of an integrated disease control strategy suitable for the climatic and socioeconomic conditions found in these regions. Although studies on this subject have been initiated at CIP (San Ramon), information concerning the highland and even more the lowland tropics is still incomplete. As the range of the potato is extended, problems caused by soft rot erwinias alone or in combination with other pests in the field and in storage have occurred and appear to be increasing. Moreover, drastic losses of valuable experimental material due to tuber decay have developed lately at the mid elevation tropical substation at San Ramon that is scheduled to become CIP's main testing station for cultivars destined for eventual cultivation in tropical zone countries.

Therefore, the Committee feels that increased support should be given to study the bacteria and the diseases they cause in developing countries with the objective to minimize losses both in the field and in storage. To that end, essentially a two pronged approach of the problem is suggested: one, based on the development of an integrated control strategy in the short term and the other, exploration of the feasibility of incorporating greater disease resistance in breeding material in the long term.

To achieve these objectives the Committee makes the following recommendations:

I. Integrated Disease Control Strategy

a) Ecological and epidemiological studies

1) Development of rapid, simple tests to detect, identify and quantify contamination by the different soft rot erwinias for use in ecological and epidemiological studies and to determine health status of plant material. Participation of specialized laboratories involved in similar studies should be encouraged in order to avoid unnecessary duplication and to ensure rapid progress.

2) Ecological studies on different sources and survival of erwinias, the role of alternative hosts in the epidemiology of the diseases, and pathways of contamination of potatoes.

3) The relative pathogenicity of the different erwinias should be investigated under warm environments.

4) Interaction of the bacteria with other pathogens and pests in the field and in storage should be examined.

b) Disease Management

1) Information obtained in I. (a) together with current knowledge should allow the development of an integrated set of recommendations aimed at controlling *Erwinia* caused diseases in the field and in storage in developing countries.

2) Adequate storage systems should be developed in order to reduce the risks of rotting.

3) Effort should be made to develop methods to produce seed with the least possible level of contamination by erwinias.

4) Special attention should be given to control the problem of tuber rot in the field at the San Ramon substation.

II. Breeding for Disease Resistance

The Committee feels that because tuber rot is one of the most important problems affecting potatoes in the warm tropics, the objective should be to breed for resistance primarily to that disease. The rationale for breeding for resistance is based on (1) the limited efficiency of agronomic control measures in the warm tropics and (2) the presence of resistance in many *Solanum* species and the demonstration of its inheritance.

a) Development of screening tests

Success of any breeding programme depends on the efficiency of the screening method used to select for the desired character. At present there are no established screening procedures for breeding for tuber resistance. Screening methods should be developed and evaluated against field results. Close collaboration should be maintained with institutions that are actively involved in similar studies.

b) Feasibility studies for the development of a breeding programme

1) Preliminary studies

It is essential to determine which of the three erwinias should be used to challenge the potato. The predominant *Erwinia* species in the tuber rot in the warm tropics should be used. In addition, it would be desirable to determine whether results obtained for tuber rot correlate with those for stem rot and blackleg.

2) An attempt should be made to identify sources of resistance and to determine its inheritance.

Consideration should be given to the possibility of correlation between resistance to *Erwinia* and interacting pathogens.

3) Advanced breeding lines for the warm tropics should be screened for resistance to tuber soft rot.

III. Complementary Actions

a) Survey of losses caused by erwinias

The Committee recommends that a survey be carried out on the extent of losses in potatoes in the field and in storage caused by erwinias in countries within CIP's regional network. Included in the survey would be the obtainment of information on the distribution of the different species of *Erwinia*, the sources of seed and any interaction with other pests. The information would be useful to guide future allocation of resources on *Erwinia* research.

b) Training and follow-up assistance

The Committee further recommends that technology transfer be promoted actively with special emphasis on national scientists to implement the disease control packages and to stimulate *Erwinia* research in developing countries.

REVIEW OF RECOMMENDATIONS OF THE JUNE 1979 PLANNING CONFERENCE

Peter Gregory

In the June 1979 Planning Conference on "Developments in Control of Potato Bacterial Diseases" recommendations were made in the areas of bacterial wilt, Erwinia, and opportunistic bacterial pathogens involved in decay of potato tubers.

The main purpose of this paper is to (1) summarize the June 1979 Recommendations and the actions taken as a result of them and (2) to illustrate the urgent need we now have for rapid, substantial progress, particularly in bacterial wilt research.

I. THE 1979 RECOMMENDATIONS

A. BACTERIAL WILT

1. Breeding for Disease Resistance

It was recommended that there should be continued emphasis on the identification and utilization of additional genes for disease resistance such as those from Solanum sparsipilum, which could be combined with those from Solanum phureja. It was pointed out that resistance from these sources did not appear to be of a general nature. Consequently it was considered important that many resistance genes from several wild species be accumulated in parental lines. It was emphasized that there was a continuing need for more sensitive procedures that would insure the identification of progeny with the highest levels of resistance to bacterial wilt. The need to consider resistance to nematodes, late blight and viruses was also pointed out.

Since the 1979 Conference a new population has been created which brings together four species including Solanum phureja, Solanum sparsipilum, Solanum chacoense and Solanum microdontum. Also more resistant wild species have been identified e.g. Solanum bukasovii. Combining bacterial wilt resistance with resistance to late blight and viruses has not been done but is currently being planned. Combination with nematode resistance is also been planned.

A more extensive effort to utilize the resistance genes available in clones of Solanum phureja was recommended. Such genes had been identified at the University of Wisconsin from the collection of Sturgeon Bay, or in such clones as 1386.15, which have a very wide range of resistance but have not been fully utilized in breeding programs. This recommendation was acted upon, and resistance to PLRV was also found, but PSTV contamination obliged us to discard these materials.

It was also recommended that emphasis should be continued on the screening of Solanum phureja collected in those geographic areas where the bacterium is present in the soil. In retrospect it appears that no relevant coevolution is apparent and that this recommendation is no longer valid.

It was considered imperative that methods of screening for disease resistance be improved. In 1979 it appeared that screening procedures were cumbersome and inadequate to process a substantial number of hybrid progeny. There was also the danger of bacterial carry-over via latent infections. In particular, the interaction of inoculum concentration and temperature was considered an important phenomenon for study. Since 1979 a new scheme (in addition to seedling tests) of testing cuttings of potentially resistant seedlings has been employed.

It was recommended that quantitative evaluations of the resistance of selected clones to different strains should be made. It was considered necessary to apply modern quantitative techniques, such as infectivity titrations, in order to obtain a critical evaluation of the resistance of different clones. It was pointed out that comparative evaluations were possible only under highly controlled environments. This recommendation has been put into effect for clones in the pathogen tested list but only to a limited extent.

It was considered necessary to establish adequate testing sites in different regions. The most valuable sites were considered those where materials could be tested rigorously against natural inoculum in the field. It was recognized that site selection and maintenance had been a recurrent problem which had to be resolved. It was acknowledged that few sites were available and, in some, nematode infestation and other problems prevented adequate selection. We now have sites in Mindanao, Sri Lanka (temporarily interrupted) and Peru, plus sites in Rwanda and Burundi, Fiji, Kenya, Bhutan and Nepal.

It was recommended that adequate amounts of clean seed of resistant clones should be made available to cooperating countries. The committee supported the efforts to provide clean seed to redistribution centers. This is still functioning but could be improved quantitatively.

It was recommended that adequate indexing procedures for bacterial and viral latent infections should be made available to redistribution centers. In response to this the latex serological kits have been developed.

2. Strain Selection and Identification

It was recommended that improved procedures should be used for selecting strains of *Pseudomonas solanacearum* for screening purposes and emphasis should be given to infectivity titrations for the selection of the most virulent isolates on a quantitative basis. The use of mixtures was encouraged but adequate criteria had to be established for the use of different cell concentrations of compatible strains from different geographical locations. Since 1979 highly virulent strains have been selected from different regions. Mixtures in greenhouse tests have not been effective.

It was recommended that serological procedures should be developed for identification of strains of *P. solanacearum* and great emphasis should be placed on the use of different antigens for the preparation of specific antisera that may allow rapid identification of unknown pathogenic strains. CIP now has serological procedures which are good for the whole species, and races can be distinguished by quantitative differences in reaction by ELISA. Strain-specific polyclonal and monoclonal antisera have been developed under contract at Wisconsin.

The recommendation that clones of *Solanum phureja* should be used as differentials for strain identification has not been acted upon.

3. Integrated Control

The Conference endorsed the concept that the most effective means for control of bacterial wilt is a combination of adequate levels of resistance and improved cultural practices. It was suggested that additional emphasis should be given to field experimentation with different methods of crop rotation, weed control, and mulching materials.

Whenever possible, other factors such as soil moisture and soil temperature should be considered. This recommendation has been followed up in some Regions, for example, in Rwanda, Burundi and the Philippines.

It was recommended that cultural practices should be designed for integrated control for the small farmer. It was recognized that procedures such as deep plowing or the use of herbicides may not be applicable under subsistence farming and that emphasis should be given to simpler procedures. This has been acted upon through studies on intercropping and spacing, and also rotation in Mindanao and Burundi.

The Conference recommended that the possible survival of P. solanacearum on roots of weed plants should be studied in detail. This has been acted upon using controlled conditions. Some field studies are underway in Mindanao.

4. Nature of Disease Resistance

It was recommended that changes in content of agglutinins (lectins) in resistant clones, following infections, should be studied in detail. This approach has not led to practical gains.

B. ERWINIA

It was recommended that efforts should be continued to determine the relative susceptibility to Erwinias of certain important breeding material such as CIP's bacterial wilt and late blight resistant clones. The Panel was in agreement with the decision not to involve personnel in a breeding program for soft rot resistance. Since 1979 screening has been done but a breeding program was started.

There was a recommendation that experiments needed to be initiated that would involve the use of true seed as well as seed tubers free of Erwinia to determine whether stem-rot, tuber decay and aerial blackleg can develop from endemic Erwinia populations. These tests were to be conducted in a few typical lowland tropical sites to determine what levels, species and pathovars of Erwinia might be present. Such experiments have been done but only in San Ramon.

The recommendation that similar studies be initiated at a few typical seed-growing sites in the tropical highlands was not followed up on.

There was a follow up of the recommendation that studies should be initiated to determine whether field symptoms induced by E. chrysanthemi or E. carotovora var. carotovora in breeding material resistant to P. solanacearum might be confused with symptoms of bacterial wilt. It was found that these problems do frequently occur together.

It was recommended that in cooperation with the seed program at CIP, efforts should be made to assist national programs directly involved in maintaining disease resistant clones free of pathogens including blackleg and soft rot bacteria. Little has been done in relation to this recommendation.

Recommended studies on evaluating the influence of CIP-recommended storage procedures on the development of bacterial soft rot in tubers in storage, in transit and also in planting, found that storage procedures were not adequate for control and that a fungal interaction occurred.

There was a suggestion that certain phases of the Recommendations listed could best be implemented by the involvement of one or more research pathologists with extensive experience with the pectolytic Erwinia. Since 1979 one of our scientists travelled twice to Wisconsin for training and another scientist joined CIP as a post-doctoral appointee.

C. OPPORTUNISTIC BACTERIAL PATHOGENS INVOLVED IN DECAY OF POTATO TUBERS

It was considered that additional knowledge on these bacteria was needed. Studies on the identity and interrelationship of Clostridia with other pectolytic bacteria has now been completed at Wisconsin. However, Clostridia is secondary to other pathogens and more of a developed country storage problem.

II. SOME URGENT ISSUES IN BACTERIAL RESEARCH

Several critical aspects of bacterial wilt research are needing urgent attention. Perhaps most important is that existing screening methodologies for resistance in clonal and TPS materials are inadequate. This implies serious problems with respect to breeding for resistance. We are not sure which inoculum to use because there are so many bacterial strains. How, where and when should we screen? Who should do it? CIP or other collaborating institutions? In TPS work we are

not sure what size of population we need to screen. Neither are we sure how to recognize a resistant TPS population. Work since 1979 suggests that greater emphasis needs to be placed on the systematic distinction (on a large scale) between resistance versus symptomless carry-over of the bacteria.

Another critical issue is how do we best achieve the much needed combining of resistance to bacterial wilt with resistance to late blight and certain viruses?

Apparently there are several possible alternatives or supplements to the control of bacterial wilt by resistance. For example, increased resistance to root-knot nematode might reduce bacterial wilt infection. Intercropping of potatoes with beans, maize or cowpea, or other crops might also reduce losses caused by the disease. Considering the enormous problems faced by those trying to develop resistance to bacterial wilt, have we considered these alternatives in a sufficiently serious manner? As bacterial wilt is a disease second only to late blight in terms of global potato agriculture (and is more important than late blight in some Regions) we must consider all reasonable approaches.

Erwinia is already an important issue in several Regions and recent reports suggest that it is increasing in importance. We need to consider whether CIP is doing enough on Erwinia and what our priority research should be on this organism.

CONCLUSION

In conclusion, most of the Recommendations of the 1979 Planning Conference were acted upon in some way, but urgent issues still face us. In this Planning Conference we need to ask: Has CIP and its collaborators done enough on some of the 1979 Recommendations? Which of those Recommendations are still high priority in 1987? Which new Recommendations are needed to guide us for the next several years in this most difficult and economically important area of research?

We have in this Planning Conference an enormous amount of collective experience and expertise on bacterial research. To maximise the benefit of this I urge each of you to speak freely, whether or not you feel your views will be popular, and to be open-minded about new approaches. These ingredients will assure us of a sucessful Planning Conference.

MASS SCREENING PROCEDURES FOR RESISTANCE TO *PSEUDOMONAS SOLANACEARUM*

E. R. French and Ursula Nydegger

Screening for resistance to *P. solanacearum* in *Solanum tuberosum* subsp. *tuberosum* was done by Nielsen and Haynes by infesting soil in pots with a bacterial suspension standardized to 97-98% light transmittance. After injuring roots by inserting a butcher's knife twice in radial cuts at the periphery of each pot, 10 ml of the suspension was added with a pipetting syringe to each cut (4). During the screening process that led to the discovery of resistance in *S. phureja*, Thurston and Lozano utilized the stem puncture method (5, 7). A similar inoculation of the aerial part of tomato plants was achieved by Valdez by cutting the base of leaves with a scalpel dipped in inoculum (6). French (1) compared five methods of inoculation utilizing 10^8 CFU/ml of bacteria: soil infestation without wounding by irrigating 10 cm pots with 40 ml of inoculum, puncturing the stem with a dissecting needle through a drop of inoculum in the leaf axil, cutting a terminal leaflet with a dissecting scissors dipped in the inoculum, infiltrating a terminal leaflet with a rubber tipped syringe, and rubbing with a cotton tip dipped in inoculum after dusting with carborundum. The last two gave inconsistent results. The scissor-cut technique was equally consistent as the stem puncture method, but the former has the advantage of being faster to perform. The soil infestation method was equally effective but symptoms took longer to develop. The stem puncture method was used in the breeding and screening work of Rowe and Sequeira at Wisconsin that led to the development of resistant cultivars, but because it was not possible to handle large populations, the more efficient mass screening method was developed with L. C. Gonzalez (3). Flats (50 x 35 cm) containing Jiffy mix were sown with true potato seed 3.2 cm apart and watered with demineralized water whilst growing at 22° C with a 14 h photoperiod for 20 days before inoculating. Watering was suspended for 2 days. The 3-5 cm tall seedlings were inoculated by watering with 5 liters per flat of *P. solanacearum* standardized to Optical Density 0.5 (600 nm). Mixtures of two isolates were used and roots were cut immediately by running a knife between and accross the rows. The inoculated seedlings were incubated in a growth room at 28° C for 2 weeks. Wilting began in 3-4 days and most deaths occurred in 4 more days. Survivors were transplanted to 10 cm pots in a greenhouse, many wilting, others growing and tuberizing. When roots weren't injured, more inoculum was needed to achieve wilting. When seedling density in flats was increased from 77 to 97 there were fewer survivors and more plants wilted after transplanting to pots.

A mass inoculation method was used by Gitaitis et al (2) in the field. Potato plants were sandblasted at a 45 degree angle with an air pressure of 550 kPa, followed by spraying a suspension of P. solanacearum of 8 x 10^7 CFU/ml. They estimated that they could inoculate at a rate of 10,000 plants per hour. All plants of a susceptible variety became infected.

At CIP a modification of the Wisconsin mass screening method has been used for the past 10 years. Flats used are plastic trays (41 x 28 cm) filled with a mix of river sand and a moss-like plant in a 1:2 proportion. Nutrition is provided with a complete fertilizer. Inoculum concentration varies from 1 x 10^7 to 1 x 10^8 depending on prior tests with each isolate, and enough volume is used to flood the trays which are not watered the day of inoculation. Initially planting was done in 10 rows with 10-20 seeds per row, but because transplant losses were great, this was reduced to 54 seeds sown equidistantly according to a template. This method is only used in the in-house process of developing resistant populations. Any clones to be field tested or exported are grown as seedlings in pots from which 3 to 5 cuttings are made for testing and subsequent incineration. The resistant mother plants are kept for tuber production.

To further improve the mass screening method an experiment is being conducted to compare two open pollinated progenies from resistant clones at two seedling densities (50 and 100 per tray) and the effect of either leaving wilting seedlings to die or removing them by pulling. Survivors have been maintained by both making a cutting and transplanting. Preliminary results indicate that the two progenies behaved similarly, that the number of wilting plants was similar for both the low and high density of planting but that the cuttings made from apparently resistant clones succumbed in greater numbers from latent wilt or poor vigor when taken from plants grown and inoculated at greater density. Removal of seedlings as they began to show symptoms greatly increased the incidence of wilt in the trays, diminished the survivability at transplanting and increased the incidence of latent wilt in cuttings, that died in greater numbers.

LITERATURE CITED:

1. French, E. R. 1986. Efficacy of five methods of inoculating potato plants with Pseudomonas solanacearum. Phytopathology 76:1078 (Abstr).

2. Gitaitis, R. D., S. R. Ghate, C. A. Jaworski, and S. C. Phatak. 1983. Evaluation of a mass inoculation method of potatoes with Pseudomonas solanacearum. Am. Potato J. 60:625-630.

3. Gonzalez, L. C., L. Sequeira and P. R. Rowe. 1973. A root inoculation technique to screen potato seedlings for resistance to Pseudomonas solanacearum. Am. Potato J. 50:96-104.

4. Nielsen, L. W., and F. L. Haynes. 1960. Resistance in Solanum tuberosum to Pseudomonas solanacearum. Am. Potato J. 37:260-267.

5. Thurston, H. D., and J. C. Lozano. 1968. Resistance to bacterial wilt of potatoes in Colombian clones of Solanum phureja. Am. Potato J. 45:51-55.

6. Valdez, R. B. 1986. Bacterial wilt in the Philippines. Pages 49-56 in G. J. Persley (ed). Bacterial wilt in Asia and the South Pacific. ACIAR Proceedings 13, Canberra.

7. Winstead, N. N., and A. Kelman. 1952. Inoculation techniques for evaluating resistance to Pseudomonas solanacearum. Phytopathology 42:628-634.

BREEDING FOR RESISTANCE TO PSEUDOMONAS SOLANACEARUM

Peter Schmiediche

Introduction

During the last five years or so the subject of "Breeding for resistance to **P. solanacearum**" has been discussed at great length on many different occasions at CIP as well as in various international settings. When this presentation was prepared the question arose whether there was anything left that had not been said or discussed somewhere and some time before, and indeed, it appeared that there was little that had not been exhaustively discussed on different occasions during the past five years. This is why this presentation will concentrate on a few things that have not been dwelt on extensively in the recent past, and it will also represent a summary of the research that has been carried out since the last planning conference on the same subject. Moreover, our latest thinking about breeding for resistance to **P. solanacearum** in potatoes will also be expressed. These thoughts and ideas are the result of at least six years of experience and close encounter with the bacterial wilt pathogen, and some of the ideas presented here took final shape only during the last year when we had to begin thinking about this Planning Conference.

2. The present situation and state of experience

Following are listed some facts about the present situation with regard to the availability and suitability of bacterial wilt resistant potato germplasm:

1. There is sufficiently resistant germplasm available for the temperate potato growing regions of the world. If agronomic measures and the production of clean seeds are combined with the use of resistant germplasm, successful potato production is, as a rule, possible under conditions of soil infestation with **P. solanacearum**. The resistant germplasm commercially available derived its resistance from the diploid cultivated species **Solanum phureja**. Having said this, I have to modify my statement almost immediately. The use of resistant material in combination with agronomic measures as well as the production and the adequate storage of clean seed does not

necessarily assure a stable disease-free potato production over long periods of time. In some regions where potato production proceeds relatively undisturbed and trouble-free for many years even with non-resistant germplasm, the disease may suddenly show up quite severely in exceptionally wet years. We have been observing this in the region of the Callejon de Huaylas in Peru during 1986.

2. The **S. phureja** based resistant germplasm is normally not resistant in what we have come to call the lowland tropics. European tuberosum germplasm, on the other hand, shows often surprising resistance in the field even if it hardly ever displays any resistance in tests conducted under controlled conditions. We have come to associate this type of pseudo-resistance with adaptation to hot conditions and short photoperiod with consequent vigorous growth of the plants. There is sufficient evidence to suspect that the expression of resistance might be a function of adaptation of the resistant germplasm to hot and humid conditions. This was one of the reasons why in the past three years we concentrated on adapting potentially resistant germplasm to the conditions of the lowland tropics by using CIP's best adapted and most advanced lowland populations in crosses with our best bacterial wilt resistant genetic material.

   However, there might be other explanations for the performance of European or North American tuberosum germplasm in the field, and I shall touch upon this phenomenon further down.

3. The last Planning Conference recommended that the genetic base of the currently available **S. phureja** based resistance should be widened by including resistance from other species which at that time could only have meant wild species as no resistance of any consequence had been found in andigena or tuberosum germplasm. This recommendation was a reflection of the concern that so far all resistance had virtually come from two clones of **S. phureja**. This is why we have been concentrating on this recommendation in a major effort in the 7 years that have passed since the last Planning Conference. The wild species **S. sparsipilum**, **S. chacoense** and **S. microdontum** were identified as new sources of resistance, and it has been successfully demonstrated that wild species can be used efficiently and effectively in a relatively short

time if the bulk of the breeding work is carried out at the diploid level. As a result of this breeding work, new tetraploid populations with at least four specific sources of resistance are now either in field tests or otherwise in various stages of development. Some of the material is being tested in the regions, particularly, however, in the Philippines and in Indonesia. Table 1 demonstrates quite clearly the superiority of the material with a broad spectrum multi-specific resistance over material that has **S. phureja** as its only source of resistance. I shall mention further down how long it will take until we will know how useful this material has been in the field.

4. There are symptomless carriers in the **S. phureja** based material, and it is not understood why some genotypes get infected with the pathogen and do not succumb to wilt. It is obvious that this type of tolerance is most undesirable as it helps to spread the disease with apparently healthy material. Susceptibility is certainly preferable to this type of tolerance. Whether the resistance based on wild species also produces symptomless carriers, we do not know yet.

5. To grow potatoes under conditions of **P. solanacearum** infestation has so far been nothing more than a more or less successful co-existence with the disease. This co-existence is aided by agronomic measures such as crop rotation, inter-cropping, specific cultural practices, as Kloos et al. (1986) have shown in Mindanao, or flooding if potatoes are cultivated after wetland rice. Climatic conditions such as prevalent in the Gangetic Plains of northern India or southern Nepal during the monsoon apparently eliminate the pathogen in the soil as well and make wilt-free potato production possible in the winter. Sometimes co-existence with the disease is possible with non-resistant, particularly, however, European tuberosum varieties that are well adapted to lowland tropical conditions.

6. Often we see mixed infection in the field. In the Philippines, bacterial wilt resistant material, which was grown under natural conditions of heavy **P. solanacearum** inoculum pressure, was found to be principally infected with **Erwinia** sp. in that particular field at that particular time. Similar observations have been made in Central Africa.

3. The nature of resistance

Rowe and Sequeira (1970), Rowe et al. (1972) as well as Zalewsky and Sequeira (1975) worked out a complex system of genetic control for bacterial wilt resistance in potato with the resistance coming from **S. phureja**. They put forward the hypothesis that resistance to **P. solanacearum** coming from **S. phureja** is controlled by 3 or 4 dominant and independent major genes (at different loci), and they also found some evidence for the presence of about the same number of modifying genes which can only act if at least one of the major genes controlling resistance is present.

Although we have not yet obtained any conclusive results regarding the nature of resistance coming from the wild species, first indications - obtained here and also in the Philippines - are that the resistance based on wild species is probably polygenic. Considering that the allels governing resistance are different in different species, the nature of resistance in a panmictic population representing at least four different species is easiest considered as polygenic in nature. The breeding strategy we have been following has therefore been that of population breeding with progeny testing. With each cycle of recurrent selection, the frequency of resistance genes has apparently increased at least judged by the results obtained in greenhouse tests.

In the **S. phureja** based material there is some intriguing evidence that selfing increases the frequency of resistance considerably. Because of the well-known problems of inbreeding depression related to selfing we have so far, with the exception of a few isolated cases, refrained from selfing.

4. Testing for resistance

Any breeding program is only as good as the testing or evaluation programs for the trait we are interested in, and more will be said about this particular problem during this conference.

Almost all the resistant material we have in the field worldwide was found with greenhouse methods of artificial inoculation and subsequent screening. However, we also know that the resistance we are looking at is rather specific and

shows specific strain/genotype/environment interaction. I think, however, that in spite of the limitations of the greenhouse screening methods, progress has been made particularly since we started screening in a system of various steps, trying to reduce the possibility of escapes. Even that does, unfortunately, not mean that the genotypes selected in the screenhouse as resistant will always turn out to be resistant under field conditions.

Some of our material with multi-specific resistance was, for instance, tried under field conditions in the Philippines. The wild ancestors of that material had been screened against four to five different strains of the pathogen. The genotypes that had shown resistant to all four or five strains of the pathogen were hybridized with diploid cultivated material, and the resulting, still diploid hybrid population was divided into two groups. One group was screened against a strain of Race I of **P. Solanacearum** from Taiwan, and the second group was screened against a strain of Race III from Peru. The survivors of this screening test were crossed with a tetraploid population which had its resistance derived from **S. phureja** in a tetraploid/diploid program of hybridization, and the resulting tetraploid hybrid population was given the code TD. Another set of characteristic we needed in this tetraploid population was adaptation to heat and earliness as we needed material that would mature in 90 to 100 days and grow under hot and humid conditions. The bacterial wilt resistant TD population was therefore crossed with CIP's best heat adapted and early maturing population available at the time. Genetically this last cross represented a backcross. The resulting heat adapted and early maturing hybrids, which combined between four and five different sources of specific resistance, were tried under natural infection pressure in the field in Mindanao, Philippines.

The first field test in Mindanao was carried out under conditions of light infection pressure. The harvest of this early maturing population took place at the beginning of 1986. The selected clones were re-planted, later in 1986, under conditions of heavy infection pressure. In this trial, a great deal of the material was lost through wilting, however, about 20 selections (roughly 10 %) were made that had withstood heavy infection pressure which was atypically severe even for Mindanao conditions. Some clones had a survival rate of 100% which is highly unusual under conditions of a high **P. solanacearum** inoculum. Surprisingly, however,

only genotypes from the group whose immediate ancestors had been screened against the relatively mild Taiwanese strain of Race I showed resistance - sometimes strongly so - under these conditions of natural soil infestation.

A second set of basically the same material, genetically only slightly different from the population just described, was planted in the middle of 1986 again in Mindanao. A field with a relatively low inoculum level had been chosen for this trial. About 35 selections (approximately 15 %) were made on the basis of yield and agronomic characters. Although very encouraging with regard to yield and agronomic characters, this material will now have to show its resistance under conditions of heavy infection in the next planting season.

5. The return of evaluation data

Experience has clearly shown that it takes about five to six years from the moment germplasm goes to the regions or national potato programs until evaluation data on the material sent return to CIP. Field data on the performance of hybrids which were produced in 1981 have only just become available in the latter part of 1986. The reasons for this relatively slow flow of information are manifold. As a rule, only small quantities of germplasm, TPS as well as clonal material can be sent. So, many national program workers prefer to go through one or various steps of multiplication before they start field testing the material. We have been encouraging steps of multiplication because of the nature of the resistance and the pathogen we are working with. Resistance is, as a rule, expressed as a percentage of survivors of a given genotype that was grown under field conditions of natural **P. solanacearum** infestation or artificially inoculated with the pathogen under controlled conditions. The higher the percentage of survival or the lower the percentage of loss, the more resistant the genotype. If only the small quantity of material we have been able to distribute is tested, eventual loss does not necessarily mean that a particular clone or genotype was really susceptible, it only means that the particular individuals tested did not survive the test and that if a large enough number of the same genotype had been tested, it might have shown resistance. If the multiplied material is then tested in various locations in different years, and if tests are repeated to confirm resistance, four to five years have, as

a rule, passed until results become available to CIP. I know of no way to reduce this period substantially. This problem is, of course, not only restricted to the breeding program for resistance to bacterial wilt.

Some of the new material with multi-specific resistance has only just left CIP, and we shall not know much about its value in the field or as parental material in breeding programs for some years to come. We know already that in some parts they would like to have the wilt resistance combined with resistance to the root knot nematode, viruses, **Erwinia**, late blight and other things.

6. Prospects of future breeding programs for resistance

Where do we go from here? Since we started to utilize the first group of resistant wild species, many more accessions of a number of wild species have shown good levels of resistance under greenhouse conditions. The most intriguing part about at least one group of wild species is that the resistance found cannot have evolved due to selection pressure because these species occur at altitudes where the pathogen does not exist or has never been found.

Why then would a high altitude species like frost-resistant **S. acaule** have evolved resistance to **P. solanacearum**? The resistance of these species must have evolved as a consequence of something else which we have not yet understood. However, it would certainly be of great value to understand the elements that are responsible for bacterial wilt resistance in germplasm that has apparently never been exposed to the pathogen.

The utilization in breeding of these species is no problem. I think we have clearly shown that we can utilize wild species very effectively in a relatively short period of time, particularly if the basic combinations are made at the diploid level.

As mentioned earlier, another intriguing aspect we have come across is the apparent resistance of some of the European germplasm under field conditions even if the same germplasm has shown no resistance under controlled conditions. We have been attributing this pseudo-resistance to superior adaptation to lowland tropical conditions, and there is a body of indirect (and it can only be indirect) evidence that suggests that adaptation has to do with the expression of resistance.

One of many characteristics that most European varieties have in common is, for instance, resistance to wart (**Synchytrium endobioticum**) or cyst nematode (**Globodera rostochiensis**). Now, we know that these two resistances are determined by a fairly straightforward system of major genes. But what do these resistances actually do for a plant not to become infected with the fungus? What is it really that prevents the fungus or the nematode from getting established in the root system of the resistant or immune genotypes? This questions is asked knowing full well that some of the mechanisms are apparently understood. Do wart or nematode resistant genotypes have "tougher" roots than susceptible ones? If so, could it be that the same characteristics or some type of interaction mechanism protects the plant up to a certain point from the invasion of the bacterial pathogen? Could this mechanism or some derivative mechanism be similar to the resistance to root knot nematode which apparently also gives resistance to **P. solanacearum**? Would it be worth our while to look into this more closely?

It has been clearly shown during the time the potato has moved into hot and humid environments that adaptation to these new conditions is an important factor for the expression of resistance at least to bacterial wilt. More research is needed to understand this phenomenon. As long as there is no general and absolute resistance to **P. solanacearum** available, we shall have to devise methods that permit a more or less successful co-existence with this pathogen.

Bibliography

KLOOS, J. P., FERNANDEZ, B. B., TUMAPON, A. S., VILLANIEUVA, L., and TULOG, B. 1986. Bacterial Wilt Research in Mindanao. CIP Circular Vol. 14, No. 3. The International Potato Center, Lima, Peru.

ROWE, P. R. and L. SEQUEIRA. 1970. Inheritance of resistance to **Pseudomonas solanacearum** in **S. phureja**. Phytopathology 60: 1499-1504.

ROWE, P. R., L. SEQUEIRA, AND L. C. GONZALEZ. 1972. Additional genes for resistance to **Pseudomonas solanacearum** in **Solanum phureja**. Phytopathology 62: 1093-1094.

ZALEWSKY, J. C. and L. SEQUEIRA. 1975. An antibacterial compound from **Solanum phureja** and its role in resistance to bacterial wilt. Phytopathology 65 (12): 1336-1341.

ADDITIONAL SOURCES OF RESISTANCE TO BACTERIAL WILT

E. R. French and Luis Sequeira

(A summary of the presentation; Planning Conference on Bacterial Diseases 16-20/3/87)

Both at the Univ. of Wisconsin-Madison and CIP-Lima programs for screening wild species of Solanum were conducted in the hope of finding general resistance that would not be as vulnerable at high temperatures as the S. phureja sources have proven to be.

At Wisconsin 1575 accessions from the Inter-Regional Potato Collection (IR-1) - Sturgeon Bay that represented 102 species were tested as approximately 50-seed lots which were planted, two per tray in jiffy mix. Seedlings grew for 21 days in a greenhouse at 22°C. Prior to inoculation they were not watered for 3 days. Inoculum was a drench with 5 liters of a mixture of Pseudomonas solanacearum strains 8 (race 1) and 81 (race 3) at concentrations of 6.0×10^5 and 1.2×10^6 CFU/ml, respectively. A knife was thrust between rows to injure roots. Incubation was for 21 days at 28°C. Survival rates ranged from 0 to 100%. The 41 most resistant accessions included 19 S. demissum, 6 S. phureja, 4 S. acaule, 3 S. commersoni, 2 S. polytrichon, 2 S. raphanifolium, and one each S. berthaultii, S. blanco-galgosii, S. boliviense, S. brachycarpum and S. chacoense. Future plans are to test the high-temperature stability of the resistance of these clones.

At CIP 85 intra and interspecific crosses between 31 tuber-bearing Solanum spp were screened by planting two trays for each with 50 seeds in a screenhouse (24 to 38°C range). After 4 weeks the soil mix was drenched with a suspension of 1×10^8 CFU/ml of Peruvian strain 204, race 3. As plants developed initial wilt symptoms they were pulled. Survival ranged from 0 to 100%. Non-wilted plants were transplanted at 8 weeks to 1-liter plastic pots, many dying soon thereafter from latent infection being expressed. Survivors (3-48 per family) were again inoculated after 4 weeks with 40 ml of inoculum per pot. Survivors of the best 16 families were grown for tuber production, many failing to tuberize. Clones that did, were inoculated with 1×10^9 CFU/ml Rwandese race 3 strain 122.

Seventeen families that had high levels of resistance involved 11 confirmed parental tuberiferous Solanum species (acaule, bukasovii, boliviense, candolleanum, coelestipetalum, leptophyes, phureja, peloquinianum, raphanifolium, sogarandinum and sparsipilum) and one tentatively identified species (tapojense). Three of the families were of intraspecific crosses involving different accessions of boliviense, peloquinianum and sogarandinum. Selected clones could not be maintained, but seven families representing the most vigorous in growth and with a high frequency of resistance, were screened again. The best clones have been placed in tissue culture for maintenance and future testing for high-temperature stability and general resistance.

The species tested at Wisconsin were accessions, whereas those tested at CIP were crosses between two accessions within or between species. Five of the species selected at the former were represented among those at the latter. Other species reported as resistant in CIP work are S. bulbocastanum, S. capsicibaccatum, S. curtilobum, S. jamessi, S. microdontum and S. stenotomum (3, 4, 5).

Literature cited

1. Barlow, E., Sequeira, L., and R. Hanneman. 1986. Screening the IR-1 collection of Solanum species for resistance to bacterial wilt. Phytopathology 76: 1137 (Abstr).

2. French, E.R., and C. Ochoa. 1986. Resistance to Pseudomonas solanacearum in several wild species of tuberbearing Solanum. Am. Potato J. 63: 424-425 (Abstr).

3. Martin, C. 1979. Sources of resistance to Pseudomonas solanacearum. Pages 49-54 in Developments in control of potato bacterial diseases. CIP, Lima-Peru.

4. Martin, C., and E.R. French. 1977. Reaction of some tuberbearing Solanum species to Pseudomonas solanacearum. Proc. Am. Phytopathol. Soc. 4: 139 (Abstr).

5. Ochoa, C., and P. Schmiediche. 1983. Systematic exploitation and utilization of wild potato germ plasm. Pages 142-144 in W.J. Hooker (ed.) Research for the potato in the year 2000. CIP, Lima-Peru.

Table 1. Seedling survival rates (~ survival-S.E.) of the 22 accessions rated as very resistant 21 days after inoculation with a race 1 and a race 3 strain of Pseudomonas solanacearum

Solanum species	Accession number	Survival rate (%)
S. polytrichon	PI 365393	98.7
S. raphanifolium	PI 473466	97.1
S. commersonii	PI 320266	95.5
S. acaule	Och 10,979	93.0
S. polytrichon	Och 14,178	92.5
S. demissum	PI 230488	91.6
S. commersonii	PI 458319	90.5
S. demissum	PI 186556	89.9
S. demissum	PI 175423	89.5
S. phureja	PI 320379	89.1
S. brachycarpum	PI 275180	89.0
S. demissum	PI 161366	88.7
S. demissum	PI 160220	88.4
S. commersonii	PI 472837	87.9
S. raphanifolium	PI 458406	87.8
S. demissum	PI 338619	86.9
S. phureja	PI 320363	86.8
S. demissum	PI 160208	86.7
S. demissum	PI 205518	86.6
S. demissum	PI 161164	86.1
S. phureja	PI 320371	85.4
S. demissum	PI 186551	85.3

Table 2. List of 42 accessions rated as resistant (survival rate 84.5-74.6) 21 days after inoculation with a race 1 and a race 3 strain of *P. solanacearum*

Solanum species	Accession number	Solanum species	Accession number
S. demissum	PI 161719	S. demissum	PI 230559
S. demissum	PI 175411	S. demissum	PI 225711
S. boliviense	PI 310974	S. demissum	PI 201850
S. demissum	PI 365381	S. stoloniferum	PI 365395
S. demissum	PI 161155	S. phureja	PI 320369
S. phureja	PI 320358	S. demissum	PI 365382
S. acaule	PI 473483	S. chacoense	PI 175401
S. demissum	PI 160222	S. chomatophilum	PI 310990
S. acaule	Och 11,336	S. demissum	PI 175408
S. demissum	PI 230579	S. phureja	PI 320365
S. phureja	PI 320381	S. chacoense	WRF 1734
S. acaule	HHA 6611	S. demissum	PI 161169
S. demissum	PI 161729	S. demissum	PI 365386
S. chacoense	PI 472827	S. phureja	PI 320367
S. berthaultii	HHA 6451	S. phureja	PI 320380
S. demissum	PI 161151	S. demissum	PI 201853
S. blanco-galgosii	PI 365368	S. demissum	PI 218047
S. demissum	PI 161365	S. commersonii	PI 320267
S. phureja	PI 320386	S. phureja	PI 320382
S. demissum	PI 205516	S. sparsipilum	PI 246536
S. demissum	PI 161149	S. chacoense	PI 320286

Table 3. Potato wild species crosses selected for resistance to race 3 of *Pseudomonas solanacearum* by screening with peruvian strain 204 at 1×10^8 CFU/ml first as seedlings in trays, and again after transplanting and a third time as clones (4-10 plants) with Rwandese strain 122 at 1×10^9 CFU/ml. Screenhouse temperatures, 24-38°C diurnal fluctuation.

Family number	Parents accession numbers & species[a]		Percent survival		
			Seedlings in trays	Transplants in pots	Clones in pots
16.1	ACL 12037	x BUK 13116	61.2[b]	25.0 (4)[c]	100.0
18.1	ACL 12073	x SOG 13336	69.1	66.7 (3)	83.3
31.3	BUK 13856	x PHU 10199	56.1	46.2 (26)	100.0
.5					100.0
33.1	BUK 13166	x COP 13667	37.5	11.1 (9)	66.7
34.4	BUK 13166	x PHU CCC 4731	59.5	32.1 (28)	100.0
.8					100.0
36.4	BUK 13176	x PHU 11211	77.8	31.8 (22)	100.0
.5					66.7
37.2	BLV 11929	x BLV 11937	82.9	13.5 (37)	83.3
.5					60.0
40.4	BLV 11935	x BLV 11938	92.0	63.0 (27)	100.0
.5					100.0
.15					100.0
.16					80.0
41.2	BLV 11938	x BLV 11935	79.4	51.6 (31)	100.0
.3					75.0
.4					75.0
.11					80.0
42.13	BLV 11938	x BLV 11937	72.6	68.0 (25)	100.0
.14					100.0
44.1	CND 11805	x CND 11814	77.3	20.0 (10)	75.0
63.5	PLQ 13231	x PLQ 13230	95.1	23.7 (38)	100.0
65.1	RAP 13759	x LPH 13542	89.6	22.5 (40)	66.7
.4					60.0
66.6	SOG 13336	x SOG 13006	100.0	41.2 (17)	80.0
.4					70.0
.5					60.0
70.4	(SPL x TAP?)11822	x (SPL x LPH) 11893	93.3	36.4 (11)	62.5
99.2	BUK 13857	x LPH 13542	58.2	50.0 (4)	100.0

a/ Codes for *Solanum* species are: ACL = acaule; BUK = bukasovii; BLV = boliviense; CND = candolleanum; COP = coelestipetalum; LPH = leptophyes; PHU = phureja, PLQ = peloquinianum; RAP = raphanifolium; SOG = sogarandinum; SPL = sparsipilum; TAP = tapojense (?).

b/ Number of seedlings that grew from 100 planted ranged from 44 to 82.

c/ In parenthesis, number of transplanted seedlings that survived for this second inoculation.

ROLE OF NEMATODES IN DISEASE EXPRESSION BY *PSEUDOMONAS SOLANACEARUM* AND STRATEGIES FOR SCREENING AND BREEDING FOR COMBINED RESISTANCE

P. Jatala, C. Martin and H. A. Mendoza

Possible association of more than one microorganism with host plant at a given time is among the factors in initiation and development of certain plant diseases. It is conceivable to assume that any potential host is constantly exposed to various root pathogens in the field and undoubtedly these pathogens affect one another, as they occupy the same ecological niche. Further, it is reasonable to suspect that infection by one pathogen may alter the host response to subsequent infection by another or even the same pathogen. Soil and root, being suitable media for microorganisms such as nematodes, fungi and bacteria, are very important sites of disease complexes.

Plant parasitic nematodes often play an important role in disease interactions. Studies concerning interrelationships of nematodes and other microorganisms in the development of disease complexes have gained considerable attention and recognition in recent years. Literature concerning various aspects of interactions of nematodes and other plant pathogens exceeds 1000 papers. The earliest published work of interaction of nematodes and bacteria was in 1901 by Hunger (1) who found tomatoes were readily attacked by *Pseudomonas solanacearum* in what might have been *Meloidogyne* infested soil.

Root-Knot nematodes *Meloidogyne* species and *P. solanacearum*, the causal agent of bacterial wilt are widely distributed in the world and are particularly favored by warm soil temperatures. These organisms have a rather wide host range and are a threat to potato production, particularly in the warm tropical areas. Common occurrence of these organisms is of concern since they readily interact on potatoes and produce disease complexes. Presence by *Meloidogyne* species apparently enhance the wilt development due to *P. solanacearum*. Results of interaction studies conducted in greenhouses indicate that the resistance of clones BR-73-40 and BR-63-76 to bacterial wilt was broken when *M. incognita* was present (3). Similar results were obtained under field conditions (5). Results of greenhouse studies indicate that root infection by nematodes as expressed by root galling index was directly correlated with bacterial wilt symptoms as expressed by percent plants wilted (3). Reaction of *Solanum sparsipilum* and *S. chacoense* (*M. incognita* resistant clones) and *S. tuberosum* ssp. *andigena* (*M. incognita* susceptible cultivar 'Mariva') to *P. solanacearum* was correlated with their reaction to *M. incognita* (2,4) Mechanism of the synergistic reaction of *M. incognita* and *P. solanacearum* is believed to be that of mechanical wounding as well as physiological changes in potato plant caused by infection of the root-knot nematodes.

Attempts have been made to incorporate the genes governing the resistance to M. incognita, M. javanica, M. arenaria and M. hapla into clones having resistance to P. solanacearum. It is interesting to note that the clones initially identified as resistant to M. incognita also had resistance to P. solanacearum. It is, therefore, conceivable to believe that such resistances may be utilized in breeding programs. Considering the global importance of P. solanacearum and its particular characteristics of sharing the same agroecological distribution and occurrence with Meloidogyne species, it is of paramount importance that breeding programs should include the incorporation of resistance to both of these organisms.

Results of recent studies indicate the synergistic relation of Pratylenchus spp. and P. solanacearum in development of disease complexes (Canto, Personal Communication). Although P. solanacearum is not a major problem in the cool climates, its relationships with Globodera pallida, principally a cool climate adapted nematode, as disease complexes has been well established. Additional studies are warranted to determine the extent of such relationship between P. solanacearum and these two important nematodes in development of disease complexes. Economic importance of their interaction needs to be determined.

Although chemical control of Meloidogyne, Pratylenchus and Globodera species can be achieved and their resultant interaction with P. solanacearum can be minimized, such measures are prohibitive particularly in developing countries of the tropics where marginal farming is common. Utilization of P. solanacearum resistant cultivars in the areas with known history of Meloidogyne or Pratylenchus infestation can only result in the loss of plants and yield. Recent progress in breeding for Meloidogyne resistance has been most promising and such breeding programs must take into account the incorporation of resistance to these two important organisms.

Because of the efficiency of Meloidogyne resistant screening method, the variability of the plant reaction to bacterial wilt organism and the inconsistencies in the P. solanacearum screening methods, it is advisable to initially screen the breeding populations for resistance to Meloidogyne species. Once the resistant progenies are selected, they can be screened for resistance to P. solanacearum. This procedure would eliminate the possibility of escape as well as the symptomless latent infection by P. solanacearum which can manifest itself at a later stage. Combination of the two organisms for screening purposes is not advisable as they may interact synergistically and as a result some material which can otherwise be of interest may be lost. Initial selection of Meloidogyne resistant progenies would reduce the workload for P. solanacearum screening. It would also allow the possibility of multiplying the material for a proper and replicated P. solanacearum screening process. Similar procedures may be used in incorporaton of resistance to Pratylenchus spp. and P. solanacearum or Globodera spp. and P. solanacearum.

Literature Cited

Hunger, F.W.T. 1901. EenBacterie-ziekteden Tomacet. Meded. PlTuin. Batavia 48: 4-57.

Jatala, P. 1978. Studies of the Interrelationships of the Plant Parasitic Nematodes and other Organisms on Potatoes. In: Developments in the Control of Nematode Pests of Potato. Report of the 2nd Nematode Planning Conference. International Potato Center, Lima, Peru. pp. 191-193.

Jatala, P., E.R. French and L. Gutarra. 1975. Interrelationships of *Meloidogyne incognita acrita* and *Pseudomonas solanacearum* on potatoes. Journal of Nematology 7: 325.

Jatala, P. and C. Martin. 1977. Interactions of *Meloidogyne incognita acrita* and *Pseudomonas solanacearum* on *Solanum chacoense* and *S. sparsipilum*. Proceedings of the American Phytopathological Society. Vol. 4: 178.

Jatala, P. and C. Martin. 1977. Interactions of *Meloidogyne incognita acrita* and *Pseudomonas solanacearum* on field grown potatoes. Proceedings of the American Phytopathological Society. Vol. 4: 177-178.

PROGRESS IN RESISTANCE BREEDING IN POTATOES AS A FUNCTION OF EFFICIENCY OF SCREENING PROCEDURES

H. A. Mendoza
Head and Senior Geneticist, Breeding and Genetics Dept.

1. Introduction

The breeding program at the International Potato Center (CIP) maximizes the utilization of the genetic resources contained in the genus Solanum. These include commercial cultivars and breeding lines, primitive cultivated species, and a set of selected wild species containing valuable traits for resistance or tolerance to stresses, pests, and diseases. All genetic information contained in the genus Solanum that is related to adaptation, yield per se, and disease, pest, and stress resistance or tolerance is distributed among the large group of clones forming the highly heterozygous and heterogeneous gene pool. Due to the large population size the frequency of genes controlling some specific attributes is low.

One of the basic goals in the management of this very large gene pool is to increase the frequency of genes controlling desirable attributes. To achieve this goal recurrent selection with progeny testing is applied on a cyclic fashion (Mendoza, 1980 and 1986a). The degree of success in altering the genotypic structure of the population, by modifying its gene frequency, is a function of the precision in identifying and isolating the individuals carrying the attribute under selection. Any errors or "escapes" during the process, depending on their magnitude, could alter the response to selection. This justifies the statement that "a breeding effort can only be as efficient as the screening procedure permits".

Considering resistance breeding, if the gene frequency of the trait under selection is low the breeder is forced to work on large populations. In this case screening performed on seedlings, when seedling and adult plant behavior are well correlated, is of extreme value. Therefore, all efforts should be made during the screening process to minimize escaping. The lack of a high correlation between seedling and adult plant screenings leads not only to inefficiency but could be dangerous. It is inefficient because it may permit a large frequency of escape that could seriously affect the results of

selection. It could be dangerous because, given the totally different physiological conditions of seedlings and adult plants, environmental conditions and inoculum potential under which the screenings are performed, it may eliminate resistant genotypes from the population. Research should be made on this subject, not only based on comparison of progeny means but also in the response of individual genotypes. This can be achieved by growing seedlings during four or five weeks, then taking cuttings and rooting them. Two weeks later the seedlings can be inoculated. Also, the rooted cuttings can be transplanted to the field and inoculated if necessary, when they are 35-45 days old. The comparison of results on an individual genotype basis as well as the comparison of progeny means would determine the real value of a seedling screening technique.

Since most of the genetic variability contained in the potato is present in diploid and tetraploid species a comparison of the effect of selection as influenced by screening efficiency, gene frequency, and the type of gene action, at both ploidy levels; will be discussed in this paper.

2. Seedling screening techniques available for resistance breeding

The availability of screening techniques is important for an efficient breeding scheme aimed to accumulate resistance genes. The elimination of susceptible genotypes from the population at an early stage is very important. Further selection for adaptation, yield, and other agronomical attributes is performed in a genetic background of resistant materials. Seedling screening techniques are available to select for resistance to the following pest, disease, and stress factors: late blight, early blight, bacterial wilt, root knot nematode, PVY, PVX, PLRV, frost, and aluminum toxicity. (Mendoza, 1986).

3. Random mating equilibrium

To make a meaningful discussion of selection applied to a population, a random mating equilibrium situation must be assumed where the genotypic frequencies will be constant from one generation to the next in the absence of selection, migration, and mutation. In diploids the equilibrium is reached in a single generation of random mating and the genotypic frequencies will be given by the expression $(p + q)^2$ where p and q are the frequencies of

the alleles A and a respectively and $p + q = 1$. Then, the genotypic array at equilibrium is p^2AA, $2pqAa$, and q^2aa. (Falconer, 1981).

In tetraploids the equilibrium stage is not reached in a single generation of random mating as in diploids but it is approached asymptotically. The diploid structure of the gametes does not permit a free assortment of the alleles, then all possible genotypic structures at the locus considered may not be present with a single generation of random mating. The genotypic array at equilibrium for a tetraploid locus with alleles A and a with frequencies p and q respectively, and $p + q = 1$ is given by the expression $(p + q)^4$, i.e. pA_4, $4p^3qA_3a$, $6p^2q^2A_2a_2$, $4pq^3Aa_3$, q^4a_4. (Kempthorne, 1957). For practical purposes one can consider that the equilibrium is reached after 4 generations of random mating. (Busbice, et al., 1972).

4. Selection

If in a given population one selects individuals presenting certain types of attributes and discard those which do not, one is attempting to modify the genotypic structure of that population. The extent to which selection modifies the genetic make up of the population depends among other factors, on the efficiency to identify and isolate the desirable individuals or discarding the undesirable ones. When the selected individuals are entered into a new reproductive cycle, the resulting population should have a different genotypic structure with respect to the original one. This is due to changes in gene frequency brought about by selection. Within the subject of this Planning Conference an important topic is being covered: breeding for resistance. The success in this task depends again on the efficiency of the screening procedure to identify the resistant genotypes and discard the susceptible ones. If the screening is perfect, i.e., no escapes occurs, then the realized progress will coincide with the expected progress. If a percent of escape takes place a certain number of susceptible individuals will be entered into the next cycle of selection and then the realized progress will decrease depending on the magnitude of escape. This is a most important consideration in the development of a resistance breeding project.

Among the pests, diseases, and stresses that affect the potato crop, resistance or immunity of the host can be controlled by a dominant gene, few genes, or be polygenic.

Immunity to potato viruses Y (PVY), and X (PVX), and resistance to wart, Synchitrium endobioticum and vertical resistance to late blight, Phytophthora infestans are monogenic. Resistances to early blight, Alternaria solani, root knot nematode, Meloidogyne incognita, and cyst nematode, Globodera pallida, are controlled by a few genes. Resistances to late blight (horizontal), bacterial wilt, Pseudomonas solanacearum, and leafroll virus (PLRV), are polygenic. Tolerance to stresses e.g. frost, heat, and aluminum toxicity also appear to be polygenic. With these heterogeneous pattern of inheritance the discussion about resistance breeding will be separated by the number of genes that control the resistances.

Potato breeding can be performed at the diploid and tetraploid ploidy levels. Therefore, both ploidy levels will be considered from the selection point of view and a comparison between them will be made under varying gene frequencies and screening efficiencies.

4.1. Selection in diploids: Changes in gene frequency (one locus model)

Let's assume a diploid population with a locus containing alleles A and a with frequencies f(A)=p and f(a)=q where p + q = 1 and A being completely dominant over a. Let's further assume that this population is at random mating equilibrium. A certain pressure of selection represented by the coefficient s will be applied against the recessive genotype aa and no selection against either AA or Aa.

Genotypes	AA	Aa	aa	Total
Genotypic freq.	p^2	2pq	q	1
Selective value	1	1	1-s	
Gametic contribution	p^2	2pq	$q^2(1-s)$	$1-sq^2$

In the initial population, the equilibrium genotypic frequencies add to 1 while the gametic contribution after selection has taken place will not add to 1 anymore but to $1-sq^2$. This means that a proportion of sq^2 recessive individuals have been selected out and will not contribute any alleles to the next generation i.e. the f(a)=q has decreased.

Therefore, after selection the new frequence of a will be q_1.

$$q_1 = \frac{pq + (1-s)q^2}{1 - sq^2}$$

Then, the change in frequency of the allele a is $\Delta q = q_1 - q$

$$\Delta q = \frac{pq + (1-s)q^2}{1 - sq^2} - q$$ which after simplification reduces to

$$\Delta q = - \frac{sq^2(1-q)}{1 - sq^2},$$ notice the - sign of the expression which indicates that $q_1 < q$

Now, if s=1, this means that the selection pressure is maximum, i.e. the screening eliminates all undesirable genotypes aa. In this case the total gametic contribution is $1-q^2$. Then the above expressions are simplified to:

$$q_1 = \frac{pq}{1 - q^2} = \frac{q}{1 + q}$$

$$\Delta q = \frac{q}{1 + q} - q = - \frac{q^2}{1 + q}$$

Selection applied against the allele a is decreasing its frequency but at the same time it is favoring the allele A which frequency f(A)=p is concomitantly increasing.

After a cycle of selection has been applied against the genotype aa, the new frequency of the allele A will be p_1.

$$p_1 = \frac{p^2 + pq}{1 - sq^2} = \frac{p}{1 - sq^2}$$ and

$$\Delta p = \frac{p}{1 - sq^2} - p = \frac{spq^2}{1 - sq^2}$$

when s=1, these expressions simplify to:

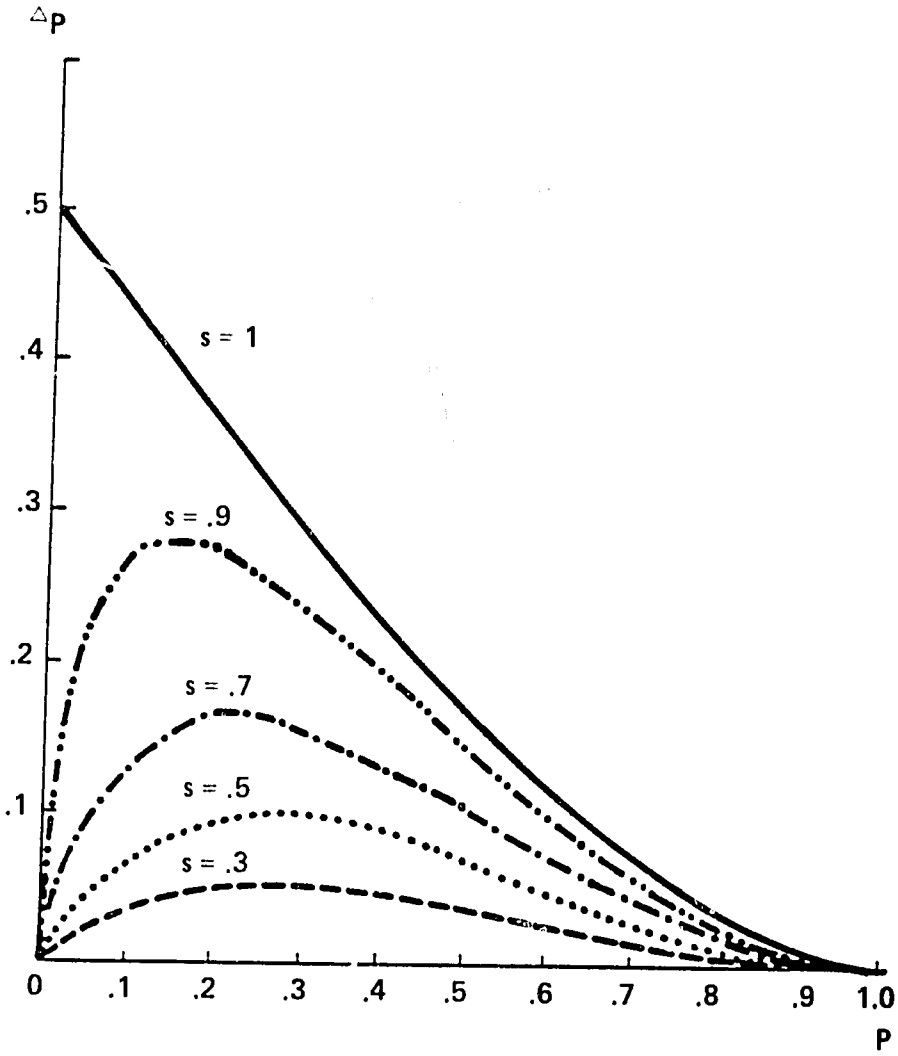
$$p_1 = \frac{p}{1 - q^2} \quad \text{and} \quad \Delta p = \frac{p}{1 - q^2} - p = \frac{pq^2}{1 - q^2}$$

Δp is positive as f(A) increases in each cycle of selection. One should notice that Δp and Δq have the same magnitude but Δp is positive and Δq negative.

Figure 1 shows the response to one generation of selection for a dominant gene A, under various gene frequencies and various levels of efficiency of screening procedures.

It is noticeable that when selection efficiency is maximum, s=1, i.e. no escape from screening, the response to selection in terms of increase in gene frequency, Δp, is also maximum. When s gradually decreases to the values .9, .7, .5, and .3 which indicate that 10%, 30%, 50%, and 70% respectively of susceptible plants are escaping from screening, the response to selection decreases drastically. With increasing rates of escape the breeding work becomes less efficient to a point that its worthiness could be seriously questioned. Also, it is important to notice when the gene frequency of the allele A is very low but at the same time s=1, the change in gene frequency Δp, is very high. If f(A)=p=.01 and selection for A is practiced, Δp=.49 which means that the low initial frequency has been brought up to .5. Beyond this frequency the next cycles of selection will still increase the frequency of A but to a much slower pace. It is also important that when the frequency of A is very small, escapes from screening will considerably reduce response to selection. With values of s=.9, .7, .5, and .3 the corresponding Δp values will be .0748, .0218, .0096, and .0041. The reason of this is very simple. At f(A)=.01, the frequency of resistant genotypes AA and Aa will be 1.99% and the frequency of the susceptible genotypes aa will be 98.01%. If the screening is made with s=.5, means that 49% of the susceptible individuals will escape and they will enter the next cycle of selection as if they were resistant lowering therefore the response to selection. With low frequency of the gene that is selected for the population size should be large.

Figure 1. Change in gene frequency, Δp, in diploids from selection for dominant phenotypes at different levels of selection efficiency, s, and gene frequency, p.



4.2. Selection in tetraploids: Changes in gene frequency (one gene model)

Let's assume a tetraploid population with a locus containing alleles A and a with frequencies f(A)=p and f(a)=q, where p + q = 1. Let's also assume that this population is at random mating equilibrium. A certain selection pressure represented by s will be applied against the recessive genotype aaaa and no selection against the remaining genotypes.

Genotypes	A_4	A_3a	A_2a_2	Aa_3	a_4	Total
Genotypic freq.	p^4	$4p^3q$	$6p^2q^2$	$4pq^3$	q^4	1
Selective value	1	1	1	1	1-s	
Gametic contribution	p^4	$4p^3q$	$6p^2q^2$	$4pq^3$	$q^4(1-s)$	$1-sq^4$

The total gametic contribution is $1-sq^4$ where sq^4 represents the proportion of recessive individuals aaaa or a_4 which have been eliminated and will not contribute <u>a</u> alleles to the next generation.

After selection and assuming that s=1 the new frequency of a will be q_1.

$$q_1 = \frac{p^3q + 3p^2q^2 + 3pq^3}{1 - q^4}$$

$$= \frac{p^3q + 3p^2q^2 + 3pq^3 + q^4 - q^4}{1 - q^4} = \frac{q - q^4}{1 - q^4}$$

$$\Delta q = \frac{q - q^4}{1 - q^4} - q = - \frac{(1-q)q^4}{1-q^4}$$

Notice that again Δq is negative which indicates the frequency of a has decreased.

When selection is not complete i.e. $s < 1$, there

$$q_1 = \frac{p^3q + 2p^2q^2 + p^2q^2 + pq^3 + 2pq^3 + q^4(1-s)}{1 - sq^4}$$

$$= \frac{q - sq^4}{1 - sq^4}$$

$$\Delta q = \frac{q - sq^4}{1 - sq^4} - q = -\frac{sq^4(1-q)}{1 - sq^4}$$

As selection against the allele a will decrease its frequency q, at the same time it will produce an increase in the frequency p of the allele A.

When $s = 1$, the frequency of A after one cycle of selection will be p_1.

$$p_1 = \frac{p^4 + 2p^3q + p^3q + p^2q^2 + 2p^2q^2 + pq^3}{1 - q^4}$$

$$= \frac{p}{1 - q^4}, \text{ and}$$

$$\Delta p = \frac{p}{1 - q^4} - p = \frac{pq^4}{1 - q^4}$$

In this case Δp has a positive value which indicates that the frequency of the allele A has increased after a generation of selection

When $s < 1$ the value of p_1 is the following

$$p_1 = \frac{p^4 + 3p^3q + 3p^2q^2 + pq^3}{1 - sq^4} = \frac{p}{1 - sq^4}$$

$$\Delta p = \frac{p}{1 - sq^4} - p = \frac{s(1-q)q^4}{1 - sq^4}$$

As in the case of diploids Δp and Δq have the same magnitude, being Δp positive, and Δq negative.

Figure 2 shows the response to one generation of selection for a dominant gene A under various gene frequencies and various levels of efficiency of screening procedures.

It is noticeable that at the tetraploid level the effect of s is similar to that discussed for diploids, as s becomes smaller the response to selection decreases. Beyond the gene frequency f(A)=p=.4 the response to election rapidly decreases as the frequency of the susceptible genotype aaaa, q^4, becomes smaller, i.e. 12.96%. Another difference is that when f(A)=p reaches the value .8 the response to selection is practically nil.

4.3. Comparison of response to selection at diploid and tetraploid ploidy levels (one gene model)

In Figure 3 a comparison between response to selection at both diploid and tetraploid ploidy levels is made. One can see that the change in gene frequency is faster at the diploid level. This is understandable if one compares the genotypic arrays at both ploidy levels i.e. p^2AA, $2pqAa$, and q^2aa for diploids and p^4A_4, $4p^3qA_3a$, $6p^2q^2A_2a_2$, $4pq^3Aa_3$, and q^4a_4 for tetraploids. This is showing that in diploids the recessive allele a is hidden by the dominant allele A in the unique heterozygous genotype Aa while in tetraploids the allele a is hidden by A in three genotypes A_3a, A_2a_2, and Aa_3. The consequence is that for any given gene frequency, the frequency of the recessive genotype aa or aaaa, subjected to selection either against or for, will be greater at the diploid level q^2 in diploid versus q^4 in tetraploids e.g. when the gene frequency is q=.5, the frequency of aa will be 25% of the population while the frequency of aaaa will only be 6.25%. Under these conditions and with s=1, the value Δp for diploids will be five times larger than that for tetraploids.

Figure 2. Change in gene frequency, Δp, in autotetraploids from selection for dominant phenotypes at different levels of selection efficiency, s, and gene frequency, p.

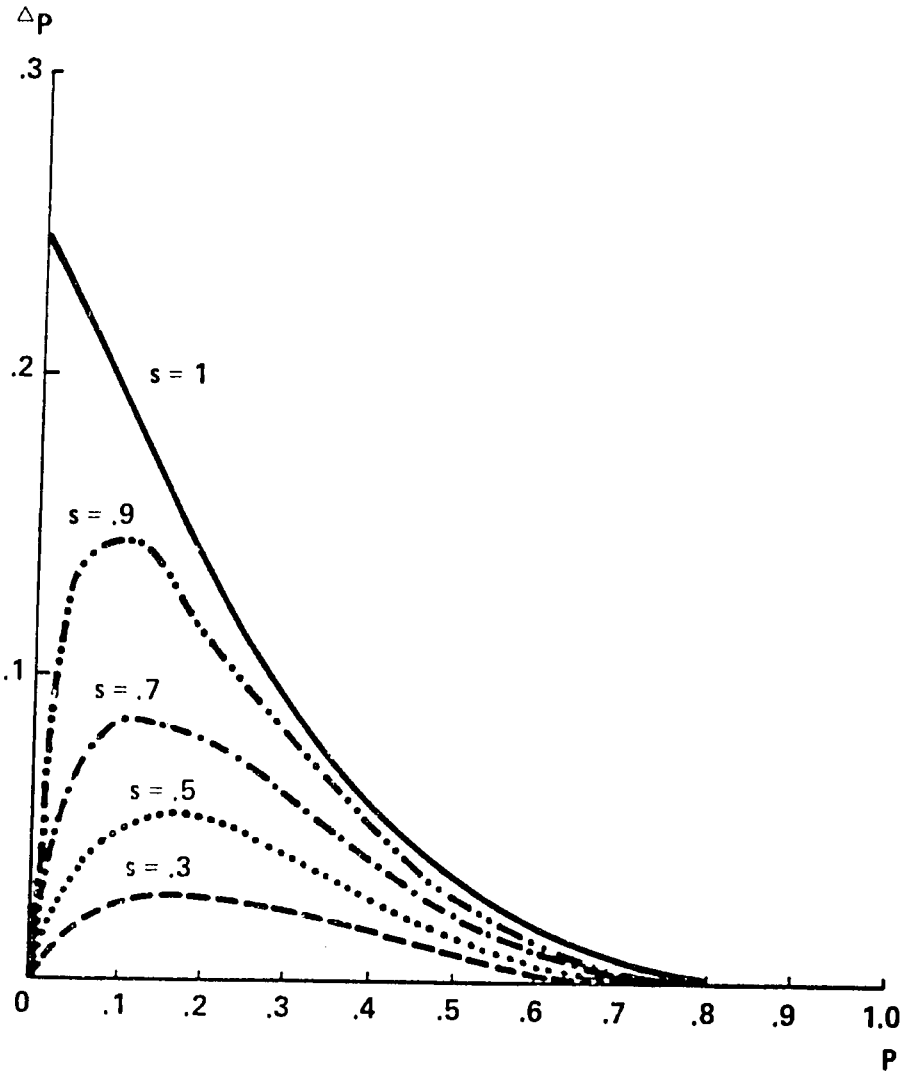
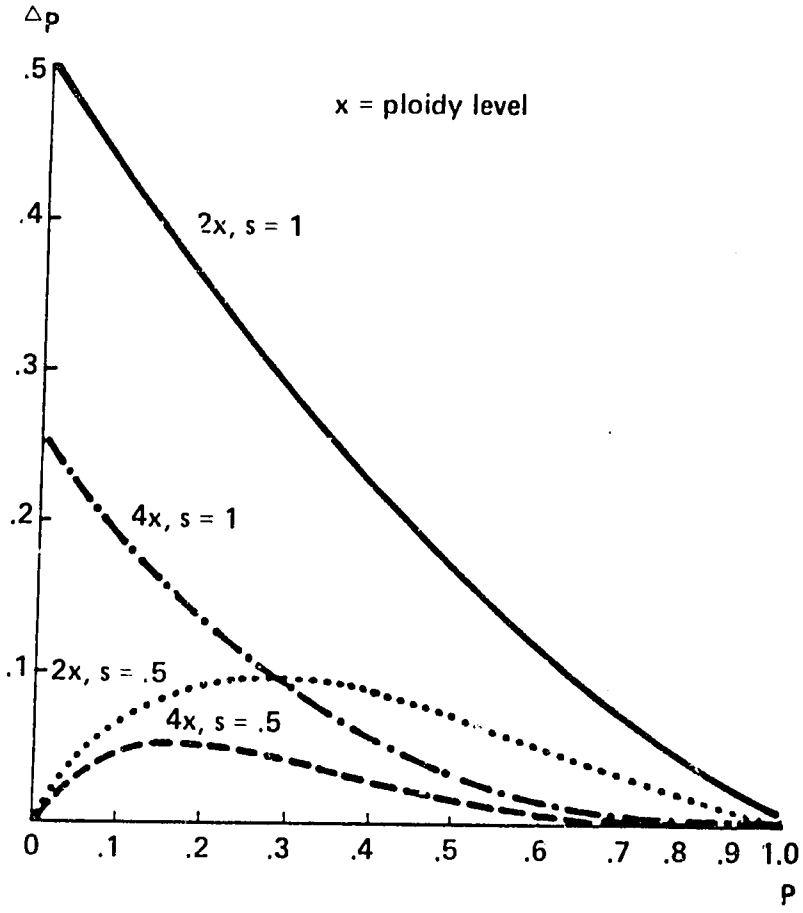


Figure 3. Comparative change in gene frequency, Δp, from selection for dominant phenotypes in diploid and tetraploid populations at different levels of selection efficiency, s, and gene frequency, p.



Assuming that the frequencies of the alleles A and a at both ploidy levels are the same as well as s, the following comparison can be established.

$$\frac{\Delta q(2x)}{\Delta q(4x)} = \frac{-\dfrac{sp^2}{1-sq^2}}{-\dfrac{s(1-q)q^4}{1-sq^4}} = \frac{1-sq^4}{(1-sq^2)q^2} > 1$$

This ratio will always be greater than 1 for all frequencies of q except for values of 0 and 1.

In the resistance breeding research the one locus model fits well in the work of PVY and PVX immunities, since each of the immunities to these two viruses are under the control of one dominant gene. It has been estimated that the efficiency of selection, in the seedling screening for PVY immunity is s=.9, i.e. there is a probability of 10% of the seedlings escaping the screening. However, during the first clonal generation the population is reduced to about 10% of the total number of surviving seedlings (immune + escapes), therefore, the escape is reduced to 1% (Mendoza, 1987). When the seedlings receive an inoculum containing both PVY and PVX the expression of symptoms becomes more severe and the frequency of escape is reduced. Hooker, 1980 carried out serological testing of 800 seedlings that remained symptomless after inoculation. He found that only 2-2.5%, according to the progenies, were infected, i.e. susceptible escapes. The breeding for resistance to these two viruses does not present major difficulties and a consistent increase of gene frequencies has been achieved at CIP as a result of a proper selection of progenitors and an efficient seedling screening technique (Fernandez et al., 1986; Mendoza, 1987).

4.4. Increasing gene frequencies in potato resistances controlled by a few genes

In this case one can not resort anymore to a simple selection situation as the one locus model discussed previously. Some biometrical measurements like means, variances, covariances, and heritabilities obtained from adequate mating

designs, have to be utilized to consider selection effects. A good fit into this category appears to be the resistances to the root knot nematode, the potato cyst nematode, and early blight.

4.4.1. Increase in frequency of genes for resistance to the root-knot nematode, *M. incognita*

Genes for resistance to *M. incognita* from the wild potato species *S. sparsipilum* and *S. chacoense* were transferred into a diploid potato population composed by *S. phureja* and *S. stenotomum*. In the base population the frequency of resistant individuals was 3.5%. After applying successive recurrent selection cycles in this population the frequency of resistance was increased to 4.5%, 27.2%, and 35.5% (Mendoza and Jatala, 1982). The screening was made on seedlings applying a technique developed at CIP (Jatala, 1982). It is considered that the efficiency of this technique is over 90%, i.e., a maximum of 10% susceptible seedlings would escape the screening. This problem is minimized since immediately after the seedling test, a second screening of cuttings of the resistant genotypes is performed and the escapes are identified and eliminated (Jatala, personal communication). A model of three dominant genes responsible for the control of the root knot nematode has been proposed (Mendoza and Jatala, 1985) and this explains the fast progress achieved under selection. Moreover, in a diallel analysis it has been found that most of the genetic variability for resistance to *M. incognita* is additive with a high estimated value for narrow sense heritability h=.78 (Gutierrez *et al.*, 1987). In conclusion, the high heritability for this trait coupled to an efficient screening have permitted a rapid progress under selection for resistance.

4.4.2. Increase in frequency of genes for resistance to the potato cyst nematode (PCN), *Globodera pallida*

Genes for a partial resistance to PCN race P_5A come from a few *S. tuberosum* sp. *andigena* cultivars. The resistance to PCN race P_4A comes from two sources: a) *S. vernei* - *S. tuberosum* sp. *tuberosum* clones breed in The Netherlands

after intensive backcrossing and b) two S. tuberosum sp. andigena primitive cultivars (Scurrah and Franco, 1985).

Screening techniques: A seedling test was utilized during a five year period and then abandoned in 1981 because of high escape rate (Gonzales and Scurrah, 1982). A test growing plants, from seed tubers, in pots and inoculating them is presently utilized. There is an escape rate variable from year to year of about 15% due to variable inoculum performance and environmental factors affecting nematode invasion and development. To minimize this escape, the pot testing is performed twice (Scurrah and Franco, 1985). A more refined Petri plate test (Mugniery and Pearson, 1976) improved accuracy and permitted quantifying the root system's ability to block nematode development. The time cost of this increased accuracy is one extra testing season that brings the selection process to three years per cycle, instead of two, of clonal evaluation. These techniques significantly ameliorated the screening efficiency reflected in an improved selection of progenitors, hence, the frequency of resistance in the progenies also increased.

Within CIP's recurrent selection breeding strategy resistance to G. pallida P_4A and P_5A races are considered. In the first selection cycle only 6% of the progeny exhibited resistance to P_5A, however, with a higher level of resistance. In the case of P_4A, 15% to 20% were rated resistant which suggests that in S. vernei fewer genes were controlling resistance. For the next cycle, clones with resistance to either P_4A or P_5A were intercrossed to combine the two resistances. After screening the progenies, 3% to 5% combined the two resistances. The recurrent selection continued adding emphasis in agronomical attributes. The fourth cycle of selection showed a significant increase of resistance: 65% to P_4A, 53% to P_5A and 35% combined resistances to both races. The agronomic performance of this population has also increased significantly (Scurrah and Franco, 1985). In 1987, a new variety has been released in Peru, Maria Huanca with high yield, partially resistant to P_4A highly resistant to P_5A and immune to PVX (Scurrah, unpublished results).

4.4.3 Increase in frequency of genes for resistance to early blight, *Alternaria solani*

In the last four years CIP has been involved in breeding at the tetraploid level for resistance to early blight. A test to screen seedlings for resistance was developed in 1985. Two identical sets of 26 seedling progenies were inoculated with an inoculum suspension of 1500-2000 spores/ml at the greenhouse in CIP-Lima and under field conditions at San Ramon with a suspension of 2000 spores/ml 45 days after seedling transplanting. Differences for resistance were substantial among progenies in both tests and the correlation between the results was significant at the .05 level of probabilities, r=.45 (Martin *et al.*, 1985). In spite of the significance of the correlation, computed on progeny means, it is necessary to count with a seedling test which results have a higher level of concomitance with the results from field grown plants. Work to improve the seedling test by reconsidering inoculation factors and environmental conditions is underway.

Genetic information from three diallel experiments carried out at San Ramon suggest that a large proportion of the genetic variability for resistance to *A. solani* in tetraploid potatoes is additive. The average narrow sense heritability estimate obtained in these experiments was h^2=.70 (Mendoza *et al.*, 1986b). In diploid potatoes, *S. phureja* and *S. stenotomum*, a narrow sense heritability estimate of h^2=.81 was obtained (Herriott and Haynes, 1984). These high heritability estimates would indicate that the control of resistance to early blight in potatoes, depends on a few genes which act additively. It also indicates that a sizable progress can be achieved under selection. The results of the early blight breeding project thru two cycles of recurrent selection are not comparable because in the second cycle, new sources of resistance were introduced. The field screenings with artificial inoculation carried out at San Ramon on transplanted seedlings, produced a uniformly distributed intense epiphytotics. Although a large number of selected resistant clones are

still late in maturity, a 5 to 10% are of medium maturity. An important success of selection, therefore, has been to break a correlation of resistance with lateness that appeared very strong.

4.5. Increasing gene frequencies in potato resistances controlled polygenically

Resistance to three of the most important potato diseases are involved in this group: late blight (horizontal resistance), bacterial wilt, and leafroll (PLRV). The inheritance of resistance to these diseases is to a variable extent, not fully understood due to their inherent complexity, strong interactions with the environment and screening methods that need to be improved. These difficulties have lead to either insufficient or conflicting genetic research results and an efficiency of selection, that, at least for two of these diseases has not been as effective as needed.

4.5.1. Increase in frequency of genes for resistance to late blight, *Phytophthora infestans*

Most of the available research reports indicate that the control of horizontal resistance to late blight is polygenic. However, when the type of gene action in the control of resistance was considered research results are conflicting. General combining ability for resistance to late blight is important (Tai and Hodgson, 1975; Malcomson and Killick, 1980). However, other authors reported that non-additive gene action is more important (Killick and Malcomson, 1973; and Vasquez, 1984). Furthermore, Landeo and Calua, 1986, using a line x tester design in a tetraploid seedling population evaluated under field conditions found a narrow sense heritability estimate for resistance of h^2 = .65. Conversely, Vasquez, 1984 in a parent-offspring mating design using diploid potatoes found that the heritability for resistance at the seedling level under growth chamber conditions was h^2 = .13. In a field evaluation using the same population the estimate was very small, i.e., h^2=.03.

Several screenhouse and growth chamber screening procedures have been attempted to select resistant individuals from large size populations. These procedures have involved entire plants, leaf discs, detached leaflets, and seedlings. Since the greatest interest at CIP is the seedling screening for large segregating populations discussion is concentrated in this topic. Guzman et al., 1966 reported a high correlation between seedling and field screening, but only on two progenies. Hooker, 1980 reported results of resistance of a seedling test of 20 progenies from blight resistant progenitors compared with resistance of adult plants obtained from the same progenies at the field in Rio Negro, Colombia. This author indicated that the positive correlation at the 95% of probability level, between resistance at these two stages suggested the value of the seedling test. Vasquez, 1984 reported a positive correlation, significant at the 95% level of probabilities, r = .385 between a seedling test and adult plant test in the same group of 42 progenies. Again, as in the case of the type of gene action the results are conflicting. As discussed previously in this paper, in spite of that, correlation coefficients of .4 or .5 might be statistically significant indicate that there is not enough degree of association between seedling and adult plant response to the disease and then one can not depend to a high degree on the seedling screening. More research on the improvement of the seedling screening to select for late blight resistance is necessary.

At CIP, selection for resistance to late blight has combined seedling screening with further exposures of the selected individuals to heavy P. infestans infections in Rio Negro, Colombia; and Toluca, Mexico. So far the results are satisfactory but could be improved as the initial selection at the seedling stage is ameliorated.

4.5.2. Increase in frequency of genes for resistance to bacterial wilt, Pseudomonas solanacearum

Potatoes are attacked by races 1 and 3 of P. solanacearum. These races differ in their ecology and host range: race 1 attacks a wide range of

species and it is present in the lowland tropics and warm temperate lands while race 3 is restricted to potatoes and a few alternative hosts. Race 3 has lower optimum growth temperature and it is present in most of the potato growing areas of the tropics and subtropics and also has strains adapted to cooler environments (French, 1985).

Scarce information is available on the genetic aspects of resistance found in S. phureja. It appears that resistance is dependant on multiple dominant genes. Although relatively few dominant genes conferred resistance to any one P. solanacearum isolate, it became evident that many genes were necessary to confer a broad spectrum of resistance (Sequeira, 1979). Because of this, the breeding for resistance to this bacterium is complex and if several strains are involved resistance can be considered a poligenically inherited trait.

The nature of resistance to P. solanacearum in the MBN (bacterial wilt, root knot nematode) population has not been investigated. This population was created by Mendoza and Jatala and screened for root knot nematode resistance in 1976-1977 and then named MBN and screened for bacterial wilt resistance by Martin in 1978. Bacterial wilt and root knot nematode resistant diploid accessions of S. phureja, S. stenotomum, S. sparsipilum, S. chacoense and to a lesser extent S. microdontum were utilized to build this new population of wide genetic basis. The rational to generate this population was that genes for resistance from these divergent species could be different and when brought together could provide a stable resistance to several strains of the bacterium.

The complex nature of the resistance to P. solanacearum is further complicated by strong environmental effects, e.g., temperature, humidity, light intensity, etc. and interaction with the root knot nematode, M. incognita.

The screening procedures also represent a problem in selection for bacterial wilt resistance. Sequeira, 1979, stated that stem

inoculation was used at first but this method proved to be cumbersome and inadequate to test large populations. He also mentioned that in 1973 a seedling screening technique was developed to rapidly screen progenies against a mixture of different strains of the bacterium. In 1977, the procedure was abandoned because some resistant seedlings surviving the inoculation could carry a latent bacterial infection. Besides Sequeira's concern about latent infection, there is insufficient information about how well correlated are the response to infection of a genotype at the young seedling and at the adult plant stages. If the correlation would be high, in spite of the latent infection, seedling screening could be useful to evaluate the parental value of prospective progenitors. However, in 1978 Mendoza, Martin, and Alvarez, unpublished results, evaluated a population from 4x-2x matings composed by 7,500 seedlings of which about 10% survived. A random sample of the surviving seedlings was grown to maturity. Plants obtained from tubers produced by these resistant seedlings were inoculated again with P. solanacearum and only a few showed some level of resistance. In 1978, Martin, unpublished results, tested 13,181 seedlings of the MBN population and selected 1,655 (12.56%) resistant seedlings surviving to the inoculation with P. solanacearum isolates 013 and 03. Later, plants from tubers produced by the surviving seedlings were inoculated with 4 or 5 isolates of the bacterium and only 81 clones were resistant (4.9%). The repeatibility of the resistance is low, hence, there is room for a question: how many of the screenhouse resistant clones will hold under P. solanacearum infected field conditions? The answer appears to be obvious: there is an urgent need to ameliorate the screening techniques in order to improve the results of selection for resistance. This was already recommended by Sequeira, 1979 during the Planning Conference about Developments in Control of Bacterial Diseases held at CIP in that year.

So far, some bacterial wilt resistant clones from the early work of Rowe, Sequeira, and French have been released as varieties. The resistance was derived from S. phureja and these clones are well adapted to the cool tropics.

On the other hand, it seems necessary to reconsider the breeding strategy to respond to the needs of the farmers of the warm and hot tropics where bacterial wilt is a serious limiting obstacle for potato production.

4.5.3. Increase in frequency of genes for resistance to the potato leafroll virus (PLRV)

Selection for resistance to PLRV is a breeding problem which shares many of the complexities of selection for resistance to bacterial wilt. The genetic information is extremely scarce and the screening techniques have not been studied sufficiently to find out correlations between seedling or adult plant screenings in the screenhouse and the behaviour of the same plants under natural field infection. Hooker, 1980, briefly described the seedling screening technique used at CIP for PLRV resistance as well as the number of seedlings subjected to the test. He also stated that efficiency and usefulness of the seedling inoculation was being evaluated by comparison with progenies exposed to and not exposed to seedling infection. To present, there is no clear cut answer to Hooker's concern about efficiency of the seedling test. An additional complicating factor in selection for PLRV resistance is the interaction of this virus with other important potato viruses e.g. PVY and PVX. This interaction means that if a PLRV resistant potato clone becomes infected with either PVY or PVX or both, its original degree of resistance to PLRV will significantly decrease. The effect of this interaction has been demonstrated at CIP and has produced a change in the breeding strategy and selection procedures in the breeding program at this institution (Mendoza, 1987).

All the above mentioned difficulties may explain the reason for which in spite of that, PLRV is the most important potato virus disease and many programs throughout the world have attempted to select for resistant varieties, very few successes have been obtained.

5. Agronomic value of selection in resistance breeding

Resistance *per se* has an important academic interest but in order to become agronomically valuable it has to be combined with other economically important attributes such as yield, quality, and a reasonable length of the growing period.

Mendoza and Rowe, 1977 proposed a strategy for potato population breeding based in the concept that the commercial value of a clones (X) may be expressed as the function:

$$X = f(A + Y + R)$$

where A, Y, and R represent the effect of groups of genes for adaptation, yield *per se*, and resistance, respectively.

This strategy is directed to situations where potatoes are grown under severe weather, and pest and disease conditions where non-genetic control measures are either insufficient or not applied. Tuber yield and quality are normally considered to measure the adaptation of a given genotype growing under a given environment. However, a more objective way to measure adaptation is by the yield per unit time and per unit area to fit into the characteristics of the growing season.

Resistance, particularly when polygenically controlled, and adaptation are closely related. The genes for resistance will express their effects in a vigorously growing well adapted crop. Conversely, resistance factors contained in an ill-fitted crop will express to a lower degree. Clear examples about this association have been observed in the cases of resistances to late blight, bacterial wilt, insects, etc.

In the process of selection for resistance, therefore, resistance *per se* has always to be combined with adaptation for the environmental conditions where the genetic material will be utilized. This underscores the importance of efficient seedling screening techniques for an early elimination of susceptible genotypes followed by a carefully planned field evaluation to confirm the resistance but linked to adaptation and good commercial characteristics.

6. Concluding remarks

The statement "a breeding effort can only be as efficient as the screening procedure permits" has become very clear throughout the discussion of the various resistances. Immunity to PVY and PVX that is controlled by monogenic dominant alleles has been introduced to several clones i.e. LT-8 and LT-9 which besides their immunity to both viruses are also heat tolerant, high yielding, and early maturing. This progress has been possible due to a simple genetic control and an efficient seedling screening which admits very few escapes. These immunities are in the process of being combined with other resistances, i.e. late blight, early blight, cyst nematode, etc.

A significant progress has also been made in resistances to root knot and cyst nematodes although, resistance was transferred from wild species and primitive cultivars. In the case of these two soil borne pests the availability of screenhouse screening procedures had a significant importance. Otherwise, it would have been necessary to depend on field testing and it is well-known that field infestation is not uniform but very patchy and this would have lead to inefficiency due to a large proportion of escape.

In the case of the blights produced by *P. infestans* and *A. solani*, although the seedling screening techniques need to be improved, the progress achieved in field selection for the resistances have been satisfactory. The choice of adequate testing sites where severe epiphytotics occur naturally or are induced by artificial inoculation have permitted to increase the frequency of genes controlling resistance. Also, it has permitted to select progenitors transmitting their resistance to a high proportion of their progenies.

Selection for resistance to bacterial wilt and PLRV still constitute serious breeding challenges. The complexities involved in these resistances, their interaction with other parasites, the specificity of resistance to *P. solanacearum* are problems to overcome. A significant improvement in the screening techniques as well as a reconsideration of the breeding strategies are indispensable to achieve success. In the particular case of bacterial wilt it seems necessary to promote a more specific selection work in the regions of CIP or even in the country programs since resistant clones selected elsewhere may totally fail in the presence of different *P.*

solanacearum strains. In selection for PLRV resistance steps have already been taken to correct the breeding strategy.

REFERENCES

Busbice, T.H., R.R. Hill, and H.L. Carnahan. 1972. Genetics and Breeding Procedures. In. Alfalfa Science and Technology. C.H. Hanson Ed. American Soc. of Agron. Madison, Wisconsin, USA.

Falconer, D.S. 1981. Introduction to Quantitative Genetics. 2nd. Ed. Longman. London.

Fernández-Northcote, E.N., H.A. Mendoza, and R. Galvez. 1986. Breeding for Potato Virus Y (PVY) Immunity Combined with Earliness and Tolerance to Heat. American Potato J. 63:422-423 (Abst.).

French, E.R. 1985. Interaction between Strains of Pseudomonas solanacearum, its Hosts and Environments. In Bacterial Wilt Disease in Asia and the South Pacific. Perseley G.J. Australian Center for International Agricultural Research (ACIAR), Camberra. 145 pp.

Gonzales, A. and M. Scurrah. 1982. Discrepancy between Seedlings and Tubers for Resistance to Potato Cyst Nematode, Globodera pallida. In. Proc. Int. Congr. on Research for the Potato in the Year 2000. Int. Potato Center. Lima, Peru. 199 pp.

Gutierrez, L.I., H.A. Mendoza, and P. Jatala. 1987. Genetic Variability for Resistance to the Root-knot Nematode Meloidogyne incognita and its Implication in Potato Breeding. (Manuscript sent for publication).

Guzman, J., H.D. Thurston, and L.E. Heidrick. 1966. Metodos de Seleccion por Resistencia Parcial a Phytophthora infestans en el Invernadero. American Potato J. 43:35-42.

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). American Potato J. 61:524 (Abst.).

Hooker, W.J. 1980. Disease Resistance Evaluations. In. Report of the Planning Conference "Utilization of the Genetic Resources of the Potato III". International Potato Center. Lima, Peru. 235 pp.

Jatala, P. 1982. An efficient Method for Screening Large Numbers of Potato Seedlings for Resistance to Meloidogyne species. In. Proc. Int. Congr. on Research for the Potato in the Year 2000. Int. Potato Center. Lima, Peru. 199 pp.

Kempthorne, O. 1957. An Introduction to Genetic Statistics. John Willey, New York, USA.

Killick, R.J. and J.F. Malcomson. 1973. Inheritance in Potatoes of Field Resistance to Late Blight (Phytophthora infestans Mont De Bay). Physiol. Pl. Path. 3:121-123.

Landeo, J.A. and L. Calua. 1986. Combining Ability Analysis for Field Resistance to Late Blight in Potato Seedlings. American Potato J. 63:438 (Abst.).

Malcomson, J.F. and R.J. Killick. 1980. The Breeding Values of Potato Parents for Field Resistance to Late Blight Measured by Whole Seedlings. Euphytica 29:489-495.

Martin, C., H. Torres, and H.A. Mendoza. 1986. Development of an Early Blight Seedling Screening Test in Potatoes. American Potato J. 63:444 (Abst.).

Mendoza, H.A. and P.R. Rowe. 1977. Strategy for Potato Population Breeding for Adaptation to the Lowland Tropics. American Potato J. 54:488 (Abst.).

Mendoza, H.A. 1980. Thrust II Philosophy, Organization, and Program Development. In. Report of the Planning Conference "Utilization of the Genetic Resources of the Potato III". International Potato Center. Lima, Peru. 235 pp.

Mendoza, H.A. and P. Jatala. 1982. Breeding for Resistance to Root-knot Nematodes. In. Proc. Int. Congr. on Research for the Potato in the Year 2000. Int. Potato Center. Lima, Peru. 199 pp.

Mendoza, H.A. and P. Jatala. 1985. Breeding Potatoes for Resistance to the Root-knot Nematode Meloidogyne species. In. An Advanced Treatise on Meloidogyne. Vol. I. Biology and Control. Sasser J.N. and C.C. Carter Editors. North Carolina State Univ. 422 pp.

Mendoza, H.A. 1986a. Advances in Population Breeding and its Potential Impact on the Efficiency of Breeding Potatoes for Developing Countries. In. The Production of New Potato Varieties: Technological Advances. Ed. Jellis G.J. and D.E. Richardson. Cambridge University Press. England. 370 pp.

Mendoza, H.A., C. Martin, R. Vallejo, and J. Espinoza. 1986b. Breeding for Resistance to Early Blight (Alternaria solani). American Potato J. 63:444-445 (Abst.).

Mendoza, H.A. 1987. Breeding for Resistance to Potato Virus Y, X, and Leafroll: Research Strategy and Selection Procedures. (Manuscript sent for publication).

Mugniery, D. et F. Pearson. 1976. Méthode d'Elevage de Quelques Nematodes a Kystes du Genre Heterodera. Sciences Agronomiques Rennes. 217-220.

Scurrah, M. and J. Franco. 1985. Breeding for Resistance to Globodera pallida at CIP. EPPO Bulletin No. 15:167-173.

Sequeira, L. 1979. Development of Resistance to Bacterial Wilt Derived from Solanum phureja. In Report of a Planning Conference on Developments in Control of Potato Bacterial Diseases. International Potato Center. 137 pp.

Tai, G.C.C. and W.A. Hodgson. 1975. Estimating General Combining Ability of Potato Parents for Field Resistance to Late Blight. Euphytica 24:285-289.

Vasquez, V. 1984. Estudio de Resistencia Horizontal a Phytophthora infestans Mont De Bary en Papas Cultivadas Solanum stenotomum Juz. et Buk. M.Sc. Thesis. Universidad Nacional Agraria, La Molina, Lima, Peru. 63 pp.

CULTURAL PRACTICES FOR MANAGING BACTERIAL WILT OF POTATOES

G. S. Shekhawat, A. V. Gadewar, V. K. Bahal, R. K. Verma
Central Potato Research Institute, Shimla 171 001, HP

The bacterial wilt or brown rot incited by Pseudomonas solanacearum is the second most important disease of potato after late blight in India. Its incidence ranges from 3-30% in the hills (below 2200 m), 4-50% in Deccan plateau and 2-20% in eastern plains (CPRI, 1984, 1985, 1986 (2), 10, 13). The disease is more severe in Karnataka (5-55%), Madhya Pradesh (10-50%), eastern plateau and eastern plains (2-26%) as compared to hills where its incidence ranges from 10-30% in Kumaon hills, 10-15% in Simla hills, 5-7% in eastern hills and 5-10% in southern hills. Maximum losses due to the disease reported are up to 55% in Kumaon hills, 50% in Karnataka and 77% in Madya Pradesh.

The P. solanacearum wilt has been a difficult disease to control. In early years of its discovery attempt were made to control it by application of soil disinfecting chemicals like chloropicrin, changing the soil pH with sulfur followed by lime application, crop rotations and use of host resistance (1, 3, 5). Application of urea at the rate of 1000 kg/ha has been found to reduce wilt incidence to a negligible level (2, 5). Success was obtained in controlling the disease by crop rotations with maize, cereals and millets and also through resistance in hosts like tobacco, groundnut, tomato and brinjal (5, 10, 12). In potato the disease has been difficult to manage. Sources of resistance were reported in many tuber bearing Solanum species (4, 9, 11, 15, 19). However, none of the sources yielded a stable resistance at least under Indian conditions.

In the absence of host resistance the bacterial wilt management through cultural practices is an effective alternative. Some of the cultural practices found to reduce bacterial wilt are discussed in this paper.

1. Sites of trials: Replicated field trials for studying the effect of cultural practices were carried out at the following locations:

a) Bhowali: In North-western mid-hills at 1800 m above msl. During testing season the minimum and maximum temperatures ranged 10-15° C and 20-30° C. The soil is gravelled loam to sandy with a pH of 6.2.

b) Shillong: Located in North-eastern hills at 1600 m above msl. During summer and autumn the maximum and minimum temperatures range 20-24° C and 13-18° C. The soil is red yellow with pH 6-6.5.

c) Ootacamund: Located in southern hills at 2200 m above msl. During summer and autumn the maximum and minimum temperatures range 18-29° C and 10-11° C. The soil is red loamy with pH 5.5-6.5.

d) Bangalore: Located in warm tropical Deccan plateau. During crop season the maximum and minimum temperatures range 27-34° C and 14-21° C. The soils are red loamy with pH 7-7.5.

2. Use of disease free seed: Replicated field trials were conducted in naturally infested soils at Shillong (eastern hills), Ootacamund (southern hills), Bhowali (western hills), Simla (North western hills) and Bangalore (Deccan plateau). The incidence of bacterial wilt was consistently high (5-25 times) in crop raised from apparently healthy tubers from a diseased crop as compared to crop raised from disease free seed (Tables 2, 3). In another trial at Simla and Ootacamund three seed lots viz., i) breeders seed ii) seed selected from brown rot free plants in a diseased crop and iii) randomly sampled seed from diseased crop were planted in infested oil. The wilt incidence in the crops at Simla and Ootacamund was 2.3 and 0.97% in the first treatment, 4.16 and 3.15% in the second treatment and 25.06 and 27.25% in the third treatment, respectively. Further, the observations during surveys showed that the disease incidence was consistantly high in Kumaon, eastern and Nilgiri hills in the farmers holdings where local seed was used. In 1985 a trial was laid out on ten farmers' fields in Kumaon hills with local and disease free seeds. The results showed that wilt incidence was high in crop grown from local seed as compared to that grown from disease free seed and that the use of disease free seed alone can reduce the disease incidence by 61-100% (Tables 2, 3).

Cut vs whole seed: Brown rot free seed was used for the trial. In one treatment seed was cut into pieces and suberised after treatment with Dithane M-45 and in the second treatment whole seed was planted in replicated trials in infested soil at Simla, Shillong and Ootacamund. At all the three locations bacterial wilt incidence was lower by 2-12 times in crop grown from whole seed (Table 4, 9). The treatment of cut pieces with fungicides does not reduce the wilt incidence (16). Therefore, the practice of use of cut seed pieces is being discouraged.

Date of planting: In the North-western and North-eastern hills, observations during 1972-1985 had shown that the disease does not appear in the summer crop before July. In Himachal and Kumaon hills, it does not appear in autumn crop. These observations indicated that if planting and harvestig dates are adjusted, probably the disease can be controlled. Trials were, therefore, conducted at Shillong to examine the possibility. The dates of plantings and harvestings taken are given in Table 5. The wilt incidence was nil to 1.6% and tuber rot was 0.8-4.4% in crop planted between 10-24 February and harveted by mid June. In delayed planting beyond 1st March and harvesting beyond June end, the incidence of both wilt and tuber rot increased. The experiment thus confirmed the contention that early planting and early harvesting reduces the bacterial wilt and tuber rot. This idea is not entirely novel. In the Himachal and Kumaon mid hills (1000-2200) where bacterial wilt is a problem, farmers plant the crop by mid-February and harvest by June end. The above planting

and harvesting dates also save the crop from late blight, and the produce fetches good market price as table potatoes because fresh potato is scarce in June.

In North-western hills, autumn crop (September-January) does not contract bacterial wilt. However, in eastern and Nilgiri hills, bacterial wilt is always more in autumn crop (August-December/January). The dates of planting for autumn crop were also examined for their effect on bacterial wilt (Table 6). It was observed that the delayed planting of autumn crop reduces bacterial wilt incidence. The maximum incidence was in crop planted on 22 July. The incidence reduced with subsequent delays in planting and was negligible-nil (1.2%) in planting done on 5th September.

Sinha et al. (17) 1986 also reported that in eastern plateau the disease incidence was low (11.2-14.7%) in crop planted on 20th August as compared to that planted earlier (25.5-32.2%).

The manipulation of date of planting, though it saves the crop from the disease, its produce cannot be used as seed because it carries incipient infection of the bacterium and gives rise to disease epidemic when planted under conditions favourable for disease development (14). Therefore, manipulation of date of planting is desirable only for ware crop.

The crop duration is reduced to about 90 days due to manipulation of planting and harvesting dates to escape the disease in both summer and autumn seasons. A short duration early maturing variety would therefore be ideal to get full advantage of this practice.

Application of stable bleaching powder: At Shillong and Bhowali application of stable bleaching powder at a rate of 12 kg/ha mixed with fertilizer in furrows at the time of planting or soil drenching before or after first earthing up reduced bacterial wilt incidence by 80% and also increased the yield (Table 7). More than one application of bleaching powder reduced the wilt incidence by 70% but suppressed the yield by 29 q/ha as compared to single application. Among furrow application and drenching, the former is a better method because of ease in application.

Straw burning in infested soil: In the eastern hills of India there is a practice called "Jhuming". The tribals clear the forest land and the cut vegetative mass is spread on the land and burnt. The bacterial wilt incidence remains negligible in Jhum lands. Experiments were carried out at Shillong, Bhowali and Rajgurunagar (Deccan plateau) in which 20 cm deep furrows were prepared in infested fields at 50-60 cm intervals. Straw was spread in these fields and was burnt. The effect of this treatment was remarkable and there was a reduction in disease by 100% (Tables 8, 9). Though the practice is cumbersome, it deserves consideration in severely infested lands.

Crop rotation: Two year rotations of potato with wheat-lupin, wheat-maize or wheat-fallow were found to reduce bacterial wilt incidence to 7% from 80% (CPRI, 1984 (2)). In trials conducted at Ootacamund and Simla the wilt incidence was reduced by 50-75% when potato was planted in rotation with maize, oats or barley (14, 16). Sohi et al., 1981 (18) found the crop rotation viz. cowpea-maize-cabbage, Hibiscus esculentus-cowpea-maize, Eleusine coracana-egg plant (Pusa-purple cluster, a wilt resistant variety) was effective in controlling the disease at Bangalore, Karnataka. At Jabalpur (MP) two-to-three years rotations, viz., onion-cowpea-garlic, wheat-sunhemp and summer ploughing and fallow were effective (6, 7). The effect of fallowing and summer ploughing is enhanced if it is followed by a non-host crop (6).

At Bangalore and Bhowali it was found that growing potato in rotation with ragi, horsegram, sorghum, wheat, cabbage, carrot, onion and garlic reduces the disease incidence by 91-94% (Table 10). The disease incidence was higher (10-36%) in potato grown in rotation with sugarcane, paddy, chillies, radish and bean (Table 10).

Blind earthing: The importance of minimum tillage for control of bacterial wilt has been very well recognised. However, post emergence earthing in potato is the most crucial operation causing the maximum injury to the roots and consequently higher chances of infection. In trials at Bhowali and Shillong blind earthing up reduced the disease incidence by 10-50%.

Hot or cold weather cultivation: Soil disturbance is deleterious for the survival of the pathogen (5). Lloyd (8) reported that the disease fails to develop in infested soil placed in pots and planted with tomatoes after 20 weeks, while the disease developed in the same soil when left undisturbed in field. One or two deep ploughings of infested soil during May-June in plains and plateaus and in October-November in hills reduced wilt incidence by about 70% (CPRI, 1985, 1986 (2), 6, 7, 14, 15). The probable mechanisms operating in disturbed soil against the bacterium are i) enhanced microbial activity resulting in decomposition of plant debris which might otherwise harbour the bacterium, ii) destroys weeds which harbour the pathogen, iii) the bacterial population which otherwise remains embeded in soil is exposed to dessicating effect of the atmosphere. In soil, the bacteria exude through roots/tubers of infected plants in copious slimy masses. This mass glued with the soil forms small pellets. These pellets in undisturbed soil remain concentrated at certain foci in soil and the bacterium gets good shelter due to polysaccharide binding which is insulating, hygroscopic and amphoteric in nature. The soil disturbance causes dispersal as well as exposure of the pellets to more adverse dessicating and thermal effects of atmosphere.

By following a combination of practices discussed above it has been possible to reduce the disease to significant low levels. Some of the traditional epidemic areas like Maharashtra state and Hyderabad in Peninsular India and central and western plains have become almost free of the disease. Similarly Kumaon hills which

used to suffer heavy losses even up to 70% now have an average incidence of only 10%. The CPRI farm located at Mukteswar in Kumaon hills had an average incidence of 40-70% in 1973. By adopting the cultural practices viz., crop rotation, use of disease free seed, blind earthing up, etc, the disease has now been reduced to 0.001%. The major contribution in reducing the disease so far has been that of disease free seed potato. If other practices, depending on local feasibility, are also practised, the disease is likely to cease to be a major threat to potato. Fortunately in India, the western and central Indo-gangetic plains and western high hills (above 2200 m msl) are free of bacterial wilt (13). Disease free seed can be produced in these areas to meet country's requirement. The cultural practices are also likely to prove additive in enhancing durability of resistance of wilt resistant/tolerant varieties wherever available.

LITERATURE CITED

1. Buddenhagen, I. and A. Kelman. 1964. Biological and physiological aspects of bacterial wilt caused by Pseudomonas solanacearum. Ann. Rev. Phytopathology 2: 203-230.

2. CPRI, 1974-1986. Ann. Sci. Reports Central Potato Research Institute, Simla, India for the years 1974 to 1984.

3. Dutt, B.L. 1979. Bacterial and fungal diseases of potato. Indian Council of Agric. Res., New Delhi 199pp.

4. French, E.R., C. Martin and H. Mendoza. 1983. Cultivars resistant to bacterial wilt and late blight for the high and mid-elevations of the tropics. In W.J. Hooker (Ed). Research for the potato in the year 2000. International Potato Center, Lima, Peru. pp. 85-86.

5. Kelman, A. 1953. The bacterial wilt caused by Pseudomonas solanacearum. A literature review and bibliography NC Agric. Exprt. Sta. Tech. Bull. 99. 194p.

6. Keshwal, R.L. 1980. Studies on bacterial wilt of Solanaceous crops caused by Pseudomonas solanacearum E.F. Smith with special reference to its control. Ph. D. Thesis, University of Jabalpur, Jabalpur, MP, India. 201 pp.

7. Kumar, S.M. 1970. Note on the effect of some cultural practices on incidence of bacterial wilt of potato (Solanum tuberosum L.). Indian J. Agric. Sci. 40: 854-855.

8. Lloyd, A.B. 1976. Bacterial wilt in a cold temperate climate of Australia. Pages 134-135 in L. Sequeira and A. Kelman (Ed). Proceedings of the Planning Conference and Workshop on Ecology and Control of bacterial wilt caused by Pseudomonas solanacearum, NC State Univ., Raleigh. 166 pp.

9. Nielsen, L.W. and F.L. Haynes. 1960. Resistance in Solanum tuberosum to Pseudomonas solanacearum. Am. Potato J. 37: 260-267.

10. Rao, M.V.B. 1976. Bacterial wilt of tomato and eggplant in India, pp. 92-94. In L. Sequeira and A. Kelman (Ed) Proc. Planning Conference and Workshop on Ecology and control of bacterial wilt caused by Pseudomonas solanacearum, NC State Univ., Raleigh, 166pp.

11. Robinson, R.A. 1968. The concept of vertical and horizontal resistance as illustrated by bacterial wilt of potatoes. C.M.I. Phytopathology paper 10: 33pp.

12. Sequeira, L. and A. Kelman (Ed). 1976. Proceedings of Planning Conference and Workshop on the Ecology and Control of bacterial wilt caused by Pseudomonas solanacearum, NC State Univ. Raleigh, July 18-25, 1976. 166pp.

13. Shekhawat, G.S., Rajpal Singh and V. Kishore. 1978. Distribution of bacterial wilt and races and biotypes of the pathogen in India. J. Indian Potato Assoc. 5: 155-165.

14. Shekhawat, G.S., V. Kishore, D.[illegible]. Singh, R.N. Khanna, Rajpal Singh and V.K. Bahal. 1979. Survival of Pseudomonas solanacearum under diverse agroclimates in India. Indian J. Agric. Sci. 49: 735-738.

15. Shekhawat, G.S., Rajpal Singh, B.R. Tyagi and F.C. Misra. 1980. Resistance to bacterial wilt in diploid Solanum microdontum Butt. J. Indian Potato Assoc. 7: 119-124.

16. Shekhawat, G.S., V.K. Bahal, V. Kishore, I.D. Garg, R.N. Khanna and Rajpal Singh. 1984. Managing Pseudomonas solanacearum wilt of potato. Indian Phytopathol. 37: 416.

17. Sinha, S.K., K. Rai and M.M. Ahsan. 1986. Effect of date of sowing on incidence of potato wilt (Pseudomonas solanacearum Smith) at Ranchi. Bacterial Wilt Newsletter, Australian Centre for Intern. Agric. Res. No. 1, Dec. 1986.

18. Sohi, H.S., M.V.B. Rao, R.D. Rawal and R. Kishun. 1981. Effect of crop rotation on bacterial wilt of tomato and egg plant. Indian J. Agric. Sci. 51: 572-573.

19. Thurston, H.D. and J.C. Lozano. 1968. Resistance to bacterial wilt of potatoes in Colombian clones of Solanum phureja. Am. Potato J. 45: 51-55.

Table 1. INCIDENCE OF BACTERIAL WILT IN INDIA IN VARIOUS CROP SEASONS

Zone/Season	Time of disease appearance	Disease incidence (%)
Himachal Hills		
Summer	Mid June	5 - 10
Autumn	Nil	Nil
Spring	May end	2 - 6
Kumaon Hills		
Summer	May June	10 - 30
Autumn	Nil	Nil
Spring	May end	5 - 11
Eastern Hills		
Summer	Mid June	3 - 10
Autumn	September	10 - 27
Spring	April	-
Nilgiris Hills		
Summer	May	
Autumn	September	0.5 - 14
Spring	May	11 - 26
Eastern Plains		
Deccan Plateau		
Nasik, Maharashtra		
Rabi	Jan. end	3 - 4
Kharif	August	11
Bangalore, Mysore		
Summer	May	10 - 50
Kharif	Sept.	5 - 25
Winter	Jan.	5 - 15
Central Plateau		
Kharif	August	10 - 50
Rabi	Jan.	5 - 20
Gujarat	Jan. end	5 - 10

Based on survey data collected during 1972-1982.

Table 2. INCIDENCE OF BACTERIAL WILT IN KUFRI JYOTI CROP GROWN IN INFESTED SOIL WITH DISEASED AND DISEASE FREE SEED (BASED ON DATA FOR 3 YEARS)

	% Wilt	% BR Tuber
SIMLA		
Seed from diseased crop	20.5	25.0
Disease free seed	1.8	0.9
SHILLONG		
Seed from healthy plants selected from a diseased crop	10.5	16.0
Disease free seed	2.9	1.5
OOTACAMUND		
Seed from diseased crop	15	15
Disease free seed	0	10

Table 3. INCIDENCE OF BACTERIAL WILT OF POTATO IN CROP RAISED FROM CPRS, MUKTESWAR SEED AND LOCAL SEED

Sr. No.	Name of the farmer	Percent incidence of wilt in crop raised from:	
		CPRS Mukteswar seed	Local seed
1.	Shri Mohan Singh	4.00	10.00
2.	Shri Satya Dev	0.00	11.00
3.	Shri Inder Ram	0.00	6.00
4.	Shri Kailash Chandra Pandey	0.00	0.00
5.	Shri Rajendra Prasad	0.00	1.00
6.	Shri Shoban Ram	6.00	12.00
7.	Shri Harish Chandra	1.00	5.00
8.	Shri Ram Singh Gorkha	0.00	16.00
9.	Shri Deep Chand Bhat	0.00	1.00
10.	Shri Diwan Singh	0.00	0.00

Table 4. INCIDENCE OF BACTERIAL WILT IN POTATO CROP RAISED FROM CUT AND WHOLE SEED (AVERAGE OF THREE YEARS)

Location	Treatment	% Emergence	% Wilt
OOTACAMUND	Cut Seed	76.5	15.5
Autumn Crop	Whole Seed	93.1	5.4
SHILLONG	Cut Seed	80.8	19.4
Autumn Crop	Whole Seed	88.6	5.1
SIMLA	Cut Seed	85.0	13.1
	Whole Seed	97.5	4.2

Table 5. INCIDENCE OF BACTERIAL WILT AND BROWN ROT IN RELATION TO DATES OF PLANTING AND HARVESTING IN EASTERN HILLS (BASED ON 3 YEARS DATA)

Planting	Harvesting	Incidence of bacterial wilt	Incidence of tuber rot
Second week of February	Second week of June	Nil	3 3
Third week of February	Third week of June	1.1	4.4
First week of March	Last week of June	5.7	10.8
Second week of March	First week of July	8.7	9.0
Last week of March	Last week of July	9.5	18.8
First week of April	First week of August	5.8	13.6

Table 6. INCIDENCE OF BACTERIAL WILT IN RELATION TO DATE OF PLANTING IN AUTUMN POTATO CROP IN HILLS

Planting	Incidence of bacterial wilt (%)	Incidence of tuber rot
August 16	23.4	-
August 26	4.0	-
September 5	0.4	-

Table 7. EFFECT OF BLEACHING POWDER APPLICATION ON INCIDENCE OF BACTERIAL WILT AND TUBER ROT (BASED ON THREE YEARS DATA)

Methods of application	Dose	Incidence of wilt (%)	% Wilt control	Tuber rot q/ha
Soil-during field preparation	12 kg/ha	4.5	35.3	8.9
In furrows alongwith fertiliser before planting	12 kg/ha	1.6	80.2	4.6
Tuber dusting	5 gm/kg	3.6	52.8	5.7
Soil drenching before earthing up	12 kg/ha	1.8	75.2	4.4
Soil drenching after earthing up	12 kg/ha	2.1	71.1	4.1
Control	-	6.3	0.0	10.5

Table 8. EFFECT OF SOIL BURNING, HILL AND PLAIN SEED ON INCIDENCE OF BROWN ROT IN POTATO CROP AT SHILLONG (BASED ON 6 YEARS DATA)

Treatment	Total No. of plants emerged	Total No. of wilted plants	Wilting % age
T1 = No burning + fertiliser + plain seed	138	4	2.2
T2 = No burning + fertiliser + plain seed	185	11	5.9
T3 = No burning + no fertiliser + hill seed	166	20	12.0
T4 = No burning + fertiliser + hill seed	173	15	8.7
T5 = Burning + no fertiliser + plain seed	180	0	0
T6 = Burning + fertiliser + plain seed	173	0	0
T7 = Burning + no fertiliser + hill seed	192	0	0
T8 = No burning + fertiliser + hill seed	185	6	3.2

Table 9. EFFECT OF SOIL BURNING ON INCIDENCE OF BROWN ROT IN CROP GROWN THROUGH WHOLE AND CUT TUBER

Treatment	Total No. of plants emerged	Total No. of wilted plants	Wilting % age
T1 = No soil burning + no fertiliser + whole tuber	378	77	20.4
T2 = No soil burning + fertiliser + whole tuber	359	70	19.5
T3 = No soil burning + no fertiliser + cut tuber	314	62	19.7
T4 = No soil burning + fertiliser + tuber	257	57	22.2
T5 = Burning + no fertiliser + whole tuber	340	0.0	0.0
T6 = Burning + fertiliser + whole tuber	347	0.0	0.0
T7 = Burning + no fertiliser + cut tuber	248	0.0	0.0
T8 = Burning + fertiliser + cut tuber	231	0.0	0.0

Based on average of six years data

Table 10. INCIDENCE OF BROWN ROT IN VARIOUS CROP ROTATIONS IN KARNATAKA (1985)

Sr. No.	Crop rotation	Percent brown rot (%)
1.	Potato - Paddy - Potato	25.3
2.	Potato - Sugarcane - Paddy - Potato	46.1
3.	Potato - Sugarcane - Potato	21.8
4.	Potato - Ragi - Potato	4.4
5.	Potato - Chilli - Potato	43.9
6.	Potato - Sugarcane - Chilli - Potato	28.6
7.	Potato - Wheat - Potato	1.6
8.	Potato - Jowar - Potato	2.5
9.	Potato - Maize - Potato	3.7
10.	Potato - Ragi - Knol Khol/onion/cabbage/carrot/ beans - Potato	2.3
11.	Potato - Beans - Potato	16.2
12.	Potato - Knol Khol/cabbage/raddish/beetroot/onion/ carrot - Potato	4.3
13.	Potato - Carrot/beetroot/onion - Potato	1.8

Note: Based on observations so far collected.

Table 11. EFFECT OF CROP ROTATION ON INCIDENCE OF BROWN ROT WHEN INITIAL INCIDENCE WAS KNOWN

	Initial BR incidence (%)	BR Incidence (%)
	Rabi 1984-85	Rabi 1985-86
Potato-carrot-onion-potato	6.0	3.8
Potato-onion-potato	6.8	2.0
Potato-carrot-radish-potato	6.6	21.2
Potato-beet root-potato	1.8	2.2
Potato-knol khol-beet root-potato	3.0	2.4
Potato-knol khol-knol khol-potato	8.8	4.2

Table 12. PREVALENCE OF BROWN ROT IN PADDY AND SUGARCANE FIELDS WHEN BREEDERS AND CERTIFIED SEED WAS USED

Nature of seed	Producing agency	Rotation crops	Place	No. of fields	% BR incidence
Breeders seed	CPRI., Simla	Potato-sugarcane-potato	Kadoli	2	12.2
Breeders seed	CPRI., Simla	Potato-sugarcane-cabbage-paddy-potato	Kadoli	1	7.2
Certified	N.S.C.	Potato-paddy-potato	Kadoli	2	25.5
Certified	N.S.C.	Potato-sugarcane-paddy-potato	Kadoli	2	50.5

Table 13. INCIDENCE OF BROWN ROT WHEN HEALTHY AND DISEASED SEED WERE USED (BANGALORE)

Crop rotation	Type of seed			
	Healthy		Diseased	
	No. of fields	% BR	No. of fields	% BR
ROTATION WITH HOST PLANTS				
Potato-beans-potato	3	25.7	2	44.0
Potato-chilli-potato	4	29.2	3	71.7
ROTATION WITH NON-HOST PLANTS				
Potato-cabbage-potato	4	3.1	1	58.0
Potato-paddy-potato	13	6.9	1	71.8

Table 14. EFFECT OF SUMMER/WINTER PLOUGHING ON BACTERIAL WILT OF POTATO

Location	Treatment	Disease incidence
Rajgurunagar	Summer ploughing	8
	Control	24
Bhowali	Winter ploughing	13
	Control	32

SOIL SURVIVAL OF PSEUDOMONAS SOLANACEARUM

S. N. de S. Seneviratne

INTRODUCTION

The mental imagery associated with the ideal situation for growing potatoes successfully, disease-wise, envisions as initial requirements, sites free of pathogenic agents and seed material of high health status, not contaminated by disease causing organisms. With the bacterial wilt disease caused by Pseudomonas solanacearum, sites without a history of cultivation with susceptible crops, or virgin land, are viewed favourably as offering good prospects for the production of potato crops. Such sites planted with clean seed potatoes, are expected to ensure good crops, free of this troublesome disease.

Yet, such a view is deceptive. The more one probes the disease, the more one is confronted with the truly catholic nature of this pathogen, its resilience in surviving in soils under a variety of conditions in apparently unfavourable locations, and in finding sanctuary in seemingly innocuous host plants. Thus, the question that is posed is not simply the soil survival of P. solanacearum, which implies the introduction of the pathogen into sites in which it did not occur before, but its presence also in sites regarded as free of it; so consciousness must be enlarged to accommodate soil presence and soil survival as facets of the occurrence of the pathogen in a given site.

In the tropical and sub-tropical regions where potatoes are grown, bacterial wilt takes a noteworthy toll of the crop and causes substantial economic losses as well as difficulties in managing planting stock. The sources of infection of potato crops with P. solanacearum are of crucial importance in the absence of conveniently applicable methods for disease control. Possible sources include infected seed, contaminated irrigation water, weed hosts harbouring the pathogen, diseased plant refuse and infected soil. Of these, soil infection is probably the most troublesome factor to contend with, excluding P. solanacearum from soil the most difficult goal to achieve. Thus, the mastery over the problem posed by bacterial wilt to potato cultivation is linked, very largely, to the success that is achieved in securing cultivable land free of P. solanacearum or sites where wilt infection is negligible so that potato crops could be raised with minimal losses on account of the disease, unless of course, acceptable bacterial wilt tolerant varieties become available.

FIELD INCIDENCE OF PSEUDOMONAS SOLANACEARUM

In Sri Lanka, for instance, P. solanacearum has been detected in a variety of situations. It has been recorded at elevations ranging from sea level to 2194 m (7200 ft), in sites with a history of cultivation with cash crops including species susceptible to the disease, as well as newly cultivated lands in the lowlands carved out from jungle areas, in new clearings of virgin montane temperate forests in the cool hill country, in flooded paddy fields where rotations of rice and vegetable crops or potatoes are practiced, and in long laid off infected sites. It is therefore a pathogen distributed widely in the soils of Sri Lanka, a situation which probably applies to other tropical countries as well.

PLANT HOSTS AIDING THE SURVIVAL OF PSEUDOMONAS SOLANACEARUM

Whatever the hazards, certain crops are frequently cultivated on the same site because of their economic value. Commonly cultivated susceptible crops include tomato, brinjal, chilli, capsicum, potato and bush bean, while bacterial wilt has also been observed in other crops like bitter gourd and winged bean. The survival and increase of the pathogen in these soils is undoubtedly facilitated by the availability periodically of such plant hosts.

In the warm lowlands, the incidence of bacterial wilt has been observed in freshly cleared jungle tracts sown with gingelly (sesame) and also in coconut plantations cultivated with ginger and turmeric. The occurrence and survival of P. solanacearum in these sites is probably influenced to a considerable extent by the abundance of weed species in them which are hosts of P. solanacearum, notably Hyptis sauveolens and Croton hirtus.

TEMPERATURE AS A DETERMINANT OF THE SOIL SURVIVAL OF PSEUDOMONAS SOLANACEARUM

The nature of occurrence of bacterial wilt in the coldest and hottest regions of Sri Lanka indicate the importance of temperature as a factor in the soil survival of P. solanacearum.

In the quest for a site that would be virtually free of aphids which are vectors of potato viruses, the ultimate sanctuary of Sri Lanka's hill country, the Horton Plains, at an elevation of 2194 m was secured for seed potato production and a potato farm carved out of this hitherto unexploited wet patanas. It was also believed at that time that bacterial wilt will not occur at elevations above 5000 ft (1523 m). However, within a short time, the incidence of bacterial wilt was observed in the Horton Plains too in 1963 and P. solanacearum biovar 2 identified as the cause. The mutilation of this unique natural reserve to produce seed potatoes outraged conservationists and their sustained

campaign resulted in the closure of the potato farm; meanwhile, the tracts where bacterial wilt had occurred in 1963 had been laid off. So it was a surprise to detect P. solanacearum in soil from the abandoned area 14 years later in 1977. Thus, the pathogen had survived in these sites for more than 14 years even in the absence of susceptible crop plants and after the land had been recolonised by the plant species that grow naturally in the Plains.

The situation prevailing in the northern plains of the hot Jaffna peninsula contrasts sharply with that of the Horton Plains. Solanaceous crops including tobacco, brinjal and chilli are extensively grown in the Jaffna District while potatoes are cultivated during the cool months of the year. Yet, in the Jaffna peninsula, bacterial wilt has not posed a problem worthy of note although a severe disease outbreak occurred there during the Maha 1982/83 season which was traced to supplies of infected seed potatoes, while the dissemination of the pathogen had also been aided by rain water.

In the mid-country, where crops such as tomato, brinjal and capsicum as well as other susceptible species are cultivated, bacterial wilt is invariably encountered to a lesser or greater extent.

The nature of the occurrence of bacterial wilt in the two contrasting situations, the Horton Plains and the Jaffna peninsula emphasise the importance of temperature as a determinant in the soil survival of P. solanacearum.

Meteorological data recorded at Sita Eliya in the Nuwara Eliya District and Thirunelvely in the Jaffna District may be considered as representative of the conditions prevailing in the Horton Plains and the Jaffna peninsula respectively.

At Sita Eliya, mean monthly minimum air temperatures ranged from 11.1 - 13.7° C and mean monthly minimum soil temperatures at a depth of 5 cm from 14.1 - 17.3° C. The lowest air and soil temperatures recorded were 2.5° C and 9.0° C respectively. Mean monthly maximum air temperatures ranged from 18.2 - 21.3° C and mean monthly maximum soil temperatures at 5 cm from 20.1 - 24.6° C. The highest air and soil temperatures recorded were 28.7° C and 35.0° C respectively. These temperatures impose no stresses at all for the survival of P. solanacearum which can remain for long periods in soils exposed to the temperatures that prevail in the cool hill country.

Jaffna in the lowland plains at near sea level has a warm-hot climate but conditions suitable for the cultivation of potatoes prevail there during the cool months of the year from December to February. Temperatures rise considerably during the hotter months of the year. At Thirunelvely in the Jaffna District where meterological data has been recorded, mean monthly minimum air temperatures ranged from 20.7 - 26.5° C and mean monthly minimum soil temperatures at 5 cm from 25.0 - 31.7° C.

The lowest air and soil temperatures recorded were 12.1° C and 17.5° C. Mean monthly maximum air temperatures ranged from 29.2 - 33.6° C and mean monthly maximum soil temperatures at 5 cm from 29.0 - 40.2° C. The highest air and soil temperatures recorded were 36.5° C and 46.9° C. During the hottest period of the year, soil temperatures rise above 43° C and remain above that temperature for several hours daily. Such conditions are not favourable for P. solanacearum as it cannot tolerate exposure to high temperatures.

The crucial factor governing the survival of P. solanacearum in these soils appears to be the highest temperatures reached during the hottest months of the year during several days when the soil temperatures remain sufficiently high to kill the pathogen. It appears that such "sterilising" conditions to eliminate P. solanacearum from these soils or at least to reduce their populations to low levels at which the pathogenic potential is inadequate to cause disease losses during the cropping season, account for the relative unimportance of bacterial wilt in the Jaffna peninsula.

DETECTION OF PSEUDOMONAS SOLANACEARUM IN INFECTED SOIL

In establishing whether P. solanacearum is present in a given soil or whether it is able to withstand exposure to applied conditions such as high temperature in soils infected with the pathogen, a method for detecting its presence is a necessary tool. A simple technique was developed to meet this need. It consisted of introducing a measured quantity of the suspect soil sample to a liquid sucrose peptone medium, incubating the flasks containing the medium at room temperature, ca. 26° C, with continuous agitation in a shaker at 60 rpm, and inoculating the liquid suspension after about 48 hr incubation, to tomato test seedlings, the susceptible host routinely used for demonstrating the pathogenicity of P. solanacearum cultures. When soil samples containing P. solanacearum were incubated in this manner and the resulting suspension inoculated to Marglobe tomato seedlings, the latter developed symptoms within 5 - 8 days. The purpose of the incubation procedure was to increase the population of P. solanacearum in test soil samples so as to induce wilt symptoms in the tomato test plants. This technique was used to advantage in studies on the effect of temperature on the soil survival of P. solanacearum.

EFFECT OF TEMPERATURE ON THE SOIL SURVIVAL OF PSEUDOMONAS SOLANACEARUM

In studying the effect of temperature on the survival of P. solanacearum in soil, time-temperature combinations were investigated using soil samples with different properties obtained from various locations. Soil samples were first sterilised and then inoculated with pure cultures of the pathogen. Test temperatures were applied for specific periods, either continuously or discontinuously with 6 hr exposure periods

alternating with 6 hr periods at about room temperature in the latter treatment. Soil samples from the Horton Plains, a black, highly acidic peaty loam rich in humic matter, and from Thirunelvely, a yellowish red near neutral sandy clay loam, were the two most interesting soil types from the point of view of soil survival of P. solanacearum included in this study.

The experimental procedure adopted was as follows: Sterilised 100 g soil samples, placed in 500 ml conical flasks, were inoculated with 100 ml of a 48 hr old liquid culture of P. solanacearum and the inoculated flasks held at controlled temperatures of 38° C, 40° C and 43° C in incubators for varying periods. Thereafter, 100 ml of sucrose peptone liquid was added to each flask, the flasks incubated at room temperature with continuous agitation in a shaker for 48 hr, and the suspension inoculated to tomato test plants which were observed for the development of symptoms of wilt, an indication of the presence of P. solanacearum in a sample subjected to a particular treatment.

On the basis of the infections induced in tomato test plants, it was established that P. solanacearum was killed in soils exposed to 43° C continuously for periods of four days and over. The pathogen was also eliminated from soil samples exposed to the discontinuous heat treatment for seven days but not always from samples treated for four days. At 40° C, the pathogen was able to survive in some samples, both with the continuous and discontinuous heat treatments at four and seven days, but not in others. These results indicate that P. solanacearum is unable to survive in soils at temperatures much above 40° C, 43° C being a critical temperature depending on the period of exposure.

EFFECT OF FALLOWING ON THE SOIL SURVIVAL OF PSEUDOMONAS SOLANACEARUM

In studying this aspect, soil from a highly infected site having P. solanacearum biovar 3 was sieved and filled into concrete rectangular containers, sunk into the soil with about 8 cm of the container wall protruding above the soil surface in order to ensure separation of the area within the container from the surrounding soil. The containers were bottomless, allowing natural drainage. Studies were done at two locations, Rahangala in the hill country dry zone (elevation 1310 m, 4300 ft), and Sita Eliya in the hill country wet zone (elevation 1889 m, 6300 ft). The containers were laid in the experimental site in two parallel rows, each with eight containers. Plantings with potato were made in them at four planting frequencies - 3, 6, 12 and 24 months with four containers for each frequency. Between successive plantings, the soil surface in the containers was kept free of any plants, weeds being manually removed no sooner they were observed. Potato plants grown in the containers were observed for the development of symptoms of bacterial wilt which indicated the presence of P. solanacearum in the container soils. The disease occurred in all frequencies of planting including the 24 months frequency in which the fallow period extended for 22 months at both Rahangala and

Sita Eliya, 100% of the potato test plants wilting at Rahangala during observation period, and 57% at Sita Eliya during the same period. These results indicate that P. solanacearum is able to survive in a soil devoid of susceptible hosts or any vegetation cover for a minimum of 22 months in the hill country of Sri Lanka.

EFFECT OF FLOODING ON THE SOIL SURVIVAL OF PSEUDOMONAS SOLANACEARUM

In the hill country dry zone of Sri Lanka as well as in other regions where rice fields are cultivated with rice only once each year during the wet season and other crops are cultivated during the season when water is a limiting factor, bacterial wilt occurs in crops such as potato and tomato which are grown during the less wet season. The incidence of the disease in such sites cannot be attributed to contaminated irrigation water or infected planting materials; it is due to the pathogen present in the soil itself. During the rice growing season, fields are flooded for six weeks or more; this flooding does not eliminate the bacterium from the soil. According to some reports by farmers, bacterial wilt occurred in rice fields which had not been cultivated with solanaceous crops for over three years. It is clear that the pathogen is able to survive in soil despite the flooding for several weeks although flooding may reduce its population.

DISCUSSION

Experiences and experimentation in Sri Lanka have strongly indicated that P. solanacearum is a constituent of the soil biota in a variety of habitats, among them some in which its presence may not be expected; it is endemic in such soils. It is able to survive almost indefinitely in infected soils, even in reduced populations. It is not eliminated from infected fields by flooding them, nor can it be removed from an infected site by a complete removal of the plant cover. The absence of susceptible host plants will reduce its populations in infected sites but not eliminate it.

P. solanacearum, however, is sensitive to temperature. It is unable to withstand exposure to soil temperatures much above 40° C and is unlikely to survive in soils subjected to temperatures of 43° C or more for about 6 hr daily for several days successively.

Flooding is a control measure adopted to clear infected soils of certain pathogens. Fumigation with chemicals, though expensive, is a method adopted in certain situations to rid infected soils of unwanted organisms. In the case of P. solanacearum, with the pathogen unable to withstand temperatures much above 40° C, there is a possibility of exploiting the pathogen's intolerance of high soil temperatures as a method for eliminating it from infected soils. Covering the soil surface with a polyethylene sheet and ensuring a measure of air tightness of the layer of air between the polyethylene and soil results in the soil

temperatures rising to a point well above that tolerated by the pathogen. Such a method might be explored especially where the land is relatively flat, the principal requirement being a covering to secure the rise in soil temperature. It is a possibility that deserves attention.

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REFERENCES

Gunawardena, Jinadarie, Udugama, Srimathie and Seneviratne, S.N. de S. (1980). New hosts of Pseudomonas solanacearum in Sri Lanka. Proc. Sri Lanka Ass. Advmt Sci., 36 (1), 34.

Seneviratne, S.N. de S. (1969). On the occurrence of Pseudomonas solanacearum in the hill country of Ceylon. J. hort. Sci., 44, 393-402.

Seneviratne, S.N. de S. (1976). Bacterial wilt in solanaceous crops grown in rice fields. Proc. First International Planning Conference and Workshop on the Ecology and Control of Bacterial Wilt Caused by Pseudomonas solanacearum. Raleigh, North Carolina, July 1976, Luis Sequeira and Arthur Kelman Editors, 95-101.

Seneviratne, S.N. de S. (1978). Survival in the soil of Pseudomonas solanacearum. Proc. Second Regional Symposium on Potato Production - Southeast Asia and the Pacific, Pathogens and Pests of Potato in the Tropics. Los Banos, Laguna, Philippines, February 1978.

Velupillai, Malarmagal and Seneviratne, S.N. de S. (1983). The spread of bacterial wilt (Pseudomonas solanacearum) of potato through the use of diseased seed. Proc. Sri Lanka Ass. Advmt Sci., 39 (1), 28-29.

LATENT INFECTIONS INDUCED BY PSEUDOMONAS SOLANACEARUM IN POTATO AND SYMPTOMLESS PLANTS

G. A. Granada

Plant Pathologist, Head Plant Pathology Program, ICA, Apartado Aéreo 233 Palmira, Colombia.

INTRODUCTION

Bacterial wilt of potatoes and other hosts caused by strains of Pseudomonas solanacearum is a well known disease in temperate, subtropical and tropical areas of the world. Most reports published register strains of race 1 and race 3 as the main cause of wilt in potatoes. In terms of Hayward's classification, biovar 2, biovar 3 (22), biovar 4 (27,40) and biovar 5 (23) can be established in potatoes. The pathogen is transmitted through tuber seed carrying latent infection, being now present in most potato growing areas of the world. Recently in China He and Hua (24) reported the spreading of the bacterium (race 1 and 3) south to north by means of latent infected tubers. This phenomenon keeps challanging researchers worldwide and up to date demand colaborative work toward comprehensive understanding of the pathogen. I am sure you agree this is not and easy task, but hopefully this meeting will enable us to learn, exchange ideas and undertake future research toward that goal.

LATENT INFECTION IN POTATO STEMS AND TUBERS

Most of the basic data reported on latent infection in potato have originated both at the University of Wisconsin - Madison and at the International Potato Center, Lima, Perú (3,6,7,8,12,13).

A. LATENT INFECTION IN STEMS

The multiplication and movement of P. solanacearum in stem tissue have been described for different species: Tobacco, tomato, and potato (3,8,25,35). In potato stem-inoculated plants, the bacterium can move upward and downward from the inoculation site, reaching the highest bacterial concentration in tissues close or next to that site (3,8). Populations up to 10^6 colony forming units per mg of tissue have been recorded in stems of resistant symptomless potato plants (3). Pippette inoculation studies have shown that movement of P. solanacearum up and down the stem is slow in resistant compared with susceptible plants (3). The ability to multiply and spread longitudinally in potato stem is similar in highly virulent

race 3 strains and less virulent race 1 strains (3,8).

Restriction for P. solanacearum to move in the stem down to the roots was found by Ciampi et al (7) in one resistant clone (BR 52.1) No stem infection was detected in plants grown at cool temperature, range: 12-22C.

The inoculation of an incompatible strain of Race 2 in potato plants resulted in considerable multiplication of the bacterium, but failed to induced wilt symptoms (3). Avirulent strains (mutants of P. solanacearum) stem inoculated in potato do not move far from the inoculation site but attain populations there that differ only by one order magnitude from those of compatible bacteria (3). Same reaction takes place in tobacco (35).

P. solanacearum (race 1 strains), multiplied less in stems of resistant (S. phureja and S. phureja x S. tuberosum) than in suceptible potato plants, being able to carry relatively high bacterial populations without symptom expression (3,8,13). This implies that in most cases we actually deal with tolerance to P. xolanacearum (3,8).

B. LATENT INFECTION IN TUBERS

1. Research under controlled conditions.

Strains of P. solanacearum differ in their ability to cause tuber latent infections in different resistant potato clones (7,13). Hybrids of S. phureja x tuberosum may yield infected tubers by one or more strains. The only reported case where it can be said relatively safe that no latent infection occurs, at least for the strains of race 1 and race 3 tested is the clone BR 53.1 (8).

2. Research under field conditions.

For more than 19 years, Colombia has been considered a good place to evaluate resistance to bacterial wilt. Data registering the reaction of potato clones to bacterial wilt (race 3) at Rionegro, Antioquia (2,120 m asl) showed that 15 out of 36 did not wilt but yielded tubers carrying latent infection. Percent of tubers carrying latent infection ranged from 0.2 to 28.6%. The rest of clones rated as susceptible (20). To illustrate the fact that inoculum potential and climatic factors (rainfall, temperature) do condition the resistance of potato to P. solanacearum, data of the evaluation of 7 clones in two consecutive years (1985-1986) at Rionegro, is presented in Tables 1,2 and 3, giving emphasis on tuber latent infection.

TABLA 1. FIELD RESISTANCE EVALUATION OF POTATO CLONES TO *P. solanacearum*: PERCENT WILTING AND ROTTING OF TUBERS IN TWO CONSECUTIVE YEARS. ICA, LA SELVA, RIONEGRO, COLOMBIA

CLONES	% WILTED PLANTS		% TUBERS WITH BROWN ROT		PRECIPITATION mm	
	1985	1986	1985	1986	1985	1986
378198-13	85.0*	30.0**	27.2	41.8	511.1	559.8
378198-3b	88.0	6.6	15.4	32.8		
378508-6	30.0	0.0	28.6	36.4		
378508-13	32.0	0.0	26.1	50.4		
378198-6	45.0	0.0	27.7	18.3		
378508-17	55.0	11.1	23.0	10.6		
378193-5	50.0	20.0	30.8	55.6		
CAPIRO	50.0	21.0	40.1	43.7		

*, ** Data based on 30 and 20 plants tested, respectively.

NOTE: Average day temperature: Max 19.1 C Min 13.4 C (1985).
Max 20.5 C Min 16.1 C (1986)

TABLE 2. FIELD RESISTANCE EVALUATION OF POTATO CLONES TO *P. solanacearum*: PERCENT ROTTEN TUBERS IN TWO CONSECUTIVE YEARS. ICA, RIONEGRO, COLOMBIA

CLONES	PERCENTAGE TUBERS WITH BROWN ROT			
	AT HARVEST		AFTER STORAGE*	
	1985	1986	1985	1986
378198-13	7.7	40.7	19.5	1.1
378198-3b	6.8	32.2	8.6	0.6
378508-6	9.8	32.2	18.8	4.2
378508-13	16.3	40.3	9.8	10.1
378198-6	3.5	14.2	24.2	4.1
378508-17	9.3	8.7	15.7	1.9
378193-5	14.3	52.6	16.5	3.0
CAPIRO	10.3	43.0	29.8	0.7

NOTE: Percent of plants wilted was higher in 1985 than in 1986. See Table 1.

* Determined cutting the tubers in two halves.

TABLE 3. YIELD OF POTATO CLONES EVALUATED FOR RESISTANCE TO BACTERIAL WILT IN TWO CONSECUTIVE YEARS. ICA, RIONEGRO, COLOMBIA

CLONES	YIELD IN GRAMS/PLANT 1985	1986	% INCREASE YIELD
378198-13	322	776	104.9
378198-3b	435	576	32.4
378508-6	388	682	75.7
378508-13	393	458	16.5
378198-6	602	653	8.4
378508-17	355	1035	191.5
378193-5	287	373	29.9
CAPIRO	379	601	58.5

The data shows that from one year to another at the same location there was significant changes in the rating of percent wilting, percent latent infection and yield, being precipitation and temperature apparently the major parameters conditioning the reaction of the potato clones studied. Such behavior implies that the evaluation of potato clones in national potato programs should include either more than one test at a given location, or different tests in different localities if more than one race is registered in the potato growing areas. This is in order to know what is the real production potential and resistance to bacterial wilt. The development of resistance to bacterial wilt derived from S. phureja has been successfull in cool but not in warm climates (13). Through the years, after a great deal of input and effort, breeding for resistance has not provided adequate control in many instances. However, in Colombia clone 7/10, one of the first clones tested in 1968, was released several years ago as cultivar ICA-Sirena. Other cultivars such as Caxamarca and Molinera have been shown to be successfull wherever grown in the highlands in Peru (13). Recent results from Kenya and Sri Lanka show promising resistant material (4,5).

As a result of the work of French and Lindo (13), it has been stated that, on the average S. phureja hybrids are more susceptible than S. phureja and more resistant than the S. tuberosum tested.

Because S. phureja is native of the highlands of the Andean Region where race 3 (biovar 2) is present, it has been pointed out that resistance in this diploid potato be more effective against race 3 than to race 1 strains (8). Supporting this statement is the fact that by the infectivity titration technique resistance to a highly virulent strain (race 3, strain 276 from Mexico) was found in the S. phureja clone 1386.12 (3). However, the same strain 276 wilted clone 1386.15 reported as resistant to all strains of P. solanacearum tested before (3).

3. Temperature in relation to susceptibility and setting of latent infection

The reaction of potato clones to a set of strains of P. solanacearum including race 1 and race 3 was dependent on the temperature regimen to which inoculated plants were exposed (6,13). Under warm temperature (28/16 C day/ night) tolerant potato plants inoculated either by soil infestation (without wounding) or by stem-puncture became disease without symptom expression, but carrying latent infection (13). No latent infection was recorded when plant infection was achieved by planting in infested soil and plants were maintained at cool (20/8 C) temperature (13). Although the reaction of potato clones at the above temperature regimes has been consistent when performed under growth chamber conditions, however, under field conditions at Rionegro, Colombia, in a relatively cool temperature regimen (average: 20/11 C, day/night) latent infection has been registered in 36 out of 38 clones tested lately (20) table 1, 2. The same situation has been reported at Umari (Peru), where at cool temperature (average: 20/6 C day/ night) 23 out of 34 potato clones exhibited latent infection (41). In Kenya at high altitude 90% of inoculated potato produced latently infected tubers (33). According to Ciampi and Sequeira (6) it appears that there is no correlation between the ability of strains to induce disease at low temperature and the growth rates in vitro. Strains of race 1 did grow at 16°C in liquid culture but did not cause symptoms at such temperature.

Because of the tremendous importance in the epidemiology of the disease, new potato breeding material combining resistance from four different sources (S. phureja, S. sparsipilum, S. chacoense, S. microdontum) in a tetraploid population (4) should be studied critically focusing the potentiality of latent infection development, to avoid the spreading of the so called "low temperature" strains to temperate zones that have remained free of P. solanacearum as did occured in Sweden (34), and race 1 and race 3 strains present in lowland infested areas.

National Programs should be encouraged to develop their own methodology for testing the resistance of potato material when no satisfactory results are obtained using standard methodology. The situation reported for Burundi, is a good example (4).

Because "latency" has become very important with the development of resistant cultivars, it is proposed tha the term "latent infection" be used only to refer to special behavior of resistant potato materials and wild and cultivated hosts, not showing symptoms during the life cycle but yielding infected tubers detected at harvest or after 3-6 weeks of storage in the case of potato or isolating the bacterium from stems and/or roots in the case of symptomless hosts. Reactions not falling within the above description should be referred as late infection, in which case we are dealing always with susceptible material, as the case reported by González (14).

Because at low temperature virulence of strains is not fully expressed, except for the "low temperature" strains, evaluation of resistance must be carried out at warm temperature. Apparently at 28 C there are no differences in pathogenicity among isolates (6). Because late and/or latent infection may be registered even at warm temperature, a safe method for diagnostic of healthy seed should be used (12).

LATENT INFECTION IN SYMPTOMLESS HOSTS

The ocurrence of P. solanacearum in virgin soils or in newly cleared land has been attributed to the presence of wild hosts in the natural flora (28,31). Evidence that wild species may act as alternative hosts of the pathogen was presented by Sequeira and Averre in 1961, who reported that native species of Heliconia and Eupatorium odoratum in virgin woodlands were infected by strains of race 2 and race 1, respectively. Several species reported as symptomless hosts for race 3 and race 1 are listed in table 4.

The study of bacterial populations in the rhizosphere of different species (Pepper, castor bean, tomato, bean, corn) showed that there was a steady decline of the population to drop to undetectable levels within a few weeks after root-dip inoculation, unless root infection occured, usuallly in a symptomless manner (18).

Since crop rotation often fails to control P. solanacearum where race 1 in present, how can we explain it remains in the soil at levels capable of causing disease, if the bacterium has relatively low survival capacity in the soil? A logic explanation for survival of P. solanacearum in the field has not been given.

TABLE 4. WILD AND CULTIVATED SYMPTOMLESS HOSTS OF *Pseudomonas solanacearum* with emphasis in Race 3

HOST	RACE	BIOVAR	ANNUAL	PERENNIAL	REFERENCE
Strawberry	1	3,4	X		15
Silene gallica	1	-	X		1
Chenopodium ambrosioides	3	-	X		1
Erodium moschatum	3	-	X		1
Rumex acetosella	3	-	X		1
Rumex crispux	3	-	X		1
Soliva anthemidifolia	3	-	X		1
Spergula arbensis	3	-	X		1
Verbena brasiliensis	3	-	X		1
Gnaphalium elegans	3	-	X		1
Galinsoga parviflora	3	-	X		1
Phyllanthus niruri	1	-	X		37
Brassica napus v. *napus*	3	2	X		34
Eupatorium cannabinum	3	2	X		34
Portulaca oleracea	1,3	-,2	X		32,36
Pepper	3	2	X		9
Soybean (*Glycine soja*)	-	-	X		42
Solanum cinereum	3	2		X	16
Solanum carolinense	3	2		X	9
Solanum capsicastrum	3	2	X		11
Datura stramonium	3	2		X	11
Solanum dulcamara	3	2		X	34
Eupatorium odoratum	1	-		X	38
Melampodium perfoliatum	1	-	X		26

- : Not reported by authors.

In some special situations the bacterium may survive for extended periods of time in plant parts, and host debris, buried in deeper soil layers (17) where microbial activity is suppose to be very low. Thus the most logical explanation for field survival is the posibble association of the pathogen with the root symtems of symptomless plants.

To corroborate this hypothesis crops such as sorghum, corn, soybean, peas, rice, sugarcane and bean were planted in infested soil (4 race 1 strains were studied individualy) and at different time intervals plants were collected, the soil removed and washed and the root system treated with 70% ethanol for 5-15 min to kill bacteria present in the rhizoplane.

The bacterium was recovered from plants tested, however, not all plants of a particular crop became infected, but the average population was relatively high. Population recovered from the root system of a given crop was lower than the one present in the soil substrate. There was no correlation between population in soil and the number of bacteria recovered from each root system of symptomless plants (19).

Latent infection in plants can be considered as the best possibility that a pathogen may have for long-term survival. In the case of P. solanacearum long-term survival, seems to be associated to physiological specialization, having race 1 the widest host range, followed by race 2 and finally race 3.

The failure of crop rotations (10,21,26) to reduce bacterial wilt incidence using corn, cowpea, sweet potato, tomato, etc., may have an explanation in plant root infections in a symptomless way, specially when dealing with race 1 strains and symptomless hosts.

In this respect, it is much more logic, because of the wide host range of the bacterium, including cultivated and wild species, to consider the pathogen as proposed by Crosse 20 years ago, that is, as a root inhabitant rather than a soil inhabitant (22).

The slow rate of colonization and disease development of symptomless infected plants, allows them to stay alive longer, serving as inoculum source for susceptible cultivated species or wild hosts, when chances put them together. Based on the knowledge we have today on the bacterium, it is expected that the presence of a strain of P. solanacearum in a symptomless host, does not imply that that particular strain will infect other plants which may be susceptible to other strains (18).

Machete used in bush-chopping oporations (2) and hoe and/or shovel in periodic removing of weeds, are probably the most common means of spreading bacteria in many cultivated plants. Periodic spreading of bacteria through mechanical means may result in heavy plant infection

in which case symptoms are observed. This plant behavior has been studied in the case of symptomless pepper plants under greenhouse conditions. After 2-3 prunnings near the crown level the symptomless plants developed characteristic wilt symptoms. By then, the vascular system was affected in a ring manner, (Granada, unpublished data). The erratic manifestation of symptoms in perennial plants, many have their explanation on the above. Observations.

The facts that either under controled or field conditions not all plants become diseased and that total populations of bacteria released from symptomless plants are not as high as the one registered on susceptible plants, explain why overall populations may decrease with time (18). In the case of potato, symptomless plants yielding latent infected tubers, may occur also at random (7).

PAST AND PRESENT CONSIDERATIONS IN RELATION TO BACTERIAL WILT RESEARCH

It is going to be 11 years since we met in North Carolina, Raleigh for the First International Planning Conference and Workshop on the Ecology and Control of Bacterial wilt. At that time Dr. Arthur Kelman (29) addressed to participans saying that after 8 decades of study of Pseudomonas solanacearum approximately 1,400 studies had been published and nevertheless heavy losses and an incomplete knowledge of the disease were still evident. Today, after 9 decades, a good number of papers have been added to the list, but still, I feel, don't know enough about the biology and epidemiology of P. solanacearum. The outcoming of latent infection with the use of tolerant potato clones complicates, unfortunately, the situation.

At the same metting Dr. H. David Thurston (39) reffered also to the necesity of considering 14 factors in evaluating resistance of plants to bacterial wilt. Althogh from time to time in open field experiments we do record data related to several of the 14 factors proposed like plant age, strain, temperature, rainfall, nematode infestation, etc., there are plenty of factors we usually know nothing and we should, at least for the plot we are working on, like: soil type, soil moisture, soil temperature, relative humidity, light intensity, inoculum potential in soil etc. Even the first 5 factors mentioned are not recorded accurately in many field tests performed.

This way we can hardly understand what is going on with the potato material under evaluation. Positive correlation for several of the above factors has been reported (30). To this we have to add differences in genetic reactions to different bacterial strains, as is the case for most promising S. phureja hybrids. It is time to: 1- evaluate results from all cooperating countries in the International Trial for disease resistance to bacterial wilt, especially from the stand point of view

of methodology and data recorded, that fully explain the reactions of materials tested. 2- Set standard methodology to be offered and practiced by every one of us, stating rules clearly of how to plant and how to record data, what to record, going from very simple experiments to difficult ones. 3- I consider that a formal Committe should lead and determine all the observations needed. Obviously research to be established in a given National Program will be in accordance to their priorities, but will be under the guidance and help of the Committe. It is about time to stop doing research and taking data at everyone's will: get together and produce data that each one of us needs, eagerly waits, believes and understands.

At the Brasilia metting in 1984 we set different topics and priorities to work with. I hope most participants are actually considering those priorities and solving them today. Through the years it has been demostrated that Bacterial wilt is not an easy task to solve alone but together. With the leadership of a Committe getting together every 1-2 years to evaluate reports, set guidelines, etc, I am sure will move faster understanding and solving, at least partially, the problem. Through the years most of the effort has been put by CIP and specially by Dr. French. Every 3-4 years few people get together, like us today, and try to help CIP's efforts to move ahead. This is OK but not enough more people from National Programs should be involved.
A newsletter on Pseudomonas solanacearum was iniciated in 1984 to report results and activities on bacterial wilt with emphasis in research carried out in Latin America. Unfortunately only one issue was published because of lack of information and interest from most Latin American researchers. Today I still believe this is a good mean of communication, which the Committe that is proposed to be created, could use satisfactorily, extended of course, to other important bacterial potato pathogens, like Erwinia and Corynebacterium.

CONCLUDING REMARKS

Incidence of latent and/or late infection induced by races 1 and race 3 in resistant and susceptible potato cultivars is a real threat to potato producing areas in the world. Such infections are extremely important from an epidemiological point of view since the pathogen can be moved from one geographical location to another via infected potato seed. The development of resistant varietics has not resolved, so far, the problem of bacterial wilt, and because of the latency phenomenom, resistant or tolerant cultivars may complicate the overall strategy of control of the disease. An integrated effort by all researchs on bacterial wilt is needed to come out with practical solutions in the near future. Short term research focusing the problem of tuber seed carrying latent infection is urgently needed.

The fact that at a great deal of plants may get infected by strains of P. solanacearum in a symptomless way is becoming more documented and accepted lately. Symptomless perennial species are expected to play a major role in longer survival than annuals in infested crop land. Fortunately host range seems to be closely related to physiological specialization, being race 3 the one with the narrower host range and the widest distribution in potato growing areas of the world. Cases where race 1 is present are much more difficult to handle because of the wide host range of strains within this race and longer capability of survival free in the soil. More work is needed on this topic of symptomless hosts.

Integrated control measures continues to be the best strategy to solve the problem.

REFERENCES

1. Belalcázar, S., Uribe, G. y Thurston, H.D. 1968. Reconocimiento de hospedantes a Pseudomonas solanacearum en Colombia. Revista ICA 3: 37-46.

2. Berg, L.A. 1971. Weed hosts of the SFR strain of Pseudomonas solanacearum causal organism of bacterial wilt of bananas. Phytopathology 61:1314-1315.

3. Bowman, J.E. 1980. The relationship of resistance to the multiplication and spread of Pseudomonas solanacearum in potato stems. M.S. Thesis. Univ. of Wisconsin. 117 p.

4. Centro Internacional de la Papa. 1986. Informe Anual del CIP, 1985. Lima, Perú. 184 p.

5. Centro Internacional de la Papa. 1985. Informe Anual del CIP, 1984. Lima, Perú. 186 p.

6. Ciampi, L. and Sequeira. 1980. Influence of temperature on virulence of race 3 strains of Pseudomonas solanacearum. Am Potato J. 57: 307-317.

7. Ciampi, L. Sequeira, L. and French, E.R. 1980. Latent infection of potato tubers by Pseudomonas solanacearum. Am. Potato J. 57: 377-386.

8. Ciampi, L. and Sequeira, L. 1980. Multiplication of Pseudomonas solanacearum in resistant potato plants and the establishment of latent infections. Am. Potato J. 57: 319-329.

9. Dukes, P.D., Jenkins, S.F., Jaworski, C.A. and Morton, D.J. 1965. The identification and persistance of an andigenous race of Pseudomonas solanacearum in a soil in Georgia. Pl. Dis. Rep-ti. 49: 586-590.

10. Drummond, O.A. 1984. Investigaciones para el combate de la Marchitez bacteriana de la papa realizadas en el período 1957 a 1982, en Río de Janeiro ** p. 83-89.

11. Fernández, M.C. 1986. Algunos huéspedes de Pseudomonas solanacearum en Chile. Agricultura Técnica 46: 101-105. In Rev. Pl. Path. 65: 354. 1986.

12. French, E.R. 1984. Diagnosis de la Marchitez bacteriana de la Papa con énfasis en latencia**. p. 51-54.

13. French, E.R. and De Lindo, L. 1982. Resistance to Pseudomonas solanacearum in potato: Specificity and temperature sensitivity. Phytopathology 72: 1408-1412.

14. Gonzáiez, L.C. 1977. Determinación de niveles de infección por Pseudomonas solanacearum en tubérculos de semilla de papa. Fitopatología (ALF) 12: 101-104.

15. Goto, M., Shiramatsu, T., Nozaki, K. and Kawaguchi. K. 1978. Studies of bacterial wilt of strawberry caused by Pseudomonas solanacearum. I Strains of the pathogen and disease tolerance of strawberry plants. Am. Phytopath. Soc. Japan 44 (3): 270-276.

16. Graham, J. and Lloyd, A.B. 1978. Solanum cinereum a wild host of Pseudomonas solanacearum Biotype 2. J. Aust. Int. Agric. Science 44: 124-126.

17. Graham, J. and Lloyd, A.B. 1979. Survival of potato strain (Race 3) of Pseudomonas solanacearum in the deeper soil layers. Austi. J. Agric. Res. 30: 489-496.

18. Granada, G.A. 1981. A selective medium for Pseudomonas solanacearum and its application in the study of survival of the bacterium in soil, rhizosphere and plant roots. Ph.D. Thesis. Univ. of Wisconsin. Madison. 101 p.

19. Granada, G.A. and Sequeira, L. 1983. Survival of Pseudomonas solanacearum in soil, rhizosphere, and plant roots. Can. J. Microbiol. 29: 433-440.

20. Granada, G.A. y Tamayo, P.J. 1986. Convenio ICA-CIP. Informe Proyecto Pseudomonas 1986. 8 p. (Mecanografiado).

21. Harris, D.C. 1976. Bacterial wilt in Kenya with particular reference to potatoes*. p. 84-88.

22. Hayward, A.C. 1978. Bacterial wilt of the potato in the Tropics. In Proc. CIP workshop on Bacterial wilt of the Potato and Second Regional Symposium of Pathogens and Pests of the Potatp in the Tropics. Los Baños, Philippines. Feb. 5-16.

23. He, L.Y., Sequeira, L. and Kelman, A. 1983. Characteristics of strains of Pseudomonas solanacearum from China. Plant Disease 67: 1357-1361.

24. He, L.Y. and Hua, J.Y. 1985. Occurrence and damage of Potato bacterial wilt in China. Plant Proteccion 11: 10-11 In Rev. Pl. Path. 65: 447. 1986.

25. Huang, A.l., Shu, S.t. and Chen, M.J. 1982. Interaction of tomato stems with highly and weakly virulent strains of Pseudomonas solanacearum. Plant Protection Bull. Taiwan 24: 235-246. In Rev. Pl. Path. 62: 198. 1983.

26. Jackson, M.T. and González, L.C. 1981. Persistence of Pseudomonas solanacearum (race 1) in naturaly infested soil in Costa Rica. Phytopathology 71: 690-693.

27. Katayama, K. and Kimura, S. 1984. Prevalence and temperature requirements of Biovar II and IV strains of Pseudomonas solanacearum from potatoes. Ann. Phytopath. Soc. Japan 50: 476-482.

28. Kelman, A. 1985. The bacterial wilt caused by Pseudomonas solanacearum. Tech. Bull. Nº. 99. N.C. Agric. Expt. Sta.

29. Kelman, A. 1976. Mission of the Conference*. p. 1-5.

30. Keshwal, R.L. 1976. Survival in the soil and virulence of Pseudomonas solanacearum*. p. 133.

31. Martin, C. 1979. Role of indigenous vegetation and crops in persistance of Pseudomonas solanacearum*. p. 63-65.

32. Moffett, M.L. and Hayward, A.C. 1980. The role of weed species in the survival of Pseudomonas solanacearum in tomato cropping land. Austr. Plant. Pathol. 9: 6-8.

33. Nyangeri, J.B., Gathuru, E.M. and Mukunga, D.M. 1984. Effect of latent infection on the spread of bacterial wilt of potatoes in Kenya. Tropical Pest Management 30: 163-165. In Rev. Pl. Path. 64: 268. 1985.

34. Olsson, K. 1976. Overwintering of Pseudomonas solanacearum in Sweden*. p. 105-109.

35. Ono, K., Hara, H. and Akazawa, J. 1984. Ecologycal studies on the bacterial wilt of tobacco caused by Pseudomonas solanacearum. V. The movement of the pathogen in tobacco plants. Bulletin Okayama Tobacco Exp. Sta. 43: 41-46. In Rev. Pl. Path. 64: 125. 1985.

36. Quimio, A.J. and Cham, H.H. 1979. Survival of Pseudomonas solanacearum in the rhizosphere of some weed and economic plant species. Philipp. Phytopathol. 15: 108-121.

37. Rao, M.V. 1976. Bacterial wilt of tomato and eggplant in India*. p. 92-94.

38. Sequeira, L. and Averre III, C.W. 1961. Distribution and pathogenicity of Pseudomonas solanacearum from virgin soils in Costa Rica. Pl. Dis. Reptr. 45: 435-440.

39. Thurston, H.D. 1976. Resistance to bacterial wilt (Pseudomonas solanacearum)*. p. 58-62.

40. Tomlinson. D.L. 1985. A preliminary study of the distribution of biovars of Pseudomonas solanacearum in Papua, New Guinea. Australian Plant Pathology 14: 8-9. In Rev. Pl. Path. 64: 529. 1985.

41. Torres, E. 1984. Situación actual de las investigaciones sobre la marchitez de la Papa en el Perú**. p. 21-26.

42. Wolf, F.A. 1922. Additional hosts for Bacterium solanacearum. Phytopathology 12: 98-99.

* In Sequeira, L. and Kelman, A. eds. 1976. Proc. First Internat. Planning Conf. and Workshop on the Ecology and Control of Bacterial wilt caused by Pseudomonas solanacearum. Raleigh, N.C. July 18-24. 1976.

** In Marchitez bacteriana de la papa en América Latina. Reunión de Planeamiento. Brasilia Agosto 31 - Septiembre 3, 1982. CIP. 1.984.

FIELD EVALUATION OF CLONES BRED FOR RESISTANCE TO *PSEUDOMONAS SOLANACEARUM*

E. R. French

Breeding for resistance to *Pseudomonas solanacearum* became possible after Thurston and Lozano detected resistant *Solanum phureja* clones in Colombia in the mid 1960's, which were utilized as of 1967 at the University of Wisconsin-Madison to develop germ plasm for distribution to tropical countries where bacterial wilt was a serious problem. Screening at Madison was done by stem inoculation first, later by a seedling inoculation assay or "mass screening". Field testing was done by interested collaborators in tropical countries where it soon became evident that late blight resistance was also needed. Thus the better yielding wilt resistant clones were crossed with blight resistant varieties developed in Mexico. Thousands of clones were distributed to more than 20 countries, with guidelines for standard field tests (10).

Greatest progress was initially made in Colombia, Costa Rica and Peru. In Colombia 1200 clones received in 1968 resulted in 38 resistant selections at Popayan (1800 m.a.s.l.), average temperature 18°C. Of these 11 were selected in trials at Rionegro (2200 m, 16°C), from which one was eventually selected for variety release. In 1970 "BR" clones that incorporated late blight resistance were received. After two consecutive field trials 34 of 672 were selected, later reduced to 24 (9).

In Costa Rica 3270 clones were screened down to 22 after five successive plantings carried out at Cot (temperature range 11-21°C) and subsequently at Alajuela (17-30°C). Of these, two became advanced selections, BR 63.15 which is being multiplied for release in Huanuco, Peru and MS 35.22 that is being multiplied in Costa Rica (3,6).

In Peru 369 BR clones of 10 families were received in 1970 and field screened in Huambos, Cajamarca Department (2450 m). The small greenhouse grown tubers were planted in family rows. Of these individual plants 67 wilted, some died from other causes or failed to tuberize. Still others had tuber symptoms at harvest or after storing for 20 days at ambient temperature. After eliminating those with primitive tuber types, 74 were replanted in a randomized block design, with five 1-plant plots per clone. Any clone that had foliar symptoms (in even one plant) or tuber symptoms at harvest or before replanting, was eliminated. After three additional tests in a randomized complete block design with 6 replications of 5 plants each, the number of selections was reduced to 20. Further testing resulted in a reduction, because

of agronomic and yield characteristics or susceptibility to late blight or other diseases, to 7 cultivars. In 1976 BR 63.74 was released as Caxamarca variety followed by BR 63.65 as Molinera in 1977. In the meantime collaborating farmers had made two more selections, BR 69.84 as Molinera 2 and an unknown BR as Amapola (5,7).

These three programs utilized naturally infested field sites in [which a heavily wilted crop had been recently harvested. Other procedures were similar, as also the climatic conditions, which allowed the selection of cultivars that had no wilt symptoms in the field, and no tubers with symptoms at harvest nor after storing them. From the results, it was apparent that adequate resistance was available for the high and mid-elevation cool to warm tropics, at least in the Americas. Based on these experiencies, the booklet "Field-screening CIP clones developed for resistance to bacterial wilt" was prepared in 1982. It is now available in English, French and Spanish (4).

With the expansion of potatoes into warmer lands it was found that the materials developed so far were not resistant under those conditions. Some clones, however, showed some tolerance but lacked adaptation, the case of MS 35.22 in Turrialba, Costa Rica (600 m). At that site, however, when 207 "tuberosum type" cultivars from the Mexican program were grown in a late blight test, only three survived bacterial wilt. Two of these succumbed in the two subsequent seasons. The lone selection with resistance to both wilt and blight was Cruza 148 (CIP 720118) which has proven resistant or tolerant elsewhere (1, 2, 8). This tolerance, however, has led to the problem of spread by latent infection (M. Potts, personal communic.).

The best selections developed in Peru were crossed to tuberosum type clones with adaptation to heat and screened for both wilt and blight. A set of 33 clones in 11 families was sent to Sri Lanka in 1980, but after multiplication so that 5 tubers of each could be planted, gaining one season over the above mentioned scheme. Because the field available for testing at Boralanda (1350 m) when planted to a susceptible variety resulted in unevenly distributed diseased plants, the randomized design with one-plant plots repeated 5 times included the alternate planting of a susceptible check variety between each test plant. This resulted in some plants being exposed to the soil inoculum potential resulting from wilting neighbours, so that of nine selections some had one wilted plant (5). Some BR clones and similar materials reported resistant elsewhere were tested also at the larger plot size test level. Some of these were resistant under these conditions but also at Sita Eliya (1900 m), the traditional seed producing District. However, when the best

clones from among the tuber families and the earlier developed cultivars (including BR 69.84, Domoni variety in Fiji) were tested in a farmer's paddy land at Boralanda, wilt occurred in all of them, with a minimum of 10% in the best performers. This was a superior performance to the local variety (11). The partial susceptibility of these materials may have been due to the effect of climate and/or specificity of strains of the bacterium (race 1).

In Rwanda a field at Ruhengeri (1850 m, 10-25°C) was heavily infested by growing potatoes and incorporating diseased tubers. During three consecutive seasons the testing of 57 cultivars and 434 clones in 14 families resulted in none being fully resistant. However, some had less percent wilted plants, the least infected was Cruza 148 with 5% (the local check had 70%). In neighbouring Burundi the same cultivar outperformed the rest in similar tests in land where potatoes were grown in three consecutive seasons prior to the tests, having 11% wilt in contrast to 100% for the check. Race 3 was involved in both countries (1,2).

Incomplete resistance or tolerance that results in few plants being infected, leads to the danger of latent infection which creates uncertainty and is a means of spread of the disease. For these situations superior resistance is needed and is being developed in a population based on resistance from four species of potato. Tests conducted at Cauday, Cajamarca Dept. and Carhuaz, La Libertad under higher inoculum potential (brought about by repeated planting of potatoes) that overcame the resistance of Molinera and Cruza 148, revealed some clones that behaved as resistant. Hopefully this new germ plasm will provide materials that under greater stress will perform as well as the prior generation of materials that were fully resistant under lesser stress of temperature and inoculum potential (see Schmiediche, these proceedings).

It should be apparent that field testing must be designed to meet the circumstances of the environment (including the strain of the bacterium) and the germ plasm being tested. Developing resistance for cooler climates, where race 3 predominates, appears feasible. It remains to be shown if more recently bred materials can do the same for warmer climates, where race 1 (that has a broader host range) predominates.

LITERATURE CITED

1. Autrique, A. 1984. Essais de contrôle du fletrissement bactérien de la pomme de terre au Burundi. Pages 22-32 dans La recherche sur le flétrissement bactérien causé par Pseudomonas solanacearum en Afrique Centrale. PRAPAC-CIP, B.P. 73, Ruhengeri, Rwanda.

2. Bicamumpaka, M. et A. Devaux. 1984. Programme de sélection au Rwanda pour l'obtention des variétés résistantes au mildiou (P. infestans) et á la bactériose (P. solanacearum). EAPR IX Conf. Trisann. p. 330-331.

3. CIP. 1985. p. 40 in Annual Report. Lima-Peru.

4. French, E.R. 1982. Field-screening CIP clones developed for resistance to bacterial wilt. Techn. Evaluation Ser. 1982-6. International Potato Center. 9 p.

5. French, E.R., C. Martin, and H. Mendoza. 1983. Cultivars Resistant to Bacterial Wilt and Late Blight for the High and Mid-elevations of the Tropics. Pages 85-86 in: W. Hooker (ed). Research for the Potato in the Year 2000. International Potato Center, Lima-Peru.

6. Gonzales, L.C., L. Sequeira, P.R. Rowe and R. Bianchini. 1972. Field resistance to bacterial wilt in hybrid potato progenies . Phytopathology 62:760 (Abstr).

7. Herrera, I.A. 1972. Progresos en la selección de resistencia a la marchitez bacteriana en el Perú. Pages 193-195 in: E.R. French, ed., Prospects for the potato in the developing world. International Potato Center, Lima-Peru.

8. Jackson, M.T., L.C. Gonzáles y J.A. Aguilar. 1979. Avances en el combate de la marchitez bacteriana de la papa en Costa Rica. Fitopatología 14: 46-53.

9. Perez, E., N. Estrada & L.A. Varón. 1972. Selección y multiplicación de variedades de papa con resistencia a "dormidera" (Pseudomonas solanacearum E.F. Smith) en Colombia. Pages 196-99 in: E.R. French, Ed., Prospects for the potato in the developing world. International Potato Center, Lima-Peru.

10. Sequeira, L. 1979. Development of resistance to bacterial wilt derived from Solanum phureja. Pages 55-62 in Development in control of potato bacterial diseases. CIP, Lima-Peru.

11. Veluillai, M. 1986. Bacterial wilt in Sri Lanka. Pages 57-64 in G.J. Persley (ed). Bacterial wilt in Asia and the South Pacific. ACIAR Proceedings 13, Canberra.

CLASSIFICATION AND IDENTIFICATION OF PSEUDOMONAS SOLANACEARUM

A. C. Hayward

Applied biologists of all kinds require systems of classification with sufficient information content to be of predictive value for the use of practitioners in the field. Classifications are a tool of basic and applied research that should bring order out of chaos, and facilitate the identifications which makes possible exchange of information. It is desirable that formal nomenclature at species and subspecies level is as stable as possible, and where informal classifications are used at the infrasubspecific level that the terminology used is universally accepted and understood. This paper assesses the present classification of Ps. solanacearum, the extent to which it meets ideal criteria, and makes proposals concerning future work on this important pathogen.

Ps. solanacearum in Relation to other Species of Pseudomonas

The pathogen of bacterial wilt is a distinct and easily recognised species. It can be stated with reasonable confidence that there are no unrecognised synonyms lurking in the recent literature, unlike the situation 35 years ago when the early work on bacterial wilt was reviewed (4). The better understanding of the relationships of Ps. solanacearum derives from studies of carbon utilization patterns (11,22), numerical taxonomy (27), and in particular the application of DNA:DNA and DNA:rRNA hybridization (8,9,23). The original finding of Palleroni and co-workers (23) that Ps. solanacearum forms one of five homology groups, on the basis of DNA:rRNA hybridization, was later confirmed and extended by others (8,9). Ps. cepacia is included in the same homology group as Ps. solanacearum yet the two species hybridize only to a mimimal extent when bulk DNA is used. Their true relationship is revealed when the ribosomal RNA, conserved during the course of evolution, is used in hybridization. The similarity in phenotype of these two species has been confirmed in several studies employing numerical taxonomy (27).

Infrasubspecific Classification of Ps. solanacearum

Ps. solanacearum has been classified into biovars, originally four and now five (12,14, Table 1), and four races (4,14, Table 2). The two classifications can be matched only to a limited extent (Table 3), however each system has made some contribution to understanding of the complexities of bacterial wilt.

It is illuminating to reexamine the results of Palleroni and Doudoroff (22) for carbon utilization patterns of the then four recognised biovars. Table 4 shows that biovars 3 and 4 are clearly the most versatile in terms of range of carbon sources which are used for growth; biovars 1 and 2 are substantially less nutritionally versatile; biovar 2 is the most uniform in nutritional spectrum and biovar 1 the most variable (see also 10,11). When the same data (22) was used as the basis of a numerical taxonomic study (27) two related but distinct sub-phenons were recognised corresponding to combined biovars 1 and 2 and combined biovars 3 and 4.

The emphasis that has been given to patterns of carbon substrate utilization in classification of Ps. solanacearum is not without point when it is considered that the substrates utilized are mostly relatively small organic molecules, such as organic acids, amino acids, hexoses, hexose alcohols and disaccharides and that most of them are plant constituents. There is a general correlation between nutritional versatility and recorded host range. Biovar 2, clearly the most specialised in terms of host range, is also the least nutritionally versatile, whereas biovar 3 which has been recorded on a far wider range of plants than any other biovar also utilizes the broadest spectrum of carbon sources. However this seeming relationship cannot be carried too far because there are examples of strains of both biovar 3 and 4 which are specialised in pathogenicity to particular hosts.

The nutritionally versatile biovar 3 also appears to have a greater capacity to survive in soil than biovar 2 (1,17,18,25). It may well be that the greater nutritional versatility of biovar 3 makes it better fit as a competitor in soil, rhizosphere and rhizoplane, where the ability to utilize low molecular weight plant exudates may be of competitive advantage.

The Present classification and Future Prospects

Few would question that a better classification of greater information content is needed for Ps. solanacearum, and a less confusing and more widely acceptable nomenclature at the infrasubspecific level. A thorough reexamination is warranted with concentration on the potato isolates. The data should be subjected to computer analysis and numerical taxonomy; from this data the information most crucial for identification can be extracted. This could be an international cooperative project in some respects similar to that conducted 25 years ago for Streptomyces, but in this case with the core study concentrated in one laboratory and more specialised studies radiating out to satellite laboratories with a

particular expertise.

An ideal classification is based upon a broad range of properties embracing morphology, physiology, ecology, biochemistry, molecular biology and genetics (Fig. 1,21). The ecology of Ps. solanacearum includes the relationship of the pathogen with the host, whether latent (5,10) or with overt symptoms; and survival in soil, or in the rhizosphere, or in an insect vector if any. Plant pathologists and plant breeders require a system of classification and identification which facilitates characterization of strains in epidemiological investigations and which relates to host range. But this is not easily arrived at. It is quite conceivable that the kind of evolution of strains that occurs in the field, perhaps analogous to the emergence of antibiotic resistant strains of bacteria in the hospital environment, which plant pathologists and plant breeders may witness from time to time when previously resistant cultivars become susceptible, is not reflected in any phenotypic property which can be readily recognised in the laboratory. There are presumably strong selective pressures acting on populations of Ps. solanacearum in the field resulting from changed cultivar use, cropping systems and cultural practices (4).

In the long term geneticists may be able to provide specific probes for the genetic determinants of host specificity, host range and virulence, but until that day more work of an international and collaborative nature is needed on the conventional characterization of Ps. solanacearum. Some of the new approaches which might be contemplated are shown in Table 5. However, it is desirable that such studies as fatty acid (15,20,26) and monoclonal antibody analysis (2), which hold promise of a remarkable degree of discrimination between strains, should be integrated with other studies of a more conventional nature so that the picture which emerges is complete rather than fragmentary. Some promising approaches which have been carried out in isolation from the needs of plant pathologists and plant breeders have also been on too limited a scale for a proper evaluation to be made of their utility (3,20,26).

Ps. solanacearum has evolved in certain ways affecting host specialization and also in ways affecting unrelated phenotypic properties. We do not know how the different biochemically specialised forms (biovars) of the pathogen have evolved but this has presumably occurred on a profoundly different time scale from many of the kinds of differences between strains which plant pathologists and plant breeders observe in the field. It is certain that Ps. solanacearum is readily transported from points of origin on planting material, particularly on potato tubers, and the risk of this occurring in latently infected tubers has increased with the

development of resistant and tolerant cultivars (5,19).

LITERATURE CITED

1. AKIEW, E.B. 1986. Influence of soil moisture and temperature on the persistence of Pseudomonas solanacearum. Pages 77-79 in: Bacterial Wilt disease in Asia and the South Pacific. G.J. Persley, ed. ACIAR Proceedings No. 13. 145 pp.
2. ALVAREZ A.M., BENEDICT, A.A., and MIZUMOTO C.Y. 1985. Identification of xanthomonads and grouping of strains of Xanthomonas campestris pv. campestris with monoclonal antibodies. Phytopathology 75:722-728
3. BAPTIST, J.N., SHAW, C.R. and MANDEL, M. 1971. Comparative zone electrophoresis of enzymes of Pseudomonas solanacearum and Pseudomonas cepacia. J. Bacteriol. 108: 799-803.
4. BUDDENHAGEN, I. and KELMAN, A. 1964. Biological and physiological aspects of bacterial wilt caused by Pseudomonas solanacearum. Ann. Rev. Phytopathol. 2: 203-230.
5. CIAMPI. L., SEQUEIRA, L., and FRENCH. E.R. 1980. Latent infection of potato tubers by Pseudomonas solanacearum. Am. Potato J. 57: 377-386.
6. CIVEROLO, E.L. 1985. Indigenous plasmids in Xanthomonas campestris pv. citri. Phytopathology 75: 524-528.
7. DAINTY, R.H., EDWARDS, R.A. and HIBBARD, C.M. 1984. Volatile compounds associated with the aerobic growth of some Pseudomonas species on beef. J. Appl. Bacteriol. 57: 75-81.
8. DE VOS, P. and DE LEY, J. 1983. Intra- and intergeneric similarities of Pseudomonas and Xanthomonas ribosomal ribonucleic acid cistrons. Int. J. Syst. Bacteriol. 33: 487-509.
9. DE VOS, P., GOOR, M., GILLIS, M. and DE LEY, J. 1985. Ribosomal ribonucleic acid cistron similarities of phytopathogenic Pseudomonas species. Int. J. Syst. Bacteriol. 35: 169-184.
10. GRANADA, G.A. and SEQUEIRA, L. 1975. Characteristics of Colombian isolates of Pseudomonas solanacearum from tobacco. Phytopathology 65: 1004-1009.
11. HARRIS, D.C. 1972. Intra-specific variation in Pseudomonas solanacearum. Pages 289-292 in Proc. Int. conf. Plant Pathogenic Bact. 3rd. H.P. Maas Geesteranus ed. Wageningen. Netherlands. 365pp.
12. HAYWARD, A.C. 1964. Characteristics of Pseudomonas solanacearum. J. Appl. Bacteriol. 27: 265-277.
13. HAYWARD, A.C. 1977. Occurrence of glycoside hydrolases in plant pathogenic and related bacteria. J. Appl. Bacteriol. 43: 407-411.

14. HE, L.Y., SEQUEIRA, L. and KELMAN, A. 1983. Characteristics of strains of Pseudomonas solanacearum from China. Plant Disease 67: 1357-1361.

15. JANTZEN, E. and BRYN, K. 1985. Whole-cell and lipopolysaccharide fatty acids and sugars of Gram-negative bacteria. Pages 145-171 in: Chemical Methods in Bacterial Systematics. M. Goodfellow and D.E. Minnikin, eds. Society for Applied Bacteriology Technical Series No. 20. Academic Press: London.

16. KAKOYIANNIS, C., WINTER, P.J. and MARSHALL, R.B. 1984. Identification of Campylobacter coli isolates from animals and humans by bacterial restriction endonuclease DNA analysis. Appl. Environ. Microbiol. 48: 545-549.

17. MOFFETT, M.L., GILES, J.E., and WOOD, B.A. 1983. Survival of Pseudomonas solanacearum biovars 2 and 3 in soil: effect of moisture and soil type. Soil Biol. Biochem. 15: 587-591.

18. MOFFETT, M.L. and WOOD, B.A. 1984. Populations of Pseudomonas solanacearum biovar 3 in naturally infested soil. Soil Biol. Biochem. 16: 57-61.

19. NYANGERI, J.B., GATHURU, E.M. and MUKUNYA, D.M. 1984. Effect of latent infection on the spread of bacterial wilt of potato in Kenya. Trop. Pest Management 30: 163-165.

20. OYAIZU, H. and KOMAGATA, K. 1983. Grouping of Pseudomonas species on the basis of cellular fatty acid composition and the quinone system with special reference to the existence of 3-hydroxy fatty acids. J. Gen. Appl. Microbiol. 29: 17-40.

21. PALLERONI, N.J. 1983. The taxonomy of bacteria. Bioscience 33: 370-377.

22. PALLERONI, N.J. and DOUDOROFF, M. 1971. Phenotypic characterization and deoxyribonucleic acid homologies of Pseudomonas solanacearum. J. Bacteriol. 107: 690-696.

23. PALLERONI, N.J., KUNISAWA, R., CONTOPOULOU, R. and DOUDOROFF, M. 1973. Nucleic acid homologies in the genus Pseudomonas. Int. J. Syst. Bacteriol. 23: 333-339.

24. PETZEL, J.P. and HARTMAN, P.A. 1986. A note on starch hydrolysis and β-glucuronidase activity among flavobacteria. J. Appl. Bacteriol. 61: 421-426.

25. RAMOS, A.H. 1976. Comparison of survival of two Pseudomonas solanacearum strains in soil columns under constant perfusion in field devoid of host cover. Pages 123-131: SEQUEIRA, L. and KELMAN, A., ed. Planning conference and workshop on the ecology and control of bacterial wilt caused by Pseudomonas solanacearum, Raleigh, North Carolina, N.C. State University.

26. SASSER, M. and MILLER, L. 1984. Identification of pseudomonads by fatty acid profiling. pp. 45-46 in: Proceedings of the 2nd Working Group on Pseudomonas syringae Pathovars. The Hellenic Phytopathological Society, Athens, Greece.

27. SNEATH, P.H.A., STEVENS, M. and SACKIN, M.J. 1981. Numerical taxonomy of Pseudomonas based on published records of substrate utilization. Antonie Van Leeuwenhoek 47:423-448.
28. TOMLINSON, D.L. and GUNTHER, M.T. 1986. Bacterial wilt in Papua New Guinea. Pages 35-39 in: Bacterial Wilt disease in Asia and the South Pacific. G.J. Persley, ed. ACIAR Proceedings No. 13. 145pp.

From: Palleroni (1983) Bioscience 33: p. 371

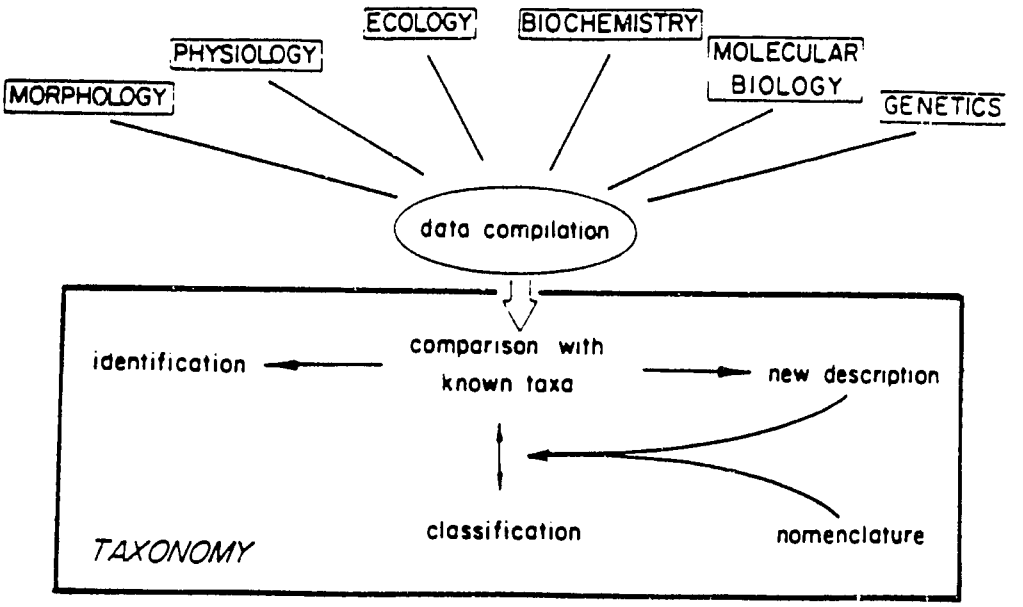


Figure 1. Flow of information in the characterization and classification of bacterial strains. Redrawn with minor modifications from Truper and Kramer (1981).

Table 1. Biovar Classification of Pseudomonas solanacearum
(He et al., (14); Hayward, (12); Palleroni and Doudoroff, (22))

		Bv.1	Bv.2	Bv.3	Bv.4	Bv.5
Utilization of	Mannitol	-	-	+	+	+
	Sorbitol	-	-	+	+	-
	Dulcitol	-	-*	+	+	-
	Trehalose	+	-	+	+	+
Oxidation of	Lactose	-	+	+	-	+
	Maltose	-	+	+	-	+
	Cellobiose	-	+	+	-	+
Gas from nitrate (Denitrification)		+/-	-	+	+	+

*Trehalose + isolates have been identified in both Indonesia (Hayward, unpublished) and Papua New Guinea (Tomlinson and Gunther (28)).
+ = positive reaction.
- = negative reaction.
+/- = some isolates give a positive reaction, others negative.
NOTE: Biovar 1 has not so far been detected in Australia or Indonesia (Hayward, unpublished data), and biovar 5 is confined to one host (Mulberry) in China (14). Biovar 2 (= race 3) is often referred to as the "potato race" or as the "low temperature" strain, which can be exported on infected, sometimes latently infected, tubers to areas (e.g., Sweden) well outside the normal distribution of bacterial wilt. Biovar 2 (race 3) is found at extremes of altitude in the tropics and at extremes of latitude in temperate regions where other races or biovars are never found.

Table 2. Race Classification of Pseudomonas solanacearum
(Buddenhagen and Kelman (4); He et al., (14))

Race 1	affects solanaceous and other plants.
Race 2	affects bananas and plantains (Moko disease) and Heliconias.
Race 3	affects potato; less often tomato; a few weed hosts.
Race 4	affects mulberry; low virulence to eggplant and potato.

NOTE: The use of the term race above is somewhat different from that of mycologists familiar with races of the wheat stem rust, for example. Races of P. solanacearum are not specialised to different cultivars of a host plant, but rather are subjective groupings of pathotypes showing more or less specialization for various host plants.

Table 3. Relationship between the Race and Biovar Classifications

Race 1 = Biovars 1*, 3 and 4
Race 2 = Biovar 1*
Race 3 = Biovar 2
Race 4 = Biovar 5

*Some race 1 (biovar 3) isolates cause wilt in banana in the Philippines and possibly elsewhere but the disease is less severe than Moko disease caused by race 2 (biovar 1).

Table 4. Nutritional Versatility of Biovars of Ps. solanacearum
(Based on data of Palleroni and Doudoroff (22), for utilization or lack of utilization of 39 carbon substrates)

	Biovar 1	Biovar 2	Biovar 3	Biovar 4
No. of strains	7	5	7	6
% Carbon substrates utilized by any strain	48	45	76	74
No. of Carbon substrates not utilized by any strain	10/39	18/39	5/39	3/39

Table 5. Some New Techniques Applicable to the Systematics of Pseudomonas solanacearum

Whole cell fatty acid analysis (15, 20, 26)
Head space analysis (7)
BRENDA (Bacterial restriction endonuclease analysis) - restriction-fragment length polymorphisms (16)
Plasmid profiles (6)
Electrophoresis of membrane proteins and enzymes (3)
Enzyme analysis using either chromogenic or fluorogenic (4-(methyl) umbelliferyl) substrates: e.g., aminopeptidases, glycosidases (13), API-ZYM galleries (24), etc.
Monoclonal antibody analysis (2)

GENETIC ENGINEERING FOR BACTERIAL DISEASE RESISTANCE IN POTATOES

P. Schmiediche, J. Jaynes and J. H. Dodds

1. Introduction

Genetic engineering of crop plants is a subject that has received a great deal of attention in recent years. Care must be exercised however in proposing the use of this type of technique to resolve agricultural problems. Many scientists have talked about the potential of genetic engineering for crop plant improvement, whilst few have shown concrete results leading to that objective.

In the case of potato concrete successes have been obtained to insert and express foreign genes, as will be described later. It is not therefore unrealistic to believe that this type of approach may be fruitful to prevent the problems of bacterial diseases.

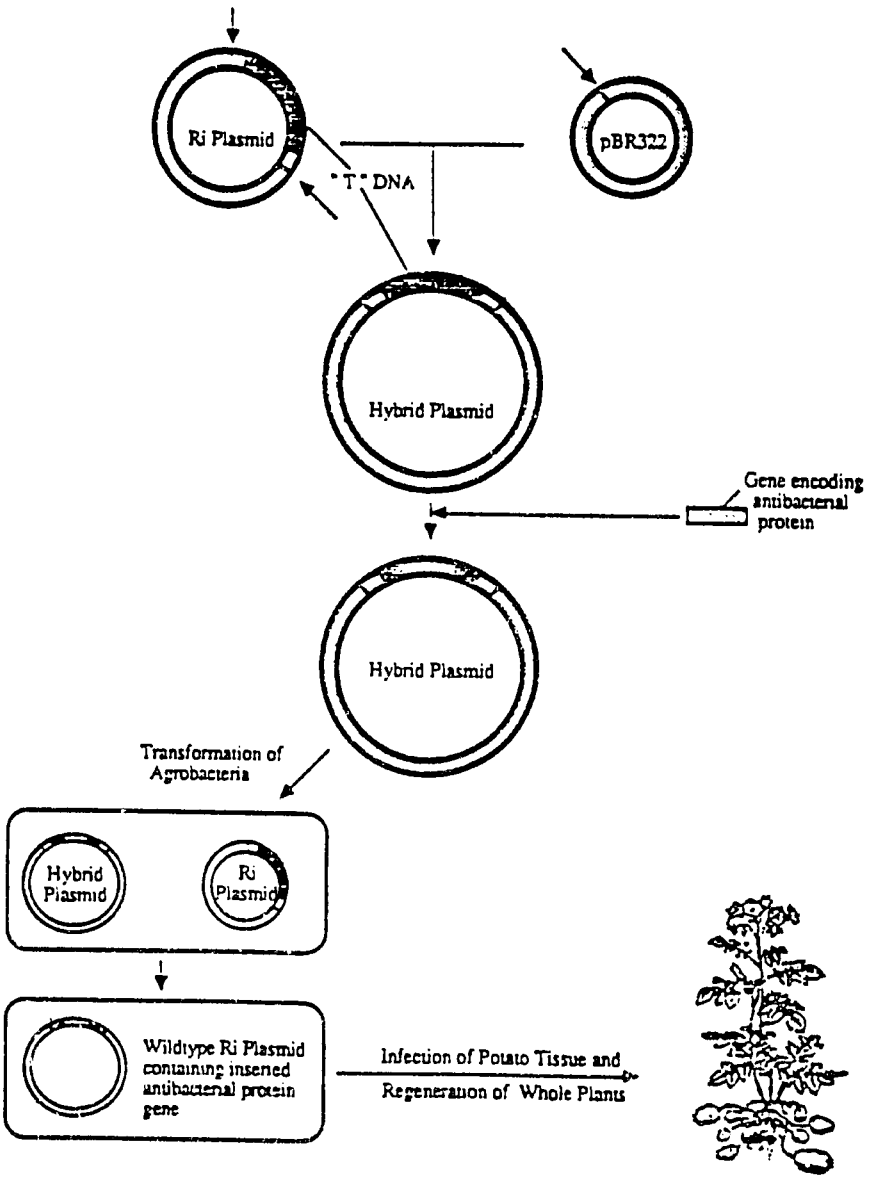
As new heat adapted potato clones are produced, allowing the potato to be grown in lowland tropical conditions, control of bacterial diseases becomes a high priority. The cultivation of genetically resistant material coupled to good field management has so far been the only effective method for the control of bacterial wilt . However, experience has shown that the available genetic resistance has a tendency to break down if the material is moved into climatic zones for which it is ill adapted. The genetic basis of resistance traditionally used in breeding is extremely small, two Colombian clones of *solanum phureja*. The reliance on this small genepool has been a concern to breeders and pathologists for a long time.

In the genetic engineering approach that we will go on to describe, we have moved away from looking for new sources of resistance genes whithin potato germplasm. The logic we have taken is that we understand little about the structure and location of genes within the potato controlling bacterial disease resistance, however, we know a great deal about the bacterial pathogens. By attacking the pathogens from our knowledge of their structure we may have a chance of success.

2. Genetic engineering of potato by use of Agrobacterium vectors: Synthetic protein as a model

We have already used *Agrobacterium rhizogenes* and *Agrobacterium tumefaciens* to insert novel genes into the potato (1,2). The basic method of gene insertion is shown in Figure 1.

GENE CLONING IN AGROBACTERIA



Through the use of restriction enzymes, modified Ri plasmids can be constructed which contain foreign genes (in this case, the genes encoding the antibacterial proteins). These new plasmids can be introduced into *Agrobacteria* and these strains can then be used to infect potato plants. The resultant transformed tissue can be regenerated into whole, reproductively viable plants and their putative increased bacterial disease resistance can be ascertained.

By a series of recombinations it is possible to insert a fragment of synthetic DNA)or a foreign gene) into Agrobacter. This gene is coupled to an antibiotic selection marker that allows the direct selection of transformed plants. The gene i also inserted under the control of a promoter sequence. The promoter is a control DNA sequence that regulates the site of gene product formation and the amount of product formed. By the use of standard detection methods we have been able to demonstrate that a synthetic protein gene can be successfully inserted into the potato genome (Figure 2a), the gene is transcribed to produce a MRNA molecule, (Figure 2b) and finally we have shown expressions of the gene, i.e. the production of the synthetic protein (Fig. 2c).

These experiments have shown that in the case of potato, genetic engineering is no longer a theoretical possibility, but is a well proven technique. It should be emphasized, however, that further studies are needed to show the control of gene expression does not affect other biochemical pathways of the plant and thus adversely affect yield.

3. Genes for bacterial disease resistance

Given from section 2 that a method does exist by which genes can be inserted into the potato genome, and those genes expressed, the next question would be, do we have genes that give products with potent antibacterial activity? The answer is yes. It has been shown that certain potent antibacterial proteins are produced in the gut of the silkmoth as a result of bacterial infection. These proteins are highly potent against bacteria as shown in Table 1. Some of these antibacterial proteins have been fully characterized (3) and their cDNA sequence determined (Figure 3,4,5).

Fig. 2

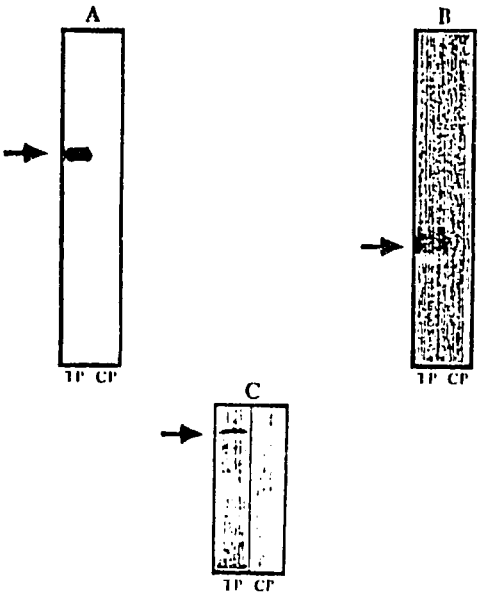


Illustration Presence of the HEAAE-DNA in potato tissue is demonstrated by the technique known as Southern transfer. DNA is isolated from the regenerated potato plants and digested with a restriction enzyme, in this case, one known as Hind III which recognizes the DNA sequence: AAGCTT and makes a double-stranded break in that region. The thousands of fragments are separated by gel electrophoresis and form a continuous smear on the gel. The DNA in the gel is treated with alkali causing the two DNA strands to separate and then is blotted to nitrocellulose filter paper where the strands of DNA become affixed to the paper. This "blot" is then dried and incubated with HEAAE-DNA (probe) which has been tagged with a radioactive phosphorous molecule. The probe DNA reassociates and binds with any complementary potato DNA bound to the filter. Unbound probe DNA can be washed away and the HEAAE-DNA bands are made visible by autoradiography. Part A shows that the probe only binds to DNA isolated from the transformed plant and not with the DNA from a non-transformed control plant, indicating that the transformation has been successful.

An additional technique, known as "northern blotting", may be used to show that the HEAAE-DNA is expressed as messenger RNA (mRNA). The mRNA is isolated from the regenerated transformed potato plants and separated in an analogous manner by gel electrophoresis. The mRNA bands are transferred to blotting paper and fixed and then incubated with the HEAAE-DNA probe. Again, the unbound probe can be washed away and the bands are made visible by autoradiography. Part B illustrates that the probe only binds to mRNA isolated from the transformed plant and not with the mRNA from a non-transformed control plant. This indicates that the gene is being copied by the mRNA synthesis enzymes of the plant cell and this message would then be ready for translation into the HEAAE-protein. Finally, to demonstrate that the HEAAE-protein is actually present in the cell, a technique known as "western blotting" is employed. Total proteins are isolated from the transformed plants, separated by gel electrophoresis and transferred to nitrocellulose paper. Previously to this, antibodies to the HEAAE-protein were prepared and are incubated with this western blot. Any spots which develop on the paper, after treatment with specific reagents, indicate the presence of the HEAAE-protein. Part C shows that only the transformed potato plants contain the HEAAE-protein in their tissue. Antibody proteins bind only to a particular shape unique to a macromolecule--in this case the HEAAE-protein and thus provide highly selective agents for the detection of expression of specific proteins (TP denotes the transformed plant while CP represents the control plant).

Table I. Antibacterial Activity on Representative Plant Pathogenic Bacteria

Organism	Lethal Concentration in μM for Cecropin A[a]
Escherichia species[b]	0.28--0.36
Pseudomonas species[c]	0.98--1.27
Erwinia species[d]	1.17--5.28
Clavibacter michiganensis[e]	5.27
Xanthomonas species[f]	1.27--5.77

a) The lethal concentration was calculated from the inhibition zones around 2mm wells in thin agar plates according to Hultmark et al[19].Synthetic cecropin A has been shown to be as potent as the natural cecropin B[20]. The data represents the average of two separate determinations. The bacteria were also tested against the basic form of the attacin. All species were found to be sensitive, however, only one concentration was used (1.5ng/μl) due to lack of sufficient attacin to conduct a lethal concentration assay.

b) Escherichia species were used as controls because of their extreme sensitivity to the cecropins.

c) Four different Pseudomonas species were assayed for their sensitivity to the toxin.

d) Three different Erwinia species were tested including the pathogen which causes soft rot.

e) Clavibacter michiganensis was included as it causes a ring rot disease in potato tubers.

f) Xanthomonas species were included as they are the causative agents of many plant diseases (citrus canker for example) although none of major significance in potato.

SEQUENCE OF MATURE LYSOZYME GENE AND PROTEIN

TGC CGT TCG CAG TTC GCT TTG CAT TGC GAT GCG AAA CGT TTC ACG AGA TGC GGG
Cys Arg Ser Gln Phe Ala Leu His Cys Asp Ala Lys Arg Phe Thr Arg Cys Gly

Partial Leader Peptide

Mature Lysozyme

TTA GTG CAG GAG CTT AGG AGA CGA GGC TTC GAT GAA ACT TTG ATG AGT AAC TGG
Leu Val Gln Glu Leu Arg Arg Arg Gly Phe Asp Glu Thr Leu Met Ser Asn Trp

GTC TGC CTT GTC GAG AAC GAA AGC GGA CGG TTT ACC GAT AAA ATC GGT AAA GTT
Val Cys Leu Val Glu Asn Glu Ser Gly Arg Phe Thr Asp Lys Ile Gly Lys Val

AAC AAG AAC GGA TCT CGA GAC TAC GGC CTC TTC CAG ATC AAT GAC AAA TAC TGG
Asn Lys Asn Gly Ser Arg Asp Tyr Gly Leu Phe Gln Ile Asn Asp Lys Tyr Trp

TGC AGT AAG GGA TCC ACT CCT GGA AAG GAT TGC AAC GTG ACT TGT AAT CAG CTA
Cys Ser Lys Gly Ser Thr Pro Gly Lys Asp Cys Asn Val Thr Cys Asn Gln Leu

CTG ACT GAC GAC ATT AGC GTG GCA GCT ACG TGC GCG AAG AAG ATT TAC AAA CGC
Leu Thr Asp Asp Ile Ser Val Ala Ala Thr Cys Ala Lys Lys Ile Tyr Lys Arg

CAC AAG TTT GAC GCT TGG TAC GGA TGG AAA AAT CAC TGT CAA CAT GGA CTG CCA
His Lys Phe Asp Ala Trp Tyr Gly Trp Lys Asn His Cys Gln His Gly Leu Pro

GAT ATT AGC GAC TGT TAG
Asp Ile Ser Asp Cys Stop

Nucleotide and deduced amino acid sequences of the Cecropia lysozyme. Part of the leader peptide is indicated by a line under the respective sequence.

SEQUENCE OF THE MATURE ATTACIN GENE AND PROTEIN

GAC GCG CAC GGA GCC CTT ACG CTC AAC TCC GAT GGT ACC TCT GGT GCT GTG GTT
Asp Ala His Gly Ala Leu Thr Leu Asn Ser Asp Gly Thr Ser Gly Ala Val Val

AAA GTA CCC TTT GCT GGT AAC GAC AAG AAT ATA GTA AGC GCT ATC GGT TCC GTA
Lys Val Pro Phe Ala Gly Asn Asp Lys Asn Ile Val Ser Ala Ile Gly Ser Val

GAC TTA ACT GAT AGG CAG AAA CTA GGC GCT GCA ACC GCT GGA GTG GCA CTG GAT
Asp Leu Thr Asp Arg Gln Lys Leu Gly Ala Ala Thr Ala Gly Val Ala Leu Asp

AAT ATA AAC GGT CAC GGA CTA AGT CTC ACG GAT ACA CAC ATC CCC GGG TTC GGA
Asn Ile Asn Gly His Gly Leu Ser Leu Thr Asp Thr His Ile Pro Gly Phe Gly

GAC AAG ATG ACA GCA GCC GGC AAA GTG AAT GTC TTC CAC AAT GAT AAC CAC GAC
Asp Lys Met Thr Ala Ala Gly Lys Val Asn Val Phe His Asn Asp Asn His Asp

ATC ACA GCG AAG GCT TTC GCC ACC AGA AAC ATG CCG GAT ATT GCT AAT GTA CCT
Ile Thr Ala Lys Ala Phe Ala Thr Arg Asn Met Pro Asp Ile Ala Asn Val Pro

AAT TTC AAC ACT GTC GGT GGC GGA ATA GAC TAT ATG TTC AAA GAT AAG ATT GGT
Asn Phe Asn Thr Val Gly Gly Gly Ile Asp Tyr Met Phe Lys Asp Lys Ile Gly

GCA TCT GCG AGC GCC GCT CAC ACG GAC TTT ATC AAT CGC AAC GAC TAC TCT CTT
Ala Ser Ala Ser Ala Ala His Thr Asp Phe Ile Asn Arg Asn Asp Tyr Ser Leu

GAC GGG AAA CTG AAC CTC TTC AAG ACT CCT GAT ACC TCG ATT GAT TTC AAC GCC
Asp Gly Lys Leu Asn Leu Phe Lys Thr Pro Asp Thr Ser Ile Asp Phe Asn Ala

GGT TTC AAG AAG TTC GAT ACA CCT TTC ATG AAG TCC TCT TGG GAG CCT AAC TTC
Gly Phe Lys Lys Phe Asp Thr Pro Phe Met Lys Ser Ser Trp Glu Pro Asn Phe

GGA TTC TCA CTT TCT AAA TAT TTC TGA TTA
Gly Phe Ser Leu Ser Lys Tyr Phe Stop Stop

Nucleotide and deduced sequences of the clone encoding the mature form of the basic attacin.

SEQUENCE OF CECROPIN GENE AND PROTEIN

ATG AAT TTC TCA AGG ATA TTT TTC TTC GTG TTC GCT TTG GTT CTG GCT
Met Asn Phe Ser Arg Ile Phe Phe Phe Val Phe Ala Leu Val Leu Ala

Leader Fepude

TCA ACA GTT TCG GCT GCA CCG GAG CCG AAA TGG AAA GTC TTC AAG AAA
Ser Thr Val Ser Ala Ala Pro Glu Pro Lys Trp Lys Val Phe Lys Lys

Mature Cecropin

ATT GAA AAA ATG GGT CGC AAC ATT CGA AAC CGT ATT GTC AAG GCT GGA
Ile Glu Lys Met Gly Arg Asn Ile Arg Asn Gly Ile Val Lys Ala Gly

CCA GCG ATC GCG GTT TTA GGC GAA GCC AAA GCG CTA GGA TAA
Pro Ala Ile Ala Val Leu Gly Glu Ala Lys Ala Leu Gly Stop

Nucleotide and deduced amino acid sequences of the clone encoding for the precursor of cecropin B. The structure of the leader peptide and the mature cecropin molecule is indicated by a line under the respective parts of the amino acid sequence.

These cDNA sequences are currently being synthesized and will then be inserted into Agrobacterium plasmid vectors. The plantlets regenerated from this transformation will then be screened against appropriate bacterial stains.

In vitro screening for Bacterial Disease Resistance

It would be most useful to screen transformed plantlets against both Pseudomonas and Erwinia in vitro. This would allow all experiments to be carried out in vitro, thus allowing easy compliance with USDA/NIH/FDA guidelines for use of recombinant organisms. Plantlets that show promise in these experiments could then be tested in quarantine growth rooms.

Current limitations to a genetic engineering approach

It is we believe important to state some of the limitations of this approach so as to stimulate further research in these areas.

(1) There exists a great need for increased efficiency in in vitro screening methods for selecting for bacterial disease resistance.

(2) There are still many effects of gene insertion on the total metabolic activity of the plant that have not been studied.

(3) Further knowledge is needed regarding the efficiency of different promoters so as to be able to obtain appropriate levels of expression.

Advantages of a genetic engineering approach

There are a number of advantages to the use of a genetic engineering approach to inserting genes for bacterial disease resistance. Firstly, by this method it is possible to insert a single gene or small group of genes into an adapted genotype or variety without major perturbations of other genetic characters. Secondly it should be possible to obtain resistance to both Pseudomonas and Erwinia in the same clone, this is something which would be extremely hard to achieve by conventional methods.

The probability of success in the short or medium term by this type of approach is low, however, the problem of bacterial disease is of such high priority and there are so many limitations to the conventional approaches that an attempt by genetic engineering technology is justified.

References

[1] Jaynes, J. M., Yang, M. S., Espinoza, N. O. and Dodds, J. H. 1986. Plant Protein Improvement by Genetic Engineering: Use of Synthetic Genes. TIBtech 4(12): 314-320.

[2] Shahin, E. A. and Simpson, R. B. 1986. Gene Transfer System In Potato. HortScience 21(5): 1199-1201.

[3] v.Hofsten, P., Faye, I., Kockum, K., Lee, J.-Y., Xanthopoulos, K. G., Boman, I. A., Boman, H. G., Engstrom, A., Andreu, D. & Merrifield, R. B. 1985. Moelcular Cloning, cDNA Sequencing, and Chemical Synthesis of Cecropin B from Hyalophora cecropia Proc. Natl. Acad. Sci. USA, 82, 2240-2243.

STRATEGIES FOR BACTERIAL WILT CONTROL

E. R. French

Bacterial wilt of the potato in the tropics is principally caused by race 3 (biovar 2) which is primarily a tuber-borne disease. The host range of race 3 is essentially limited to the potato, but it infects tomatoes when on rare occasions these are grown in rotation, as also in some locations eggplant or one or two different weeds at different sites. This upland wilt also occurs in the higher latitudes, Southern Sweden and Southern Argentina at the extremes. With the increased expansion of potatoes into warmer tropical lands the incidence of lowland wilt caused by race 1 (biovars 1, 3 and 4) has increased greatly. Race 1 has a very broad host range and is usually limited to the tropical lowlands (though found rarely in potato in the highlands) and subtropical lowlands such as southern USA (8, 10,30). Because of the differences between upland wilt and lowland wilt of the potato which will become even more apparent in subsequent paragraphs, these will be considered as two distinct diseases, the control measures for which require different treatment.

Upland wilt can under certain circumstances be eradicated, in others it can at least be brought under control with relative ease. It was successfully eradicated from the cool Northern Tablelands of Dorrigo, New South Wales - Australia where it became endemic after introduction from warmer lands, where presumably it is indigenous. Eradication was achieved with a pasture rotation of two and a half years minimum and control of tuber seed health (23). In the tropical mid-elevation highlands of Peru where an outbreak occurred presumably through introduced diseased seed at Umari, Huanuco Department (2400 m elevation), eradication was achieved after four years of rotation with maize or fallow which resulted from the prohibition of growing potatoes. However, experimental results demonstrated that a six months fallow followed by the planting of resistant variety Molinera diminished the inoculum potential so that a susceptible variety that followed had no wilt. It is considered that unknown suppressive factors occurred (9). Suppresiveness occurred to varying degrees in parts of the highlands of Peru and in coastal valleys, being great in the latter (11). In Rwanda a one crop rotation with any one of the five other principal crops grown there, greatly reduced wilt incidence, longer rotations showing no advantage (4). When rotation was followed by use of a resistant potato variety wilt incidence was further reduced (Devaux, personal communication).Conduciveness, however, has been reported for large geographical areas such as in Chile and Uruguay. In the latter, spread by mechanized equipment and by cattle was observed (7, 14).

In Colombia race 3 damage was greatest with continuous potato or rotation of potato and tree tomato, *Cyphomandra betacea*. The latter is also susceptible. Rotating maize (8-10 months growth) with either of these crops gave good control. Investigating several crops in rotation after heavily infected potato, onion or carrot resulted in the least wilt of a

subsequent potato crop (Granada and Tamayo, personal communication). In Honduras, good control was achieved by rotation with a maize-bean intercrop (25). In Burundi intercropping potatoes with beans resulted in less spread of P. solanacearum than with maize intercrop (Autrique and Potts, personal communication).

Delaying the date of planting in India, reduced wilt incidence (34). This was attributed to decreasing temperatures; maximum temperatures dropped from 31.6-33.2 to 29.3-29.6 °C in each of two years by delaying planting for two months. A similar report of a 3 week delay in autumn (fall) planting in Japan was accompanied by similar conclusions (19). However, increased time till planting involves a longer rest period in which soil suppressiveness may also act.

Fallow, exposing plowed fields to the summer heat and its drying effect, and burning straw in 20 cm deep furrows 40-50 cm apart, or both, greatly reduced wilt incidence in India (33).

Positive and negative selection for symptoms of wilt in Central Africa permitted the establishment of low infection seed in Burundi and Zaire (2, 26). Selecting small seed from healthy looking plants and storing them at high temperature to promote symptoms in the latently infected tubers, was most effective. The use of latex serology kits distributed by CIP has permitted a further refinement (M. Potts, personal communication).

Resistance, as mentioned above, has proven useful to control race 3, and it is potentially the most effective way (see Schmiediche in these proceedings). Apart from the cultivars bred for resistance, which are resistant under some field conditions and in greenhouse tests, there are some varieties that were not bred for resistance that behave as resistant or tolerant in specific situations. Red Pontiac was more resistant than Kennebec in Uruguay (13). Achat was very resistant in Brasil (24). In Mexico, variety Molinera that was bred for resistance and variety Lopez that was not behaved as equally resistant (3). In Papua New Guinea Ontario and clone BR 63.76 (bred for resistance) were both resistant (35). In Rwanda several varieties behaved as resistant or tolerant (18).

Lowland wilt has been investigated less than upland wilt, because it has until recently not been an apparent problem. The disease has undoubtedly curtailed the planting of potatoes in many lowland tropical and subtropical situations, but this fact has rarely breen recorded and published. With the recent thrust to increase potato production in response to population pressure and because of the commitment of governments to utilize warmer lower lands, P. solanacearum has surfaced as a limiting factor in the tropics. In the lowland subtropics of the Southeastern USA it has been controlled by avoidance of infested land or by the use of tolerant varieties (that yield well in spite of a low percentage of infected plants). Varieties with this tolerance or partial resistance in the USA are Ontario, Sebago, Katahdin and Green Mountain (17, 27, 29, 37) Nielsen and Haynes (27) found that Prisca was the most resistant tuberosum type potato among 9000 evaluated. It remains today one of the major varieties in

Madagascar, presumably because of this resistance. Lalmahomed (22) found varieties Marylin and Greta tolerant in Mauritius where only race 1 is known. The resistance derived from S. phureja that contributes significantly to the control of upland wilt, so far appears to be a lesser potential component of an integrated control approach (8, 12). Other sources of resistance will hopefully contribute more.

Rotation as a means of control indicated that a preceding rice crop diminished wilt in a tomato crop in Brasil, much more than other crops and fallow (31), a result that can be extrapolated to potato. In Turrialba, Costa Rica, rotation with a maize and beans intercrop, or sweetpotatoes, did not diminish the soil's inoculum potential, but the use of a herbicide and minimal till of the crop or a 5 months bare fallow with weeds controlled by a herbicide, reduced wilt incidence (16).

Intercropping potato with maize or cowpea served to contain the spread of wilt from diseased to healthy plants when initial infection was either due to latent infection of tuber seed or to soil infestation in Mindanao, Philippines. Wide spacing also reduced spread and the incidence of latent infection in progeny tubers. A shallow planting depth of 2 cm resulted in less wilt from soil infestation, but yield was less with more greened tubers than when 5 and 10 cm depths were used (21).

The control of race 1 in Southern USA and in Japan has been successful with the application of the fumigant chloropicrin at a rate of 300-326 L/ha covered by either a polyethylene film or a water seal whilst another 7 chemical fumigants did not give full season control (5, 20). Solarization (use of a polyethylene film alone) was effective during summer in Japan for race 1, but not for race 3.

A practical method (bioassay) for determining the wilt potential of soils in Mauritius consisted of growing tomato seedlings in sampled soils placed in large cans (6). The use of trays gave similar results at CIP. Another useful method consists of passing water through soil in a funnel with a Whatman No. 4 filter paper, dipping a scissors in the collected liquid and cutting the tip of a potato leaflet. Making this method quantitative is being explored (French and Nydegger, unpublished).

Because producing tuber seeds free of latent wilt infection is difficult in warm climates, two alternate methods of reproducing the potato could be used that reduce this risk. One is planting true potato seed directly or in beds for transplant. Another is to reproduce by cuttings from in vitro stocks, which has been succesfully done on a large scale in Vietnam (36).

The most relevant and/or recent information that relates to the control of both upland (race 3) and lowland (race 1) wilt has been presented. Additional pertinent information was summarized by French (8, 10) and by Persley (30). Considering

all of this information, a listing of the principal factors to be considered in developing a strategy for control of bacterial wilt is shown in Table 1. Each factor has been rated with a value of 1 to 7, the greater the figure the more the factor is judged to contribute to good control of the disease. According to circumstances determined of judged at each location, these values would be reset. If the sum or any of these reaches or surpasses 10, good control should be judged probable. Below are three examples.

1. In the locality of Umari, Huanuco in Peru, an upland wilt situation, it has been shown that soils are suppressive (4 points) that resistance is effective (3 points) and that potatoes are the only susceptible plant amongst crops and weeds. Thus, by roguing volunteer potatoes (4 points), these three factors that sum 11 points should be sufficient to give control. If a susceptible variety is planted, then the use of clean seed (3 points) would also give a total of 11 points. For greater security an additional 4 points can be added by a one-crop rotation (9).

2. In Mauritius potatoes are grown in between the rows of sugarcane after it is cut for harvest or at replanting time. Race 1 causes wilt, but it is minimal by use of healthy seed (3 points), choice of a tolerant variety (1 point), planting potatoes at intervals of several years (rotation, 1 point), planting in winter (which because of an island climate close to the Tropic of Capricorn, is cooler than many tropical lowland situations, would rate at least 2 points for time of planting), and the choice of the most suppressive soils (2 points) by testing them through a bioassay (6). A total of 9 points is still 1 short of the 10 suggested as necessary for adequate control. Practicing minimal tilling by applying herbicides for weed control (if feasible) or by earthing up with soil distant from the roots and stolons, might make the difference between having little or no wilt.

3. Wilt free soils are found in both the highlands and lowlands (7 points). Once identified, the use of healthy seed (3 points) would result in avoidance of the disease (11).

It is apparent that this exercise is subjective, but going through it will help focus on the factors to be considered and even some research that may need to be planned. In future years it is probable that better resistance for the lowland tropics will be available and that other means of control will be developed, such as biocontrol (1, 15, 32). These will have to be added to the factors considered in Table 1. It must be emphasized that a strategy is site specific, and must take into account not only the possible means of control that scientists can demonstrate feasible and effective, but the socioeconomic factors that influence decision making amongst the local farmers.

Table 1. Factors to be weighted in developing a strategy for bacterial wilt control. Figures shown for upland and lowland wilt, ranging from 1 to 7, are those considered to be generally applicable, but must be judged for each location. Generally, a sum of 10 would be adequate for good control.

UPLAND WILT, race 3		LOWLAND WILT, race 1
3	Resistance	2
2	Tolerance	1
3	Healthy seed	3
7	*P. solanacearum* free soils	7
4	Suppressive soils	2
4	Short rotation	1
3	Intercropping	2
3	Date of planting	1
2	Nematode control, resistance	4
2	Dry/heat soil	3
3	Solarization	1
4	Roguing volunteers	2
2	Roguing wilted plants	1
4	Cuttings, TPS	3
5	Fumigants	3
2	Water flow control	1
1	Minimal till	2
Often	ERADICABLE	Seldom

LITERATURE CITED

1. Aspiras, R. B. and Angela R. de la Cruz. 1986. Potential biological control of bacterial wilt in tomato and potato with Bacillus polymyxa FU6 and Pseudomonas fluorescens. Pages 89-92 in G.J. Persley (ed.). Bacterial wilt disease in Asia and the South Pacific. ACIAR Proceedings 13, Canberra.

2. Autrique, A. 1980. Essai de reconstitution d' un lot de plançons de pome de terre libres de bactériose (Pseudomonas solanacearum) à partir d' un matériel de plantation fortement infecté. Note Technique No. 2, Inst. Sc. Agron. Burundi. 7 p.

3. Camacho-Guerrero, J. S. 1980. Efecto de Globodera rostochiensis (Woll. 1923) Mulvey y Stone 1976 ("nemátodo dorado" de la papa) y Pseudomonas solanacearum E. F. Smith (causante de la "marchitez bacterial") inoculados en forma aislada y asociados, sobre diferentes variedades de papa. Tesis M.C. Chapingo, México. 90 p.

4. Devaux, A. et M. Bicamumpaka. 1984. Les methodes de controle limitant le developpement de la bactériose (Pseudomonas solanacearum) au Rwanda. EAPR 9th Trienn. Conf. Abstrs. p. 27-28.

5. Enfinger, J. M., S. M. McCarter and C. A. Jaworski. 1979. Evaluation of chemicals and application methods for control of bacterial wilt of tomato transplants. Phytopathology 69:637-640.

6. Felix, S. and C. Ricaud. 1978. A practical method for the assessment of the bacterial wilt potential of soils in Mauritius. Pages 139-159 in Pathogens and pests of the potato in the tropics. CIP, Los Baños, Philippines.

7. Fernández M., Carmen. 1984. Determinación de la marchitez bacteriana causada por Pseudomonas solanacearum E. F. Smith en papa. Agricultura Técnica (Chile) 44:173-174.

8. French, E. R. 1979. Progress in the integrated control of bacterial wilt. Pages 72-81 in: Developments in control of potato bacterial diseases. International Potato Center, Lima-Peru.

9. French, E. R. 1984. Situación actual y perspectivas futuras en el control de la marchitez bacteriana de la papa en América Latina. Pags. 9-11 en Centro Internacional de la Papa. Marchitez bacteriana de la papa (Pseudomonas solanacearum) en América Latina. CIP, Apartado 5969, Lima 100-Peru.

10. French, E. R. 1986. Interaction between strains of Pseudomonas solanacearum, its hosts and the environment. Pages 99-104 in G. J. Persley (ed.) Bacterial wilt disease in Asia and the South Pacific. ACIAR Proceedings 13, Canberra.

11. French, E. R., L. Gutarra and G. Vilchez. 1975. Field survival of Pseudomonas solanacearum race 3 in Peru. Europ. Assoc. Potato Res. VI Trienn. Conf. Papers p. 96.

12. French, E. R., and L. de Lindo. 1982. Resistance to Pseudomonas solanacearum in potato: Specificity and temperature sensitivity. Phytopathology 72:1408-1412.

13. García, Stella. 1976. Status of potato brown rot in Uruguay. Pages 78-83 in: L. Sequeira and A. Kelman, ed., Planning conference and workshop on the ecology and control of bacterial wilt caused by Pseudomonas solanacearum. North Carolina State University, Raleigh.

14. García, Stella, C. Crisci y J. Carbonell. 1979. Consideraciones para el control de Pseudomonas solanacearum (Smith) Smith, grave enfermedad de la papa en Uruguay. Pags. 29-32 en Revista Divulg. Tecn. Centro Invest. Agrícolas Alberto Boerger (Uruguay) No. 1.

15. Holloway, B. W., A. R. St. G. Bowen and A. Kerr. 1986. Genetics of Pseudomonas solanacearum and prospects for biological control. Pages 93-98 in G. J. Persley (ed.). Bacterial wilt disease in Asia and the South Pacific. ACIAR Proceedings 13, Canberra.

16. Jackson, M. T. and L. C. González. 1979. Persistence of Pseudomonas solanacearum in an inceptisol in Costa Rica. Pages 66-71 in Developments in control of potato bacterial diseases. International Potato Center, Lima-Peru.

17. Jaworski, C. A., R. E. Webb, R. W. Goth and S. C. Phatak. 1980. Relative resistance of potato cultivars to bacterial wilt. Am. Potato J. 57:159-165.

18. Joseph, W. 1983. Essai de Triage de varietés resistantes de pomme de terre (Solanum tuberosum L.) au flétrissement bactérien causé par Pseudomonas solanacearum E. F. Smith. Bulletin Agricole du Rwanda 16:150-163.

19. Katayama, K., S. Kimura and K. Hama. 1983. Influence of temperature on development of bacterial wilt on potato in fall cropping. Proc. Assoc. Pl. Prot. Kyushu 29:15-18.

20. Katayama, K., S. Kimura and T. Shinsu. 1984. Evaluation of solar heating (solarization) and chemicals for the control of bacterial wilt of potato. Proc. Assoc. Pl. Prot. Kyushu 30:21-24.

21. Kloos, J. P., B. B. Fernandez, A. S. Tumapon, L. Villanieuva and B. Tulog. 1986. Bacterial wilt research in Mindanao. CIP Circular 14(3):1-7.

22. Lallmahomed, G. M. 1976. Strains of Pseudomonas solanacearum and current research on bacterial wilt in Mauritius. Page 104 in: Planning conference and workshop on the ecology and control of bacterial wilt caused by Pseudomonas solanacearum. North Carolina State University, Raleigh.

23. Lloyd, A. B. 1976. Bacterial wilt in a cold-temperature climate of Australia. Pages 134-135 in Planning conference and workshop on the ecology and control of bacterial wilt caused by Pseudomonas solanacearum. North Carolina State University, Raleigh.

24. Lopes, C. A. & L. de B. Giordano. 1983. Avaliação da resistencia de oito clones e três cultivares de batata (Solanum tuberosum L.) à murcha bacteriana causada por Pseudomonas solanacearum. Hort. Bras. 1:33-35.

25. Mateo, N., D. Aguilar, W. Fiallos, J. Trejo y J. Salgado. 1982. Efecto de períodos de descanso de suelo en el rendimiento e incidencia de Pseudomonas solanacearum. Fitopatología 17:24-29.

26. Mundundu, N. et. B. N. wa Baseko. 1984. Aperçu sur nos connaissances actuelles sur la bactériose de la pomme de terre au Zaire et reduction du taux d'infection par selection. Pages 46-51 dans La recherche sur le flétrissement bactérien causé par Pseudomonas solanacearum en Afrique Centrale. PRAPAC-CIP, B.P. 73, Ruhengeri, Rwanda.

27. Nielsen, L. W., and F. L. Haynes. Resistance in Solanum tuberosum to Pseudomonas solanacearum. Am. Potato J. 37:260-267.

28. Navarro, R. & G. A. Granada. 1978. Problemas de marchitez bacterial en el Departamento de Antioquia-Colombia. ASCOLFI Informa 4(2):7-8.

29. O'Brien, Muriel and A. E. Rich. 1976. Potato Diseases. Agric. Handb. 474. USDA, ARS, U.S. Gov. Printing Office, Washington, D.C. 79 p.

30. Persley, G. J. 1986. Ecology of Pseudomonas solanacearum, the causal agent of bacterial wilt. Pages 71-76 in G. J. Persley (ed.). Bacterial wilt disease in Asia and the South Pacific. ACIAR Proceedings 13, Canberra.

31. Robbs, C. F. 1960. Influencia da rotação de cultura na incidencia da "murcha" bacteriana de tomateiro (Pseudomonas solanacearum). Pag. 35-38 in Bacterioses Fitopatogenicas no Brasil. Serie Divulgação de Pesquisas No. 2. Univ. Rural, Itaguai, RJ-Brasil.

32. Sequeira, L. 1983. New approaches for control of bacterial wilt of potato. Pages 174-175 in Research for the Potato in the Year 2000, W. Hooker, ed. CIP, Lima-Peru.

33. Shekhawat, G. S., B. B. Nagaich, V. Kishore, V. K. Bahal and I. D. Garg. 1982. Managing Pseudomonas solanacearum wilt. Chemrawn II Conference Hadbk. & Abstrs. PS.A-31, p. 62. International Conference on Chemistry and World Food Supplies: The New Frontiers, Manila, Philippines Dec. 6-10, 1982.

34. Sinha, S. K., K. Rai and M. M Ahsan. 1986. Effect of date of sowing on the incidence of potato wilt (Pseudomonas solanacearum E. F. Smith) at Ranchi. ACIAR Bacterial Wilt Newsletter No. 1, Dec. 1986: p. 4-5.

35. Tomlinson, D. L. and M. T. Gunther. 1986. Bacterial wilt in Papua New Guinea. Pages 35-39 in G. J. Persley (ed.). Bacterial wilt disease in Asia and the South Pacific. ACIAR Proceedings 13, Canberra.

36. Vander Zaag, P. 1986. Potato production under Pseudomonas solanacearum conditions: sources and management of planting materials. Pages 84-88 in G. J. Persley (ed.). Bacterial wilt disease in Asia and the South Pacific. ACIAR Proceedings 13, Canberra.

37. Weingartner, D. P., and J. R. Shumaker. 1985. Bacterial wilt and tuber brown rot as a potential threat to potato production in Northeast Florida. Proceedings of the Florida State Horticultural Society (1984) 97:198-200.

ECOLOGY OF ERWINIAS CAUSING STEM AND TUBER DISEASES

M. C. M. Perombelon

Potato, traditionally a cool temperate crop, is increasingly grown in warmer climatic regions because of its excellent nutritional qualities and potential high yield per unit area and time. Although serious losses in the field and/or in storage can be caused by a wide range of pathogens, few are virulent under all climatic conditions. Of these the soft rot erwinias stand out as being the most important. They usually macerate parenchymatous tissue causing rots affecting both stems and tubers but can also cause wilting and desiccation of the plant. In addition to losses attributed to lower yields in the field and rots in storage which can affect both table and seed crops, infection can also reduce the value of the latter when they fail to meet health standards in seed certification schemes. In addition, recent studies (Pérombelon & Hyman, 1986) suggest that, even in the absence of disease, significant yield losses of up to 20% can occur in crops grown from latently infected seed tubers.

Partly because disease development is more dependent on environmental conditions than on the presence of the bacteria and is therefore unpredictable, and partly because control cannot be achieved by traditional straightforward measures such as chemotheraphy or seed certification, the industry has tended to live with the problem and absorbed the losses when they occur. However, falling profit margins in farming, greater pressure to maximise yield by increased irrigation, growth of modern cultivars with often low resistance and the spread of potato cultivation to warmer and wetter climates, mean that these losses can less and less be tolerated.

Disease control through the breeding for resistance in the traditional manner or by genetic engineering is unlikely to bear fruit in the near future and would require first the development of a meaningful test to screen for resistance based on a sound understanding of host resistance and pathogen pathogenicity. Until then, some degree of control can be achieved by adopting a series of measures which interfere with the cycle of infection in the field or in storage. Development of such an integrated approach to disease control must be based on a sound knowledge of the ecology of the pathogens, sources, survival and pathways of contamination, and of their pathogenicity in the different environments potatoes are grown.

This paper reviews the ecology of soft rot erwinias in relation to their paghogenicity to potatoes. It emphasises development which has taken place since the subject was last reviewed (Pérombelon, 1978; Pérombelon & Kelman, 1980). Certain aspects of this subject have also been reviewed previously (Graham, 1964; Starr & Chatterjee, 1972; Pérombelon, 1981, 1982; Stanghellini, 1982).

The pathogens

Three soft rot erwinias, Erwinia carotovora ssp. carotovora (Ecc), E. carotovora ssp. atroseptica (Eca) and E. chrysanthemi (Echr) have been associated with potatoes. They are members of the family Enterobactericeae and belong to the carotovora group and their main characteristic is their ability to produce pectic enzymes and rot plant tissues. They are gram negative, non-spore forming prototrophic facultative anaerobes. Although similar in many respects, they differ sufficiently in their physiological and serological characters to justify separation into species and sub-species (Lelliott & Dickey, 1984). Their different optimal temperatures for growth could reflect their geographical distribution and their relative virulence to potato. Thus, all three erwinias grow at temperatures 37°C but Eca cannot grow at higher temperatures while Echr can grow at temperatures > 38°C but most Ecc strains cannot do so (Pérombelon & Kelman, 1980).

The three soft rot erwinias have a worldwide distribution but with contrasting host ranges and apparent host specificities which probably are reflected in their temperature characteristics and geographical distribution. It is notable that the number of different crops suffering from frequent and severe attacks is significantly lower than the actual number of host species that can become infected. Ecc strains have a wide distribution in both the temperate and tropical zones and are pathogenic to a wide range of plants. In contrast, Echr is pathogenic to a wide range of plants in tropical and subtropical regions and is also found in certain glasshouse crops grown in temperate regions as well as a few field-grown crops such as maize (Zea mays). The host range of Eca is restricted mostly to potatoes which have traditionally been grown in temperate regions. Its occasional detection on other crops probably indicates contamination from sources associated with potatoes.

It is notable that some two decades ago Eca was the only erwinia commonly associated with potato stem infection in the field and infection by Echr was unknown or went unnoticed. But now all three erwi-

nias are recognised to be pathogenic under field conditions to potato but not necessarily in all countries. This situation could have arisen through a combination of several factors and it is difficult to say which were operative in each case. First of all, clarification of the taxonomy and increased selectivity and specificity of media have made isolation and identification of the different erwinias easier. Then, increased trade between countries often in different climatic regions as well as the spread of potato cultivation to warm regions have led to either the introduction of certain erwinias in hitherto free potato seed production areas (eg Echr in several continental European countries) or have given the opportunity to endemic erwinias to spread to and infect recently introduced potato crops (eg Echr in potatoes grown in tropical Peru), or again have allowed virulence of certain erwinias (Ecc or Echr) to be expressed when high temperatures prevailed. On the other hand, Eca is not pathogenic and cannot survive unless repeatedly reintroduced on imported infected seed in hot countries. It is unavoidable that sooner or later all three erwinias would contaminate potato stocks in most temperate countries. Just as Ecc is present in most seed stocks and yet rarely causes blackleg because of prevailing low temperatures, similarly Echr can survive but is pathogenic only when the seed is grown under warm conditions.

The diseases: symptoms and terminology

A confusing range of names is used to describe the infection of potatoes by soft rot erwinias, especially the field diseases. In an attempt to bring some order, Pérombelon & Kelman (1987) have argued that there is a strong case for the concept that different erwinias actually cause only one disease when the inoculum source is the seed tuber. Symptoms caused by the three erwinias are often indistinguishable, the biology of infection is similar, more than one *Erwinia* can be present in a diseased plant and physiologically the bacteria are very similar. On this basis, one name only, blackleg, could be used to describe all manifestations of the disease. In the case of aerial stem rots caused by direct infections found usually late in the growing season especially in fields subjected to frequent overhead irrigation during periods of high temperatures, the term aerial stem rot can be used. Because seed contamination by erwinias may not be a direct or primary contributing factor, because infection is initiated above ground from external sources and because the disease is not economically important in most seed growing areas, it is justifiable to distinguish aerial stem rot from blackleg; otherwise, seed health status would be a meaningless concept. The nomenclature of potato diseases caused by erwinias, singly or in any combination is given in Table 1. It should be stressed, however, that certain of the symptoms listed could also be attributed to other causes.

Table 1. Terminology of potato diseases caused by the soft rot erwinias: Erwinia carotovora sub sp. carotovora, E. carotovora sub sp. carotovora and E. chrysanthemi

In the field		
Non emergence or blanking	:	caused by decay of seed tubers or early sprout death.
Blackleg	:	infection of the stem originating from seed tubers; symptoms variable ranging from dark stem rot to wilting and plant desiccation only.
Aerial stem rot	:	dark wet rot initiated on exposed stem and not originating at point of attachment to seed piece or mother tubers.
Stolon end rot	:	affect progeny tubers of blackleg plants, usually results in decay of central portion of tuber.
Tuber soft rot of progeny tubers	:	characterised by a cream to tan colour with a soft granular consistency; brown to black pigments often develop at margins of decayed tissue. Erwinia populations often overrun by other saprophytic bacteria which are responsible for the characteristic evil smell.
In storage/transit		
Tuber soft rot	:	as above and in addition can lead to a soft rot pocket in bulk stored potatoes.
Lenticellular hard rot or pit rot	:	sunken hard dry brown to black lesions where lenticels became infected when tubers were wet but decay arrested after drying of tuber.

Blackleg symptoms caused by the different erwinias may vary not only from one climatic region to another, but also within a region according to local and annual variations in weather conditions. Although symptoms may appear at any time between emergence and harvest, they usually develop during the first half of the growing season. Broadly speaking they can be grouped into two classes depending on rainfall or irrigation and relative humidity but regardless of temperature; under wet conditions a stem rot develops and under dry conditions the plant is stunted, wilts and under extreme conditions desiccation of the leaves occurs starting at the leaf margins. However, different combinations and variations of these symptoms can also occur especially under fluctuating environmental conditions.

Methodology

The considerable progress achieved during the past two decades in the epidemiology of diseases caused by soft rot erwinias can be attributed to the vastly improved methodology available for the isolation, identification and enumeration of the bacteria even when present in low numbers. Latent or cryptic infection or contamination of tubers can be detected by an enrichment procedure in which the tubers are induced to rot (Pérombelon, 1972b, 1979a; De Boer & Kelman, 1975). Low populations in soil, leaves and water can also be detected by enriching in a pectate basal salt solution incubated anaerobically(Stanghellini & Meneley, 1976; Burr & Schroth, 1977; Elphinstone & Pérombelon, 1986b). Following preferential growth of soft rot erwinias, as well as other pectolytic facultative and obligate anaerobes, erwinias can be detected by plating on several selective pectate based diagnostic media of which that of Cuppels & Kelman (1974) (CVP) is the most efficient. The bacteria can be identified by testing for certain characteristic physiological characters (Graham, 1972) after purification of single colony isolates or directly without the purification step and according to the pattern of cavity formation after spotting bacteria from individual colonies on CVP with or without erythromycin added and incubating at 27, 33.5 and 37.5°C (Pérombelon & Hyman, 1986). Population of erwinias can be identified and quantified rapidly and directly from plant material and other sources by dilution plating on CVP followed by velvet-replicating on to fresh CVP plates with or without erythromycin and incubating at differential temperatures as above (Pérombelon *et al.*, 1987).

In the past various serological procedures based on the use of somatic antigens, fluorescent antibody and immuno-diffusion tech-

niques (Pérombelon & Kelman, 1980) have been used to identify erwinias but they are often misleading because of cross reactions between different erwinias and interference by other microorganisms. De Boer et al. (1979b) has identified as many as 36 serogroups based on immunodiffusion techniques in Ecc and Eca and more have been found since. In contrast, Echr appears to be more homogeneous; only 3 or 4 serogroups or serovars were detected (Schaad, 1979). However, current research in the production of specific monoclonal antibodies in several laboratories coupled with the development of new immuno-sorbent and -fluorescence identification methods (Van Vuurde, 1987) could be of great value.

Lastly, studies on the relative pathogenicity of erwinias in the field and on the relationship between inoculum level and blackleg development have benefitted from the development of methods to inoculate seed tubers by vacuum infiltration to simulate natural infection and to quantify the level of seed contamination (Pérombelon et al., 1987b).

Factors affecting disease development

Erwinias, like most bacterial pathogens, can gain access into plants through natural openings (eg lenticels on tubers) or via wounds caused by wind or farm implements on stems and tubers. The numbers of erwinias found in potential infection courts are usually small and the bacteria may remain dormant for a long time even when in contact with the living cells. Disease develops only when populations have reached a critical level which is c. 10^7 cells per site. The transition from a latent or quiescent to an active stage occurs when favourable environmental conditions prevail which reduce host resistance mechanisms and at the same time allow multiplication and expression of the pathogenicity traits of the bacteria. The precise nature of both potato resistance and the determinants of pathogenicity in erwinias is still not clear, but current studies on the molecular biology of the interaction should help to clarify the situation. What is known however are the conditions necessary for disease development.

Naturally infected tuber will rot when anaerobic conditions prevail, free water covers the tuber surface, the temperature is above the minimum required for growth of the pathogen and physiological factors such as high tissue water potential are present (Pérombelom & lowe, 1975). It is clear that local oxygen concentration undoubtedly favours disease initiation by affecting oxygen-dependent host resistance mechanisms and possibly favouring bacterial pathogenicity expression. However, rotting will not occur solely under anaerobiosis.

There is some evidence that readily available nutrients are also needed for bacterial multiplication, such as solutes leaked from turgid cells when cell membrane integrity is lost under anaerobiotic conditions. Under these conditions, only small numbers of bacteria ($<10^2$ cells) are required to initiate a lesion, the time for decay to develop being temperature dependent (Pérombelon, 1982a; De Boer & Kelman, 1978).

Decay of tubers can occur in the field, in transit or in storage when they remain wet for some time. Tubers in storage become wet as a result of water condensation or liquid oozing from decayed tubers (particularly those infected by species of Pythium or Phytophthora). Similarly, early decay of seed tubers or seed pieces resulting into extensive non-emergence or blanking can occur when the soil is waterlogged, for example after heavy rain or flood irrigation. Although less common, progeny tubers may rot even when not attached to blackleg affected plants usually when the soil is wet and temperatures high. A continuous water film on the tuber surface results in rapid depletion of oxygen within the tuber, eg 2.5 h at 22°C (Burton & Wigginton, 1970) and at the same time increases tuber tissue water status, two essential conditions for rot initiation. Although other pectolytic soil borne bacteria including species of the genera Clostridium, Bacillus and Pseudomonas, are usually present, rotting is usually initiated by soft rot erwinias which may subsequently be succeeded by the other bacteria more adapted to grow in a rotted tissue environment (Pérombelon et al., 1979b). There is some evidence that under hot conditions (> 30°C), Ecc and Eca are less pathogenic than the other bacteria (Shekawat & Pérombelon, 1986). Furthermore, bacterial infection may follow on fungal attack of the tuber especially in soil.

Blackleg is invariably associated with decay of the mother tuber which more often than not is contaminated by more than one erwinia type. Although it is not a necessary sequel to tuber rot, conditions in the soil which favour tuber decay also favour blackleg development. Stem infection can occur at any time after multiplication of the pathogen/s in the rotting mother tuber through the vascular system, and once in the stem, the bacteria may remain dormant or may cause an infection if conditions are favourable. Most mother tubers sooner or later will rot and not surprisingly most healthy stems are latently infected with one or more erwinias usually the same type present in the rotting mother tuber. Similarly, more than one type of erwinia is often present in diseased stems although only one type usually predominates and is probably responsible for symptom development (Pérombelon et al., 1987). Stems can also become infected by bacteria in the soil via the

root system but practical experience suggests that it is unlikely to be of importance except perhaps under exceptional circumstances.

Several factors have been identified which favour blackleg development, the most important being soil water status. In wet soils, mother tubers become anaerobic and rot. Under Scottish conditions (soil temperature < 20°C) blackleg develops during or soon after periods of 10-14 days when soil water deficit has been brought to near zero by daily irrigation (Pérombelon & Lowe, 1984). For similar reasons, the reputedly higher disease incidence in crops grown in the mediterranean area than in Scotland can be attributed to the fact that soil is often wetter in the former as a result of frequent irrigation than in the latter region. Temperature during an important part of the growing season in the two countries is not very different because of different planting times.

It has been demonstrated beyond doubt that incidence of both non emergence or blanking and blackleg is correlated with the inoculum level on the seed tubers (Aleck & Harrison, 1978; Pérombelon & Lowe, 1978). Because disease development is also affected by other factors in particular soil water status, it is not possible to predict the disease level in a crop on the basis of the seed contamination level. Nevertheless, more disease is likely to develop if the seed is heavily than lightly contaminated in any given situation. Recent work has shown that provided weather conditions in one climatic region are not abnormal there is a threshold level of seed contamination below which blackleg is unlikely to develop for different cultivars depending on their resistances (Bain & Pérombelon, 1987).

In addition to these two major factors, blackleg expression is also affected by several others which although relatively minor may occasionally play an important role. These are treatments or conditions that damage the seed tuber or reduce its resistance, i.e. use of cut-seed under unfavourable conditions, seed damaged by freezing or overheating (black heart) during storage, seed affected by various fungal diseases (late blight, dry rot, gangrene) and by lenticellular hard rot. Disease is also affected by factors which affect host metabolism such as soil nitrogen level with which it is inversely related. Soil texture and topography can affect soil water holding capacity hence mother tuber rotting and blackleg development. There is also some evidence that there is a mutual synergistic effect between Verticillium dahlia and Eca. Blackleg incidence also depends on the virulence of the pathogen; different Eca strains inoculated in similar numbers in seed tubers can give to widely different levels of blackleg (M.C.M. Pérombelon, unpublished).

Although most contaminated mother tubers rot and most stems become latently infected, blackleg incidence is usually low in most potato growing areas. The mechanisms that trigger the disease are still unknown. Preliminary results (M.C.M. Pérombelon, unpublished) suggest that disease develops following a sudden influx of large numbers of the bacteria from the rotting mother tuber in the stem provided conditions are favourable for their survival and multiplication in the stem. The fact that disease frequently develops in only one of the several systemically infected stem of a plant tends to support this view.

Soft rot erwinias can be described as vascular pathogens when they infect stems because partly of the wilt symptoms they can cause are similar to those of the vascular pathogens such as Verticillium spp. and partly because the way they can spread systemically via the vascular system before symptoms appear. Spreading lesions failed to develop when soft rot erwinias were inoculated into the inter-fascicular regions of stems but not when inoculated directly in the vascular bundles (Hellmers & Dowson, 1953). Under wet conditions, the bacteria move out of xylem vessels to infect adjoining parenchymatous tissue giving rise to a rot. However, under dry conditions, the bacteria are restricted to the vascular system which become blocked either by the bacteria themselves and/or by gumlike material and tyloses with the result that water flow is impeded and wilt and desiccation symptoms develop.

Pathogenicity and temperature interactions

Temperature is the main factor affecting the relative virulence of soft rot bacteria and its level may determine which organisms predominates in a lesion. When tubers were inoculated with Ecc and Eca, Ecc and Echr and Eca and Echr in a 1:1 ratio and incubated at different temperatures, Eca tends to predominate in the rot lesion at 15°C, Ecc at 25°C and Echr at > 30°C (Pérombelon et al., 1979b; unpublished data). A similar pattern was obtained when the relative pathogenicity of the bacteria assessed by the effective medium dose (ED50) for induction of decay was determined; Eca and Echr were more virulent at 15°and 35°C respectively and Ecc was intermediate. Essentially similar results were obtained when stems of potted plants were inoculated. Although this appears to be the general rule, different results may be obtained when using weakly or highly virulent strains. There is some evidence to suggest that this relationship could be explained in terms of (a) the absolute growth rate of the different er-

winias in vitro and (b) the regulation and production of different pectic enzymes (M.C.M. Pérombelon, unpublished data).

In field experiments in Israel, rotting mother tubers previously inoculated jointly with two erwinias in different combinations yielded more Eca early and Echr late in the February-sown growing season whereas Ecc was present at all times (Pérombelon et al., 1987a). In contrast, in Scotland where Echr was not used, mother tubers equally infected with both Ecc and Eca at all times during the growing season. These results can be interpreted in terms of the mean weekly maximum soil temperatures at tuber depth which were 20°C after April in Israel and never exceeded 20°C in Scotland. When blackleg stems were analysed, Eca predominated at all times in Scotland but only until early May when the mean weekly maximum air temperatures were < 25°C and Echr later in the season. In contrast, Ecc was apparently not pathogenic in both countries although there was some indication that it could cause blackleg in the September sown crop in Israel when soil and air temperature during the first half of the growing season was > 25 and 30°C respectively. It is notable that Ecc was found to be the main causal agent in aerial stem rot in both countries and was probably brought in by rain from the air, by insects or in irrigation water. Molina & Harrison (1980) also found that Ecc was pathogenic when soil temperatures were > 25°C.

The relative ability of the different erwinias to cause blackleg could therefore be explained in terms of prevailing temperatures during the growing season. However, it is not clear at present whether this apparent differential effect of temperature is a result of a direct influence on multiplication within the mother tuber and the dominant erwinia infecting the stem or indirectly on the pathogenicity of the bacteria in the stems.

Epidemiology: sources and pathways of contamination

It appears from what is known of blackleg etiology that the disease is seed borne. Therefore it is important to understand how seed contamination occurs.

(a) Role of the mother tuber

Until fairly recently, it was commonly accepted that the source of inoculum for blackleg from one potato generation to the next was either the seed tubers from blackleg-affected plants or from the bacteria surviving freely in the soil. By the mid sixties most European

and some American workers believed that survival of the bacteria in soil was limited and clearly less than the time lapse (c. 3-5 yrs) from one potato crop to another in the average crop rotation system. The concept that the pathogen was transmitted in latently infected progeny tubers from diseased plants led to the development of seed certification schemes, based on roguing out diseased plants and their progeny tubers, to produce cleaner crops, a process which had been used successfully to control many viral diseases. However, these measures have patently failed to have a significant impact on blackleg control. The present lower disease incidence in certified crops than in the past can be explained in terms of improved hygiene on farms and use of more resistant cultivars. It is common knowledge that seed from blackleg-free crops can produce blackleg plants and the converse is also true (Bonde, 1950; Conroy, 1952). Furthermore, no explanation was proposed regarding the source of Ecc which typically does not cause blackleg in temperate regions, although it is frequently present on tubers.

Since the incidence of blackleg in temperate regions is relatively low, the number of tubers with latent infection by Eca was also presumed to be low. However, large scale surveys in Scotland and elsewhere have shown that a high proportion of tubers in commercial stocks regardless of the presence or absence of blackleg in the parent crops were contaminated with both Ecc and Eca (Pérombelon, 1972c; Naumann et al., 1976; Nielsen, 1978). In sound tubers, the bacteria are located either on the surface where they tend to die out in storage under dry conditions or in lenticels where they persist in undiminished numbers for a long time. They are rarely present in the vascular ring except in tubers taken from blackleg affected plants (Pérombelon, 1973). Moreover tuber lenticel contamination level can vary widely from stock to stock.

There is little doubt that in most temperate areas a high proportion of seed tubers at planting could be contamined by both Ecc and Eca. Therefore it was not surprising that seed tubers were shown to be a major source of contamination for the progeny tubers (Pérombelon, 1974). Progeny tuber contamination occurs after rotting of the contaminated mother tubers when the bacteria are liberated in the soil and are transmitted to the tubers mostly by soil water. Erwinias are moved readily in soil water vertically and horizontally over a distance of several metres in the soil profile (Graham & Harper, 1967; Pérombelon, 1976). Breakdown of mother tubers depend on several factors; erwinia contamination level, cultivar tuber resistance to rotting and especially soil water status. In dry soils, fewer mother tubers will rot and

and there is little or no transmission of the bacteria to progeny tubers because of discontinuity of water film around soil particles (Elphinstone & Pérombelon, 1986a). These results could explain the failure of other workers (Harris & Lapwood, 1977; Webb & Wood, 1974) to find a clear association between breakdown of mother tubers and contamination of progeny tubers.

It is notable that the soil and tuber lenticel contamination levels do not gradually increase during the growing season, but instead can fluctuate markedly; they tend to be low during dry spells but tend to increase after heavy rain (Pérombelon, 1976; De Boer et al., 1978). Soft rot erwinias, unlike many soil bacteria, are apparently sited superficially on soil particles (Kikumoto & Sakamoto, 1970) and they can be displaced by soil water subjected to evapotranspiration forces. The condition of the tuber lenticels may also be involved in this variation in bacterial cell numbers (Pérombelon, 1976); in wet soils, tuber water potential is high and lenticels tend to open (proliferate) allowing entry of the bacteria and in dry soils, tuber water potential is low and lenticels tend to close (suberise). The bacteria present are presumably excluded when the complementary cells are sloughed off (Adams, 1975; Bétencourt & Prunier, 1965; Pérombelon & Lowe, 1975).

Although this relationship between rotting mother tuber, soil water movement and progeny tuber lenticel condition and contamination is a simplified view of a more complex situation, the concept that the contaminated mother tuber is an important source of contamination of progeny tubers is now generally accepted. It also explains, at least in part, the different levels of contamination of potato stocks and possible tuber contamination by other erwinias which do not cause blackleg in a given country. This concept of blackleg epidemiology has also been a contributing factor in attempts to produce seed free of seed borne diseases from stem cuttings or micropropagated plants, first in Scotland and subsequently in the USA and Australia. However, surveys carried out on commercial farms in Scotland showed that a high proportion of stem cutting-derived stocks were contaminated after only the first multiplication cycle and most were extensively contaminated after the third multiplication cycle (Pérombelon et al., 1976; 1980). Once contamination has occurred, at however low a level, it can spread throughout stocks by the rotting mother-daughter tuber pathway even in the absence of further contamination.

In addition to mother tubers, several sources of erwinias and methods of dispersal and spread within and between crops have now been identified and some indication of their relative importance has been obtained .

(b) Airborne erwinias

The role of insects as vectors of soft rot erwinias has long been known (Chiu et al., 1958) and has been examined in relation to potatoes more recently in Scotland and the USA (Graham et al., 1976; Harrison et al., 1977; Philips & Kelman, 1978; Molina & Harrison, 1980; Brewer et al., 1980). Contaminated insect species, mostly by Ecc, belonging usually to the genus Diptera were trapped generally after June in Scotland near potato and vegetable refuse dumps. It has been shown that these can transmit marker strains of erwinias from potato dumps to a nearby potato crop. They appear to be particularly attracted by damaged stem tissue which subsequently can develop aerial stem rot.

Aerial transmission by wind-blown rain has long been implicated in the spread of several plant bacterial diseases; but the recognition that long distance dispersal of soft rot erwinias can occur in aerosols is a relatively recent development (Graham et al., 1977, 1979; Pérombelon et al., 1979; Quinn et al., 1980). Aerosols containing erwinias are generated by rain impaction on diseased stems or on any other contaminated substrate such as leaves and surface water and when potato stems are pulverised before harvest as is commonly done in Scotland. Estimates derived from data obtained in a wind tunnel which allowed isokinetic sampling showed that the numbers of bacteria released into the air by rain (assuming a 2% blackleg incidence) or stem pulverisation were similar at c. 10^8 per ha. When air was sampled in Scotland even with no potato crop visible, both Ecc and Eca were readily detected, albeit in low numbers, more often on rainy than dry days and not before August. Although survival of the bacteria captured on microthreads was poor under conditions similar to those prevailing at harvest time in Scotland (September-October), 50% remained viable for 5-10 min, which would be long enough for them to be blown for several hundred metres. Under optimal atmospheric conditions, c. 100 cells per m^2 could be deposited at 100 m down-wind from a ground level point source of 10^8 cells and more probably would be scrubbed down by rain depending on drop size and rain intensity.

It is clear that potato stems and leaves can become contaminated mostly during the second half of the growing season by erwinias, insect borne or in aerosols. Because the density of deposited bacteria is low, the practical significance of these sources will depend on survival and multiplication before they can contaminate the progeny tubers.

(c) Role of potato leaves

Contamination mostly by Ecc of leaves of potato plants and of other crops, weeds and trees in Scotland was detected by an enrichment procedure mostly after August when rain was more frequent and air humidity greater (M.C.M. Pérombelon, unpublished). Field and controlled environment studies indicated that survival and multiplication of Ecc and Eca on potato leaves occurred only as long as the leaf surface remained wet but bacterial numbers were $< 10^2$ cells per cm^2 leaf area. Low erwinia numbers could be attributed to competition whith phyllopla ne microflora and low levels of nutrients on leaf surfaces (Pérombelon, 1979).

When leaves of field-grown plants were inoculated by spraying with bacterial suspensions of marker strains of Ecc and Eca in August on a dry day, bacterial numbers fell rapidly; populations of both erwinias could not be enumerated after 24 h and only Ecc could be detected by an enrichment procedure until the end of the growing season, probably because the bacteria grew intermittently on leaves when they were wetted by rain or dew formation. The bacteria failed to survive on leaves inoculated a month earlier when conditions were dry and evapotranspira tion potential higher than in August. Even when August-inoculated leaves were wet for days in September, erwinias were not present in numbers high enough to be enumerated. Therefore, it is unlikely that leaf-borne erwinias could be transmitted directly by rain from leaf surfaces in numbers high enough to significantly increase progeny tuber contamination. However, bacterial multiplication occurred in the rotting leaf debris on the soil surface to reach between 10^3-10^4 cells/g debris under wet conditions in September, a situation akin to that found when Chinese cabbage leaves rotted when in contact with the soil (Kikumoto & Sakamoto, 1969a). Progeny tuber contamination occurred at the same time. Tubers of micropropagated plants also became contaminated at about the same time by only wild type airborne Ecc after multiplication in leaf debris (Elphinstone & Pérombelon, 1987). Because leaves in non-inoculated plots also became contaminated late in the season by the marker bacteria inoculated on leaves of the treated plots, probably by rain splash or transmitted by insects, it is likely that even if under natural conditions contamination might not be initially widespread, spread of the bacteria within a crop and progeny tuber con tamination could occur as discussed above.

(d) Soil

Since the first studies on soft rot erwinias early this century, longevity of the bacteria in soil has been and still remains a controversial subject. Much of the early studies have to be viewed with caution because of misconceptions about blackleg epidemiology especially the many ways tubers can become contaminated, inability to detect low numbers of the bacteria in non-sterile soil and insufficient attention paid to past cropping history of fields. When a sensitive method to detect $< 10^1$ erwinia cells per g soil was developed, the problem was re-examined by several workers (Meneley & Stanghellini, 1976; Burr & Schroth, 1977; De Boer et al., 1979a; Gudmestad & Secor 1983; Mc Carter Zorner et al., 1985). The frequent detection of the bacteria, Ecc more than Eca, in many fields with different cropping histories led Stanghellini (1982) in his review of the subject to conclude that E. carotovora is a true soil-borne organism surviving in the rhizosphere of suitable weeds and crops and that it is unlikely that nutrients would be a limiting factor in its survival particularly in heavily and continually cropped agricultural areas. However, not enough attention was paid to the effect on survival of the bacteria of such factors as field cropping history (many fields were only one season removed from potato or other host crops), seasonal variation and the use of contaminated irrigation water. On the basis of their studies in the field and in vitro, Pérombelon & Hyman (1987) also concluded that erwinias may have an apparently protracted soil phase but for different reasons.

Survival of Ecc and Eca inoculated in field soil was more affected by temperature than soil moisture content, longevity decreased gradually with increasing temperature but fell sharply at 20°C, Ecc surviving marginally better than Eca. It was difficult to define longevity in absolute terms because it varied with the inoculum level; suffice to say that it could be measured in weeks or even months at 20°C but only in days at higher temperatures up to 35°C.

Since the bacteria can survive for extended periods in sterile soil (Lazar & Bucur, 1964), the limiting factor affecting survival in non sterile soils is not temperature per se. Accumulation of energy rich reserve substances common in many soil bacteria has not been reported in erwinias, therefore survival is likely to be affected mostly by their temperature dependent rate of endogenous metabolism. Erwinias, as many plant pathogenic bacteria adapted to grow in nutrient rich plant tissues, cannot gear down their metabolic rate to a sufficiently low level to persist in the relatively poor soil environment. Addition of nutrients to the soil extended longevity of Ecc and Eca

significantly, even when the numbers of indigenous bacteria also increased which would suggest that antibiosis was not an important factor.

When erwinia survival was examined in rhizosphere soil in situ, survival was only marginally better than in soil. Otherwise, essentially similar results were obtained regarding moisture level and temperature except that longevity of Ecc was significantly greater than that of Eca. Survival was not affected by the different crops including potatoes and weeds tested with the notable exception of brassicas but only with Ecc. In Japan, Ecc survival was also found to be favoured by cruciferous plants (Kikumoto & Sakamoto, 1969b).

Fields in Scotland which have last been under potatoes for different lengths of time on farms where irrigation was not practised were monitored over a 3 year period for contamination by erwinias using an enrichment procedure. As mentioned before, the soil in potato fields at harvest time in September-October was heavily contaminated by both Ecc and Eca. However, contamination level fell sharply by the following spring and was rarely present in summer, regardless of the crop grown, usually cereals or grass, but often increased sharply for a short period sometime during the autumn or early winter before falling to a near nil level. From then on and so long as potatoes or brassicas were not grown, contamination was only rarely detected during most of the year except that the same seasonal and transitory sharp increase was sometimes found. The increase could be explained by (a) the multiplication and spread, which could take place for unknown reasons at that time of the year, of a residual surviving erwinia population too low to be detected at other times. However, it is difficult to see how bacterial numbers could increase or fall so rapidly in the absence of any obvious change in environmental conditions; (b) the bacteria could survive below the top arable 25 cm layer which was sampled, as Gudmestad & Secor (1933) found in North Dakota, USA and de Mendonca & Stanghellini (1979) in Arizona, USA. The presence of large numbers of erwinias ($> 10^3$ cells/l) in underground drain water from fields several years since last under potatoes in Scotland tends to support this view. It is feasible therefore that vertical upward movement of the bacteria could occur but it is not a likely event at a time when evapotranspiration forces are least except perhaps in the rare cases of a sudden transitory rise of the water table; (c) the bacteria could have originated from the air. The irregular ocurrence of erwinia-bearing air masses coupled with a requirement for rain for efficient deposition,

could explain the seasonal, erratic and shortlived widespread soil contamination. It is notable that rain water puddles in the fields were almost invariably contaminated at that time of the year. This explanation is also not at odds with the apparent inability of the bacteria to survive in soil for any extended period when determined in vitro.

The question of whether erwinias have a permanent or a more or less protracted soil phase has not been and probably is not likely to be resolved readily and could well be irrelevant. Regardless of whether they are endemic in soil or not, erwinias are constantly introduced from a wide range of sources. Their longevity may be prolonged when protected in crop debris after harvest, such as groundkeepers (an estimated 100,000 tubers per ha may be left in the field after harvest (Pérombelon, 1975) and infected stem debris, for as long as the plant material is not completely decomposed (Logan, 1968). Alternatively, populations may be maintained by continuous cropping in the same fields with a susceptible host crop as is often the case with Chinese cabbage in the Far East (Togashi, 1972; Mew et al., 1976) or perhaps the rapid infestation of fields after harvest with solanaceous alternative host weed species as can happen in some subtropical areas.

(e) Water

Less than a decade ago little was known of the presence let alone the ecology of soft rot erwinias in natural water sources although several reports suggested that they probably had been repeatedly isolated but rarely fully characterised. Therefore, it was not unexpected to detect erwinias in water when they were specifically looked for, but what was unexpected was their widespread distribution in surface water. A high proportion of random water samples from lochs, streams and rivers in Scotland were found to be contaminated by erwinias, mostly Ecc (Pérombelon & Lowe, 1981) and similar results were obtained by Mc Carter-Zorner et al., (1874) in Scotland and Colorado, USA and by Gudmestad and Secor (1983) in North Dakota, USA. The bacteria were also commonly found in river and irrigation canal water in The Netherlands, France, Spain, Israel and tropical Peru (Lumb & Pérombelon, 1985; J.G. Elphinstone, personal communication). The numbers varied considerably from one country or even from one area within a country to another probably a reflection of the degree of pollution present. They can range from 0 to 10^6 cells/1. Water from deep wells was usually erwinia free.

Pérombelon & Hyman (1987a) on the basis of their studies on the ecology of erwinias in river water in eastern Scotland suggested that

the erwinias were probably of allochthonous origin in agreement with the generally held view that most bacteria in rivers are washed in from surrounding land. Rivers flowing through uninhabited moorland were found not to be contaminated at any time during the year but they became increasingly so when they flowed through cultivated, inhabited land, with greater erwinia numbers in late summer and early autumn than at other times.

The bacteria failed to persist when introduced into the previously uncontaminated section of a river by dumping infected potatoes, hence it is unlikely that the mud at the bottom of rivers is an important source as suggested by Mc Carter-Zorner et al. (1984) It is more likely that they originate from field drainage water which showed a temporal contamination pattern and contamination levels similar to those of river water. Drainage water from fields presently and those which had been in the recent past under potatoes but not those which had not been planted with potatoes for a long time or else permanent pastures were contaminated. Ecc was 4-5 times more common in both river and drainage water. Further potential sources of erwinias are potato discards and vegetable refuse, often dumped in the proximity of rivers, in which the bacteria multiply when they rot, and usually contaminated sewage effluent discharging in rivers.

Although a greater number of strains of both Ecc and Eca survived better in dilute phosphate buffer at pH 5.7 than 7.7 and at 15°C than at 5°C, survival was equally good under all conditions in filter sterilized river water. Whereas the number of viable cells of both Ecc and Eca in sterile distilled water fell sharply within 24 h, the bacteria multiplied, Ecc more than Eca, in both sterile and non-sterile river water. Apparently nutrients and other suspended matter in adequately oxygen rich river water enable the bacteria to survive long enough and they may even multiply to some extent depending on the temperature while they are transported over long distances. However, in fast moving rivers, contamination is more likely to result from a continous influx of the bacteria from different sources along the river length.

(f) Mechanical handling equipment

Mechanical grading has long been thought to cause spread of erwinias because there was more soft rot (Scholey et al., 1968; Naumann et al., 1978) and blackleg (Boyd & Penna, 1967; Graham & Hardie, 1971; Henriksen, 1976) in crops grown from graded than in those from ungraded tubers. The higher disease levels were attributed to movement of

the pathogens from soft rotting to healthy tubers, either by direct contact or via bacterial slime left on the grader. Using placement rotting tubers infected with a marker strain of Ecc, Elphinstone & Pérombelon (1986a) have tried to evaluate the importance of this source of contamination. Bacteria from one rotting tuber can spread to c. 17% of a total of 600 kg tubers during grading, although only the first 50 kg of tubers were heavily infected usually by $> 10^4$ cells per tuber. Therefore, if a stock contained on average one or more rotting tubers per 50 kg and surveys have shown that this level is common in UK, then contamination following grading would be extensive. It follows that whenever tubers are mechanicallly handled at planting time, during harvest and store filling, contamination is also likely to occur if rotting tubers are present. Most soft rotting tubers in stored potato stocks contain large numbers of erwinias, more often Eca than Ecc because of the low prevailing temperatures favour the former organism, even if the initial cause of rotting is of fungal origin such as late blight.

More importantly was the finding that survival of the bacteria after grading was related to the extent and nature of external damage incurred. Tuber wounding is unavoidable and the incidence and type of wounds depends on the design of the machinery and the operating speed and on cultivar susceptibility to wounding. The bacteria tended to die out within $<$ 1 month in cold storage in shallow ($<$ 1 mm) wounds but they can survive in large numbers for $>$ 2 months in deeper wounds where they would be better protected from desiccation especially after wound healing.

Relative importance of contamination sources

Of the different factors which can affect disease development, tuber contamination level can be manipulated more readily to achieve better disease control. Contamination can occur from different sources and through different pathways. An assessment of their relative importance would be essential in the development of an integrated approach to disease control.

The relative importance of different sources of erwinias in relation to progeny tuber contamination level, hence to blackleg in the following generation crop under Scottish conditions is given in Table 2. Crops harvested early, before mid August, are less likely to be contamined with air, insect and leafborne contamination than late harvested crops because widespread leaf contamination and conditions (wet

Table 2. Relative importance of erwinia sources in relation to tuber contamination and blackleg

Source	Source contamination*	Dominant erwinia	Importance: tuber cont.	Importance: blackleg	Importance: aerial stem rot
Air	+	Ecc	+	+	+
Insects	+	Ecc	+	-	++
Water	++	Ecc	+	-	+++
Leaves	+	Ecc	+	-	+
Leaf debris	+++	Ecc	+++	-	-
Mother tubers	+++	Ecc/Eca	+++	+++	-
Harvesters/graders	+++	Ecc/Eca	+++	+++	-

* Relative number of erwinias.

weather) favouring rotting of fallen leaves tend to occur late. Moreover, contamination is mostly by Ecc which is not the causal agent of blackleg in temperate areas. In contrast, erwinias from mother tubers can contaminate progeny tubers at an earlier time in the growing season, as soon as conditions favour rotting of the mother tubers and spread of the bacteria in soil. Mother tubers tend to rot at any time between July and August depending on tuber contamination level but especially on soil water status. The fact that the mother tuber can be a source of both Ecc and Eca further increases its importance relative to leaves in the contamination of tubers.

Although water favours rotting and erwinia multiplication in leaf debris and mother tubers, hence the relative contribution of these sources at different times during the season, and can promote both blackleg and aerial stem rot, it is not apparently a major source of tuber contamination per se. Results obtained in Israel and Spain where irrigation water is often heavily contaminated, mostly by Ecc and frequently with $> 10^6$ cells/l (Lumb & Pérombelon, 1986; M.C.M. Pérombelon, unpublished data) suggested that the risk of increased blackleg level was low and that progeny tuber contamination was not very great. But water containing even low numbers of Eca could have serious consequences when used to irrigate high grade erwinia-free seed crop because once introduced, the bacteria would spread during stock multiplication.

Only contamination brought about during mechanical handling of tubers could possibly explain the spread of Eca in stocks derived from stem cuttings especially after the third multiplication cycle when, because of the bulk, more mechanical handling is involved. As discussed before, Eca is rarely found in the environment, air, water and soil and on potato leaves and should be also rare on progeny tubers in crops derived from stem cuttings. Buth when the few Eca infected tubers rot in storage, the organism tends to predominate in the rot because it is more pathogenic than Ecc at low storage temperatures and it is therefore more likely than Ecc to spread within and between stocks during grading to high contamination levels.

Disease control

The rationale for studies on the ecology and pathogenicity of soft rot erwinias and on the diseases they cause is to enable the development of disease control strategies. It is clear that an integrated approach to disease control is needed because of the complexity

of the contamination process. A range of measures can be advocated, some more appropriate to the seed producer or the table potato producer than others. But the main emphasis is on the production of 'clean' seed. It is however impossible to quantify the economic impact of individual measures; their commercial integration and evaluation will take years and will be subjected to market forces.

In the field contamination can be reduced by early harvesting, before extensive decay of the contaminated mother tuber, but it incurs the penalty of serious reductions in yield. In countries where early harvesting is practised as a means of avoiding aphid disseminated virus infection, progeny tuber contamination tends to be low. Measures which favour a delay in mother tuber rotting should be fostered, such as use of seed with low contamination level, land preparation for good drainage, avoiding over irrigation etc.

Haulm pulverisation prior to harvest is hazardous and should be discouraged. Apart from generating large numbers of aerosols containing erwinias which may subsequently contaminate crops downwind from the source, bacteria may multiply in wet weather in the debris spread over the pulverised field to contaminate progeny tubers. Prophylactic application of long lasting bactericides, such as copper oxychloride, could help in reducing the importance of leaf debris as a source of the bacteria (Elphinstone & Pérombelon, 1987). This treatment is more appropriate on high grade seed crops grown from micropropagated plants than on low grade seed crops which are exposed to far more contamination from other sources.

Dry storage conditions are paramount to reduce tuber conditions even if some loss in weight through drying occurs. Under moist conditions the bacteria in lenticels may not only survive but may also multiply (Pérombelon, 1973).

Immediately after grading, the contaminating bacteria are on the tuber surface or in open wounds and are therefore exposed to liquid disinfectants. Their numbers can be drastically reduced by chemical sprays but only if total tuber surface coverage can be achieved. Although the latent bacteria well protected as they are in lenticels and in old healed wounds are not affected directly by the chemical, their multiplication is triggered by anaerobiosis induced by the water film on the tuber's surface and will cease only when the tuber surface dries. It is therefore essential to ensure rapid drying of sprayed

tubers for success. More promising is thermotherapy of tubers which is based on the findings of Mac Kay & Shipton (1983) that almost total control of tuber contamination can be achieved by a hot water treatment. The latest development is for a continuous treatment of prewashed tubers in water at 50-55°C for 3-5 min depending on the amount of tuber damage followed by thorough drying of the tubers (M.C.M. Pérombelon, unpublished).

Seed certification has long been the traditional approach to control blackleg. It is however clear now that tuber contamination by the different erwinias and still less the number of bacteria present are not related to the presence of blackleg in the parent crop. It follows therefore that seed certification schemes which rely on field inspections for blackleg caused in many seed producing countries by only Eca cannot provide an indication of seed health status. The apparent failure of such certification schemes to control blacleg can be attributed to the fact that they are based on an erroneous concept of blackleg epidemiology. Production of seed stocks derived from stem cuttings or micropropagated plants is helpful but largely because by restricting the life of high seed grades it tends to reduce the risks of contamination build-up in these stocks. An assessment of seed health based on tuber contamination level by the different erwinias would be more accurate and recent development in serology and molecular biology should allow the development of a commercially viable method for that purpose.

A wide range of resistance appears to exist even in the relatively narrow genetic base of modern cultivars. There is some indication that resistance to blackleg does not match necessarily resistance to tuber soft rot. Two distinct resistance/susceptibility mechanisms are apparently involved with blackleg, one concerned the mother tuber and the other the stem. It is notable that the ranking orders of cultivars resistant to tuber soft rot and to blackleg are different (M.C.M. Pérombelon, unpublished). In the case of tubers, too often an attempt is made to distinguish between tuber tissue resistance and what can be called field resistance which encompasses such factors as susceptibility to wounding and lenticel receptivity to infection. Recent studies (Bain & Pérombelon, 1987) have shown that the ranking order of cultivar resistance to tuber soft rot by Eca varies with inoculation method used and the incubation conditions. Until a meaningful screen test is developed, breeding for resistance is not advisable.

Conclusions and future outlook

Advances made in the past two decades in our understanding of the ecology of the soft rot erwinias have led to the development of new concepts as to the etiology and epidemiology of the diseases caused by bacteria on potatoes. Is is now clear why seed certification schemes have failed to control blackleg, and why attempts to produce 'clean' seed from stem cuttings or microporpagated plants as initial vegetative reproduction material have not been successful. The identification of several hitherto little known or unsuspected contamination sources has not only opened up new vistas on certain aspects of the ecology of the bacteria but has also led indirectly to establish the relationship between tuber contamination level and disease incidence. Presence of the bacteria is not enough, numbers greater than a given threshold are necessary for pathogenesis. Moreover, all three erwinias can be pathogenic and even the symptoms they cause can be similar under conditions optimal for expression of the pathogenicity of the different bacteria. As a result, disease control is now a distinct possibility and an integrated approach is being developed which is based on this new knowledge. Control of seed contamination could be achieved by adopting both preventive and curative measures.

Most of this research has been done in temperate regions where potatoes until now have been grown traditionally. Because of the wide differences in climate and of the environment taken in its broadest sense, the results cannot be directly applied to tropical or sub-tropical regions. For one, the priorities are different; emphasis in the tropics is likely to be placed on disease prevention rather than on the production of 'clean' seed tubers as has been the case in temperate countries. It is likely that seed material in the tropics will continue to be imported either in the form of seed tubers or botanical seed in the foreseeable future.

In addition to further research in non-temperate countries on the ecology of soft rot erwinias for practical local reasons and to obtain a better knowledge of the subject on a global level, several fields of study which have hardly been touched so far need to be explored. It is becoming clear that erwinia-caused diseases, as well as no doubt other diseases, rarely develop on their own. The weakening of the host plant by one pathogen may encourage the development of another or even of opportunistic pathogens which in turn may have a synergistic effect on the primary causal agent especially if the infective process is si-

milar. Blackleg development has long been associated with other diseases such as Verticillium or Fusarium wilt or Rhizoctonia infection. The resistance or the tolerance of a cultivar to one disease may break down in the field if it is susceptible to another pathogen when dual infection occurs. For example, any synergism between erwinias and other important pathogens such as Pseudomonas solanacearum, the causal agent of bacterial wilt, would affect strategies for breeding for disease resistance.

There has been an increased interest recently in biological control of diseases partly for environmental reasons and partly because of the improved understanding of microbial ecology. This approach is more likely to succeed when the infection court normally contains an important microflora in addition to the pathogen and the inoculum level (latent infection) is an important factor in pathogenesis as is the case with erwinia infection of potato lenticels and wounds. Erwinia populations on tubers could be controlled by the addition of selected naturally occurring or genetically engineered antagonists well adapted to survive in that special ecological niche.

Although the interaction between erwinias and their hosts has been studied for a long time, most studies have centered mainly on the resistance mechanism of the host. It is clear now that host resistance is multifactorial, involving both performed and induced components which moreover could be either oxygen dependent or independent. However, little is known of pathogenicity determinants of the erwinias and the same could be said for most other bacterial pathogens with the notable exception of Agrobacterium tumefaciens, the causal agent of Crown Gall. But recently, with the development of powerful molecular genetic tools, such as transposon mutagenesis, gene cloning and sequencing,etc,it is now possible to study the production, regulation and function of pathogenicity determinants in erwinias which are switched on or off by the host and environmental conditions. Such studies should complement those on host resistance and the combined results would be of value in resistance breeding.

Soft rot erwinias could be viewed more as opportunistic than as specialised true plant pathogens (Pérombelon & Kelman, 1980). Although they apparently can behave as vascular pathogens able to cause systemic infections, they tend to behave differently when they rot parenchymatous tissue especially that of storage organs. Rotting occurs when host resistance is reduced to such a low level that single cells

of most pectolytic bacteria can initiate infections. In the case of potatoes for example, association of erwinias with tuber rots could be attributed to ecological factors which in the first place enable the bacteria to survive in the environment and infect tubers and also favour their growth and the expression of their pathogenicity, notably the rapid production of large quantities of cell wall dissolving enzymes. By the same token, in the case of blackleg, the large numbers of the bacteria in the rotting mother tuber could be viewed simply as an inoculum equivalent to that used when stems are inoculated by wounding. The infection of the vascular system of the stem at the point of attachment to the mother tuber is unavoidable and also facilities rapid invasion and spread of the pathogen in the stem. Other plant species without the equivalent of a mother tuber acting as a source of the bacteria can only become infected when the stems are wounded. Disease development is usually not common because the inoculum level is generally low and favourable environmental conditions for multiplication of the bacteria are also usually not common.

The host specificity which can be found notable with Eca and Echr may be more apparent than real. Once again, ecological factors could be invoked to explain the apparent host specificity. It could be argued that Eca for example is restricted to potatoes in temperate regions because it survives poorly in the environment and only potatoes, because of its vegetative method of reproduction, provides a favourable niche. Given the opportunity, it can infect other crops such as turnips, broccoli in the field and tomato plants in the greenhouse (M.C. M. Pérombelon, unpublished). Similarly, Echr strains from different hosts and believed to exhibit some host specificity, can rot tubers and cause blackleg under field conditions (V.M. Lumb & M.C.M. Pérombelon, unpublished). However, there are some known instances of true host specificity, for example, only Echr from corn can infect that crop and a variant of Ecc of uncertain identity which is specific to the giant cactus (Carnegiea gigantea) in Arizona, US.A. Moreover, a not fully described soft rot erwinia, E. betavasculorum, which has a very restricted geographical distribution in south west USA, apparently infects only sugar beet. It is not clear to what extent these host specificities are real or reflect a lack of other susceptible hosts in those environments and methods of transmission. Therefore, if it exists, host specificity in the common soft rot erwinias is at best poorly developed, and by inference, so probably would be host recognition systems, a situation which contrasts sharply with that found in many specialised bacterial plant pathogens such as Pseudomonas spp., Xanthomonas spp., and Corynebacterium spp.

References

Adams, M.J. 1975. Potato tuber lenticels: susceptibility to infection by Erwinia carotovora var. atroseptica and Phytophthora infestans. Ann. Appl. Biol. 79: 275-82.

Aleck, J.R., Harrison, M.D. 1978. The influence of inoculum density and environment on the development of potato blackleg. Amer. Potato J. 55: 479-94.

Bain, R.A. & Pérombelon, M.C.M. 1987. The influence of method of inoculation on the tuber resistance of potato cultivars to soft rot caused by Erwinia carotovora sub sp. atroseptica (in preparation).

Betencourt, A. & Prunier, J.P. 1965. A propos de la pourriture seche lenticellaire des tubercules de pommes de terre provoquee par Erwinia carotovora (Jones) Holland. Eur. Potato J. 8: 230-42.

Bonde, R. 1950. Factors affecting potato blackleg and seed-piece decay. Maine Agric. Exp. Sta. Bull. 482, 31 pp.

Boyd, A.E.W. & Penna, R.J. 1967. Seed tuber disinfection. Proceedings of the Fourth British Insecticide and Fungicide Conference pp. 294-302.

Brewer, J.W., Harrison, M.D. & Winston, J.A. 1980. Differential attraction of Drosophila melanogaster Meig. to potato tissue infected with two varieties of Erwinia carotovora. Am. Pot. J. 57:

Burr, T.J. & Schroth, M.N. 1977. Occurrence of soft rot Erwinia spp. in soil and plant material. Phytopathology 67: 1382-87.

Burton, W.G. & Wigginton, M.J. 1970. The effect of a film of water upon the oxygen status of a potato tuber. Potato Res. 13: 150-86.

Chiu, W.F., Yuen, C.S. & Wu, C.A. 1958. On the overwintering and dissemination of soft rot organisms, Erwinia aroideae (Towns) Holland. Acta Phytopath. Sin. 4: 8-15 (in Chinese).

Cuppels, D. & Kelman, A. 1974. Evaluation of selective media for isolation of soft rot bacteria from soil and plant tissue. Phytopathology 64: 468-75.

Conroy, R.J. 1952. Blackleg of potatoes in New South Wales. The question of seed transmission. Agric. Gaz. N.S.W. 63: 534-536.

De Boer, S.H. & Kelman, A. 1975. Evaluation of procedures for detection of pectolytic Erwinia spp. on potato tubers. Am. Potato J. 52: 117-23.

De Boer, S.H. & Kelman, A. 1978. Influence of oxygen concentration and storage factors on susceptibility of potato tubers to bacterial soft rot (Erwinia carotovora). Potato Res. 21: 65-80.

De Boer, S.H., Allan, E. & Kelman, A. 1979a. Survival of Erwinia carotovora in Wisconsin soils. Amer. Potato J. 56: 243-52.

De Boer, S.H., Copeman, R.J. & Vruggink, H. 1979b. Serogroups of Erwinia carotovora potato strains determined with diffusible somatic antigens. Phytopathology 69: 316-19.

Elphinstone, J.G. & Pérombelon, M.C.M. 1986a. Contamination of potatoes by Erwinia carotovora during grading. Plant Pathology 35: 25-33.

Elphinstone, J.G. & Pérombelon, M.C.M. 1986b. Contamination of progeny tubers of potato plants by seed- and leaf -borne Erwinia carotovora. Potato Res. 29: 77-93.

Elphinstone, J.G. & Pérombelon, M.C.M. 1987. Control of contamination of potatoes with air-borne Erwinia carotovora by foliar application of copper oxychloride. Ann. Appl. Biol. (in press).

Gudmestad, N.C. & Secor, G.D. 1983. The bionomics of Erwinia carotovora in North Dakota. Am. Potato J. 60: 759-771.

Graham, D.C. 1964. Taxonomy of the soft rot coliform bacteria. Ann. Rev. Phytopathol. 2: 13-42.

Graham, D.C. 1972. Identification of soft rot coliform bacteria. 273-79. In Proc. Int. Conf. Pl. Path. Bact. 3rd, Wageningen 1971. ed. H.P. Maas Geesteranus, Centre Agric. Publ. Docum. PUDOC. Wageningen, The Netherlands. 365pp.

Graham, D.C., & Hardie, J.L. 1971. Prospects for control of potato blackleg disease by the use of stem cuttings. Proc. Br. Insectic. Fungic. Conf., 6th 1: 219-24.

Graham, D.C. & Harper, P.C. 1967. Potato blackleg and tuber soft rot. Scott. Agric. 46: 68-74.

Graham, D.C., Quinn, C.E. & Bradley, L.F. 1977. Quantitative studies on the generation of aerosols of Erwinia carotovora var. atroseptica by simulated raindrop impaction on blackleg-infected potato strains. J. Appl. Bacteriol. 43: 413-24.

Graham, D.C., Quinn, C.E. & Harrison, M.D. 1976. Recurrence of soft rot coliform bacterial infections in potato stem cuttings: an epidemiological study on the central nuclear stock production farm in Scotland 1967-74. Potato Res. 19: 3-20.

Graham, D.C., Quinn, C.E., Sells, I.A. & Harrison, M.D. 1979. Survival of strains of soft rot coliform bacteria on microthreads exposed in the laboratory and in the open air. J. Appl. Bacteriol. 46: 367-76.

Harris, R.I. & Lapwood, D.H. 1977. The spread of Erwinia carotovora var. atroseptica (blackleg) from degenerating seed to progeny tubers in soil. Pot. Res. 20: 285-94.

Harrison, M.D., Quinn, C., E., Sells, A. & Graham, D.C. 1977. Waste potato dumps as sources of insects contaminated with soft rot coliform bacteria in relation to recontamination of pathogen-free potato stocks. Pot. Res. 20: 37-52.

Hellmers, E. & Dowson, W.J. 1953. Further investigations of potato blackleg. Acta Agric. Scand. 111: 103-12.

Henrikson, J.R. 1976. Influence of the condition under which seed potato tubers are handled on blackleg infection. EPPO Bul. 6: 309-313.

Kikumoto, T. & Sakamoto, M. 1969a. Ecological studies in the soft rot bacteria of vegetables. VI. Influence of the development of various plants on the survival of Erwinia aroideae added to soil. Ann. phytopath. Soc. Japan 35: 29-35 (in Japanese).

Kikumoto, T. & Sakamoto, M. 19[illegible]. Ecological studies on the soft rot bacteria of vegetables. VII. The preferential stimulation of the soft rot bacteria in the rhizosphere of crop plants and weeds. Ann. Phytopath. Soc. Japan 35 : 36-40 (in Japanese).

Kikumoto, T. & Sakamoto, M. 1970. Ecological studies on the soft rot bacteria of vegetables. X. The distribution of soft rot bacteria within the soil aggregates. Ann. Phytopath. Soc. Japan 36 : 207-13 (in Japanese).

Lazar, I. & Bucur, E.L. 1964. Recent research in Roumania on blackleg and bacterial soft rot of potato. Europ. Potato J. 7: 102-11.

Lelliot, R.A. & Dickey, R.S. 1984. Genus VII. Erwinia in Berge's Manual of Systemic Bacteriology Vol 1, pp. 469-476; eds. N.R. Kreigh & J.G. Holt. Williams and Wilkins, Baltimore, U.S.A.

Logan, C. 1968. The survival of the potato blackleg pathogen over winter. Rec. Agric. Res. Minist. Agric. Nth. Ir. 17: 115-21.

Lumb, V.M., Pérombelon, M.C.M. & Zutra, D. 1986. Studies of a wilt disease of the potato plant in Israel caused by Erwinia chrysanthemi. Plant Path. 35: 196-202.

Meneley, J.C. & Stanghellini, M.E. 1976. Isolation of soft rot Erwinia spp. from agricultural soils using an enrichment technique. Phytopathology 66: 367-70.

Mew, T.W., Ho, W.C. & Chu, L. 1976. Infectivity and survival of soft rot bacteria in Chinese cabbage. Phytopathology 66: 1325-27.

Molina, J.M. & Harrison, M.D. 1980. The role of Erwinia carotovora in the epidemiology of potato blackleg. II. The effect of soil temperature on pathogen activity. Amer. Pot. J. 57:

de Mendonca, M. & Stanghellini, M.E. 1979. Endemic and soil-borne nature of Erwinia carotovora var. atroseptica, a pathogen of mature sugar beets. Phytopathology 69: 1096-99.

Mc Carter-Zorner, N.J., Franc G.D., Harrison, M.D., Michand, J.E., Quinn, C.E., Sells, I.A. & Graham D.C. 1984. Soft rot Erwinia bacteria in surface and underground water in Southern Scotland and in Colorado, United States. J. Appl. Bact. 57: 95-105.

Mc Carter-Zorner, N.J., Harrison, M.D., Franc, G.D., Quinn, C.E., Sells, I.A. & Graham, D.C. 1985. Soft rot Erwinia bacteria in the rhizosphere of weeds and crop plants in Colorado, United States and Scotland. J. Appl. Bacteriol. 59: 357-368.

Mackay, J.M. & Shipton, P.J. 1983. Heat treatment of seed tubers for control of potato blackleg (Erwinia carotovora sub sp. atroseptica) and other diseases. Plant Path. 32: 385-393.

Naumann, K., Zielke, R., Pett, B., Stachewicz, H. & Janke, C. 1976. Umfang und Bedeutung des latenten Befalls von Kartoffelknollen mit Pectobacterium carotovorum var. atrosepcticum. Tag-Ber., Akad. Landwirtsch. -Wiss. DDR. Berlin. 140: 231-41.

Naumann, K., Zielke, R. & Peter, K. 1978. Die Belastung der Kartoffelknollen mit dem Erriger der Schwarzbeinigkeit und Knollenassfaule, Erwinia carotovora var. atrosepctica (Van Hall) Dye, und anderen Nassfaule verursachenden Bakterien unter industriemassign Produktions bedingungen. Archio fur Phytopathologie und Pflanzenschutz 14: 151-161.

Nielsen, L.W. 1978. Erwinia species in the lenticels of certified seed potatoes. Amer. Potato J. 55: 671-76.

Pérombelon, M.C.M. 1972a. A quantitative method for assessing virulence of Erwinia carotovora var. carotovora and E. carotovora var. atroseptica and susceptibility to rotting of potato tuber tissue. In Proc. Int. Conf. Pl. Path. Bact. Wageningen, 3rd, 1971 pp. 299-303. ed. H.P. Maas Geesteranus, Wageningen, The Netherlands. Centre Agric. Publ. Docum. PUDOC.

Pérombelon, M.C.M. 1972b. A reliable and rapid method for detecting contamination of potato tubers by Erwinia carotovora. Pl. Dis. Rep. 56: 552-54.

Pérombelon, M.C.M. 1972c. The extent and survival of contamination of potato stocks in Scotland by Erwinia carotovora var. carotovora and E. carotovora var. atroseptica. Ann. Appl. Biol. 71: 111-117.

Pérombelon, M.C.M. 1973. Sites of contamination and numbers of Erwinia carotovora present in stored seed potato stocks in Scotland. Ann. Appl. Biol. 74: 59-65.

Pérombelon, M.C.M. 1974. The role of the seed tuber in the contamination by Erwinia carotovora of potato crops in Scotland. Potato Res. 17: 187-99.

Pérombelon, M.C.M. 1975. Observations of the survival of potato ground keepers in Scotland. Potato Res. 18: 205-15.

Pérombelon, M.C.M. 1976. Effects of environmental factors during the growing season on the level of potato tuber contamination by Erwinia carotovora. Phytopath Z. 85: 97-116.

Pérombelon, M.C.M. 1979a. Factors affecting the accuracy of the tuber incubation test for the detection of contamination of potato stocks by Erwinia carotovora Potato Res. 22: 63-8.

Pérombelon, M.C.M. 1979b. Contamination of potato crops by airborne Erwinia carotovora. Proc. Int. Conf. Plant Path. Bact. 4th, Angers 1978. pp. 63-65. ed. Stat. Path. Veg. Phytobacteriol. Angers, France INRA 978 pp.

Pérombelon, M.C.M. 1979c. Ecology of soft rot erwinias in relation to potatoes. In Development in Control of Potato Bacterial Diseases. Rep. Planning Conference, Lima, June 1979.

Pérombelon, M.C.M. 1981. The ecology of erwinias on aerials plant surfaces. In Microbial ecology of the phylloplane pp. 44-431. Academic Press, London.

Pérombelon, M.C.M. 1982. The impaired host and soft rot bacteria. In Phytopathogenic Prokaryotes Vol 2, pp. 55-69. Eds M.S. Mount & G.M. Lacy, Academic Press, New York.

Pérombelon, M.C.M., Fox, R.A. & Lowe, R. 1979a. Dispersion of Erwinia carotovora in aerosols produced by the pulverization of potato haulm prior to harvest. Phytopathol Z. 94: 249-60.

Pérombelon, M.C.M., Gullings-Handley, J. & Kelman, A. 1979b. Population dynamics of Erwinia carotovora and pectolytic clostridia in relation to decay of potatoes. Phytopathology 69: 167-73.

Pérombelon, M.C.M. & Hyman, L.J. 1986. Blackleg etiology and epidemiology. Scot. Crop Res. Inst. Ann. Rep. 1984, p. 97.

Pérombelon, M.C.M. & Hyman, L.J. 1986. A rapid method for identifying and quantifying soft rot erwinias directly from plant material based on their temperature tolerances and sensitivity to erythromycin. J. Appl. Bacteriol. 60: 61-66.

Pérombelon, M.C.M. & Hyman, L.J. 1987a. A study of the frequency of Erwinia carotovora in rivers in eastern Scotland and of the sources of bacteria (in preparation).

Pérombelon, M.C.M. & Hyman, L.J. 1987b. Survival of soft rot erwinias in soil (in preparation).

Pérombelon, M.C.M. & Kelman, A. 1980. Ecology of the soft rot erwinias. Ann. Rev. Phytopathol. 18: 361-387.

Pérombelon, M.C.M. & Kelman, A. 1987. Blackleg and other potato diseases caused by soft rot erwinias: a proposal for a revision of terminology. Plant Diseases (in press).

Pérombelon, M.C.M. & Lowe, R. 1975. Studies on the initiation of bacterial soft rot in potato tubers. Potato Res. 18: 64-82.

Pérombelon, M.C.M. & Lowe, R. 1981. Survival of E. carotovora on leaves, in soil and in water. Scot. Hort. Res. Inst. Ann. Rep. 1980 pp. 28-29.

Pérombelon, M.C.M. & Lowe, R. 1984. Blackleg etiology: field studies. Scot. Crop Res. Inst. Ann. Rep. 1983 pp. 99-100.

Pérombelon, M.C.M., Lowe, R. & Ballantine, E.M. 1976. Contamination by Erwinia carotovora of seed potato stocks of stem cutting origin in the process of multiplication. Potato Res. 19: 335-347.

Pérombelon, M.C.M., Lowe, R., Quinn, C.E. & Sells, A. 1980. Contamination of pathogen-free seed potato stocks by Erwinia carotovora during multiplication. Results of a six-year monitoring study. Potato Res. 23: 413-425.

Pérombelon, M.C.M. & Lowe, R. 1978. Gangrene, blackleg and soft rot and contamination. IUTSC seed potato stocks. Scotl. Hort. Res. Inst. Ann. Rep. 1977. pp. 84-85.

Pérombelon, M.C.M. Lumb, V.M. & Zutra, D. 1987a. Pathogenicity of soft rot erwinias to potato plants in Scotland and Israel. J. Appl. Bacteriol. (in press).

Pérombelon, M.C.M., Lumb, V.M. & Hyman, L.J. 1987b. A rapid method to identify and quantify soft rot erwinias on potato seed tubers. EPPO Bul. 17 (in press).

Phillips, J.A. & Kelman, A. 1978. Tracing the dissemination of Erwinia carotovora from an inoculum source with a direct fluorescent antibody technique. Amer. Potato J. 55: 389 (Abstr.).

Quinn, C.E., Sells, I.A. & Graham, D.C. 1980. Soft rot Erwinia bacteria in the atmospheric bacterial aerosol. J. Appl. Bacteriol. 49 : 175-181.

Schaad, N.W. 1979. Serological identification of plant pathogenic bacteria. Ann. Rev. Phytopathol. 17: 123-47.

Scholey, J., Marshall, C. & Whitbread, R. 1968. A pathological problem associated with pre-packaging of potato tubers. Pl. Path. 17: 135-39.

Shekawat, G.S. & Pérombelon, M.C.M. 1986. Pathogenicity of soft rot bacteria. Scot. Crop. Res. Inst. 1985 p. 99-100.

Stanghellini, M.E. 1982. Soft rotting bacteria in the rhizosphere. In Phytopathogenic Prokaryotes Vol 1, 249-261. Academic Press, New York.

Starr, M.P. & Chatterjee, A.K. 1972. The genus Erwinia: enterobacteria pathogenic to plants and animals. Ann. Rev. Microbiol. 26: 389-426.

Togashi, J. 1972. Studies on the outbreak of the soft-rot disease of Chinese cabbage by Erwinia aroideae (Towns.) Holland. Rep. Inst. Agric. Res. Tohoku Univ. Japan 23: 17-52.

Van Vuurde, J. 1987. New approach in detecting phytopathogenic bacteria by combined immunoisolation and immunoidentification assays. EPPO Bul. 17 (in press).

Webb, L.E. & Wood, R.K.S. 1974. Infection of potato tubers with soft rot bacteria. Ann. Appl. Biol. 76: 91-98.

SOFT ROT AND BLACKLEG (ERWINIA SPP.) WARM CLIMATES

O. Hidalgo

INTRODUCTION

In the warm tropics, very little attention has been given to Erwinia diseases, probably because bacterial wilt (R. solanacearum) has traditionally attracted and received more attention. Importance of bacterial wilt is not questioned, since it is still the most important potato disease in warm areas, but its unchallenged position is due to the lack of emphasis that National Programs and CIP have given to Erwinia diseases in the past. It is a fact, but unfortunately undocumented, that these diseases have slowly increased through the years to levels that could be considered serious and dangerous, especially in the warm areas of those countries that grow potatoes. In some countries Erwinia diseases are now as important as, and sometimes more important than, bacterial wilt, because of the heavy losses in the field and in storage due to high levels of contamination in the seed stocks and in the subsequent ware crops.

In this paper the etiology and epidemiology of these diseases in the warm climates are described, and the role of seed certification in Latin America discussed. Some conclusions and perspectives are given in view of the actions that would be needed from the management and technical aspects to reduce the disease incidence.

THE ORGANISMS AND THE DISEASES IN WARM CLIMATES

In warm climates, soft rot and blackleg bacterial diseases are induced by the same organisms as in temperate zones: Erwinia carotovora subsp. carotovora (Ecc) and E. carotovora subsp. atroseptica (Eca); in addition, E. chrysanthemi (Ech) is also frequently involved. In warm climates, Ecc and Ech are prevalent (Robbs, 1981; Robbs et al, 1981; Takatsu, 1983), Ecc being the more important; in temperate zones (Perombelon, 1979) Eca is more prevalent than Ecc. It appears that Eca is normally introduced in imported seed tubers from temperate zones and it is believed that the organism is "detected or causes damage only during the initial years (2 or 3) of multiplication of the imported seed" (Robbs, 1981; Takatsu, 1983), but in addition it has been suggested (Robbs, 1981) that "soil survival of the organism (Eca) under Brazilian conditions seems improbable." If this is the case, then other survival mechanisms, especially through the seed tuber, need to

be operating since Eca has been found consistently in the Southern areas of Brazil (Jabuonski et al, 1986A and 1986B) associated with locally multiplied seed originating within the country. It has been reported, however, that Eca is present in hosts other than potato in the states of Amazonas, Pará and Pernambuco in the warmer areas of Brazil. In these places Eca has been isolated from two tropical plant species Carica papaya and Manihot esculenta; in addition Eca has been found in 15 other plant species in Brazil (mostly horticultural crops) including potatoes and tomatoes from which it is more commonly isolated. (Jabuonski et al, 1986A). Some mechanisms of adaptation in the bacteria may be operating in warm climates to facilitate survival. Eca is a subspecies normally found in cool environments in temperate zone countries, but is is also present in similar environments in some areas of in Brazil, Uruguay and Argentina, where it and Ecc are responsible for the most important disease of potato.

The two other organisms Ecc and Ech are much more important than Eca in causing diseases in the warm areas of the tropics. Sometimes Ech has not been definitely associated (identified) with the disease, but it is highly probable than it is also involved in inducing blackleg and soft rot in more cases than is recognized at present. Temperature requirements for disease development with Ecc and Ech are much higher than for Eca (Graham and Harrison, 1985). It is common, however, to find the three organism (Ecc, Eca and Ech, in that order of importance) present in the same areas; the incidence of the different species depends on local environmental conditions (temperature, relative humidity, soil moisture, etc.).

Both Ecc and Eca have been traditionally associated to blackleg and soft rot respectively. Studies have shown already that both subspecies and Ech are capable of producing either disease. Both diseases induced by the two (Ecc and Eca) or the three organisms are known to be present in most developing countries. This assumption is based on visual symptoms in the field, but in a few cases the organisms involved have been adequately identified and reported. In the case of Latin America, Eca and Ecc have been correctly identified in only 6 out of 19 countries: Brazil, Peru, Ecuador, Chile, Colombia, and Mexico. In addition Ech has been reported in Brazil and Peru affecting potatoes (Cartin and Fucikowski, 1981; Chiampi, 1981; French, 1977; French and Lindo, 1979; Hidalgo et al, 1968; Jabuonski et al, 1986A and 1986B). Furthermore, Ecc and Ech have been found associated to a great number of cultivated species in warm climates (Daengsubha 1974; Hidalgo, 1981; Jabuonski et al, 1986A and 1986B; Kelman, 1979; Robbs, 1980 and 1981; Robbs et al, 1981; Takatsu, 1983).

EPIDEMIOLOGICAL STUDIES IN WARM CLIMATES

Relatively few epidemiological studies have been conducted on Erwinia diseases in the warm tropics and most of the knowledge that has been used in these diseases under these conditions, is being extrapolated from studies conducted in temperate zones. Identification and epidemiological studies are urgently needed in order to understand the disease and develop better control measures in warm climates. CIP scientists have initiated interesting studies on the epidemiology of Ecc and Ech in San Ramon (800 m a.s.l) which will be reported in this conference. In a study on the survival of Eca and Ecc in Mexico (Cartin and Fucikouski, 1981) it was found that Eca survived for three weeks in the soil surrounding the tubers and for three months in the lenticels of a Mexican cultivar. In the Toluca vulcano potato producing area, however, Eca was able to survive the winter and induce blackleg (4%) in the subsequent growing season. Yield reduction of 29% was recorded when inoculated tubers were planted in the field. This is the only known published study, not including those conducted at CIP, that has relation to the epidemiology of blackleg and soft rot in potato in Latin America.

There are, however, some undocumented facts that are very important in the disease expression that consistently occur during production process in the field and in storage. Seed tubers imported from temperate countries usually come contaminated with Erwinia spp (Graham and Harrison, 1985) and the tuber soft rot appears when the tubers are stored poorly during the transportation (increase of temperature) or badly handled at the arrival port and during subsequent transportation to the field. Disease appearance is also very common with imported or locally produced seeds when tubers are cut. Blacking (underground rotting), blackleg, and other symptoms in the field, and tuber soft rot at harvest and in storage are the major consequencies of the rapid contamination of seed tubers pieces with Erwinia. Usually very little care is taken during the cutting process. Contamination of daughter tubers in crops planted with cut seed is frequently high. This situation is readily observed in the field especially when soil conditions are unfavorable for the potato crop (drought, high temperature, excessive rain, etc.). Contaminated tubers are presumed to be the major source of inoculum for disease development in warm climates (Peronbelon, 1979; Kelman 1979; Booth, 1979). Cultivated host plants and weed species probably play a more important role in the survival of the pathogens and as sources of inoculum than in temperate areas. Further epidemiological research is required so that appropriate control measures can be formulated.

SEED CERTIFICATION TOLERANCES AND ERWINIA DISEASES IN WARM CLIMATES

There is a continuous controversy on the value of certification programs for tuber seed potatoes (Graham and Harrison, 1985) in regards to Erwinia diseases. In one side, these diseases are considered uncertifiable because the seeds are continually exposed to blackleg soft rot bacteria and because there does not exist a clear cut correlation between affected plants in the field and the subsequent level of tuber infections of the next generation. For these and other reasons some programs have stopped certifying for blackleg/soft rot diseases. In Table 1 official tolerance levels from some Latinamerican and known developed countries are shown. The wide range of tolerances for these diseases indicates the relative importance that those programs give to the certification tolerances. There are, of course, those, such as the Seed Certification Program of The Netherlands, that still consider it important to maintain strict low tolerances, which are associated with the actual reduced levels of disease appearance.

Levels of infection with Erwinia diseases in Latinamerican countries in which potato grow in warm areas has increased slowly but steadily through the years, especially in those countries (Argentina and Uruguay) in which cutting tuber seed is a usual practice. It is highly probable that the high tolerance levels permitted in those countries have been responsible for the actual high levels of contamination of the seed stocks. Because of the high tolerance levels, crops registered for certification are very rarely rejected because of the blackleg symptom appearance alone, usually low, but for sure with high proportion of contaminated tubers which mostly was reflected in lost (rot) of seed pieces before the plant emergence. Then the increase of the inoculum in the soil in the same season and the further daughter-tubers contamination facilitate the easy expression of the different forms of the diseases, especially when very severe adverse conditions are present in the following season.

There are at the present time renewed hopes of reducing infection levels by using <u>in vitro</u> Erwinia-free material coped with rapid multiplication of pre-basic seed under controlled conditions. Most of the Latin American countries indicated in Table 1 and others now have pre-basic seed production programs mostly especially to reduce--in the long term--the actual high levels of Erwinia contamination. The certification programs of these countries have reduced significantly the virus problems, but not the bacterial diseases which are now causing higher losses in the field and in storage than virus diseases.

Actual "higher" tolerance levels should be reviewed in the light of the new Erwinia-free material being produced and incorporated into the seed certification process and reduce them adequately to more "adecuate" figures. Percentages adopted for tolerances for these and other diseases in developing countries sometimes indicate a lack of sufficient knowledge of the disease or lack of sufficient alternatives to cope with them. Since the disease has increased slowly over a long period of time, it would be expected to decrease equally slowly if clean material is continually added to the seed production system and if cultural practices associated phytosanitation and potato handling are also improved. Fortunately disease resistance is now being considered at CIP and by some National Programs, as an additional component for integrated control of the disease. Some research has been already conducted on disease resistance, with promising results (Graham and Harrison, 1985; Hidalgo, 1981; Thurston and French, 1972).

Table 1. Tolerances (*) for blackleg and soft rot (Erwinia spp) for basic and certified potato seed in some selected countries.

	% blackleg			% tuber soft rot
Country (State)	Basic seed (Elite or nuclear)	Certified Seed (Lowest categ.)		Certified Seed (Low categ.)
Holland	(-) - 0.0	"C"	(-) - 0.02	-
Canada	0.25 - 0.1**	"Cert"	3.0 - 2.0 **	0.1
Mexico	0.5 - 0.0	"Cert"	0.5 - 0.9	-
Chile	(-) - 0.5	"C-3"	(-) - 2.0	0.0
Uruguay	(-) - (-)	"B"	5.0 - 2.0	0.5
Argentina	0.0 - 2.0	"B"	5.0 - 4.0	0.0 (?)
Brazil (SC)	(-) - 2.0	"B"	7.0 - 5.0	1.0
(Federal)	(-) - 2.0	"B"	10.0 - 8.0	2.0
USA (Oregon)	3.0 - 1.0	Depend on Winter Test ***		

* Official regulations for certification.
** Total blackleg, wilts, and viruses.
*** Blackleg is counted in the field, recorded, and data published, but this is no indication that this is a true value for the amount of blackleg in seed tuber rot.

(Compiled by O. Hidalgo, Jan., 1987).

CONCLUSIONS AND PERSPECTIVES

In order to face the problems generated by the increase of Erwinia diseases in warm areas, it is proposed that CIP and National Programs analyze, promote, and adopt the following political and technical corrective measures:

1 Recognize the appropriate importance that blackleg and soft rot diseases now have in the warm areas.

2 Identify and map the distribution of the organisms involved, in each country as well as in areas within the countries.

3 Initiate and/or promote epidemiological studies in order to have better knowledge of the interactions between the species and the cultural practices in warm environments and to understand better the pathogen's sources of inoculum and their probable different survival mechanisms. These and other studies are urgently needed.

4 In the long term, promote the use of new, non-contaminated sources of seed generated from pre-basic seed produced from in vitro cultures.

5 Determine and adapt better agronomical practices and urge the use of better storage conditions.

6 Continue with the identification of better resistant or tolerant cultivars as part of integrated control measures to reduce the incidence of Erwinia diseases.

Increased involvement of CIP and National Programs in future work on these diseases in warm areas, would be highly desirable.

LITERATURE CITED

BOOTH, R.H. 1979. The importance of bacteria in potato storage. In: Developments in control of potato bacterial diseases. Report of a Planning Conference. International Potato Center. Lima, Peru. pp. 120-124.

CARTIN, L.F. and L. FUCIKOUSKI. 1981. Distribución de la pierna negra de la papa en México y la supervivencia de las bacterias causales. Fitopatología 16 (2): 55-59.

CHIAMPI, L. 1981. Taxonomy and etiology of soft rot Erwinia affecting potatoes in Southern Chile. In: Proceedings of Fifth International Conference Plant Pathogen of Bacteria. CIAT. Cali, Colombia. pp. 369-373.

DAENGSUBHA, W. 1974. Vegetable soft rot bacteria in the Philippines. Ph.D. Dissertation. Univ. Philippines.

FRENCH, E.R. 1977. Enfermedades bacterianas de la papa en Latinoamérica. Fitopatología 12 (2): 87-96.

FRENCH, E.R. and L. DE LINDO. 1979. The Erwinia of potatoes in Peru. In: Developments in control of potato bacterial diseases. Report of a Planning Conference. International Potato Center. Lima, Peru. pp. 88-93.

GRAHAM, D.C. and M.D. HARRISON. (Eds.). 1985. Report of the international conference on potato blackleg disease. Potato Marketing Board. The Potato Association of America. Edinburgh, June 26-29, 1984. 95 p.

HIDALGO, O.A. 1981. Studies on the resistance of potato to tuber and stem rot and on its inducer: Erwinia chrysanthemi. Ph.D. thesis. North Caroline State University. 60 p.

HIDALGO, O.A., L. NIELSEN and E.R. FRENCH. 1968. Pierna negra (Erwinia sp.): Nueva enfermedad de la papa en el Perú. Fitopatología 4: 15-18.

JABUONSKI, R.E., A. TAKATSU and F.J.B. REIFSCHNEIDER. 1986A. Levantamento e identificaçao de especies de Erwinia de diferentes plantas hospedeiras e regiones do Brasil. Fitopatologia Brasilera 11: 185-195.

JABUONSKI, R.E., A. TAKATSU and F.J.B. REIFSCHNEIDER. 1986B. Avaliaçao de patogenicidade de bacterias do genero Erwinia isoladas de batateira, tomateiro e de outras plantas hospedeiras. Fitopatologia Brasileira 11: 587-597.

KELMAN, A. 1979. Present and future status of bacterial diseases of potatoes in the lowland tropics. In: Developments in control of potato bacterial diseases. Report of a Planning Conference. International Potato Center. Lima, Peru. pp. 20-27.

PEROMBELON, M.C.M. 1979. Ecology of soft rot Erwinias in relation to potatoes. In: Developments in control of bacterial diseases. Report of a Planning Conference. International Potato Center. Lima, Peru. pp. 94-118.

ROBBS, C.F. 1980. Erwinia chrysanthemi: agente de una "podridao mole" de cebolinha [green onion]. Fitopatologia Brasileira 5: 453 (Abstract).

ROBBS, C.F. 1981. Taxonomia, bio-ecologia e principais representantes do Genero Erwinia no Brasil. Fitopatologia Brasileira 6: 304-305 (Abstract).

ROBBS, C.F., J.R. NETO, R.L.D. RIBEIRO and O. KIMURA. 1981. Annotated list of bacterial plant pathogens in Brazil. In: Proceedings of Fifth International Conference on Plant Pathogenic Bacteria. CIAT. Cali, Colombia. pp. 601-613.

TAKATSU, A. 1983. Erwinia do grupo carotovora no Brasil. Fitopatologia Brasileira 8: 535-536 (Abstract).

THURSTON, H.D., E.R. FRENCH. 1972. Major pathological resistance needed for adapting the potato to the lowland tropics and the possibility of obtaining them. In: French, E.R. (ed.). Prospects for the potato in the developing world. International Potato Center. Lima, Peru. pp. 236-246.

METHODS OF CONTROL OF ERWINIA DISEASES OF THE POTATO

John Elphinstone

INTRODUCTION

Erwinia diseases of potatoes have, historically, been difficult to control for the following reasons:

(i) The three soft rot erwinias, Erwinia carotovora ssp. atroseptica (Eca), E. carotovora spp. carotovora (Ecc) and E. chrysanthemi (Echr), have varying temperature requirements for growth and pathogenicity so that Erwinia diseases can occur in all climates where potatoes are grown. Furthermore, Ecc and Echr both have wide host ranges and may be widely dispersed throughout the environment (10).

(ii) Potato seed tubers are almost always latently infected with Erwinia (9). Furthermore, latently infected tubers appear healthy so that the extent of infection is often only observed later, after the crop is planted, when symptoms appear.

(iii) Latent erwinias may be located on the tuber surface but are also harboured in suberized lenticels or in the vascular system where they are protected from liquid disinfectants (3).

(iv) No effective bactericide with systemic action is commercially available.

CONTROL STRATEGIES

Control strategies according to Lapwood (6) are:

(i) To decrease known sources of inoculum.

(ii) To minimize the opportunities for spread of the pathogen.

(iii) To minimize the damage to the growing and harvested crop.

The extent to which control strategies are applied will depend on the type of crop to be grown. For the production of seed tubers more stringent control measures are required since latent infection of progeny tubers must be kept to a minimum. Crops grown for consumption, however, can tolerate a degree of latent infection provided storage facilities, if used, are adequate. Blackleg disease, except in extreme cases, does not greatly affect yield and therefore may also be tolerated in a consumer potato crop, whereas, in a seed crop, certification procedures

may impose severe penalties (especially in developed countries in temperate lands). Bacterial soft rot of potato tubers occuring either as pre-emergence in the field, or pre or post-harvest, can cause significant yield loss and requires careful control in both consumer and seed crops.

Methods of control of Erwinia diseases may be categorised as follows:

(i) Avoidance of the pathogen.

(ii) Avoidance of the disease.

(iii) Eradication of the pathogen.

(iv) Development of resistance.

Development of resistance will be discussed elsewhere in this publication (5); the first three categories will be discussed below.

(i) Avoidance of the pathogen

This is the most desirable form of control, particularly for seed tuber crops, since latent infection would theoretically be avoided. At present, seed production schemes mostly rely on the propagation of seed tubers from nuclear stocks which are free from Erwinia spp. and which are planted in Erwinia-free soil.

Planting material free from Erwinia may be produced as cuttings from tested plants or by rapid multiplication techniques under aseptic conditions. True potato seed is also usually Erwinia-free, particularly if disinfected with sodium hypochlorite solution before use.

Unfortunately, such schemes based on avoidance of the pathogens are difficult to manage on a practical basis. Seed stocks quickly become latently contaminated, often after only the first multiplication cycle (11). Erwinia from sources outside of the crop are transmitted by insects, in wind-blown aerosols, or in irrigation water, to contaminate the growing crop (10).

Certain practices could reduce the entry of bacteria from outside of the crop as follows:

(a) Disease-free material should be planted in Erwinia-free soil where adequate crop rotation has eradicated the organisms or where host plants have never been grown. Volunteer plants and weed species harbour the bacteria and should be eliminated during the rotation period.

(b) Irrigation water should be free from the pathogens.

(c) Insects, which disseminate the bacteria, should be controlled.

(d) Refuse dumps containing diseased or waste potatoes or other vegetables should be destroyed since they represent inoculum sources.

(e) Strict hygiene practices should be observed before entering the crop e.g. the cleaning of machinery and footwear.

(f) Nuclear potato stocks should be grown as far away as possible from other potato crops.

(g) Harvesting should be as early as possible to reduce the risk of contamination of the crop by the pathogen.

(ii) Avoidance of disease

If latently infected tubers are to be planted, then certain procedures may be implemented to reduce disease impact:

(a) The choice of cultivar is important since they vary widely in resistance to blackleg and soft rot.

(b) Planting should be into well drained soil.

(c) Seed tubers should not be cut since they rot more easily than whole tubers.

(d) Movement through the growing crop should be restricted to avoid damaging the crop and spreading disease.

(e) Over-irrigating should be avoided. Sprinkle irrigation is probably preferable to the flood method since water logging is less likely and the spread of bacteria along the furrow is decreased.

(f) The removal of blackleg plants is advisable in seed crops to reduce inoculum sources, but is probably not practical in consumer crops.

(g) Damaging tubers increases their susceptibility to rotting, therefore, mechanical damage during harvesting and handling, heat or cold damage, and insect or fungal attacks, should all be minimized to the extent possible.

(h) Tubers should be adequately dried before storage and maintained so during the storage period. Forced draft ventilation in the store helps prevent condensation on the tuber surface, as does avoidance of temperature fluctuations.

(i) Calcium fertilization

Mc Guire and Kelman (8) found that the calcium content of the potato tuber could influence it's resistance to bacterial soft rot. Furthermore, in field trials conducted in low calcium soils, progeny tuber calcium levels could be increased by pre-planting incorporation of a calcium source, and tubers from the enriched calcium soils were found to be more resistant to soft rot. Tzeng (13) found that calcium sulphate applied as gypsum gave the most consistent results. However, the effectiveness of calcium applications varied with soil type; increases in tuber calcium and reductions in tuber susceptibilities to soft rot were only consistently observed in low cation exchange capacity, low calcium soils (less than 400 ppm Ca).

(iii) Eradication of the pathogen

Since the likelihood of a latently infected tuber giving rise to a diseased plant is correlated with the inoculum density of *Erwinia* spp. (1), a method to eradicate or reduce latent erwinias is needed.

(1) Chemotherapy

Many chemicals have been shown to be effective in controlling *Erwinia* spp. *in vitro* (4). However, no consistently effective method of disinfection of potato tuber is known. Recently, Sun (12) suggested that spraying tubers with formaldehyde, followed by forced-air drying, prevented blackleg in the following crop. Bartz and Kelman (2) reduced the potential for bacterial soft rot through disinfection with sodium hypochlorite solutions or organic acids such as citric acid (1%) followed by air-drying. The treatments were most effective when the bacteria were on the tuber surfaces rather than in lenticels; attempts to infiltrate the bactericides into lenticels did not increase the level of control. An experimental bactericide CGA 78039 gave the best control of soft rot although it did not completely prevent the disease. It was concluded that air drying, coupled with further provisions for keeping tuber surfaces free from moisture, remains the most effective means of reducing losses to bacterial soft rot.

(2) Heat therapy

In Scotland, heat treatment of tubers, by immersion in hot water at 50-55°C for 5-10 minutes, significantly reduced contamination by *Erwinia* and subsequent blackleg in the field without adverse effects on growth or yield (7). Further investigation (14) showed that temperatures of 44.5 for 30 minutes also reduced

contamination, particularly if followed by forced-ventilation. However, contamination levels were observed to rise again during subsequent storage. Interestingly, ventilation alone significantly reduced contamination levels and was especially effective when applied 30 days after harvest.

LITERATURE CITED

1. Aleck, J.R. and Harrison, M.D. 1978. The influence of inoculum density and environment on the development of potato blackleg. Am. Potato J. 55: 479-494.

2. Bartz, J.A. and Kelman, A. 1986. Reducing the potential for bacterial soft rot in potato tubers by chemical treatments and drying. Am. Potato J. 63: 481-493.

3. Graham, D.C. and Volcani, Z. 1961. Experiments on the control of blackleg disease of potato by disinfection of seed tubers with mercury compounds and streptomycin. European Potato J. 4: 129-137.

4. Harris, R.I. 1979. Chemical control of bacterial soft rot of wounded potato tubers. Potato Research 22: 245-249.

5. Huaman, Z., de Lindo, L. and Elphinstone, J.G. 1987. Resistance to blackleg and soft rot and its potential use in breeding. Proceedings of Planning Conference on Bacterial Diseases of the Potato. CIP, Lima, Peru.

6. Lapwood, D.H. 1985. Assessment of means of control through agronomic measures in the growing crop. Report of the International Conference on Potato Blackleg Disease (eds. D.C. Graham & M.D. Harrison) p. 61-62. Potato Marketing Board, Oxford.

7. Mackay, J.M. and Shipton, P.J. 1983. Heat of seed tubers for control of potato blackleg (Erwinia carotovora sub sp. atroseptica) and other diseases. Plant Pathology 32: 385-393.

8. Mc Guire, R.G. and Kelman, A. 1984. Reduced severity of Erwinia soft rot in potato tubers with increased calcium content. Phytopathology 74: 1250-1256.

9. Pérombelon, M.C.M. 1972. The extent and survival of contamination of potato stocks in Scotland by Erwinia carotovora var. carotovora and E. carotovora var. atroseptica. Annals of Applied Biology 71: 111-117.

10. Pérombelon, M.C.M. and Kelman, A. 1980. Ecology of the soft rot erwinias. Annu. Rev. Phytopathol. 18: 361-387.

11. Pérombelon, M.C.M., Lowe, R., Quinn, C.E. & Sells, A. 1980. Contamination of pathogen-free potato stocks by Erwinia carotovora during multiplication. Results of a six-year monitoring study. Potato Research 23: 413-425.

12. Sun, M. 1985. Formaldehyde treatment for potato blackleg disease control in Montana. Report of the International Conference on Potato Blackleg Disease (eds. D.C. Graham & M.D. Harrison) p.62. Potato Marketing Board, Oxford.

13. Tzeng, K.C. 1985. Calcium nutrition of potato plants in relation to bacterial soft rot susceptibility and internal brown spot of potato tubers. PhD Thesis, University of Wisconsin-Madison, 150 pp.

14. Wale, S.J. and Robinson, K. 1986. Evaluation of large scale hot water dipping and forced ventilation of seed potatoes to reduce tuber contamination with blackleg bacteria (Erwinia spp.). 1986 British Crop Protection Conference - Pests and Diseases, p. 1137-1142.

CONTROL OF ERWINIA DISEASES IN SAN RAMON

John Elphinstone, Liliam de Lindo
and E. R. French

A) INTRODUCTION

In the CIP mid-elevation experimental station at San Ramon (800 m.a.s.l.), all manifestations of the *Erwinia* diseases which affect potatoes have been observed. Both *Erwinia carotovora* ssp. *carotovora* and *Erwinia chrysanthemi* are pathogenic (1) in both the wet (November-March; more than 1000 mm rainfall per season) and the irrigation-supplemented, dry (May-August; less than 500 mm rainfall per season) seasons. Blackleg disease occurs almost entirely in crops grown from seed tubers, and is most prevalent if the seed is produced at source when pre-emergence rotting of the seed can also be very damaging. Crops grown from seed tubers imported from the highlands usually exhibit less than 5% blackleg although levels can increase to 20% and above when soil moisture is in excess, which more commonly occurs during the wet season. Stem soft rot (or 'aerial soft rot') is also observed and has affected transplanted potatoes (less than 3% of plants) grown from true potato seed (TPS). Latent infection of tubers at harvest time is often as high as 100%, even in TPS crops, and rotting of progeny tubers in the field (up to 20%) is common, particularly if flood irrigation is used. Bacterial soft rot has caused total losses in rustic potato stores, either when associated with damage by the potato tuber moth (*Phthorimaea operculella*) or when evapourative water-cooling of stores has been used in conjunction with poor ventilation.

B) CONTROL BY CALCIUM NUTRITION OF THE GROWING CROP

Development of control of *Erwinia* diseases in San Ramon has mainly focussed on the use of calcium to increase resistance of potato tubers to bacterial soft rot, as originally determined in Wisconsin (3, 4).

Incorporation of calcium sulphate as gypsum into the soil prior to planting of seed tubers did not significantly reduce the incidence, in wet or dry seasons of a) pre-emergence soft rot of seed tubers, b) blackleg, c) soft rot of progeny tubers before harvest, or d) soft rot during 6 months storage in diffused light (see Table 1).

The mean soil calcium level (0-30 cm depth) in San Ramon was 1519 ppm; in Wisconsin consistent effects of calcium nutrition were only observed in soils with less than 400 ppm Ca (4).

Significant differences, with respect to incidence of Erwinia diseases, were observed between the different clones of potato grown (Table 2).

In contrast, results from experiments with TPS crops demonstrated that progeny tuber soft rot at harvest time could be significantly, although not completely, reduced by pre-transplant incorporation of calcium sulphate into the soil (Table 3). Furthermore, tubers harvested from calcium enriched soils had higher concentrations of calcium in the periderm (Table 4) and were more resistant to soft rot, 4 days after inoculation and soft rot induction (2), than tubers from control soils (Table 5).

C) POST HARVEST TUBER DUSTING

Dusting of potato tubers with Calcium sulphate had no effect on soft rot during storage. However, dusting with the insecticide phenthoate ('Cidial'), which controlled damage by the Potato Tuber Moth, highly significantly reduced the incidence of soft rotting during 6 months storage in diffused light rustic stores (Table 6).

D) POST-HARVEST TUBER DISINFECTION

Disinfection of highly latently infected potato tubers, by dipping in sodium hypochlorite solution (1% active Cl_2) for 10 minutes, was examined in the laboratory. The dipping treatment reduced the incidence of tuber disease by half when determined 4 days after rotting was artificially induced by wrapping tubers in moist paper and 2 layers of plastic film and incubating at 25° C (Table 7). A pre-dipping treatment of 48 hours at 95% R.H. and 5° C further decreased rotting, presumably because lenticels were proliferated allowing contact between bactericide and latent bacteria. In a similar way, vacuum infiltration of the disinfectant (-86 kPa for 5 minutes) reduced rotting.

E) REDUCING IRRIGATION WATER CONTAMINATION

Surface irrigation water in San Ramon is constantly contaminated throughout the year with more than 100,000 viable cells of Erwinia carotovora ssp. carotovora per liter. Deep well water (50 m) was found to be free of the pathogen. Contamination levels of the surface water were decreased by 94.44% by filtration through 75 cm of fine river sand, or 99.99% by the addition of 5 ppm of chlorine to the water (however, this level was at times phytotoxic).

F) RECOMMENDATIONS

(i) That studies on the interactions between soft rot, fungal infections and insect infestations be continued in order that control measures may be directed, more efficiently, at the primary pathogen or pest to control secondary *Erwinia* diseases.

(ii) That disinfection of potato tubers immediately after harvest, when lenticels are proliferated and non-suberised and latent bacteria are more exposed, be thoroughly examined.

(iii) That the effects of *Erwinia* in irrigation water on the growing, harvested and subsequent crops be studied.

(iv) That simple methods to detect *Erwinia* in water be developed for field use in order to identify water sources free from contamination.

G) LITERATURE CITED

(1) De Lindo, L. and French, E.R. 1981. Erwinias que afectan papa en los trópicos húmedos en el Perú. Fitopatología 16: 69-74.

(2) De Boer, S.H. and Kelman, A. 1975. Evaluation of procedures for detection of pectolytic *Erwinia* spp. on potato tubers. Am. Potato J. 52: 117-123.

(3) Mc Guire, R.G. and Kelman, A. 1984. Reduced severity of *Erwinia* soft rot in potato tubers with increased calcium content. Phytopathology 74: 1250-1256.

(4) Tzeng, K.C. 1985. Calcium nutrition of potato plants in relation to bacterial soft rot susceptibility and internal brown spot of potato tubers. Ph.D. Thesis, University of Wisconsin-Madison, 150pp.

Table 1. Effect of calcium sulphate fertilization on the incidence of *Erwinia* diseases in crops produced from tubers in San Ramon, during the wet season (Dec.-Mar. 1984-85) and dry season (May-Aug. 1985)[1]

Disease incidence	Calcium sulphate rate (t/ha)					
	Wet season (4 clones)			Dry season (3 clones)		
	0	12	24	0	12	24
% pre-emergence soft rot of seed	16.5a	12.0a	11.5a	4.8a	3.2a	3.6a
% blackleg	24.3f	26.8f	19.7f	5.4f	4.9f	4.9f
% soft rot at harvest	5.3q	3.4q	3.8q	1.0q	0.7q	0.6q
% soft rot after 6 months storage[2]	37.1x	28.3x	28.2x	32.0x	31.0x	27.3x

(1) Within rows and seasons, numbers followed by the same letter are not significantly different, p = 0.05, according to Duncans multiple range test.

(2) Tubers stored in diffused light rustic stores in San Ramon.

Table 2. Incidence of Erwinia diseases in different clones produced from seed tubers in San Ramon during the wet season (Dec.-Mar. 1984-1985) and dry season (May-Aug. 1985)[1]

Disease incidence	Wet season			Dry season			
	Desiree	Revolucion	Rosita	Desiree	Revolucion	Rosita	DTO-33
% pre-emergence rotting	3.1b	20.5a	16.4a	3.1b	11.0a	0.9b	0.5b
% blackleg	12.9g	28.8f	29.1f	1.8g	6.8f	3.9f	6.2f
% soft rot at harvest	4.8q	3.5q	4.2q	0.3r	1.0q	1.1q	0.7qr
% soft rot after 6 months storage[2]	17.4y	43.2x	32.9x	12.7y	28.7xy	38.3x	20.7xy

(1) As for Table 1, p = 0.05.

(2) As for Table 1.

Table 3. Effect of calcium sulphate fertilization on the incidence of *Erwinia* diseases in crops produced from true potato seed in San Ramon during the wet season (Dec.-Mar. 1984-85) and dry season (May-Aug. 1985). Combined means of two families[1]

Disease incidence	Wet season				Dry season			
	0[2]	6	12	18	0	6	12	18
% blackleg	2.5a	1.7a	2.2a	1.9	3.2a	2.9a	2.9a	1.9a
% plants with soft rot tubers at harvest	10.9x	6.1y	8.0xy	6.1y	40.5x	29.9xy	18.2y	16.5y
% soft rot (by weight) at harvest	3.7f	1.2g	1.6g	1.1g	5.4f	3.7f	2.6f	2.3f

(1) As for Table 1, p = 0.05.

(2) Calcium sulphate rate in t/ha.

Table 4. Concentration of calcium in tuber periderm (mean of 20 samples) in p.p.m., when crop grown with different rates of application of calcium sulphate

Family	Rate of Calcium sulphate (t/ha)			
	0	6	12	18
Atzimba x R128	175	205	245	245
DTO-33 (O.P.)	205	230	300	285
Average[1]	190a	217ab	272b	265b

(1) Numbers followed by the same letter are not significantly different, p = 0.05, according to Duncans multiple range test.

Table 5. Percentage weight of soft rotted tissue from tubers, 4 days after soft rot induction (mean of 5 replications), following harvest from plots with different rates of fertilization with calcium sulphate[1]

Family	Application rate of Ca SO_4 (t/ha)			
	0	6	12	18
Atzimba x R 128	23.0ab	18.2ab	16.8ab	14.1 b
DTO-33 (O.P.)	24.9a	18.6ab	14.1 b	16.3ab
Average	24.0a	18.4ab	15.4 b	15.2 b

(1) As for Table 4, p = 0.05.

Table 6. Effect of post-harvest dusting of potato tubers with calcium sulphate ($Ca\ SO_4$), and/or Cidial insecticide, on the percentage soft rot during 6 months storage in diffuse light

Post-harvest dust		Wet season (3 clones)	Dry season[3] (4 clones)
$Ca\ SO_4$[1]	Cidial[2]		
-	-	57.5a[4]	46.7x
-	+	6.5b	1.7y
+	-	55.0a	50.7x
+	+	5.8b	1.7y

(1) 60 g. per 100 tubers

(2) 30 g. per 100 tubers

(3) As for Table 1

(4) Within columns, numbers followed by the same letter are not significantly different, p = 0.0001, according to Duncans multiple range test.

Table 7. Number and percent tubers induced to rot out of 15 (mean of 6 replicates) disinfected by different procedures with sodium hypochlorite (Na O Cl) solution (1% active chlorine)1.

Disinfection procedure	Number of rotted tubers	Percent rotted tubers
Control (no treatment)	15.0a	100
Na O Cl dip, 10 minutes	7.3b	48.7
Vacuum infiltration with Na O Cl	4.0c	26.7
5°C, 95% R.H. for 48 h + Na O Cl dip	3.0c	20.0

(1) As for Table 4, p = 0.005.

ERWINIAS AND OTHER PATHOGENS AND PESTS: POSSIBLE INTERACTIONS IN THE WARM CLIMATE OF SAN RAMON

V. Otazu, J. Elphinstone, H. Torres

INTRODUCTION

Potatoes have been grown at CIP-San Ramon Exp. Station in Peru for over 10 years. Pathological problems at these mid elevation tropics (800 m.a.s.l.) are peculiarly complex. An initial survey reported the presence of Erwinia and other fungal organisms associated with disease symptoms in leaves, stems and tubers (8). Subsequent reports focused on the incidence of blackleg caused by Erwinias (3, 4). Presently it is known that Erwinia is not only present in potato plants but also on the rhizospheres of other hosts, in irrigation water and obviously in the soil (4).

There are 2 distinctly different growing seasons in San Ramon, one dry and the other wet. During both seasons extensive early-dying of plants is observed. Although most dying plants exhibit wilting, close examination reveals different kinds of symptoms including: typical wilts, basal rots (blackleg), girdled stems, aerial rots, cutworm damage, etc. Even though Erwinia is isolated from a large proportion of dying plants, some other organisms, especially fungi are also isolated, very often from the same samples. Possible pathogenic interactions were suspected between Erwinia and other organisms such as have been reported previously in the literature (1, 2, 5, 7, 9). In order to explore the possibility of Erwinia/fungal interactions, careful observations and isolations of different organisms were performed from diseased plant material during 4 growing seasons. Results of this study are reported.

TPS SEEDLING DAMPING-OFF

Erwinia was not associated with seedling damping-off. Fungi, especially Rhizoctonia solani were consistently isolated (Table 1). TPS seedlings seem to be resistant to Erwinia at this stage. Experimentally, only high concentrations of Erwinia (which in nature is unlikely to occur) produced disease in young seedlings.

WILT PROBLEMS IN THE FIELD

The number of wilting or dying plants was considerable during both growing seasons (Figs. 1 and 2). The problem was visibly higher in continuously cropped land. One year rotation (although insufficient) was shown to lower the incidence of dying plants (Figs. 1 and 2). It was also evident that plant survival was higher in plants originated from seed tubers than those from TPS, especially during the dry season (Fig. 3).

During the wet season, Pythium and Pseudomonas were associated with dying TPS transplants in the field (Table 2). A high proportion of Erwinia was also obtained from a group of samples for which enrichment medium was used. However, it is probable that these small Erwinia populations were epiphytic only since stem-rotting was not observed. No apparent interactions were observed and Pseudomonas incidence was considered important, especially during the later part of the season. The pathological picture in plants grown from seed tubers was somewhat different (Table 3). Pseudomonas and Erwinia were mostly isolated from dying plants. Most dual infections occurred in plants showing typical wilt symptoms caused primarily by Pseudomonas. A possible interaction of Erwinia and Pseudomonas is difficult to visualize in wilted plants since healthy appearing stems were also latently contaminated with Erwinia. In general latent stem infections by Erwinia were higher than those reported for cooler growing areas (6). Latent Pseudomonas infections were low.

Rhizoctonia solani is mostly associated with wilted plants grown from TPS during the dry season (Table 4). Control experiments using TOLCLOFOS METHYL, an efficient fungicide used against Rhizoctonia confirmed our isolation results. Significant insect damage was also evident, mostly involving cut worms and stem borers. In plants grown from seed tubers, Erwinia was most frequently associated with wilted plants (Table 5), causing mostly blackleg symptoms. Possible interactions were difficult to identify.

SOFT ROT AT HARVEST

At harvest, tuber soft rot was apparent in 4-10% of tubers. Erwinia and Pythium organisms were mostly isolated (Table 6). Indirect evidence indicates that an interaction may exist between Erwinia and Pythium. Control experiments using Metalaxyl (that is specific against Phycomycetous fungi, to which Pythium belongs and non-toxic to Erwinia in vitro), produced lower percentage of soft rotted tubers (Table 7) indicating that Pythium probably infects first, followed by Erwinia. A similar relationship in stems was reported by Abo-El-Dahab (1). This effect persisted even after storage.

ROT DURING STORAGE

During storage, a high proportion of tubers rotted with no apparent seasonal differences (Table 8). Rot symptoms were variable from soft rot to a typical dry rot. Fusarium organisms were mostly involved (Table 8). F. oxysporum, F. solani and other Fusarium spp. were present in frequencies of 60, 27 and 13% respectively. More than 50% of Fusarium isolates showed pectolytic activity in crystal violet pectate medium. Such isolates, when artificially inoculated in tuber slices showed symptoms resembling those caused by Erwinia. The significance of this phenomenon is unclear. Strong indications of an interaction between rotting agents and the Potato Tuber Moth were observed:

In treatments where the tuber moth was controlled by insecticide applications the proportion of rotted tubers was significantly lower (Table 9). Larvae of PTM may act as a vector for spreading the rotting agents or they may stimulate conditions for infection by previously latent-bacteria. Literature on these kinds of interactions in storage is scarce; further research on this subject is presently underway at CIP.

CONCLUSIONS

1. The pathological panorama of potatoes in San Ramon varies according to the growing season and to the type of propagation.

2. One year rotation leads to greater plant survival than in no rotation at all.

3. There is greater survival for plants grown from seed tubers than those grown from TPS.

4. During the rainy season, *Pythium* and *Pseudomonas* were mostly associated with dying TPS transplants and Erwinia and *Pseudomonas* were present in plants grown from seed tubers.

5. During the dry season, *Rhizoctonia solani* and insects caused most damage in TPS transplants and Erwinia was more important in plants grown from seed tubers.

6. *Fusarium oxysporum* and *Fusarium solani* were mostly involved in rotted tubers in storage.

7. Possible interactions were detected at harvest, in soft rotted tubers. *Pythium* and Erwinia are probably interacting.

8. There are strong indications that the tuber moth may be spreading or stimulating the rotting organisms in storage.

Figure 4 summarizes some of these conclusions in schematic form

LITERATURE CITED

1. Abo-El-Dahab, M. K.; El-Goorany, M.A. and El-Masry, M. H. 1978. Association of Erwinia carotovora var. carotovora and Pythium butleri in causing blackleg symptoms on potato stems. Int. Congr. Plant Pathology, 3rd. Munich p. 77.

2. Davis, J. R.; L. H. Sorensen, and G. S. Corsini. 1985. Synergistic effect of Fusarium sambucinum on the expression of infection by Erwinia carotovora subsp. atroseptica on potato. In Report of the International Conference on Potato Blackleg Disease. (D.C. Graham and M. D. Harrison, Eds.) Edinburgh, Scotland, p. 23.

3. Lindo, L. de y E. R. French. 1981. Erwinias que afectan papa en los trópicos húmedos en el Perú. Fitopatología 16:69-74.

4. Lindo, L. de, and E. R. French. 1985. The Erwinias causing blackleg in a jungle soil of Peru. In Report of the International Conference on potato blackleg disease (D.C. Graham and M. D. Harrison, Eds.). Edinburgh, Scotland. pp 30-31.

5. Logan, C. and R. B. Copeland. 1979. The effect of time of planting inoculated tubers on the incidence of potato blackleg and gangrene. Ann. Biol. 93:133-140.

6. Otazú, V., M. D. Harrison, G. Caero, M. Coca, y W. M. Brown. 1985. Incidencia de Erwinias pectolíticas en cultivos y almacenes de papa en Bolivia. Fitopatología 20:12-16.

7. Stanghellini, M. E. and J. D. Russel. 1971. Induction of bacterial seed piece decay by various soil-borne fungi. Phytopathology 61:1324. (Abstr).

8. Vessey, J. C. 1975. Some potato diseases of the peruvian humid tropics. Plant Dis. Reptr. 59:1004-1007.

9. Zink, R. T. and G. A. Secor. 1982. Interaction of fungal wilt pathogens and potato blackleg. Plant Disease 66:1053-1056.

Table 1. Isolation frequency (%) of different organisms from 56 dampped-off TPS seedlings in San Ramón.

Rhizoctonia	Fusarium	Pythium	Insects + Others
52	12	4	32

Fig. 1. Survival of TPS transplants during the rainy season in San Ramón, 1985-86. Bars represent standard error.

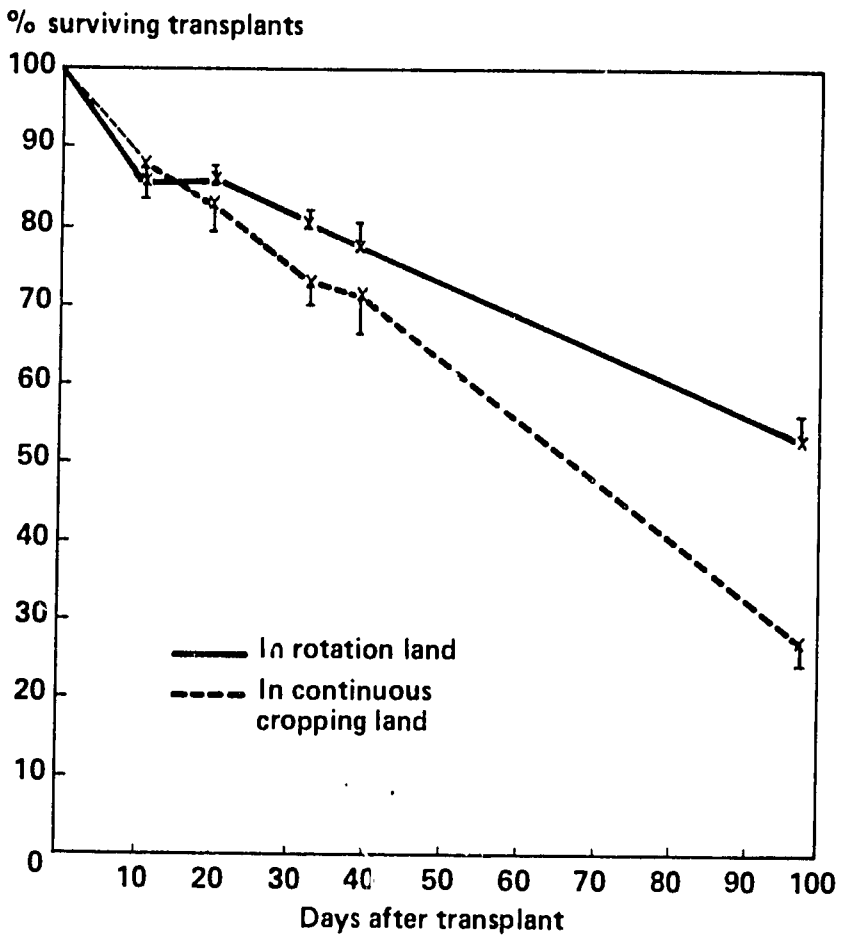


Fig. 2. Survival of TPS transplants during the dry season in San Ramón, 1986. Bars represent standard error.

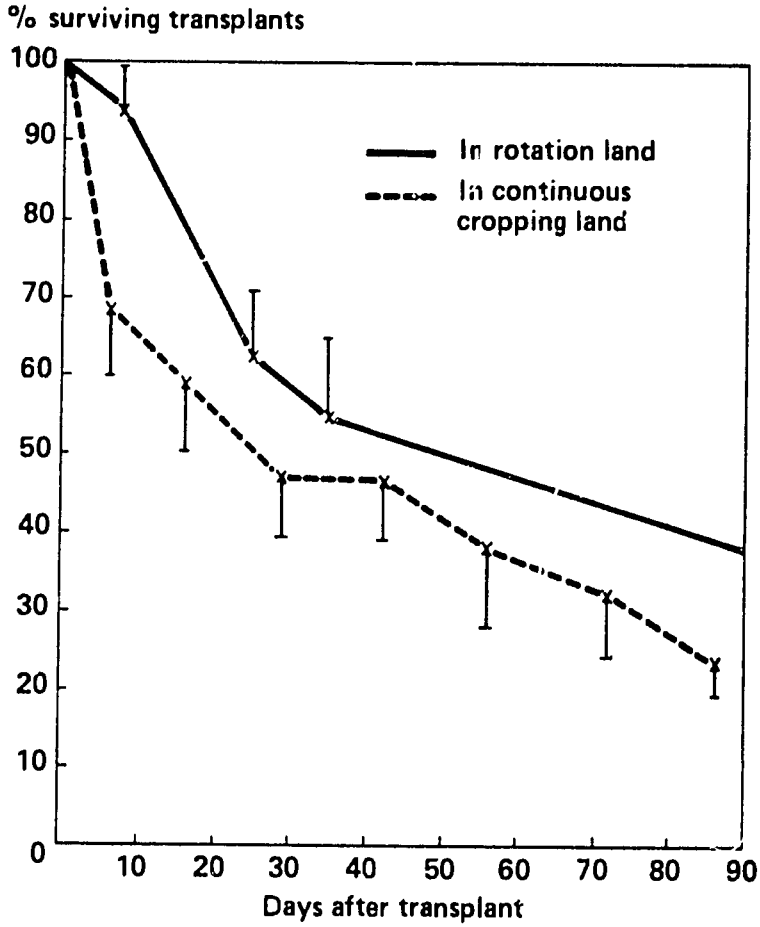


Fig. 3. Survival of plants originated from seed tubers and TPS during 2 different growing seasons in San Ramón.

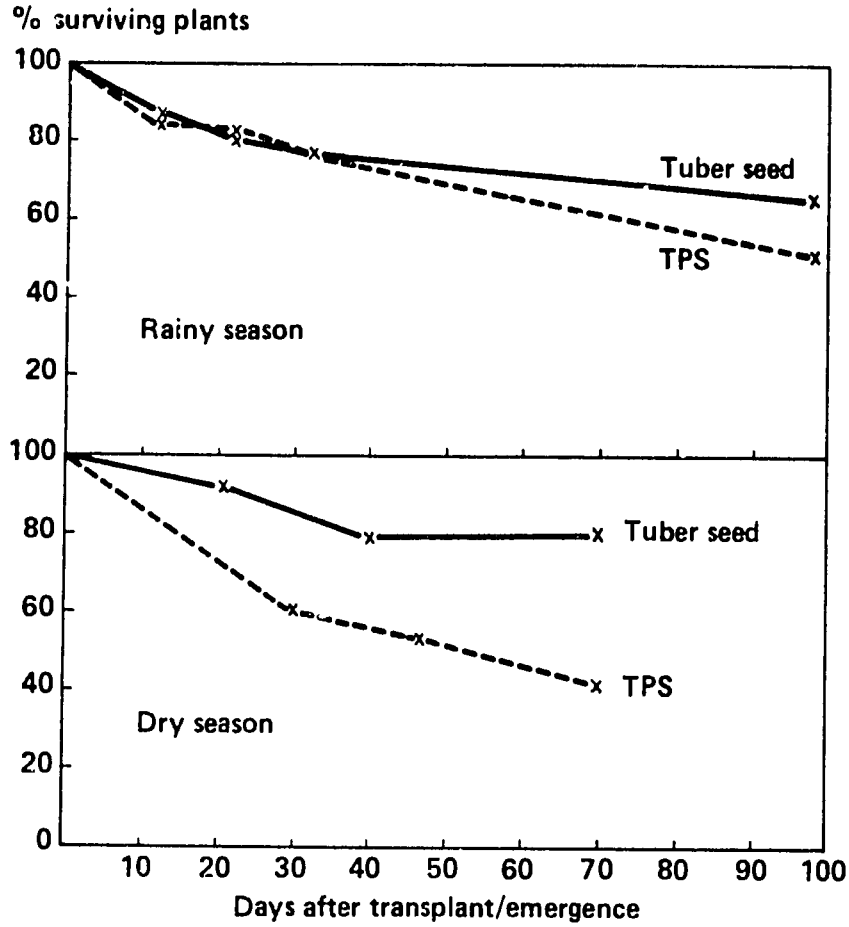


Table 2. Pathogens[a] isolated from wilting plants grown from TPS during the rainy season in San Ramon.

Field	No. Processed Plants	Isolation Frequency (%)				
		E	Ps	E+Ps	P	E+P
1	28	3.6	0	0	21.4	75.0[b]
2	23	-	47.8	-	-	-

[a] E = Erwinia, Ps = Pseudomonas, P = Pythium,

[b] enrichment medium was used for Erwinia isolation.

Table 3. Pathogens* isolated from wilting plants grown from seed tubers during the rainy season in San Ramon.

No. Processed Plants	Isolation Frequency (%)				
	E	Ps	F+Ps	F	Insects + others
82	41.5	32.9	4.9	2.4	11.0

* E = Erwinia, Ps = Pseudomonas, F = Fusarium

Table 4. Pathogens* isolated from wilting plants grown from TPS during the dry season in San Ramon.

Field	No. Processed Plants	Frequency of isolation (%)							
		E.	E+F	E+R	E+Ps	R	R+F	P	Insects
1	32	0	0	3.1	0	46.9	12.5	0	25.0
2	46	17.4	6.5	37.0	4.3	19.6	2.2	0	10.9
3	52	1.9	5.8	1.9	1.9	26.9	1.9	7.6	38.5
4	111	0	4.5	9.9	0	31.5	27.9	0	9.9

* E = Erwinia, F = Fusarium, R = Rhizoctonia, Ps = Pseudomonas

Table 5. Pathogens* isolated from wilting plants grown from seed tubers during the dry season in San Ramon.

Field	No. processed plants	Frequency of isolation (%)								
		E	F	R	E+R	E+F	R+F	E+Ps	P	I
1	84	35.7	7.1	11.9	7.1	13.1	2.4	0	6.0	13.1
2	44	56.8	0	0	20.5	6.8	0	6.8	2.3	0

*E = Erwinia; F = Fusarium; R = Rhizoctonia; Ps = Pseudomonas; P = Pythium; I = Insects

Table 6. Isolations from tuber soft rot at harvest time; 1985 dry season.

Organism isolated	% rotting tubers from which organism isolated*
Erwinia sp.	91.5
Fusarium sp.	13.7
Pythium sp.	21.4
Phytophthora erythroseptica	3.4

* 117 soft rot tubers tested.

Table 7. Influence of soil fungicide treatments, before transplanting, on the incidence of tuber soft rot at harvest time in crops grown from true potato seed in San Ramon

Pre-transplanting soil treatment	Weight of soft rot tubers per plant (g)	% Soft rot tubers
Control (No treatment)	14.3	4.24
PCNB	21.0	4.86
Metalaxyl	4.2	0.95
Copper Oxychloride	18.3	4.96
Tolclofos methyl	19.0	6.70
Benamyl	23.5	6.61
L.S.D. (p = 0.005)	12.0	3.99

Table 8. Isolation of pathogens[a] from rotted tubers stored for 4 months in San Ramon.

Season Harvested	Seed planted	No. processed tubers	Frequency of isolation (%)						
			E	F	P	E+F	Ps	Ps+F	Others
Rainy	Tubers	139	12.1	78.7	3.0	1.5	-	-	3.0
Dry	Tubers	71	5.6	85.5	1.4	2.8	-	-	1.4
Rainy	TPS	28	0.0	89.3	3.6	0.0	0.0	0.0	-
Dry	TPS	120	0.0	87.4	0.0	0.0	8.33	0.83	2.54

[a] E = Erwinia; F = Fusarium; P = Pythium; Ps = Pseudomonas.

Table 9. Incidence of potato tuber rotting during storage in San Ramon

Growing season	Storage period (months)	% tubers rotting (by weight)	
		Without PTM control	With PTM control
1983/84 (wet)	6	83.8	5.1
1984 (dry)	2	14.5	7.6
1984 (dry)	4	48.0	11.1
1984 (dry)	6	87.3	15.1
1984/85 (wet)	2	5.0	2.0
1984/85 (wet)	4	22.4	3.5
1984/85 (wet)	6	57.3	6.1

Figure 4.
Summary of principal organisms associated with pathological problems during the different stages of the potato in San Ramón

Dry season		Rainy season	
Tuber seed plants	TPS plants	Tuber seed plants	TPS plants
Field stands — Erwinia	Seedlings — Rhizoctonia, Pythium	Field stands — Erwinia, Pseudomonas	Seedlings — Rhizoctonia, Pythium
Harvest — Erwinia	Field transplants — Rhizoctonia, Insects	Harvest — Erwinia	Field transplants — Pythium, Pseudomonas, Rhizoctonia
Storage — Fusarium, PTM	Harvest — Erwinia*, Pythium	Storage — Fusarium*, PTM	Harvest — Erwinia
	Storage — Fusarium*, PTM		Storage — Fusarium*, PTM

* Indicate possible interaction

RESISTANCE TO BLACKLEG AND SOFT ROT AND ITS POTENTIAL USE IN BREEDING

Z. Huaman, L. de Lindo and J. Elphinstone

INTRODUCTION

Bacterial soft rot of tubers is becoming a serious problem not only in the cool, temperate but also in the warm, tropical potato-producing regions of the world. Rotting of stems (blackleg) and/or the tubers is caused by Erwinia carotovora pv. carotovora (Ecc) and pv. atroseptica (Eca), and Erwinia chrysantemi (Ech). The optimum temperature for the growth of these Erwinias have been reported to be at or above 27° C [12]. Therefore, in the tropics the incidence of the disease is greater because of the high temperatures and excess moisture in the soil which allow rapid multiplication of the bacteria.

The fact that at present there is no effective chemical control of the disease, makes difficult the reduction of the incidence of bacterial soft rot in the potato crop. Therefore, farmers depend on other non-chemical methods for control. In the long term, reliance must be placed on resistance breeding [25].

In this paper a revision is made of all the different components needed in breeding for blackleg and soft rot resistance.

SOURCES OF RESISTANCE

The identification of sources of resistance to any disease requires a reliable method for assessing the level of resistance in the plant. Many methods have been described to determine the susceptibility/resistance of potato cultivars to the Erwinias. Numerous sources of resistance to either or both blackleg and soft rot reported in the literature have been determined using different methods. Therefore, their parental value should be interpreted with caution because the method of evaluation has a significant effect on the intensity of symptom development and consequently in the degree of separation between resistant and susceptible genotypes [39]. More reliance should be

placed on those sources identified with inoculations of bacterial suspensions in the stems or the tubers since they have generally produced clear and consistent differences in blackleg or soft rot incidence [11,18].

European and North American potato cultivars reported as resistant to blackleg or soft rot caused by Eca or Ecc are listed in Table 1. As far as possible those cultivars with contradictory reaction in the literature have been excluded from the list.

Tubers of one cultivar of the native S. tuberosum ssp. tuberosum Chilean potato collection has been reported to produce resistance response when inoculated with either Ecc (strain 22) and Eca (strain 9) ([6].

Resistance of S. tuberosum ssp. andigena to Eca has been reported in a potato collection in the USRR. Some andigena cultivars also combined resistance of the tubers and stems [37]. Within the potato collection of native Andean cultivars maintained at the International Potato Center (CIP), sources of resistance to Erwinia chrysanthemi (Ech) have also been identified in andigena and andigena x tuberosum cultivars by infectivity titration inoculation of tubers with concentrations of 4.4 x 10^6, 8.8 x 10^5 and 1.76 x 10^5 cells/cc [11].

Soft rot resistance has also been found in diploid populations involving S. stenotomum, S. goniocalyx and S. phureja developed at North Carolina State University in collaboration with CIP. Some of these sources produced also unreduced gametes which facilitated the production of tetraploid offspring by 4x x 2x crosses. Some 4x clones that combined high resistance to both soft rot and blackleg were selected [19].

Search for sources of resistance has recently been made at CIP among 76 hybrid progenies involving tuberosum, andigena adapted to long photoperiods (neo-tuberosum) and phureja. These progenies combine adaptation to the lowland tropics, earliness and high yields. From 6,840 genotypes evaluated, 308 clones where selected which had no rotten tubers at harvest in a naturally infected field with Ech and Ecc at CIP's mid-elevation tropics in San Ramon, Peru. Ten to 30 tubers per each clone were planted again in the field to enhance expression of latent infection in the tubers.

Table 1 - European and North American potato cultivars reported as resistant to blackleg or soft rot caused by *Erwinia carotovora* pv. *carotovora* or pv. *atroseptica*

Cultivar	(Reference)	Cultivar	(Reference)
Adretta	[15, 21]	Nova	[40]
Ambassador*	[4, 20]	Pentland Crown*	[18, 23, 24, 25, 29, 34]
Belorusskii Rannii*	[4]	Pentland Dell*	[18, 24, 34]
Butte	[31]	Pentland Envoy	[42]
Cajka	[8]	Pentland Squire*	[18, 25, 34]
Cara	[24, 29, 34]	Pola	[15]
Carnea	[36]	Polonia	[40]
Croft	[34]	Priobskii	[36]
Dorshta	[20]	Rektor	[20]
Drayton	[25]	Record*	[3, 25]
Epoka*	[4]	Rod 1008	[20]
Fecula	[20]	Roslin Castle	[36]
Flava*	[7, 40]	Royal Kidney	[42]
Fridolin	[42]	Russet Burbank	[31]
Giewont	[27]	Saphir*	[7, 8, 40]
Hasia	[42]	Sickingen*	[7, 40]
Home Guard	[25]	Sirius	[40]
Humalda	[20]	Siskin	[20]
Kameraz	[36]	Smothi Rural	[42]
Ker Pondy	[42]	Sokol	[15]
Koretta	[21]	Sputnik	[36]
Krasava	[8]	Stormont Enterprise	[3, 25]
Laverta*	[7]	Tempo	[36]
Lekkerlander	[20]	Tourist	[36]
Linzenhof	[36]	Ultimus	[42]
Loshitskii*	[4, 36]	Vanessa	[34]
Maris Piper*	[18, 23, 24, 34]	Varmas*	[4]
Maritta*	[32]	Vltava	[20]
Mars	[42]	Wanda	[20]
Norgold Russet	[31]		

* Indicates resistance to blackleg

During plant development, numerous plants did not emerge because the mother tubers were rotten, others died soon after emergence or showed blackleg symptoms. At harvest, 107 clones were selected and their tubers were incubated in an anaerobic environment at 26° C for six days. Clones that produced none or very low percentage of rotten tubers by natural infection were inoculated with Ech by vacuum infiltration of the lenticels with a suspension of 4.4×10^6 cells/cc. Again great differences were obtained among clones. Finally, tubers of all promising clones were tested by inoculation with a suspension of Ech at a concentration of 8.8×10^5 cell./cc. So far, 19 clones with no blackleg symptoms, 9 clones with some resistance to soft rot and 10 clones that combine both desirable traits have been selected. Crosses in different combinations have been made for the next cycle of selection and genetical studies.

High levels of resistance to blackleg has been reported in wild potato species such as S. chacoense (synonym of S. boergeri, S. horovitzii, S. subtilius, S. garciae, S. laplaticum, and S. schickii) and S. stoloniferum (synonym S. antipovitzii) [10]. Among cultivated potato species S. phureja comprised cultivars with high levels of resistance to blackleg followed by andigena cultivars from Peru, Argentina and Colombia [22].

It appears that the levels of resistance to either or both soft rot and blackleg in some of these sources might not be high. This is particularly true in those European cultivars that are very closely related and therefore, it was more difficult to detect clear cut differences between those considered resistant and susceptible genotypes. More reliance should be placed on those sources that are identified in potato germplasm that includes a broader genetic base. Thus, Bushkova [5] reported that higher frequency of resistant genotypes to inoculation of stems and tubers have been found in andigena (57.9% of tested clones) and S. chacoense (25.5%). Relatively few resistant genotypes (12.5%) were found among inter-specific hybrids and virtually none among tuberosum cultivars.

FACTORS OF RESISTANCE

There appear to be several mechanisms of resistance to blackleg and soft rot and several correlations between resistance and other factors have been reported. However,

many of these factors are either strongly influenced by the environment or their inheritance has not been determined.

Since the rotted mother tuber is assumed to be the main source of contamination, several tuber characters have been studied to determine their role in resisting rotting. The following have been reported as the most important relationships:

1. Resistance to tuber rot caused by Eca appear to be correlated with dry matter content. Resistant genotypes combine high dry matter content with low levels of electrolyte leakage, total sugars, and reducing sugars in the tubers [17].

2. Resistance to Eca decay in the tubers has been associated with high calcium content in the medullary and periderm tissue [31,38].

3. A host-mediated response affecting pectic enzyme substrate or activity rather than direct inhibition of bacterial growth may be involved in resistance of potato tubers to bacterial soft rot under aerobic conditions [30].

4. The resistance to bacterial soft rot increases with the increasing resistance of the tuber flesh to mechanical damage [42].

5. The lenticels on potato tubers are possibly the entry points for several pathogens. Environmental factors such as high temperatures and wet growing conditions favours their proliferation thus increasing the susceptibility to infection by Erwinia. Furthermore, the readiness with which lenticels proliferate varies with tuber age and possibly cultivar [1]. However, the differential responses to infection or penetration of the potato cultivars studied showed that lenticel infection occurs principally on growing tubers and that all the differences in resistance cannot be attributed to a single anatomical factor [2].

6. Phenolics of potato tubers such as chlorogenic, caffeic and ferulic acid, and terpenoid phytoalexins such as rishitin and phytuberin, have been shown to have an antibacterial action against Eca [14].

INHERITANCE OF RESISTANCE

Breeding for resistance to blackleg has been undertaken for several years. Thus, Dobias [7] in 1977 reported that selection for resistance to blackleg caused by Eca should be effective and that studies on combining ability were necessary. He obtained higher percentages of resistant F1 plants in progenies of resistant x resistant and resistant x susceptible crosses than in susceptible x susceptible crosses. More recently in 1984, Munzert [33] pointed out that although a reliable method for testing was not available, selection for resistance is worthwhile especially because the correlation between blackleg and the proportion of totally rotten tubers is r=0.57. However, in the same year Wastie and Mackay [39] did not share that optimism. They considered that "as yet no-one would claim any success in attempts to improve the level of resistance to blackleg, or that, with currently available techniques for detecting resistance, such attempts were worth making".

Pfeffer et. al. [35] in 1974 suggested that the relative resistance to soft rot in the tubers could be raised by resistance breeding because they found sufficient genetic variation in reaction to soft rot.

Progeny tests using open pollinated seeds obtained from some *andigena* cultivars from CIP's potato collection which reacted as resistant to soft rot caused by Ech have recently been made at CIP. The results are shown in Table 2 and indicate that this resistance is transmitted to the offspring. Some progenies showed various levels of resistance. The range of genotypes with resistance was from 8 to 41% depending on the resistance level of the parents. Higher levels of resistance (up to 84%) in progenies from self-pollinations made in some of the same *andigena* cultivars have been reported by Hidalgo and Echandi [16]. Furthermore, within these progenies of *andigena*, high levels of resistance to dry rot caused by *Fusarium oxysporum* and *F. solani* have also been found (B. Tivoli, personal communication). Some genotypes were resistant to both *Erwinia* soft rot and *Fusarium* dry rot. Crosses in different combinations have been made for genetic studies and their use in breeding. Some of these *andigena* clones resistant to Ech have also been tested to Ecc and Eca. Preliminary results shown in Table 3 indicates that two of them might be

resistant to the three Erwinias and six to combinations of two of them [13]. It is important to note that infectivity titration inoculations showed Ech most aggressive followed by Ecc and Eca [28].

Table 2 - Percentage of resistant genotypes to soft rot caused by *Erwinia chrysantemi* obtained from open pollinated progenies of resistant *andigena* cultivars. (H = highly, M = moderately).

CIP Number	Percentage of Resistant Genotypes			
	RH	R	RM	Total
700532 (=JAK72)	0	23	61	84*
702440	0	17	38	55*
700031 (=OCH5708)	3	10	28	41
700031 (=700718)	0	11	29	40*
700114 (=JAK109)	0	6	19	25
702734 (=CUP48)	0	7	17	24
700726	0	7	17	24
700729 (=OCH6400)	0	0	20	20
703855 (=OCH6114)	0	3	15	18
703660 (=JAK113)	2	4	12	18
701488	0	0	17	17
702870	0	3	12	15
700614	0	0	14	14
703861 (=OCH6175)	0	0	13	13
704193 (=OCH5331)	0	0	11	11*
703264 (=OCH6112)	0	0	8	8

* self-pollinated families [16]

Table 3 - Reaction of *Solanum tuberosum* ssp. *andigena* cultivars previously selected for resistance to *Erwinia chrysanthemi* to inoculation of tubers with the three Erwinias. (R=resistant, S=susceptible).

CIP Number	*Erwinia chrysanthemi*	*E. carotovora* pv. *carotovora*	*E. carotovora* pv. *atroseptica*
700532 (=JAK72)	R	R	R
703667 (=JAK228)	R	R	R
700575 (=JAK119)	R	R	S
703855 (=OCH6114)	R	R	S
702535	R	R	S
704194 (=OCH10032)	R	R	S
700953 (=701026)	R	S	R
701131 (=HUA560)	R	S	R

Lellbach [26] made diallel crosses with three cultivars and found that general combining ability was the most important determinant of resistance. He suggested that resistance to soft rot was controlled by minor gene effects whose additive action would allow resistance levels to be increased. He also considered that the influence of the environment was very high. However, these results should be taken only as a reference because a diallel with only three parents may be insufficient to make any sound inference.

Zadina and Dobias [41] have reported a positive correlation (r=0.647) between resistances to soft rot and blackleg caused by Eca. The difference in average degree of resistance between single progenies from selfings of resistant and susceptible cultivars and from hybridization of resistant with resistant, susceptible with susceptible and resistant with susceptible was highly significant. These results suggest that there is heritable variation for combined resistance. Dobias [9], later on confirmed previous reports and reported that the resistance to both blackleg and soft rot is transmitted to the progeny and it can be expected in combinations of crosses between resistant parents to each of the diseases. He also considered that cultivars that are resistant to only one of the diseases are not suitable as starting material for breeding for resistance to both diseases because the hybrids show different reactions to each one of the diseases.

BREEDING FOR RESISTANCE

Most of the pre-requisites to develop resistant cultivars to blackleg and soft rot caused by the Erwinias are at present available. Different sources of resistance to the disease have been found within the potato genetic resources. There is enough evidence that this resistance is genetically inherited. Although no significant correlation between resistance to blackleg and soft rot have been found among European potato cultivars, the positive correlations so far reported (r=0.57 [33], r=0.647 [41]) indicate that it should not be difficult to select genotypes that combine both resistances. Therefore, we propose that selection pressure should be applied to discard the most susceptible genotypes to stem blackleg and then select for tuber soft rot resistance among survivors.

We coincide with Wastie and Mackay [38] in that what is lacking is a reliable screening method to identify effectively resistant and susceptible genotypes among segregating populations. A seedling test that produces blackleg symptoms in the most susceptible genotypes and that allows to save the remaining genotypes to be tested in the field could be extremely useful. An in-vitro test for screening of potato seedlings to Erwinias is under development at CIP. Blackleg symptoms have already been obtained in young seedlings.

Although our breeding program for resistance to the Erwinias is in its early stages of development, we consider that progress is quite feasible especially if the sources of resistance to this disease come from a broad genetic base.

Our breeding plans include:

1. The assembling of sources of resistance to blackleg and soft rot reported in the literature to complement those found within genetic resources available at CIP.

2. The re-evaluation under the same environment and using the same method of evaluation of all sources obtained from outside CIP.

3. Progeny tests of parental materials to select those that transmit the highest levels of resistance in high frequencies.

4. Studies of inheritance of resistance to soft rot and blackleg.

5. The development of lowland tropic adapted populations from parental materials with resistance to blackleg, soft rot or a combination of both, selected among advanced materials adapted to that environment. These populations will also include other sources from tuberosum, neo-tuberosum, and advanced diploid materials with adaptation to long photoperiods and production of unreduced gametes.

6. Development of a highland population based on sources of resistance found in andigena. Crosses with resistant genotypes from the lowland population will be made to improve agronomic traits and to incorporate adaptation to the lowlands.

REFERENCES

[1] Adams, M. J. 1975. Potato tuber lenticels: Development and structure. Ann. Appl. Biol. 79:265-273.

[2] Adams, M. J. 1975. Potato tuber lenticels: susceptibility to infection by Erwinia carotovora var atroseptica and Phytophthora infestans. Ann. Appl. Biol. 79:275-282.

[3] Bourne, W. F., D. C. McCalmont and R. L. Wastie. 1981. Assessing potato tubers for susceptibility to bacterial soft rot (Erwinia carotovora var atroseptica). Potato research 24:409-415.

[4] Bushkova, L. N. 1983. (Resistance of potato varieties to the blackleg pathogen.) In: Potato Abstracts 1985, 10(3):26.

[5] Bushkova, L. N. and N. M. Vlasov .1983. (Resistance of potato to Erwinia atroseptica.) Kartofel'i Ovoshchi 11:17. In: Potato Abstracts 1986, 11(5):70.

[6] Ciampi, L. and N. Andrade-Soto. 1984. Preliminary evaluation of bacterial soft rot resistance in native Chilean potato clones. Am. Pot. J. 61(2):109-112.

[7] Dobias, K. 1977. (The possibility of breeding potatoes for resistance to blackleg Erwinia carotovora). Rostlinna Vyroba 23(3):255-260. In: Potato Abstracts, 1978 3(1):1.

[8] Dobias, K. 1977. (Artificial infection tests for Erwinia carotovora resistance in potatoes). Ochrana Rostlin 13(2):101-106. In: Potato Abstracts 1978 3(3):63.

[9] Dobias, K. 1977. (The relationship between resistance to blackleg and tuber wet rot in progenies from crosses of potato varieties which differ in resistance). Ved. Pr. Vyzk. Slechtitelskeho Ustavu Bramborarskeho v Havlickove Brode 6:75-83. In: Potato Abstracts 1978. 3(4):75.

[10] Dobias, K. 1978. (Resistance of some potato species to blackleg). Ved. Pr. Vyzk. Slechtitelskeho Ustavu Bramborarskeho v Havlickove Brode 7:53-61. In: Potato Abstracts 1981. 6(4):66.

[11] French, E. R. and L. de Lindo. 1979. The Erwinias of potatoes in Peru. In: Report Planning Conference on Developments in Control of Potato Bacterial Diseases, International Potato Center, p. 88-93.)

[12] French, E. R. and L. de Lindo. 1980. Resistance to Erwinia chrysanthemi in tubers of Solanum tuberosum ssp. andigena. Fitopatología 15(1):34.

[13] French, E. R. and L. de Lindo. 1985. Sources of resistance in tuberiferous Solanum spp. to soft rot by Erwinias. In: Report of the International Conference on Potato Blackleg Disease held at the Royal Society of Edinburgh. (Ed. D. C. Graham and M. D. Morrison) p 79-80.

[14] Ghanekar, A. S., S. R. Padwal-Desai and G.B. Nadkarni. 1984. The involvement of phenolics and phytoalexins in resistance of potato to soft rot. Potato Research 27:189-199.

[15] Gotz, J., H. Henniger, E.Ratuszniak and J. Komorowska-Jedrys. 1984. (Joint inoculation with Erwinia carotovora and Fusarium species, taking wound reaction into account in the determination of rot resistance in potato varieties). Ziemniak 1983/84,19-32. In: Potato Abstracts, 1985. 10(8):124.

[16] Hidalgo, O. A. and E. Echandi. 1982. Evaluation of potato clones for resistance to tuber and stem rot induced by Erwinia chrysantemi. Am. Pot. J. 59(12):585-592.

[17] Hidalgo, O. A. and E. Echandi. 1983. Influence of temperature and length of storage on resistance of potato to tuber rot induced by Erwinia chrysanthemi. Am. Pot. J. 60(1):1-15.

[18] Hossain, M. and C. Logan. 1983. A comparison of inoculation methods for determining potato cultivar reaction to blackleg. Ann. Appl. Biol. 103:63-70.

[19] International Potato Center. 1984. Annual Report CIP. 1983. Lima, Peru. p.47.

[20] Kiselev, E. P. and A. K. Novoselov. 1974. (The resistance of potato varieties to bacterial rot of the tubers in the Primor'e region). Nauch. tr. NII kartof. kh-va 20:121-124. In: Potato Abstracts 1977 2(6):79.

[21] Kleinhempel, D. and J. Gotz. 1984. Problems in assessing susceptibility of potato varieties to tuber soft rot. Abstracts 9th Triennial Conference EAPR: 386-387.

[22] Koromyslova, M. I. 1972. (Initial material for breeding potatoes for resistance to blackleg). Byulleten Vsesoyuznogo Ordena Lenina Instituta Rastenievodsta Imeni N.I. Vavilova 22:35-38.

[23] Lapwood, D. H., P. R. Legg. 1983. The effect of Erwinia carotovora subsp. atroseptica (blackleg) on potato plants. I. Growth and yield of different cultivars. Annals of Applied Biology 103 (1):71-78.

[24] Lapwood, D. H., P. J. Read and P. T. Gans. 1985. The field susceptibility of potato varieties to blackleg caused by Erwinia carotovora subsp. atroseptica. Jour. Nat. Inst. Agr. Botany 17(1):107-116.

[25] Lapwood, D. H., P. J. Read and J. Spokes. 1984. Methods for assessing the susceptibility of potato tubers of different cultivars to rotting by Erwinia carotovora subspecies atroseptica and carotovora. Plant Pathology 33:13-20.

[26] Lellbach, H. 1978. (Estimating genetic parameters derived from diallel crosses in cases of susceptibility to soft rot in the potato). Archiv fur Zuchtungsforschung 8:193-199. In: Potato Abstracts 1979, 4(6):97.

[27] Lewosz, W. 1981/1982. (Factors which affect the development and virulence of Erwinia carotovora var atroseptica and methods for the detection of the agents of soft rot in potato tubers). In: Ziemniak Bonin, Poland; Instytut Ziemniaka 161-184. In: Potato Abstracts 1983, 8(12):219.

[28] Lindo, L. de y E. R. French. 1981. Erwinias que afectan papa en los trópicos húmedos en el Peru. Fitopatología 16:69-74)

[29] Logan, C. and L. R.Cooke. 1984. Potato diseases. In: Annual Report on Research and Technical Work of the Department of Agriculture for Northern Ireland 1983.

[30] Maher, E. A. and A. Kelman. 1981. Relationship of oxygen status of potato tubers to pecticenzyme maceration of tissue. In: 5th Int. Conf. Plant Pathology Bacteria. CIAT, Cali-Colombia. (Ed. Lozano J.C.).

[31] McGuire, R. G. and A. Kelman. 1983. Susceptibility of potato cultivars to Erwinia soft rot. Phytopathology 73:809 (Abstr.).

[32] Munzert, M. 1975. A method of testing the resistance of the potato plant to the blackleg pathogen (Erwinia carotovora var atroseptica (van Hall) Dye). Potato research 18:308-313.

[33] Munzert, M. 1984. (Breeding chances for improvement of resistance to blackleg (Erwinia atroseptica). Abstracts 9th Triennial Conference EAPR, p.384.

[34] Pérombelon, M. C. M., R. Lowe, L. J. Hyman, V. M. Lumb, J. G. Elphinstone, R. A. Fox and E. P. Dashwood. 1984. Epidemiology and etiology of fungal and bacterial diseases of the growing potato plant. In: Report of the Scottish Crop Research Institute, 1983. Invergowrie, Dundee, U. K.: 99-106

[35] Pfeffer, C., H. Lellbach, B. Pett and K. Schuler. 1975. (Some problems of breeding for resistance to the most important storage diseases of potato). Ziemniak 1974, 39-59.

[36] Serdyuk, L. S. and B. N. Dorozhkin. 1980. (Evaluation of varieties and forms of potato for resistance to Erwinia atroseptica.) Kartofel'i Ovoshchi 3:14-15. In: Potato Abstracts 5(9):163.

[37] Tomchuk, N. G., S. I. Bel'skaya and I.V. Generalova. 1978. (Selection of initial forms for resistance to blackleg).Kartofel'i Ovoshchi 3,39. In: Potato Abstracts 3(8):146.

[38] Tzeng, K. C., A. Kelman, K. E. Simmons and K. A. Kelling. 1985. Relation of calcium content in potato tuber periderm to bacterial soft rot susceptibility, internal brown spot and subapical necrosis of sprouts. APS Abstracts No.801, Annual Meeting. Reno, Nevada.

[39] Wastie, R. L. and G. R. Mackay. 1985. Breeding for resistance to blackleg: the present and the future. In: Report of the International Conference on Potato Blackleg Disease held at the Royal Society of Edinburgh. (Ed. D.C. Graham and M.D. Morrison) p 75-77.

[40] Zadina, J. 1974. (Breeding potato for resistance to storage diseases in the Czechoslovakian SSR.) In Ziemniak. Bonin,Poland; Instytut Ziemniaka 60-61. In: Potato Abstracts, 1976 1(1-3):11.

[41] Zadina, J. and K. Dobias. 1976. (Possibilities of breeding for resistance to tuber rot in potatoes.) In: Potato Abstracts 1977, 2(8):120.

[42] Zadina, J., K.Dobias and V. Horackova. 1983. (Potato resistance to Fusarium dry rot and bacterial soft rot in relation to tuber resistance to mechanical damage). Ved. Pr. Vyzk. Slechtitelskeho Ustavu Bramborarskeho v Havlickove Brode 9:135-151. In: Potato Abstracts, 1984 9(7):105.

Planning Conference

on

Bacterial Diseases of the Potato

A G E N D A

March 16, MONDAY

14:00 - Tour of CIP facilities

March 17, TUESDAY

8:00 - Inaugural comments
Richard L. Sawyer - Director General

8:15 - Statement of objectives
Peter Gregory - Director of Research

FIRST TOPIC. Bacterial wilt caused by Pseudomonas solanacearum.

Chairperson, morning session: Kenneth Brown

8:30 - Mass screening procedures for resistance to P. solanacearum.

Ed French and Ursula Nydegger

9:00 - Breeding for resistance to P. solanacearum.
Peter Schmiediche

9:30 - Additional sources of resistance to bacterial wilt.
Ed French and Luis Sequeira

10:00 - Break

10:30 Role of nematodes in disease expression by P. solanacearum and strategies for screening and breeding for combined resistance.
Parviz Jatala, Carlos Martin and Humberto Mendoza.

10:50 - Effect of breeding as a function of screening procedures. Humberto Mendoza

11:10 - Discussion

12:30 - Lunch

Chairperson, afternoon sessions: Primo Accatino

13:30 - Producing potatoes in P. solanacearum infested sugarcane cropping land. Jean Claude Autrey

13:50 - Soil survival of P. solanacearum S. Nisanka de S. Seneviratne

14:10 - Latent infection in stems and tubers of potatoes and in symptomless hosts. Gustavo Granada

14:30 - Break

15:00 - Field evaluation of clones bred for resistance to P. solanacearum. Ed French

15:20 - Discussion till about 17:00 hours.

March 18, WEDNESDAY

Chairperson, morning session: Luis Sequeira

8:00 - Classification and identification of P. solanacearum. A. Chris Hayward

8:40 - The potential of controlling bacterial diseases by gene insertion. John Dodds and Peter Schmiediche

9:00 - Strategies for bacterial wilt control. Ed French

9:30 - Break

10:00 - Discussion

11:00 - Discussion on direction of future research on *P. solanacearum*.

12:30 - Lunch

13:30 - Discussion continues till about 17:00 hours.

SECOND TOPIC: Bacterial Soft Rots and Interacting Fungi

Chairperson, morning session: Humberto Mendoza

8:00 - Ecology of the Erwinias causing stem and tuber rots.
Michel Pérombelon

8:30 - Soft rot and blackleg in warm climates.
Oscar Hidalgo

8:50 - Control of Erwinia diseases in San Ramon.
Liliam de Lindo and John Elphinstone

9:10 - Methods of control of Erwinia diseases of the potato.
John Elphinstone

9:30 - Discussion

10:00 - Break

10:30 - Importance of interaction of Erwinias and other pathogens and pests in the warm climate of San Ramon.
Víctor Otazú, Hebert Torres, John Elphinstone

11:00 - Discussion

12:15 - Lunch

Chairperson, afternoon session: Arthur Kelman

14:00 - Resistance to blackleg and soft rot and its potential use in breeding.
Zósimo Huamán and Liliam de Lindo

14:30 - Discussion on direction of future research on the Erwinias.

March 20, FRIDAY

8:00 - A committee of four will work during the morning, drawing up recommendations.

14:00 - Discussion, modification and approval of the recommendations

PARTICIPANTS

Invited

Jean Claude Autrey
Leopoldo Fucikovsky
Gustavo Granada
A. Chris Hayward
Arthur Kelman
Carlos A. Lopes
Michel Pérombelon
S. Nisanka de S. Seneviratne

CIP

Directors

Richard L. Sawyer
José Valle Riestra
Peter Gregory
Kenneth Brown
Primo Accatino

Staff

John Elphinstone
Edward French
Anton Haverkort
Oscar Hidalgo
Zósimo Huamán
Parviz Jatala
Jeroen Kloos
Liliam de Lindo
Carlos Martin
Humberto Mendoza
Ursula Nydegger
Víctor Otazú
Michael Potts
Hebert Torres
Peter Schmiediche