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Potato Bacterial Diseases

Report of a Planning Conference

Held at
International Potato Center
Apartado 5969 — Lima, Peru
June 12-15, 1979

Participants in the Planning Conference on Developments in the Control of Bacterial Diseases of Potatoes held at the International Potato Center, Lima, Peru, June 12-15, 1979. (Left to right): M. Jackson, P. Jatala, M. Perombelon, A. Kelman, E. French, L. Sequeira, L. Fucikovsky, O. Page, H. Mendoza, G. Granada, C. Martin, L. Cartin.

COVER -- A single row of Molinera potatoes growing in a field of Mariva variety as a farmer's trial on the Peruvian highlands. Molinera is a bacterial wilt and late blight resistant variety released in 1977 by the Peruvian Potato Program. (Photograph by Dr. V. Moreno, Universidad Nacional Agraria, La Molina).

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INTERNATIONAL POTATO CENTER

REPORT OF THE

PLANNING CONFERENCE

ON THE

DEVELOPMENTS IN THE CONTROL

OF

BACTERIAL DISEASES OF POTATOES

Held at CIP - Lima, Peru
June 12-15, 1979

F O R E W O R D

This is the eighteenth Planning Conference sponsored by the the International Potato Center (CIP) to develop recommendations to serve as guidelines for CIP's research. Recommendations are projected for a five-year period although Planning Conference relating to a particular area of research are held every three years.

During the Planning Conference particular emphasis was placed on bacterial wilt caused by *Pseudomonas solanacearum*, the most serious bacterial disease of potatoes in the lowland tropics. Attention was also focused on two soft rotting genera of bacteria, *Erwinia* and *Clostridium*, of potential importance under certain conditions in the tropics. A review paper on *Corymbacterium sepedonicum* is included in the Report.

O. T. Page

June 1979

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International Potato Center

PLANNING CONFERENCE ON THE DEVELOPMENTS IN THE CONTROL OF BACTERIAL DISEASES OF POTATOES

AGENDA

Tuesday, June 12

	Chairman, morning session. O.T. Page
08:30	Welcome and opening remarks. R.L. Sawyer, Director General
09:00	Tour of CIP's research facilities. C. Martin
10:00	Break
10:15	"Present and Future Status of Bacterial Diseases of Potatoes in the Lowland Tropics." A. Kelman
	REVIEW OF PROGRESS IN BACTERIAL WILT RESEARCH
11:15	"Classification, Distribution and Origin of *Pseudomonas solanacearum*." E. R. French
11:45	Discussion: "A Practical Classification Scheme for *Pseudomonas solanacearum*." Leader: E.R. French
12:00	Lunch

Tuesday afternoon

	Chairman, afternoon session. H. Mendoza
13:30	"Methods of Diagnosing *Pseudomonas solanacearum*." L. Fucikovsky
14:00	"Sources of Resistance to *Pseudomonas solanacearum*." C. Martin
14:30	Break

Tuesday afternoon (continued)

14:45	"Development of Resistance to Bacterial Wilt Derived from *Solanum phureja*." L. Sequeira
15:15	Discussion: "General vs. Specific Resistance Breeding." Leader: H. Mendoza

Wednesday, June 13

	Chairman, morning session. L. Sequeira
08:30	"Role of Indigenous Vegetation and Crops in Persistence of *Pseudomonas solanacearum*." C. Martin
09:00	"Persistence of *Pseudomonas solanacearum* in an Inceptisol in Costa Rica". M.T. Jackson and L.C. González
09:30	"Progress in the Integrated Control of Bacterial Wilt." E.R. French
10:00	Break
10:20	"*Pseudomonas* - *Meloidogyne* Interactions." P. Jatala
10:40	"Problems in the Utilization of Resistance by Developing Country Potato Programs: The Colombian Example." G.A. Granada
11:00	Discussion: "Preliminary Recommendations for Research Directed toward the Control of Bacterial Wilt: a) Breeding for Resistance b) Cultural Practices c) Pathovar Identification Leader: O.T. Page
12:15	Lunch

Wednesday afternoon

	Chairman, afternoon session. K.J. Brown

Wednesday afternoon (continued)

ERWINIS DISEASES OF POTATOES

13:30	"The Erwinias of Potatoes in Peru." E. R. French and L. de Lindo
14:00	"Ecology of Soft Rot in Relation to Potatoes." M.C.M. Pérombelon
15:00	Break
15:15	"The Importance of Bacteria in Potato Storage." R.H. Booth
15:30	Discussion: "Future Emphasis in Soft Rot and Blackleg Research." Leader: K.J. Brown

Thursday, June 14

Chairman, morning session.
R. Wurster

OTHER BACTERIAL DISEASES OF POTATOES

08:30	"The Role of Clostridia in Bacterial Soft Rot of Potato." A. Kelman
09:00	"The Importance of *Corynebacterium sepedonicum* to Potato Production in the Developing World." M. Velupillai
09:30	Discussion: "Status of Distribution of Bacterial Wilt Resistant Clones to CIP Regions." Leader: K.J. Brown
10:15	Break
10:30	Discussion: "Relative Emphasis that CIP Should Give Different Potato Pathogenic Bacteria." Leader: L. Sequeira
12:00	Lunch

Thursday afternoon

FORMULATION OF RECOMMENDATIONS

A Committee of three will formulate recommendations arising from presentations and discussions during the first three days of the Conference.

Participants not involved in developing recommendations will have the opportunity for discussions with CIP staff or visiting other CIP facilities.

Friday, June 15

	Chairman O.T. Page
10:00	Discussion and approval of recommendations.

RECOMMENDATIONS

BACTERIAL WILT

It is the general feeling of this Committee that bacterial wilt will continue to be the major bacterial disease of potato in developing countries. As the range of the potato is extended to the humid, lowland tropics, bacterial wilt will become increasingly important.

Although considerable progress has been made in the development of potato clones with resistance to bacterial wilt, numerous problems remain. To resolve some of these problems, this Committee recommends that: a) the study of this disease and its control retain high priority within CIP's plant pathology program, and b) the following measures be implemented in the near future:

1. Breeding for Disease Resistance

CIP (PERU)

A. That emphasis should be continued on the identification and utilization of additional genes for disease resistance, such as those from *Solanum sparsipilum*, which could be combined with those from *S. phureja*. Because resistance from these sources does not appear to be of general nature, it is important that as many resistance genes from several wild species be accumulated in parental lines as is possible by appropriate crossing procedures. Breeding procedures must consider resistance to nematodes, late blight and viruses as well. We must emphasize, also, the continuing need for more extensive screening procedures that would insure the identification of progeny with the highest level of resistance.

B. A more extensive effort must be made to utilize the resistance genes available in clones of *S. phureja*, such as those identified at the University of Wisconsin from the collection at Sturgeon Bay,

or in clones such as 1386.15, which have a a very wide range of resistance but have not been utilized fully in breeding programs. Emphasis should be continued in the screening of S. phureja collections from those geographic areas where the bacterium is present in the soil.

WISCONSIN

A. It is imperative that methods of screening for disease resistance be improved. Present screening procedures appear cumbersome and inadequate to process a substantial number of hybrid progeny without the danger of bacterial carry-over via latent infections. In particular, the interaction of inoculum concentration & temperature must be established with accuracy.

B. Quantitative evaluations of the resistance of selected clones to different strains should be made. Modern quantitative techniques, such as infectivity titrations, must be applied to obtain a critical evaluation of the resistance of different clones. Comparative evaluations are possible only under highly controlled environments.

CIP REGIONAL PROGRAMS

A. Adequate testing sites must be established in different regions. The most valuable component of a breeding program is a site where materials can be tested rigorously under natural inoculum in the field. It is apparent that site selection and maintenance have been recurrent problems that must be given attention; very few sites are available and, in some, nematode infestation and other problems prevent adequate selection.

B. Adequate amounts of clean seed of resistant clones must be made available to cooperating countries. The experience of the past 10 years indicates that small numbers of tubers cannot be tested adequately, or increased without viral contamination, in most countries. We support present efforts to provide clean seed to redistribution centers. Adequate indexing procedures for bacterial and viral latent infections should be made available to these centers.

2. Strain Selection and Identification

CIP (PERU) — Improved procedures should be used for selecting strains of Pseudomonas solanacearum for screening purposes. Emphasis should be given to infectivity titrations for the selection of the most virulent isolates on a quantitative basis. The use of mixtures is encouraged but adequate criteria must be established for the use of different cell concentrations of strains from different geographical locations.

WISCONSIN

A. Serological procedures should be developed for identification of strains of P. solanacearum. Greater emphasis must be placed on the use of different antigens for the preparation of specific antisera that may allow rapid identifications of unknown pathogenic strains.

B. The use of clones of S. phureja as differentials for strain identification should be explored. Initial results suggest that this method may provide a convenient means for strain identification.

3. Integrated Control

CIP REGIONAL PROGRAMS

A. The Conference endorse the concept that the most effective means for control of bacterial wilt is a combination of adequate levels of resistance and improved cultural practices. The present program at Turrialba (Costa Rica) is promising. Additional emphasis should be given to field experimentation with different methods of crop rotation, weed control, and mulching materials. Other factors to be considered are the control of soil moisture and soil temperature, at locations where such work is feasible.

B. Cultural practices for integrated control should be designed for the small farmer. It is evident that procedures such as deep plowing or the use of herbicides may not be applicable under subsistence farming. Emphasis should be given to alternative, simpler procedures.

WISCONSIN

A. The possible survival of P. solanacearum on roots of weed plants should be studied in detail. This objective is entirely dependent on the development of adequate procedures (selective media, enrichment media, immunofluorescence, etc.) for detection of P. solanacearum in rhizosphere soil.

4. Nature of Disease Resistance

WISCONSIN

Changes in content of agglutinins (lectins) in resistant clones, following infection, should be studied in detail. Although concentrations of lectins in potato plants, prior to infection, do not appear to be correlated with resistance to bacterial wilt, there is a possibility that concentrations of these substances do change after infections. These changes should be explored in an effort to explain the inability of virulent strains to multiply extensively in resistant plants.

E R W I N I A

At present knowledge is very limited on the biology, ecology and epidemiology of the Erwinia species that attack potato in both the lowland and highland tropics. Although research on these pathogens in the temperate zones of potato production has provided a depth of information on certain aspects of transmission and soil survival and many useful techniques, most of these procedures have not been applied to obtain a better understanding of the diseases caused by these bacteria in developing countries of the world. In a recent review of the literature on bacterial soft rot of potatoes with a bibliography of over 210 publications (Lund, 1979), no papers were cited which provided research data on the ecological or epidemiological aspects of this disease in either the lowland or highland tropics. This does not reflect an oversight on the part of the author, but emphasizes the paucity of information on the diseases caused by the soft rotting Erwinias in the developing countries of the world.

There are many reports that seed potatoes grown in Western Europe and North America have on some occasions produced crops with high incidence of seed-piece decay and blackleg. Although there was presumptive evidence

that Erwinia carotovora strains were involved as primary agents in the disease problems that have been developed in the past, definitive studies of the causal factors have not been made in a majority of such cases that have occurred. Since this pattern of heavy losses from Erwinia has occurred under environmental conditions similar to those that may occur in the lowland tropics, an assessment of the potential threat of the soft rotting Erwinias is essential if projected plans for increased production are to be implemented successfully.

Initial studies at CIP and at the University of Wisconsin-Madison, indicate that potato strains of E. chrysanthemi are extremely virulent when inoculated into the stems of Russet Burbank plants. In contrast other strains of E. chrysanthemi were moderately to weakly virulent or avirulent. These Peruvian strains also appeared to be more destructive than strains of Erwinia carotovora that were tested under the same conditions. Other than preliminary studies on the Peruvian strains and one report of E. chrysanthemi on potato from Brazil and Japan, there is no information on this particular disease or pathogen on potato in the lowland tropics.

It should be pointed out that symptoms of bacterial stem rot caused by Erwinia on potato plants (i.e. wilting, stunting and vascular discoloration) show certain similarities to the symptoms caused by Pseudomonas solanacearum and it is essential to be certain that individuals making evaluations of wilt-resistant clones being tested at locations in the lowland tropics can recognize and differentiate these two diseases. It also should be noted that E. chrysanthemi has a very wide host range and has been reported mainly on a variety of tropical ornamental plants usually grown in greenhouses or at high temperatures and humidity.

Certain country programs which could be extremely helpful in testing advanced lines for wilt and late blight are seriously hampered by the prevalence of Erwinia carotovora strains in their foundation seed stocks. Assistance to these Programs in resolving problems appears to be an essential component in the overall evaluation and testing of resistant material. It is also a requisite for maintaining disease-free seed stocks for increase of the most productive lines in the future.

QUESTIONS NEEDING RESOLUTION

The objectives of projected work at CIP and related studies should serve to answer the following questions:

1. What are the species and pathovars of Erwinia that can be expected to cause problems?

   a) Are they endemic in the soil or on the native flora of the lowland tropics?

b) Are they maintained as epiphytes or in the rhizosphere of non-host plants?

2. Are the strains of E. carotovora that are introduced on seed potatoes likely to cause a higher risk of seed-piece decay in the lowland tropics? Once introduced, are these strains capable of surviving?

3. Will true seed or tubers free of Erwinias produce plants that can be readily attacked by strains of E. chrysanthemi or E. carotovora?

4. Do the clones in CIP's seed program with high resistance to P. solanacearum and Phytophthora infestans vary significantly in relative susceptibility to E. chrysanthemi and/or E. carotovora var. carotovora and atroseptica?

5. Will CIP's current storage management procedures minimize decay in storage and planting losses resulting from seed decays?

RECOMMENDATIONS

1. Current efforts should be continued to determine the relative susceptibility to soft rotting Erwinias of tubers of CIP's important breeding material such as wilt and late blight resistant clones. The panel was in agreement with the decision not to involve personnel in a breeding and screening program for soft rot resistance at this time.

2. Experiments need to be initiated that will 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 should be conducted in a few typical lowland tropic sites to determine what levels, species and pathovars of Erwinia may be present.

3. It is recommended that similar studies be initiated at a few typical seed-growing sites in the tropical highlands. It will be important to determine whether breeding materials selected for increase are likely to become contaminated with Erwinias at upland seed growing stations.

4. 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.

5. In cooperation with the seed program at CIP, efforts should be made to assist those country programs directly involved in maintaining disease resistant clones free of other pathogens including blackleg and soft rot bacteria.

6. Studies should be made to evaluate the influence of current CIP recommended storage procedures on the development of bacterial soft rot in tubers in storage, in transit and also after planting.

7. It is suggested that certain phases of the recommendations listed above can best be implemented by the involvement of one or more research pathologists with extensive experience with the pectolytic Erwinia. Short trips for the purpose of collection and isolation of strains can be scheduled. Identification and characterization of strains can best be done in the laboratories of an investigator who may have essential techniques that are used routinely in his research. Serological techniques, selective media, and enrichment procedures are available and can be applied to these problems. Additional studies are needed to provide seed producers with techniques to detect low-levels of contamination with Erwinia in seed potatoes.

OPPORTUNISTIC BACTERIAL PATHOGENS INVOLVED IN DECAY OF POTATO TUBERS

Various bacteria which are normally saprophytic can invade potato tubers which are placed under adverse environmental conditions. In particular the presence of water films over tubers and temperatures above 20-25°C creates conditions favorable for decay not only by Erwinia strains but also by certain anaerobic bacteria such as certain pectolytic Clostridia.

Currently studies in progress at the University of Wisconsin, Madison, are concerned with identity and interrelationship of Clostridia with other pectolytic bacteria in decay problems. In view of the prospect of increased production of potatoes in the lowland tropics and the evidence that Clostridia potentially are more important than Erwinia is causing tuber decay at temperatures above 20° C, additional knowledge is needed on these bacteria.

In view of the prospect that proper storage management and related practices can minimize the losses from these pathogens and the use of true seed may minimize possible losses from seed-piece decay problems, support by CIP of research on clostridia should be re-evaluated once these initial studies are completed.

PLANNING CONFERENCE ON THE CONTROL OF BACTERIAL DISEASES

R.L. Sawyer

Thanks for taking the time from your busy schedules to help us. Some of you have been here before. For some of you this is the first time. To bring you quickly up-to-date on the history of this institution, I hope you all will take time early in your days here to read through the booklet "Profile, International Potato Center 1970-1995." The components of this Profile are:

1. A research center of expertise in the area of origin of the potato.
2. A global research network reacting to priority problems and utilizing cells of expertise wherever they exist.
3. A global capability for the transfer of potato research from CIP as well as among countries.
4. A training program to provide aid and advice to national programs so that a number of tropical countries can quickly develop their own capabilities for producing and transferring the production-oriented potato research sufficient for their national needs.

The Profile describes the stages of CIP's development up until now and sets goals and gives steps required to reach those goals by 1995.

The Profile

before 1970	A number of scientists had recognized the need of a center of expertise in the center of origin of the potato. As early as 1967 Peru had by official decree established its interest in such an entity.
1970-1972	Long-term funding interest was secured and CIP was accepted as the fifth member in the network of centers funded through the Consultative Group on International Agricultural Research. A planning money grant from USAID to the author of the profile made this possible.
1972-1976	Facilities were constructed, major staffing accomplished, and the present research program established which is a combination of both CIP conducted and contract research at many centers of expertise around the world.

1974-1977	The seven regional research and training units were established in association with selected national programs around the world. These units receive genetic material for multiplication, evaluation and transfer to countries in the region and conduct production-oriented training programs. Approximately 50% of CIP's core budget is for Regional Research and Training.
1976-1978	The "third dimension" approach at CIP was established which basically states that CIP does not expect or desire to accumulate special projects for country programs. CIP believes its comparative advantage is in research and training and backstopping with technology national programs and their bilateral interests. CIP does not believe it has a comparative advantage in managing national program special projects.
1979-1980	CIP is implementing its strategy for testing CIP material in growers'fields in developing countries, and is becoming involved operationally in an acceptable manner with national potato workers. This is the final stage of the research transfer system from scientists, wherever applicable technology is being developed, to the fields of growers in countries where there is a need.
1980-1990	During this period it is essential that a few tropical countries develop their own research strength for solving national production problems. Prepared national programs must be in place in each region to do the kinds of research with immediate application to growers such as: 1) clean seed multiplication and maintenance of clean stocks of local varieties; 2) variety trials; 3) fertilizer trials; 4) local applied breeding programs for selected characteristics using populations and resistances coming from other sources; and 5) chemical control of diseases and pests. No addition or major reduction in CIP core funded staff is contemplated from 1980 to 1990. As soon as a sufficient base of knowledge is developed on tropical potato production for both the highlands and the lowlands, priorities will change as individual problems are solved to the point that CIP no longer has to be involved.
1990-1995	1. CIP should have transferred to national programs all traditional potato research efforts for the upland and lowland tropics. Some of this may still be CIP funded by contracts to national programs in developing countries.

2. CIP's program in Peru should concentrate on those things for which there is a long-term, essential need and for which CIP has a comparative advantage. These are:

   a. Maintenance and utilization of the world collection and the distribution of material resulting from research with the collection.

   b. Global communication network for the transfer of potato information.

   c. Training for mid-career scientists who need short term, intensive exposure to new research techniques with potatoes.

At this planning conference, you should keep in mind the long-term comparative advantages of CIP which are: 1. collection, maintenance, utilization and distribution of the world collection of tuber bearing Solanums, 2. a global network of communications on potato information and the accompanying transfer system for material, and a training ability in new research techniques with the potato.

For about 50% of the work we are now doing, we have no comparative advantage. Think about this as you discuss bacteriology programs. What can other institutions do equally well? What has to be done in the country where the research is to be utilized? What does CIP have to do now because no one else is doing it and how soon can CIP pass on the responsibility?

These planning conferences should accomplish the following:

1. Plan a 5-year program for CIP.

2. Integrate the work CIP is doing on the particular subject with that which other institutions are doing.

3. Acquaint the scientists of the world working with potatoes with the fact that there exists here in Peru a center of expertise with potatoes with which scientists from all areas in the world where potatoes are important should be associating using their own state and national funds.

I look forward to the year 1995 when this institution should have only about 1/2 the present number of CIP staff. The facilities would be used to a maximum by scientists coming from institutions such as yours on your own national and state funds to utilize the world collection for your own

selfish interests and in the process exploiting the collection for the good of the world.

Please take the time while you are here to learn about what we are doing and offer your suggestions freely. Find out where we are with the development of the tropical potato, the technology to produce consumer potatoes from true seed instead of tubers, and the development of CIP's global transfer network.

Finally, it is urgent that CIP and bilateral agencies associate with national programs and their development so that in the next ten years, approximately ten countreis will emerge which are capable of conducting their own production-oriented research and have a capability of horizontally transferring technology and training to their neighbors if funding is available from a third party.

We welcome the opportunity to associate with your countries and your bilateral technical assistance programs to help make this possible. CIP can then orient its work to those things for which this institution has a comparative advantage.

Once again, thanks for taking the time from your busy schedules to help us. I shall be available for individual discussions with any of you who so desire throughout the week.

Present and Future Status of Bacterial Diseases of Potatoes in the Lowland Tropics

A. Kelman

In development of long-range research programs on a plant disease it is appropriate to assess current and future economic impact of the disease. Unfortunately, estimates of economic loss or yield and quality reduction of a crop resulting from attack of a given pathogen are often highly inaccurate, not available, or extremely difficult to obtain. This is certainly true with respect to data on losses resulting from attacks of bacterial pathogens on potatoes, particularly in semi-tropical and tropical regions of the world. In these areas, estimates of disease damage under field conditions, combined with post-harvest and storage losses, frequently range from 30-40% of total yield (Van der Zaag, 1976).

Most of the potatoes produced in the tropics are consumed relatively soon after harvest and relatively few areas in tropical regions have adequate facilities for large-scale or long-term storage. Thus, the great bulk of the loss may occur prior to harvest.

One possible approach in making an assessment of the relative importance of specific potato diseases is to determine frequency of literature citations in a major abstracting journal. Thus, a survey was made of the number of papers on bacterial diseases of potatoes that were abstracted in the Review of Plant Pathology from 1972 to 1978 (Table 1). The relative number of papers published on ring rot disease (*Corynebacterium sepedonicum*) and bacterial wilt (*Pseudomonas solanacearum*) remained at a relatively constant level throughout this period except for *P. solanacearum* in 1978. The largest number of papers reported in five of the six years were those on bacterial soft rot (*Erwinia carotovora*). It is of interest to note that for several years the number of papers on bacterial soft rot has increased from 38% in 1972 to 69% in 1976. These data indicate that bacterial soft rot continues to rank as the major research subject for studies on bacterial diseases of potatoes. This is an indirect indicator that it is still considered a major cause of economic loss.

In a survey of economic impact of bacterial diseases, soft rot and ring rot were ranked second and fourth, respectively, in a listing of the eight most important bacterial diseases of plants (Alcorn and Kennedy, 1978).

Table 1.

Ranking of the Importance of Bacterial Diseases of Potatoes Based on Numbers of Research Papers Published in a Given Year [a]

Bacterial pathogen	Percentage of total number of papers published/year						
	1972	1973	1974	1975	1976	1977	1978
Erwinia carotovora	38	51	59	54	69	68	55
Streptomyces scabies	43	35	14	25	21	20	17
Corynebacterium sepedonicum	8	8	18	13	5	6	8
Pseudomonas solanacearum	10	6	9	8	5	6	20
Total number of papers published on bacterial diseases	62	49	22	36	39	45	36

[a] Based on information obtained from Annual Review of Plant Pathology.

The various approaches to determine current ranking of bacterial diseases with respect to economic impact may not be applicable when assessing present and future destructive potential of specific pathogens in the lowland tropics. In part, this reflects the small number of plant pathologists in areas where production can be increased. It also reflects past experience; farmers do not plant susceptible crops in fields where major losses occur and there can be an apparent decline in the relative severity of damage from the disease in question because of shifts in cropping patterns. Also, we cannot be certain that minor or previously unrecognized pathogens will not become major problems. In this connection, it is of interest to note that strains of Clostridium have been involved in decay of potatoes in storage in the U.S. (Lund and Kelman, 1977). Prior to this work, these anaerobes had not been reported as potential causes of economic loss on this crop in the USA.

The Environment of the Lowland Tropics

On the basis of our knowledge of types of environmental conditions prevalent in selected lowland tropical regions, it should be possible to make a rational projection of the types of diseases and possible impact of these diseases if extensive plantings of potatoes are made.

Environmental conditions in the lowland tropics will inevitably enhance development of certain bacterial pathogens and be relatively unfavorable to others. The conditions that characterize the lowland tropics may be described as follows:

1. Elevation of less than 1,000 meters.
2. Mean temperature above 25° C.
3. Rainfall ranging from 1,500 to 5,000 mm/year.
4. Light intensity 10,000 ft candles or above.

In Peru, one of the sites that typifies the lowland tropics is Yurimaguas. It has a minimum annual rainfall of 375 mm, air temperature of 36° C (maximum) and 19° C (minimum) and a growing period of 60 days. At San Ramon, environmental conditions can be characterized as follows: rainfall (minimum), 673 mm; air temperatures, 34° C (maximum) and 15° C (minimum); and length of growing period, 90 days.

On the basis of the above conditions, it is possible to predict which bacterial pathogens may cause more problems in the lowland tropics of Peru and other similar areas than in temperate areas of the world.

Projected Changes in Relative Importance of Bacterial Pathogens in the Lowland Tropics

In view of optimal temperatures for disease development and other factors, it is likely that *Erwinia carotovora* var. *atroseptica* (blackleg), *Streptomyces scabies* (common scab), and *Corynebacterium sepedonicum* (ring rot) will be significantly less important in lowland tropics than in temperate zones in which major potato production areas are located (Table 2).

In particular, the acid soils, high temperatures, and high rainfall would not be conducive to infection or survival of the scab organism. If the plan for large-scale use of true seed succeeds, certainly the threat of ring rot and blackleg would be markedly reduced or possibly eliminated.

The disease that would represent a major threat to potatoes in lowland tropics is bacterial wilt caused by *Pseudomonas solanacearum*. The pattern of planting potatoes at high elevations throughout the semi-tropical and tropical regions of the world reflects, in part, the fact that potatoes have not survived when planted at the low elevations where soils may be heavily infested with the wilt pathogen.

In contrast, certain soils high in salt content in the lowland tropics are apparently not favorable for *P. solanacearum*. This is based on observations made by CIP investigators.

Relationship of Environmental Conditions to Severity of Bacterial Diseases

The importance of temperature in determining relative susceptibility to *P. solanacearum* of potato selections and lines was emphasized by Sequeira and Rowe (1969) in their studies on inheritance of resistance to bacterial wilt. All the data support their conclusion that high levels resistance available in some breeding material may not be expressed under conditions of high temperatures and/or periods of low light intensity.

On the basis of observations made in temperate regions under conditions of high temperatures, *E. carotovora* var. *carotovora* often becomes the dominant and most prevalent organism. This has been demonstrated by tests *in vitro*. When mixtures of *E. carotovora* var. *atroseptica* and *E. carotovora* var. *carotovora* were injected into potato tubers and held at temperatures above 22° C, *E. carotovora* var. *carotovora* became the dominant component of the population (Pérombelon et al., 1979; Pérombelon, 1979). Heavy losses from seed-piece decay have occasionally resulted when seed potatoes from temperate zone seed-growing areas were planted in subtropical or tropical areas. These losses have been attributed to attack by *E. carotovora* var. *carotovora* originating from latent infections.

Table 2.

Possible Changes in Relative Importance of Bacterial Pathogens Associated with an Increase in Production of Potatoes in the Lowland Tropics

Pathogens that may become more important	Pathogens that may become less important
1. *Pseudomonas solanacearum*	1. *Erwinia carotovora* var. *atroseptica*
2. *Erwinia carotovora* var. *carotovora*	2. *Streptomyces scabies*
3. *Erwinia chrysanthemi*	3. *Corynebacterium sepedonicum*
4. *Pseudomonas marginalis*	
5. *Bacillus polymyxa* [a/]	
6. *Clostridium* spp. [a/]	

[a/] Opportunistic pathogens that may become involved in decay of tubers in storage, in transit, or as causes of seed-piece decay.

One pathogen which may have a high potential for destructive impact on potato in the tropics is Erwinia chrysanthemi. The features that distinguish the E. chrysanthemi group from other soft-rotting Erwinias is the relatively high temperature requisite for optimal growth and disease development. This is even well above the optimum for E. carotovora var. carotovora.

In an extensive study of E. chrysanthemi, Dickey (1979) was able to obtain isolates from a very broad range of host plants in many different plant families and from most of the warm temperate and tropical regions of the world. Many of the hosts for E. chrysanthemi are ornamental plants which have been introduced into temperate growing regions from tropical areas. Very few authentic isolates of E. chrysanthemi have been studied with respect to pathogenicity to potato. Strains from Peru tested under greenhouse conditions in Madison, Wisconsin, caused more rapid death of potato plants than any of the isolates of Erwinia carotovora var. carotovora or E. carotovora var. atroseptica with which they were compared (de Lindo, French, and Kelman, 1978). In view of evidence of a rather broad host range and destructive capabilities of many of the strains of E. chrysanthemi, it is necessary to rank this pathogen high on the list of bacteria that represent serious threats to potato in the lowland tropics.

Under high soil temperatures (above 28°C) and high moisture conditions, decay of seed tubers is greatly enhanced. This increased hazard can result from attack not only by strains of Erwinia carotovora var. carotovora adapted to high temperatures, but possibly also to strains of E. chrysanthemi such as those recently isolated in the area of San Ramon in Peru (de Lindo, French, and Kelman 1978). It is possible also that certain strains of clostridia and species of Bacillus could be involved under such conditions (Lund and Kelman, 1977; Lund, 1979).

The major factor contributing to loss of potatoes in storage and transit relates to bruising that occurs during handling and failure to provide adequate conditions for wound-healing after damage. This damage is usually most severe at low temperatures. It is likely that bruising damage associated with use of mechanical equipment and subsequent high potential for decay will not be a problem in the lowland tropics as long as hand labor and simple harvesting procedures are used.

The washing of potatoes either prior to storage or prior to shipment also enhances prospects of decay from the diverse species of bacteria that have a capacity to decay tuber tissue. This decay is greatly facilitated by the presence of water films on the tuber in storage that prevent the normal healing process.

If current storage design and management practices developed and recommended for farmers in developing countries by CIP are accepted and applied, it is likely that losses in storage may be a minor concern. This will depend naturally on the volume of potatoes and the handling procedures that may evolve if production is increased to the magnitude of the north temperate zones. At present, it may be questioned whether it will be practical to construct facilities with costly refrigeration and humidity controls that are currently common in the U.S.A. and Europe.

In summary, it appears that temperature relationships may be the primary factor determining the shift in importance of certain bacterial plant pathogens as production in the lowland tropics increases (see Table 2). On this basis, it is likely that the major threats to production will be *Pseudomonas solanacearum* and *Erwinia carotovora* var. *carotovora*. Much more work will need to be done to determine distribution and potential importance of *E. chrysanthemi* as a cause of potato loss. In the case of all of these pathogens, it is unfortunate that our knowledge of their ecological relationships is so limited. It will be essential to gain a better understanding of this aspect of the biology of these pathogens in order to develop rational and effective control.

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CLASSIFICATION, DISTRIBUTION AND ORIGIN OF *PSEUDOMONAS SOLANACEARUM*

E. R. French

INTRODUCTION

Pseudomonas solanacearum, the causal agent of bacterial wilt or brown rot of the potato, is also a pathogen of numerous crops in the tropical belt of the world and subtropical lands of hot summers (Buddenhagen and Kelman, 1964; Kelman, 1953). The potato strain (race 3) has a much greater distribution into both higher latitudes of the globe and greater altitudes in the tropics (French & Martin, 1977; French, Martin & Nydegger, 1977; Hayward, 1979; Olsson, 1976). Apart from this potato race which is easy to define, other strains that affect potatoes are pathogens of numerous crops and weeds, and still others do not affect potatoes. However, it has not so far been possible to design a classification system adequate to readily characterize isolates and derive knowledge of the host range. More information is needed to accomplish this objective which is essential to establishing meaningful control programs utilizing resistance.

CLASSIFICATION

Two classification systems proposed, based on host range in one case and on biochemical properties in the other, complement each other to some extent (Buddenhagen & Kelman, 1964; Hayward, 1964, 1976). In addition, other tests have proven of some use in characterizing certain populations (Hayward, 1979). The first system recognizes three races: race 1, that attacks a large number of useful and wild plants causes severe losses in solanaceous crops of several other families. Race 2 attacks musaceous hosts (banaba, plantain, abaca and Heliconias). Race 3 is primarily a pathogen of potato, but also of tomato, apparently only when following a wilted potato crop. It can infect a few weeds but these are not usually significant in perpetuating the bacterium. A difficulty with this system based on hosts in nature, is that unless the history of a disease in a given field is known, the host in which it is found may be misleading. To experimentally determine the host range on the other hand requires that conditions simulate those in nature, which is hard to accomplish. Stem inoculations are of no value for this, but even soil infection may not be easily carried out so as to simulate nature.

The second system classifies isolates into four biovars (originally called biotypes) according to their capacity to utilize three disaccarides, to oxidize three hexose alcohols or to do one or the other, or neither (Hayward, 1976). The biovar (Bv) II coincides with race 3 in all instances so far, but other Bv (I, III and IV) do not appear to have a natural relationship to pathogenic capabilities. The methods used in biovar determination are given in Appendix A.

The race system has been complemented with observations of correlation of host range with other characteristics such as colony size, tirosinase production, infiltration of tobacco leaves, antibiotic specificity and optimum temperature for growth (Hayward, 1979). The Bv II coincidence with race 3 appears useful but further host range studies must be conducted.

DISTRIBUTION

Utilizing published information and data on hosts of origin, geographic location and climate and Bv determination for 170 isolates, the following distribution for the races and Bv was arrived at.

Race 1 is complex in its host range and encompasses Bv I, III and IV. In the Americas Bv I is very common within race 1 and is found on potatoes with the same frequency as Bv II (race 3) in the Amazon Basin within Peru (150-1000 m) (Martin & French, 1977). Bv I also occurs in race 2 in Central and South America. Bv III within race 1 has been isolated from the Amazon Basin on tomato (Brazil and Peru) and from Costa Rica on tomato and wild plants (it also occurs in race 2).

Race 1/Bv I occurs on potato and tobacco in tropical Africa. In Nigeria it is considered to have been introduced with the potato during World War II (Ofuya & Wood, 1978). It has not been recorded elsewhere.

Race 1/Bv III occurs in East Africa, Asia, S.E. Asia, Australia and the Pacific Islands in addition to Central and South America.

Race 1/Bv IV is known to occur in East Africa, India, S.E. Asia, Australia and Pacific Islands affecting Solanaceae or ginger.

Race 2 (Bv I and III) has been until recently restricted to Central and South America, increasing its distribution in the past two decades into the upper Amazon Basin in Peru (French & Sequeira, 1968), Southern Mexico (Fucikovsky, 1978) and half way around the world into southern Philippines (clearly taken there by man).

Race 3/Bv II is considered a low temperature race since some isolates have been shown to have a lower optimum temperature for growth (Graham

& Lloyd, 1978; Moraes, 1947; Thurston, 1963) and symptom development (Ciampi, 1979) and it is found at the higher altitudes in tropical countries. In Costa Rica it overlaps with race 1/Bv I between 900 and 2500 m (French & Martin, 1977); in Peru the overlap is between 150 and 3300 m, but always race 3 predominates at the higher altitudes. Furthermore, it has spread during the past two decades to the higher latitudes of 37.5°S in Argentina to 56°N in Sweden (Olsson, 1976). However, we have found Bv II along with Bv I in the same potato fields and even the same potato plant in the lowland Amazon Basin location of Yurimaguas at about 170 m elevation. These sites had never been planted with potatoes before, some of the lands were virgin forest, and the tubers used for planting were not contaminated.

Race 3 occurs in all the continents. In the Americas it has spread to higher lands in Colombia (Granada, 1976) and Peru and to cooler latitudes in Southern Brazil, Uruguay (García, 1976), Argentina and Mexico (Fucikovsky, 1978). A report from U.S.A. is considered unsubstantiated. In nearly all instances man is clearly involved in this spread. It is in East and West Africa (Harris, 1976; Ofuya & Wood, 1978), all around the Mediterranean and has apparently recently been introduced in commercial shipments to northern Europe (El-Goorani, 1976; Olsson, 1976). Records of occurrence are common to most countries in Asia and S.E. Asia that received the potato from Europe, as also Australia (Hayward, 1964; Seneviratne, 1969). Combining the records of Hayward (1964) and our own, race 3 has been found to occur in 25 natural geographic regions of the world. This compares to 11, 12 and 6 regions for Bv I, III and IV, respectively.

ORIGIN

The information is insufficient to establish the origin of P. solanacearum, but a few conclusions can be drawn and other ideas postulated for further study.

Race 2 (Bv I and III) is unquestionably of American origin since the disease arose in Central America and Northern South America in bananas and plantains which were introduced from Asia where the disease did not occur. The strains affecting Musa spp. apparently existed on Heliconia spp. or underwent slight change in adapting to these new hosts (Buddenhagen, 1960; Sequeira, 1960; Sequeira & Averre, 1961).

Race 3 is found in virgin soil of the Amazon basin. The fact that race 1 occurs there also, and that an unusual strain that does not attack tobacco has been found further down the Amazon (French & Sequeira, 1970) all point to an area of great genetic diversification for this bacterium (Martin & French, 1977). It is therefore conceivable that centuries ago potatoes, which were the staple crop of the heavily populated highlands,

were periodically grown in the upper reaches of the jungle where a few surviving infected tubers were then taken to higher lands, planted and thus remained with latent infection. It would seem that this race did not evolve along with the evolving potato of the cooler lands, but (as in the case of race 2 was present in jungle soils and upon the introduction of the exotic potato, it was infected. In this case, however, there appears to be no need for genetic change. Its original hosts are not known. Tubers with latent infection were then taken to other parts of the world, probably through northern Europe by the colonial powers that adopted the potato as a staple crop. Latent infection would mean nothing centuries ago, and even recently little to a plant pathologist who would not expect such an occurrence. Throughout the "colonies" this unusual strain, race 3, appeared (Shrestha, 1977) even "in virgin soils" (Seneviratne, 1969), so it is now the most widespread race of P. solanacearum in the world.

Race 1 remains last to tackle since it is the more difficult one. Race 1/Bv I has been recorded only throughout the Americas from southern U.S.A. to southern Brazil and in East and West Africa. At CIP we recently found this race/Bv damaging potatoes in the jungle when the seed came from the highlands at 3300 m. There was latent infection in the following crop of that highland seed. It is therefore also conceivable that race 1/Bv I (which is less frequent on potatoes than race 3/Bv II in South America where the potato has its center of origin) was taken to a cool climate country of northern Europe and was then distributed to its colonies in East and West Africa, the only other parts of the world it has been recorded. When it comes to Bv III and IV this sequence cannot be substantiated. I can only postulate two alternatives: 1) that although race 1/Bv III-IV has not been found on potatoes at the center of origin for the crop, it nevertheless does occur and has been transported as latent infection in tubers to S.E. Asia, Asia, East Africa, Fiji and Hawaii (Lallmahomed, 1976; Quimio & Tabei, 1978; Shekhawat, 1976; Shekhawat, et al., 1978; Zehr, 1969) either by western migration across the Pacific Ocean or via Europe (the so called native potatoes of these lands are generally considered old European introductions) or, 2) that P. solanacearum existed before continental drift split up the massive single continent into the ones we know today are still drifting apart. Bv III and IV which are rare in the Americas may have been mostly replaced in the evolutionary process by Bv I and II. Unfortunately, we do not know the significance of the traits that distinguish biovars and what role they may have in the fitness of a given Bv, if any.

CONCLUSIONS

The race concept for classifying P. solanacearum is a natural system, but it requires more precise definition since first enunciated briefly in an abstract in 1962 and then restated in 1964 (Buddenhagen & Kelman, 1964).

Emphasis must be given to natural hosts under field conditions or carefully simulated field conditions (soil infestation), whereas stem inoculations should be reserved for the use of plants as indicators that can complement other information. Biovar determination is a useful complement to race determination, but the assumption that Bv II is race 3 must be put to the test periodically with new isolates from additional regions under true or simulated field conditions. Further accumulation of information on these and other characteristics such as colony size, formazan pigmentation in tetrazolium medium, salt tolerance, tyrosinase production, antibiotic sensitivity, etc., should eventually lead to a better system of classification and a better understanding of the origin of *P. solanacearum* which then would permit a rational approach to control of bacterial wilt by breeding for resistance.

So far the resistance sources available for control of bacterial wilt of the potato have not been general against all pathovars, not even those in one race. The use of these different resistance sources, or different genes from a given source (we do not know if these genes are common to different clones or species) to develop a pathovar scheme might eventually lead to a new race concept and the presently established races might then be reclassified as different species. Races would then fit the customary designation for this term as used for most pathogens.

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APPENDIX A

CULTURE MEDIA FOR *PSEUDOMONAS SOLANACEARUM* ISOLATION, IDENTIFICATION AND MAINTENANCE

A. KELMAN's TZC AGAR (Kelman, 1954): Useful for isolation and purification, to distinguish virulent from avirulent cultures.

TZC Stock Solution: Dissolve 1 g of 2, 3, 5, triphenyl tetrazolium chloride (TZC) in 100 cc of distilled water, place in a dark or light proof capped bottle, and autoclave for only 8 min., or sterilize by filtration. Store under refrigeration if possible.

Basal medium:

Dextrose	10 g (2.5 g)*
Peptone	10 g
Casamino acids (Difco)	1 g
Agar	18 g
Water (distilled)	1000 cc

* Modification by reducing the amount to 2.5 g, results in better growth rate, especially of the potato race (biovar 2 = race 3). Sucrose can be used as a substitute.

This medium alone (without adding TZC) is useful for multiplication of inoculum free of formazan pigment.

Preparation for plating: The basal medium can be autoclaved and stored, then melted as needed. To each liter of the melted somewhat cooled agar basal medium add 5 cc of the TZC solution to give a final concentration of 0.005% (aliquots of 200 cc are recommended for ease of handling; to these 1 cc of TZC solution is added).

Plating and storing: Pour about 20 cc per petri plate. When the gel is set, store inverted. Keep 1-2 days before use to permit surface drying (longer storage may result in poor growth).

B. CARBOHYDRATE MEDIA FOR BIOVAR DETERMINATION (Hayward, 1976): The determination of biovars of *P. solanacearum* is based on the utilization of the disaccarides lactose and maltose, and the oxidation of the hexose

alcohols mannitol and sorbitol; three of each were utilized by Hayward (1964) but since no differences among them have been encountered, the costlier dulcitol and cellobiose have been dropped.

Basal medium:

Ammonium dihydrogen phosphate ($NH_4H_2PO_4$)	1.0 g
Potassium chloride (KCl)	0.2 g
Magnesium sulphate ($MgSO_4 \cdot 7H_2O$)	0.2 g
Peptone	1.0 g
Bromothymol blue	0.08 g
Distilled water	1.0 liter
Agar	3.0 g

Adjust the final pH of the medium to 7.0-7.1 (an olivaceous green color) by dropwise addition of 40% w/v Na OH solution. Melt the agar by steaming or heating in double boiler with constant stirring (alternatively weigh the agar per flask and add dry prior to dispensing the rest of the media). Dispense 90 cc aliquots into flasks and autoclave at 121°C for 20 minutes.

Prepare 10% solutions of the carbohydrates in 10 cc amounts. Heating may be needed to dissolve the sugars. The hexose alcohols mannitol and sorbitol are relatively heat stable and can be autoclaved at 110 C for 20 minutes. The disaccarides lactose and maltose are heat-labile and should be sterilized by Seitz or membrane filtration (0.22 millipore) into pre-sterilized containers or by Tyndilization (steaming for 20 minutes on 3 successive days).

To the melted basal medium cooled to about 60°C add the carbohydrate solution. Dispense 3-4 cc into previously sterilized test tubes (150 mm x 10 mm size or similar) using a sterile cotton stoppered pipette.

Prepare about 4 ml of a milky inoculum suspension from 2-day old cultures. With a sterile Pasteur pipette add 3 drops to each tube. Use 3 repetitions and a control with no carbohydrate. Incubate at 30°C and examine at 3, 7 and 14 days for change to acid pH (yellow color) from the top downwards. Hexose alcohols usually take 3-5 days, disaccarides may take a few days longer.

Biovar determination is based on the following:

- Biovar 1 = utilization and oxidation tests negative.

- Biovar 2 = utilizes disaccarides; does not oxidize the alcohols.

- Biovar 3 = utilization and oxidation both positive.

- Biovar 4 = utilization of disaccarides negative; oxidizes the hexose alcohols.

The biovar classification correlates to races in the following manner: biovars 1, 3 & 4 if isolated from non-musaceous hosts are race 1; biovar 2 is race 3; musaceous host isolates are the "banana" race 2 (Buddenhagen & Kelman, 1964).

C. WATER FOR STORAGE (Kelman & Person, 1961): Cultures are maintained in their wild type form best when stored in distilled, deionized or tap water (boiled to eliminate chlorine) in screw-cap test tubes. Two loopfulls of bacteria from a composite of about six individual 2 day old colonies are transferred to 5 cc of sterile water. These should be streaked on Kelman's TZC Agar every 6 months and purified if mutants are numerous.

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METHODS OF DIAGNOSING PSEUDOMONAS SOLANACEARUM

L. Fucikovsky

Diagnosis of plant diseases is an art as well as a science (Streets, 1972, Subcom. Pl. Path. 1968). It is based tentatively on symptoms and positively on identification of the causal agent.

Our attention is drawn to a potato plant affected by Pseudomonas solanacearum E.F. Smith, one of the bacterium that is particularly damaging in tropical and subtropical regions of the world. This bacterium causes wilt and eventual death of the plants. Distribution of this pathogen is extensive throughout the world.

One of the ideal methods of diagnosing P. solanacearum in potatoes is by field symptoms, however, some problems may arise when other agents produce similar symptoms. If one wants a correct diagnosis, Koch's postulates and several biochemical tests should be performed. In reviewing the methods of diagnosing P. solanacearum in potatoes, we shall divide the work into two parts. One, field diagnosis, includes the aid of symptoms and certain rapid diagnostic tests that can be performed directly on the plant patient, potato. The other is laboratory work; confirming or sometimes carrying out a correct diagnosis.

Diagnosis in the Field

The aerial symptoms described by investigators such as Kelman (1953), O'Brien and Rich (1967) are the following: the first symptom in the potato plant is a partial wilting of the upper leaves. These become pale green and even yellow. A slight yellowing may be observed on the lower leaves. These symptoms might be confused with that of Corynebacterium sepedonicum where the leaves become mottled. Plants affected by brown rot disease frequently show wilting of only one branch with a leaf wilt, and later a progressive wilting, stunting and death of the whole plant. If the disease progresses quickly, the leaves may turn brown without other color change and finally die. Kelman (1953) states that under certain environmental conditions a true wilt symptom may fail to develop, but dwarfing and stunting may occur.

Other symptoms in the form of dark narrow stripes beneath the epidermis of the stems may appear, which correspond to the affected vascular bundles. In a cross section of a stem these bundles show brown coloring. If the disease is sufficiently advanced a white ooze forms on the cut surface in a few minutes.

The underground symptoms, especially those of the roots, may easily be overlooked. At advanced stages, roots turn dark and decay. The tubers are excellent material for observation. In the early stage of disease development, the tubers are or may appear completely healthy on the surface or even when they are cut. Herein lies the danger since very marked symptoms are not observed until a few months later in storage.

Slightly infected tubers on being cut may show under a stereoscopic microscope very tiny milky droplets of bacterial ooze that can usually be overlooked. Tubers in a more advanced stage of infection frequently present a slimy, whitish exudate from the somewhat sunken "eyes" to which soil adheres. This is a symptom, however, which does not occur by C. sepedonicum. Moreover, tubers affected by C. sepedonicum frequently have star-shaped cracks on their surfaces. These differences serve as a means of distinguishing between two diseases (Kelman, 1953). A tuber in a more advanced stage of bacterial wilt disease, cut crosswise, may usually show browning of the vascular ring even though exceptions are known, such as in Portugal and Kenya.

These symptoms are also observed with Verticillium and Fusarium infections. In brown rot, when a tuber is slightly pressed, the bacterial ooze in a form of milky white droplets comes to the surface of the cut vascular bundles (this does not happen with Verticillium or Fusarium infections). Mexican farmers have baptized it "Vaquita", which means a little cow. These symptoms lend themselves to somewhat simple confirmatory tests that could be performed directly in the field assuring a better diagnosis.

Practical Field Tests

Sequeira and Averre (1961) employed a simple, but elegant, test suspending P. solanacearum diseased tissue in water and observed the bacterial ooze coming from the vascular bundles. This can be easily distinguished between bacterial and fungal infection and can be performed in the field.

For a confirmation of diagnosis of P. solanacearum in the plant, two methods have been employed, serological and biochemical ones. Both demand a small amount of bacterial exudate from the plant. Escudie and Digat (1965) described and tested on microscope slides a highly successful sero-agglutination method, which gives results in few minutes. The reaction is specific and unequivocal.

In most serological work a relatively long time is needed to prepare the antiserum, before any tests can be made. This applies unless the antiserum was prepared or obtained beforehand.

The biochemical tests that can be performed within a short time (seconds to a few hours) are lysis with 3% KOH (Ryu, 1938 and Gregersen, 1978), oxidase and urease reaction. The first method can be distinguished between gram-positive and gram-negative bacteria without a microscope. After stirring P. solanacearum in KOH for 5-10 seconds the suspension becomes viscose, and on drawing it with a loop, it forms a very fine thread of slime 1/2 to 2 or 3 cm in length. The oxidase reaction, which takes only 10-30 seconds has been tested directly on diseased tubers of Alpha variety with good results, the aim being an early detection of symptomless tubers (Fucikovsky, 1978). The substances employed are N, N-dimethyl-p-phenylenediamine reagent or its tetramethyl form in 1% aqueous solution. In addition to Alpha variety, other varieties such as Greta, Prevalent, Patrones, Lopez and Murca also respond well to this test, however, healthy tubers of varieties such as White Rose, Lopez, Prevalent and Murca present a coloring reaction on the unaffected vascular bundles, very similar to the oxidase reaction. The reason for this is unknown. In addition, the urease reaction, using the micromethod of National Collection of Type Cultures (Cowan, 1974), has been useful as a confirmatory test incubating a loop of bacteria in a urea medium for approximately 4 hours at 28°C.

The four tests are simple to perform and do not require an expensive laboratory or experienced person. Although these procedures may function under many conditions, problems of mixed reactions with other contaminating microflora may arise or samples may be too small. For this reason a trained pathologist is needed with good laboratory facilities.

Laboratory Tests

Once diseased potato plant samples reach a laboratory, the plant pathologist must correctly assess in the shortest possible time if serious problems, like excessive contamination or scarcity of good samples, arose in the field relative to diagnosis. For this reason growing and purifying the bacteria is essential.

Another interest may be in preserving the pure specimen. If only diagnosis is desired, serological methods of high specificity could be used in combination with other methods.

Serology

Serology for diagnosis of P. solanacearum is advantageous because of its rapidity and specificity, but great care should be taken in preparation of antigens and interpretation of results. Moraes (1947) was the first to use the slide agglutination technique for diagnostic purposes. Likewise, Gehring (1962) used agglutination with success for

the same purpose, identifying the bacteria in imported potatoes. Perez (1962) satisfactorily used a "ring test" in the tube precipitation technique, extracting the bacteria with Fuller's formamide. The whole process takes only an hour, but the extraction is tedious for general use (Morton et al., 1966). Morton et al. (1965, 1966) used a better serological technique of micro-agglutination combined with a rapid method of expressing sap from plant stems, thus saving time. They claim that the antisera were race specific and that samples can be evaluated in less than 20 minutes. These investigators also used fluorescent antibody and agar-gel diffusion tests, both very specific methods of identification, but from the point of practicality the micro-agglutination is preferred. The preparation of antigens is important for Digat and Escudie (1967) and Kojima and Buddenhagen (1969), who found the somatic antigens heterogeneous. Undoubtedly, serological methods will be very useful in future diagnostic work. Perhaps standardization is necessary.

Media

Growing bacteria on different specific, selective and sometimes non-specific media, colony type and morphology can easily be observed and identified, although waiting time, usually 3 to 4 days, is necessary. Thus, in the past, various excellent media have been used routinely in laboratories for isolation of P. solanacearum from diseased plants and soil. In general, the growth of P. solanacearum in GPG or PDA is typical, since after a few days of growth the colonies start to become more fluid and loose their round shape.

Kelman (1954) first devised trifenil tetrazolium chloride medium, eliminating many contaminants, but letting both virulent and avirulent types of P. solanacearum grow. These can be easily identified by their colony appearance.

Harris (1976), studying strains of P. solanacearum from potato, tomato and soil, improved Kelman's medium adding 4,000 ppm of trifenyl tetrazolium chloride (TTC), 100 ppm of actidione and 200 ppm neomycin. However, isolated from plants, the increased TTC concentration may be sufficient, without other antibiotics. The complete medium, however, proved more sensitive, accurate and convenient for following population trends of P. solanacearum in soil, than methods involving indicator plants. Using the well-known characteristic of production of pectic enzymes of this bacterium, the media designed by Cuppels and Kelman (1974) and Burr and Schroth (1977) can be used. In the first one, the bacteria produce broad pits, and in the second, a clear zone is formed around the colony on addition of 1% aqueous solution of cetyltrimethylamonium bromide (cetrimide). Very selective media have been designed by Karganilla and Buddenhagen (1972) and also recently by Nesmith and Jenkins (1979) using the principle of selective exclusion. These media are

very useful for soil samples and Nesmith and Jenkins claim that colonies of P. solanacearum could be recognized within 36 to 48 hours. The investigators report that their medium is helpful in studying inoculum density, population dynamics, survival and saprophytic behavior of P. solanacearum in soil. A disadvantage of this medium is the large number of antimicrobial compounds and salts it contains and that are not always available in developing countries. Also Drigalski's modified medium (Tanaka, 1979) was recently used with success for isolation of P. solanacearum from diseased tobacco and soil. As claimed, the virulent strain of P. solanacearum could be easily distinguished from other bacteria on the medium, because of its peculiar colony appearance, such as irregularly round margin and milky white color with blue center. In general, the media mentioned are good for specific studies of P. solanacearum.

Indicator Plants

Since the use of the hypersensitive reaction technique in tobacco leaves, there has been a number of attempts (Lozano and Sequeira, 1970, Tanaka, 1977, Graham and Lloyd, 1978) to use this system or a similar one to increase accuracy, speed and sensibility in diagnosing isolates of P. solanacearum from plants and soil. Under specific environmental conditions, Lozano and Sequeira (1970) were able to differenciate three known races of P. solanacearum by infiltrating tobacco leaves with bacterial suspensions. Tanaka (1977) also successfully infiltrated tobacco leaves for detection of low quantities of cells of P. solanacearum from soil (10^1-10^2 cells/gm of soil) in a period of 5 to 20 days. Dark brown necrotic lesions were produced. The work of Graham and Lloyd, (1978) is of importance at this point, since they use seedlings of Sebago potato variety with small tubers as indicator plants. These plants grown in naturally infested soil even at low temperatures (10°C) produce characteristic wilt symptoms in 10 to 14 days.

Other Diagnostic Methods

Kelman (1953) relates that Gram staining, methylene blue bipolar staining, red litmus paper test and browning of potato plugs were among the characteristics used by early investigators for diagnostic work. He also notes that bacterial ooze from diseased tissue is very alkaline and will cause red litmus paper to turn blue on contact. This has also been observed in our laboratory. After applying a few drops of a 1% aqueous solution of bromo-cresol purple or phenol red to a cut surface of potato tubers, the bacterial ooze from the vascular bundles will turn purple blue or red respectively in 10 to 30 minutes. These will stand out sharply against the greenish or yellowish background of the other potato tissue. This indicates again a presence of highly alkaline exudate and an efficient and simple diagnostic procedure where no laboratory equipment is necessary.

Browning of potato plugs has been considered to be an important diagnostic feature of the species related to the production of tyrosinase by the bacterium (Hayward, 1976). However, this characteristic may be somewhat variable. Hayward (1960) mentions that since almost pure cultures of P. solanacearum can be obtained from vascular bundles of infected plants, this feature can be used, taking and staining directly the bacteria with Sudan Black in order to reveal the massive inclusions of poly - B - hydroxybutyric acid. This is a specific enough characteristic to differentiate from C. sepedonicum and E. carotovora var. atroseptica, both potato pathogens. These are some of the additional, but very useful characteristics for diagnosis of P. solanacearum that can easily be performed in the laboratory.

Kelman (1953) cites independent Japanese workers that used specific bacteriophages for testing of P. solanacearum. These methods if developed for practical use could be very valuable in the future.

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SOURCES OF RESISTANCE TO PSEUDOMONAS SOLANACEARUM

C. Martin

Bacterial wilt or brown rot is the most serious disease problem of potatoes in warm regions (tropical and subtropical areas).

It has been early recognized by many potato pathologists that the use of varieties resistant to P. solanacearum would be the most practical and useful means of control (4, 7, 8, 9). However, the number of species found to be resistant have been scarce despite the high number screened.

Probably one of the first workers to search different possible sources of resistance to bacterial wilt of potatoes was Thung in 1947 (6). He studied the resistance to S. andigenum, S. caldasii, S. antipoviezii, S. chacoense and S. demissum, and hybrids derived from crosses of these with S. tuberosum. Although the wild species had good levels of resistance, he found that the hybrids were much less resistant than the original parents.

Nearly 9,000 clones, mostly of Solanum tuberosum, were evaluated for resistance to P. solanacearum with field and laboratory procedures by Nielsen and Haynes from 1947 to 1959 (5). They found that all potatoes studied were susceptible to bacterial wilt. However, in some clones disease symptoms appeared at a later date, and the disease progressed more slowly than in the susceptible control. This slower development of disease was interpreted as resistance, even though the proportion of wilted plants was sometimes equal, at a later date, to that of the susceptible control. By using this assumption they were able to find three resistant clones: Prisca, 2983 and 2777. However, because of the late maturing characteristic of those clones, they did not use them extensively in breeding programs.

The first important and useful source of resistance was found by Thurston and Lozano (8). After screening 1,061 clones they found that of the 190 clones of S. phureja tested, six consistently showed a high degree of resistance to race 3. Further evaluations of the six clones indicated a high level of resistance when they were tested at different temperatures (20, 25 and 30°C), and against races 1 and 2 under greenhouse conditions. The original six S. phureja clones were:

CCC	1339	PI 310489
CCC	1350	PI 310490
CCC	1386	PI 31091
CCC	1388	PI 310492
CCC	1395	PI 310492
CCC	1449	

A program was then initiated in Wisconsin by Sequeira and Rowe in 1967, using the resistant Colombian S. phureja lines (6). During the first step, over 250 accessions of S. phureja and 300 S. phureja x S. tuberosum hybrids were screened in the greenhouse for resistance to P. solanacearum (isolate K60). These series of crosses and screenings were made in 1968 with the objective of combining wilt resistance and acceptable agronomic characters. A second series of crosses in 1970 combined these characters with late blight resistance derived from Mexican clones. From this program many series of different clones were developed and widely distributed around the world. The series of clones known as BR, MS, PS and PSY are probably the best known. CIP has also been using this source of resistance trying to develop clones with more adaptability to highland and lowland tropics. I do not want to get involved in this topic because Dr. Sequeira in his presentation will talk specifically on the development of resistance to bacterial wilt derived from S. phureja.

Over 147 accessions from 63 wild species have been screened for resistance to P. solanacearum in CIP during the last two years (2, 3). Table 1 summarizes the best 12 resistant species after inoculation with isolate 013, race 3 of P. solanacearum. Only six accessions had less than 20% susceptible plants; the best were S. stenotomum, S. stoloniferum, S. jamesii and S. sparsipilum. Further tests involved the screening of the best resistant species to different isolates of P. solanacearum (Table 2). Because of immunity to the root-knot nematode, S. sparsipilum is already being used in a breeding program at CIP. It is expected that a series of clones with resistance to bacterial wilt and root-knot can be developed.

In tests to present the resistance found is not immunity to bacterial wilt infection. Furthermore, results tend to indicate a high specificity between host and isolates of the bacteria. This further complicates the search for a good source of resistance and therefore, the development of clones that could maintain resistance at different locations. Although not immune, S. jamesii seems to have a better level of resistance than the other wild species to the five different isolates tested at CIP (Table 2). Contacts have been established through Mendoza that will allow Hermsen, in Wageningen, to use S. jamesii (PI 275265) in his breeding program.

At a site in Turrialba, Costa Rica, a clone has been found to have a good level of resistance after three consecutive plantings in a heavily infested field. The clone Cruza 148, sent by the Mexican Potato Program, is probably S. tuberosum x S. demissum selection (Montserrate x PI ?). It has a good adaptation and moderate yield under Turrialba conditions (620 m elevation) and at the present is being freed of virus at CIP for further distribution and evaluation.

Table 1. Best Resistant *Solanum* spp. Out of 147 Accessions From 63 Species Screened Against *Pseudomonas solanacearum*, Isolate 013 (race 3).

Solanum spp.	Accession Number	No. Plants Tested	% Susceptible Plants
S. bulbocastanum	PI 243511	40	27.5
S. chacoense	CIP 760917.1	32	18.7
S. demissum	PI 160221	0	22.2
S. jamesii	CIP 760476	93	17.0
S. jamesii	PI 275266	32	40.6
S. polytrichon	PI 275241	64	17.5
S. stenotomum	OCH 3520	7	14.5
S. stenotomum	OCH 3571	25	32.0
S. stoloniferum	PI 161178	16	25.0
S. stoloniferum	PI 161170	38	15.7
S. sogarandium	PI 230510	20	30.0
S. sparsipilum	CIP 760147.7	26	15.0
BR 63.76		49	65.3
Var. Ticahuasi		40	80.0

Table 2. Susceptibility of Different *Solanum* spp. to Different Isolates of *Pseudomonas solanacearum*.

Solanum spp.	Accession Number	ISOLATES				
		052 (1)*	092 (1)	075 (3)	048 (3)	03 (1)
S. bulbocastanum	PI 243511	11/12**	-	-		-
S. chacoense	CIP 760917.1	8/20	-	11/17	14/24	5/7
S. jamesii	CIP 760476	10/84	1/12	2/7	20/85	24/77
S. jamesii	PI 275266	2/29	0/12	1/5	18/33	16/30
S. polytrichon	PI 275241	2/11	1/5	-	6/12	1/7
S. stenotomum	OCH 3571	2/11	0/3	-	6/15	8/12
S. stenotomum	PI 365344	0/13	0/13	12/25	6/7	-
S. stoloniferum	PI 161170	5/11	2/3	-	4/4	-
S. sogarandium	PI 230510	6/15	2/10	-	2/10	-
S. sparsipilum	CIP 760146.7	13/49	-	12/25	26/54	22/31

* Number in parenthesis denotes race.

** Number of wilted over healthy plants.

Recently, Opeña from the Asian Vegetable Research and Development Center (AVRDC) selected a clone that combines heat tolerance and bacterial wilt resistance (1). According to his information (personal communication) the clone AVRDC 1282-19 has been found almost immune to bacterial wilt under lowland tropical conditions. The selected clone originated from a cross between G 69191 x G 6652, whose origins are:

G 69191 = *S. tuberosum* ssp. andigena x 3 wild spp. - *S. chacoense*, *S. sogarandinum* and *S. raphanifolium*.

G 6652 = Tuberosum cultivar

Tubers from clone 1282-19 have been sent to CIP-Lima for further evaluation.

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DEVELOPMENT OF RESISTANCE TO BACTERIAL WILT DERIVED FROM SOLANUM PHUREJA

L. Sequeira

Bacterial wilt is one of the major limiting factors to potato production in the tropical and subtropical areas of the world. Although many attempts to locate resistance to this disease among Solanum tuberosum cultivars have been made (Nielsen and Haynes, 1960), adequate levels of resistance have not been detected. The discovery that certain Colombian clones of the cultivated diploid, S. phureja, carried high resistance to local isolates of the bacterium (Thurston and Lozano, 1968) stimulated further interest in the possibility of breeding for resistance.

A program designed to examine the range of resistance in Colombian clones of S. phureja, to determine the inheritance of resistance, and to develop germ plasm for use in local breeding programs was initiated in Wisconsin in 1967. Initially, the program was supported by the Rockefeller Foundation, but, since 1972, the International Potato Center has provided the major source of funding. The project has become an international enterprise; thousands of hybrid clones have been distributed throughout the tropics and extensive laboratory investigations have been carried out at Wisconsin, Peru, and elsewhere by many dedicated scientists. It is appropriate, after twelve years of effort, to attempt to evaluate the progress that has been made.

Because bacterial wilt remains a major problem in many tropical countries (e.g. Mexico, where a recent epidemic has caused serious problems) it is evident that breeding for resistance has not provided adequate control in many instances. On the other hand, the release of resistant cultivars in Peru, and encouraging developments in other cooperating countries, indicate some measures of success. This article will consider: a) the major accomplishments, b) the stumbling blocks that have reduced the impact of the program, and c) additional progress that could be made by appropriate, concerted action.

It is encouraging to note that most of the initial objectives of the program were reached. A brief summary of these accomplishments follow:

1) Procedures for adequate, reproducible screening for disease resistance were developed

Stem inoculation was used at first but it proved cumbersome and inadequate to test large populations. The development of a seedling inoculation

assay (Gonzalez, et al., 1973), in which progeny could be screened rapidly against a mixture of different strains of the bacterium, was a very significant improvement. Thousands of potentially resistant clones were developed by this procedure; it was a major factor in the increased efficiency of clonal development. Unfortunately, the procedure had to be abandoned in 1977 when it was discovered that some resistant seedlings, which survived the initial inoculation, could carry latent bacterial infections. To prevent any possible carryover of bacteria, we adopted a new procedure in which seedlings were grown until they could be propagated vegetatively. One plant of each clone was stem-inoculated with a mixture of P. solanacearum strains and, if it survived, tubers from the uninoculated plants were retained. This is a cumbersome, slow procedure. In addition, very few plants survive the stem inoculation procedure. Although the present procedures are adequate to prevent bacterial carryover, it is important that more efficient procedures be implemented in the near future.

2. Resistance was transferred to Solanum tuberosum.

The most resistant clones of S. phureja were crossed with 24 and 48-chromosome clones of S. tuberosum. Analysis of the F_1 progeny indicated that resistance was a dominant trait that was transmitted to the progeny at a high frequency (Sequeira and Rowe, 1969; Rowe and Sequeira, 1972). Hybrid clones that were reported to be resistant in the tropics were added to the crossing program. The majority of the clones that were selected in the tropics were from the tetraploid populations, presumably because they were more vigorous and had better type and yield than the diploids. No barriers to the transfer of resistance were detected.

3. Resistance was shown to be dependent on multiple, dominant genes.

Further investigation of the resistance in S. phureja indicated that clones differed in their reaction to diverse isolates of the bacterium (Sequeira and Rowe, 1969). Clones that were resistant to a series of 10 isolates were found, but most were susceptible to one or more of the isolates. Although relatively few, dominant genes conferred resistance to any one isolate, it became evident that many genes were necessary to confer a broad spectrum of resistance. From analysis of segregation in progeny from intercrosses of the S. phureja clones, it was determined that resistance to two isolates (K-60 and S-123) was each controlled by three dominant, independent genes, but only one gene appeared to be in common (Rowe and Sequeira, 1970, Rowe, et al., 1972). Similar studies involving a race 3 isolate, S-206, led to the hypothesis that four major genes controlled resistance (Zalewski, 1974). Numerous attempts have been made to determine how these three genes systems are interrelated. Four separate experiments were carried out in which up to 100 clones were developed each time from the hybrid family 1386.12 x 1339.28 (R x S).

One plant of each of these clones was inoculated with either S-206, K-60, or S-123. Clones were found to be resistant or susceptible to one, two, or three of these strains; all eight possible segregation patterns were obtained. Computer analysis was used to test the goodness of fit of these results to the expected values for various gene combinations. These analyses did not provide a clear choice between a system in which only one gene is common or one in which all genes are independent. Unfortunately, the four separate tests were not in agreement with each other; the reasons for this lack of reproducibility are not at all clear. Because of the high cost and laborious nature of these experiments, work on the genetics of resistance was discontinued. Because of the very large number of strains of the pathogen that exist, however, it is unlikely that independent systems for resistance to each strain have evolved.

4) Resistance was not dependent on specific bacterial growth inhibitors or agglutinins.

When inoculated in resistant potato clones, *P. solanacearum* grows at a relatively slow rate and does not spread rapidly from the initial point of inoculation. In susceptible clones, in contrast, the bacterium grows rapidly and kills the plant in a few days. Extensive efforts have been made to determine if the reduction in bacterial multiplication is caused by specific compounds present in the resistant tissues. Initially, it was determined that ethanolic extracts from wilt-resistant clones of *S. phureja* contained a bacterial growth inhibitor at concentrations substantially higher than in susceptible *S. tuberosum* (Zalewski and Sequeira, 1973). The compound, a terpenoid, has been partially characterized. Clones derived from segregating hybrid progenies of *S. phureja* were used to test the correlation between resistance and the presence of the compound. Disease reaction segregated independently from content of the inhibitor (Zalewski and Sequeira, 1975).

More recently, extensive studies were conducted to establish the relationship between lectin content and disease resistance. Crude extracts from Katahdin potato tubers contain substances (lectins) that cause rapid agglutination of avirulent (B1) cells of *P. solanacearum*. Because virulent cells were not agglutinated, this provided the basis for the hypothesis that the inability of certain strains to multiply in potato tissues was due to the presence of particular lectins. Procedures for complete purification of potato lectins were developed.

The lectin from Katahdin potatoes (PL1) agglutinated bacterial cells of all 33 avirulent isolates tested; all 55 avirulent isolates tested either failed to agglutinate or agglutinated only weakly or at much higher lectin concentrations (Sequeira and Graham, 1977). In contrast, the lectin from the breeding line, P13, which has resistance originating from *S. phureja*, was found to contain a second lectin, PL2, capable of aggluti-

nating several virulent, slime-forming isolates of P. solanacearum, suggesting a possible role in resistance. The pattern of resistance of P13 to different isolates of the bacterium was correlated, except in a few instances, with the pattern of agglutination obtained in vitro. These encouraging early results stimulated further work on this problem. We selected six resistant clones of widely diverse genetic background (T2, MS 38.14, MS 45.3, MS 70.23, and MS 35.4) and extracted the lectins. These lectins then were tested against five strains of the pathogen (K60, K60-B1, S-123, S-206, and S-210) that represent a wide spectrum of races and biotypes. The results indicated that only isolates B1 and S-210 were agglutinated by all lectins; all other strains were agglutinated by some but not by all lectin preparations. Unfortunately, the pattern of agglutination did not fit the pattern of pathogenicity of the strains under greenhouse conditions.

The possible involvement of agglutinins in disease resistance was reexamined with the use of four clones of S. phureja (1386.1, 1386.5, and 1386.9, 1386.15) and six strains of the bacterium (B1, K60, S212, S206, S123, S213) which provided a highly cross-linked system, representing a wide combination of resistant and susceptible host-parasite combinations. Lectin preparations were obtained from all potato clones and specific agglutination activities were determined for all possible combinations (Cantrell, unpublished). The results indicated that there were wide differences in specific agglutination among the many lectin-bacteria combinations, but there was no discernable pattern that would correlate agglutination with resistant or susceptible interactions. Differences in agglutinations could be correlated with the amount of slime produced by each individual bacterial strain, rather than with its inherent pathogenicity on any particular clone.

5) Resistance was shown to be altered by environmental factors.

As is the case with many other systems in which resistance is controlled by several genes, that originating from S. phureja was found to be affected by high temperatures and low light intensity. Differences were found in the proportion of resistant to susceptible plants when the same progenies were tested at different times of the year in the greenhouse (Sequeira and Rowe, 1969). Temperatures in excess of 30°C (constant) or low light intensities (1,300 ft.-c or below) eliminated resistance under growth room conditions. Less vigorous plants of a resistant clone, particularly if exposed to low light intensities and short days in the greenhouse, succumbed to stem inoculation. The breakdown of resistance at high temperatures necessarily imposed limitations as to the geographical locations where clones could survive when planted in infested soils.

6. Clones with multiple resistance were developed.

Soon after field testing of the wilt-resistant clones was initiated in the tropics, it became evident that in most areas where *P. solanacearum* was a problem, late blight (*Phytophthora infestans*) and virus diseases (potato viruses X, Y, A, and S, Potato Leaf Roll Virus) also were severe. To deal with the first problem, some of the better-yielding clones that were resistant to bacterial wilt were crossed with late blight-resistant varieties developed in Mexico. The progeny of these crosses were tested for their reaction to late blight as part of the testing program of the International Potato Center at Toluca, Mexico. The material selected in Mexico ("BR" and "MS" series) has been evaluated in various countries, including Peru, Costa Rica, Colombia, and Nigeria; several clones that were resistant to late blight also were resistant to bacterial wilt.

The wilt and blight-resistant clones then were crossed with virus-resistant lines (of German origin) and procedures for screening progeny against potato virus Y were developed with the assistance of Professor R. W. Fulton. The uniform resistance or susceptibility of the parental clones was confirmed. The virus inoculation was carried out either before or after seedlings were screened for resistance to bacterial wilt. As a result of field testing initated in 1976, several clones ("PSY" series) that combined resistance to all three diseases have been selected in various countries. These clones constitute the most promising materials for future use in tropical areas.

7. Resistant clones for most potato-producing countries are now available.

The collaboration with workers in more than 20 different countries throughout the tropical areas of the world has been an essential part of this program. Based on their reports, clonal material of potential usefulness for each participating country is being maintained at the Sturgeon Bay Experiment Station in Wisconsin. Thousands of clones have been distributed to collaborators; guidelines for standard field tests were provided to key collaborators, particularly in Peru, Colombia, Costa Rica, and Brazil. Most countries have reported finding material with resistance to bacterial wilt and, as expected, different clones were selected in different countries. The wide variety of strains of the pathogen, and local preferences for different types, were the major factors that dictated the selection procedures in different countries.

It is evident that the major objective of this program, to develop germ plasm that would be useful to potato breeding projects in countries, has been achieved. In Peru, two varieties that combine resistance to bacterial wilt and late blight, Caxamarca and Molinera, have been released as a result of selections made among the "BR" series of clones shipped

there in 1970. Approximately 30 resistant clones are being maintained in Mexico, and others have shown promise in Costa Rica, Colombia and Nigeria. The testing program has not been universally successful, however. Several collaborators have not submitted reports, or the materials were tested under inadequate conditions. Several countries (including Brazil, Mexico and Mauritius) reported that the levels of resistance in the clones they had selected were inadequate when potatoes were grown at the lower elevations, where mean temperatures would be expected to be relatively high. In a few instances, however, some clones (e.g. MS 35.22) have been found to give adequate yields at relatively low elevations, such as Turrialba, Costa Rica (M. Jackson, personal communication). Within the same country, as in Costa Rica, some clones (e.g. MS 26A.7) have survived well at one location but not at another, presumably because of the prevalence of different strains at the two locations (González, personal communication).

In general, the results of ten years of extensive field testing give adequate evidence that clones with resistance originating from S. phureja can provide excellent control of the disease in many highland production areas. Where extremely virulent strains of the pathogen exist (as in Mexico) or under extremely hot, humid conditions (as in Brazil) it is unlikely that clones presently available will be adequate for control of the disease. These facts have important implications in terms of present efforts by the International Potato Center to extend the range of cultivation of the potato.

Present Outlook and Guidelines for the Future

The resistance to bacterial wilt derived from S. phureja has been used effectively to control the disease in limited areas. It is evident also that the program has not had the impact that could be expected, and it is appropriate that we consider some of the possible reasons. The following may be listed:

1) The complex genetic system that controls resistance to P. solanacearum in S. phureja and the wide range of variability of the pathogen.

These militate against the possibility that one particular hybrid clone will be resistant at all locations. Clones that are resistant in one country are not likely to be resistant in another. However, in our experience, one S. phureja clone (1386.15) is resistant to all strains of the pathogen, including extremely virulent isolates from Mexico and Sri Lanka. Given appropriate selection procedures, it should be possible to identify hybrid clones that carry as many genes for resistance as this parent. The nature of the genetic system, however, complicates the process of selection of the hybrids because there is no adequate substitute for extensive testing in the field. There are limits to the selection program that can be carried out at either Wisconsin or Peru.

2) The instability of resistance at high ambient temperatures.

Because all parental clones of S. phureja succumb to bacterial wilt at temperatures above 30°C, it is evident that this source of resistance will not be adequate in programs to extend the range of the potato to the tropical lowland areas. Additional sources of resistance, including the clones of Solanum sparsipilum and S. chacoense being tested by Martin in Peru, may be more stable under high temperature conditions.

3) The lack of adequate follow-through in many cooperating countries.

Our experience with standard field testing trials, and those that have been done on an ad hoc basis in the tropics, is that the initial results are encouraging but that follow-through is lacking. Almost invariably, clones that appear promising at first do not give adequate yield later, or are eventually rated as susceptible. Many of these problems can be traced to lack of adequate testing sites, viral contamination, nematode infection, or similar field problems that could have been controlled under adequate supervision. The lack of an adequate clean seed program in most tropical countries compounds the problem of maintaining clones in a reasonably healthy condition until their resistance to bacterial wilt can be established with some degree of certainty. For some time this problem was circumvented by increasing resistant clones at the Toluca Station in Mexico, but regulations no longer permit movement of these materials into or out of Mexico at present. Alternative means for the increase of resistant clones, perhaps by commercial potato seed growers in the United States or elsewhere, must be established.

In summary, it is evident that the continued success of a program to control bacterial wilt of potato is dependent on efforts to resolve some of the problems that were listed above. Although the resistance derived from S. phureja is not adequate under all possible conditions, we know enough about the potential and limitations of the system to assure us that renewed efforts will yield practical results. Although work on other sources of resistance should be intensified, the overall program must continue to rely on resistance derived from S. phureja because it is the most likely to pay adequate dividends in the immediate future.

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ROLE OF INDIGENOUS VEGETATION AND CROPS IN PERSISTENCE OF PSEUDOMONAS SOLANACEARUM

C. Martin

The extensive number of plants affected by Pseudomonas solanacearum include not only important economic hosts but also a number of ornamentals and a very diverse group of weeds. Kelman in 1953 reported an extensive number of hosts from more than 33 families to be affected by the bacterium. A large proportion of these hosts belong to the Solanaceae family. However, during the last 25 years a large number of plant species have been added to that list, most of them belonging to other families than the Solanaceae. The list could probably be greatly increased if further studies were done on a large number of plant species that do not show wilt symptoms under field conditions, but in which the bacteria is latent inside the host. I believe that the situation would be even more dramatic if studies were conducted in plant species that host bacteria in their rhizosphere. These statements suggest the importance of indigenous vegetation and crops in the persistence of the bacteria and therefore in controlling the disease.

The importance of indigenous vegetation has been clearly established when potatoes have wilted in fields never previously planted with solanaceous hosts or planted in virgin soils. In many instances the incidence of bacterial wilt has decreased after the first planting with a susceptible host. Probably the mechanics of removing the top soil layer after first planting has played an important role in decrease of soil inoculum. The occurrence of a high level of bacterial wilt in a potato field never before planted with solanaceous hosts at Turrialba, Costa Rica, is an excellent example of the important role of indigenous vegetation in this host-pathogen interaction problem. Studies conducted by Jackson revealed later that the bacterium was native to the area and that two weeds were the principal reservoir of inoculum. The bacterium was repeatedly isolated from two common weeds, Melampodium perfoliatum and Bidens pilosa, which had wilt symptoms. However, the bacterium was also isolated from plants showing no symptoms at all.

Most researchers working on bacteria have agreed over the years that two of the principal means of controlling the disease are rotations and use of disease-free seed. However, in order to establish a rotation program, it is imperative to know the host range of the pathogen. As early as 1939, Smith, working on host range, stated very clearly that the main purpose of these experiments was to control the disease by crop rotation. A lot of research has been done on host range, most studies by artificial

inoculation. However, although artificial inoculation has been useful for virulence and strain determination, it has not been a good index for natural host range. According to Smith, the term "host plant" loses its practical significance if plants so listed do not develop the disease in the field and do not play an active role in the persistence and spread of the causal organism. When the organism is introduced through wounds into the stems of certain plants their mechanism of resistance is also by-passed.

It has been observed many times that rotation not always reduces the soil inoculum. It seems that certain soils tend to maintain certain levels of inoculum. On the other hand, it has been also noted by some workers that continuous cropping with a susceptible host has not always resulted in increased losses, especially in virgin areas opened up to that crop. I will not discuss this topic because there is a presentation that will cover much of this information.

It is impossible to mention in a few minutes the large amount of reports on new hosts and their importance in the persistence of the bacteria. However, let me mention two brief cases that have been reported during the last five years:

a) In 1976 Olsson reported (N.C. Workshop) that P. solanacearum can overwinter in S. dulcamara in Sweden which is far from the tropics at 59°N. S. dulcamara was demonstrated to contain the bacteria in 1974 and 1975 in plants growing by the riverside. Potato fields irrigated with river water where naturally infected S. dulcamara are growing have repeatedly been infected with P. solanacearum. Two other hosts were shown to be infected latently with the bacteria: Eupatorium cannabinum and Brassica napus.

b) During the Bacterial Wilt Workshop held at the Philippines in early 1978, Felix and Ricaud reported that in Mauritius bacterial wilt is endemic in sugar cane fields. No sugar cane plants have been found to show wilt symptoms but they suspect that the rhizosphere of the plant root is the principal reservoir of the pathogen from one season to the next.

During the planning conference and workshop on the Ecology and Control of Bacterial Wilt held at North Carolina in 1976, more than 13 new hosts, principally weeds, were reported from different countries around the world.

The susceptibility of indigenous vegetation under natural field conditions was used by Buddenhagen et al. in 1962 to separate P. solanacearum in three different races. Because host distribution is also closely related to ecological zones, races are more often found in relation to certain hosts in a given ecological zone. Thus, generally race 1 affects solanaceous hosts in places with low elevations and higher temperatures.

The opposite is generally also true for race 3. However, there are some exceptions and probably many more will be found. Despite these exceptions, P. solanacearum undoubtedly originated in the tropics, probably as race 1 only and later came to affect potatoes at higher elevation and as a result of this new adaptation a new race 3 was born. This might explain the wide host range of race 1, especially in Africa and Southeast Asia, where almost 90% of isolates are race 1 (Biovars I, III and IV). So far Biovar IV has never been found in Central or South America.

After these few statements we can easily understand how important and obvious is the role of host range on the persistence of the bacterium.

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PERSISTENCE OF PSEUDOMONAS SOLANACEARUM IN AN INCEPTISOL IN COSTA RICA

M.T. Jackson and L.C. González

Bacterial wilt, caused by Pseudomonas solanacearum E.F. Smith, is undoubtedly one of the most serious plant diseases throughout the tropics and subtropics. Its host range is wide, including non-solanaceous crops as well as weed species from other taxonomic groups. As a disease of potatoes it is widespread throughout Central America and race 3 is the most commonly found variant, particularly in cool highlands.

In Costa Rica, the farming system in the main potato growing area includes rotations with pastures and other vegetable crops, and even in marginal areas, farmers have managed to control the disease through rotations and the planting of healthy seed. However, at altitudes lower than 1000 meters, many soils appear to be infested naturally with race 1 of the bacterium, even where there is no recent history of cultivation of susceptible crops. With the geographical and ecological extension of potato cultivation towards the hot humid lowland tropics, the problem of race 1 is a limiting factor. A number of valuable clones have been selected from material produced at the University of Wisconsin, which have undergone extensive testing in Costa Rica against race 3. Until resistance to bacterial wilt is widely available in potato varieties adapted to lowland tropical conditions, however, methods of decreasing the severity of the disease, other than resistance, will be important.

We have been studying the persistence of race 1 of Pseudomonas solanacearum over a three year period, and the amount of bacterial wilt in potatoes after different crop rotations and weed management practices. All experimental work was carried out in the La Montaña experimental field of the Tropical Agricultural Center for Research and Training (CATIE), Turrialba, Costa Rica, which lies at an altitude of approximately 600 meters. The average maximum temperature is 27°C and the minimum 18°C, with little variation from month to month. Annual precipitation is in excess of 2500 mm, and there is an ill-defined short dry season during February and March. The soil is an inceptisol with stony and normal phases, but its other physical and chemical characteristics are the same.

The level of bacterial inoculum was determined by the percentage of wilted and non-wilted plants of wilt-susceptible and tolerant potatoes. The number of wilted plants was counted weekly, and average percentage wilt (APW), which gives an indication of both total wilt and the rapidity of its development, was calculated at harvest. In 1979, a severity index

at 11 weeks (SI_{11}) based on a scale of 1-6, was calculated to differentiate levels of tolerance among different potato genotypes. Analyses were carried out using the angular transformation of percentage data.

Two experiments were planted to study the effects of crop rotations on the bacterial population. One, over three growing seasons, had maize and beans in 1977 and maize and sweet potatoes in 1978 as rotation crops. In December 1976, the variety Atzimba was planted in a randomized complete block design with three replicates, and plot size of 8.10 x 5 meters. In subsequent potato plantings after crop rotations, the relative tolerance of the varieties Atzimba, Rosita and MS35-22 was compared in 1977-1978, and Atzimba and MS35-22 in 1978-1979, both in a split plot design. Fallow periods between potatoes and rotation crops lasted up to three months.

In the other experiment, the effects of continuous cropping between two plantings of potatoes one year apart was studied. There were five treatments, replicated four times in plots of 3 x 3 meters. Continuous plantings of susceptible potatoes was one of the treatments. The others were maize followed by cowpea, resistant tomato by the same, cowpea by sweet potato and a maize-potato association followed by the same.

During the course of the study, the importance of several local weeds became apparent, especially Melampodium perfoliatum and Bidens pilosa, from which the bacterium was isolated and the pathogenicity of the isolates demonstrated. We compared the amount of wilt in two plantings of Atzimba one year apart in plots in which weeds were allowed to grow with those in which they were controlled by tillage with residue incorporation and through the use of a contact herbicide, paraquat, over a five month period, after a cowpea rotation.

Most potatoes emerged at two weeks, and the maturity of each variety was variable, but never exceeded 90 days; there were some differences between years. In all plantings of potatoes, the first wilted plants appeared between four and five weeks after planting.

The amount of bacterial wilt in potatoes was not reduced significantly after any crop rotation, although there were slight changes from year to year in the experiment in which different potato varieties were compared. For example, the amount of wilt in Atzimba in 1978-1979 was lower than in the previous planting, although APW values in both years were considerably higher than for the first planting of potatoes in 1976, whereas the amount of wilt in MS35-22 increased from 1978 to 1979, despite the rotation crop planted in between. Factors such as soil humidity could well account for these minor differences.

In the other experiment, none of the crop rotation treatments reduced the level of bacterial inoculum. The disease also developed much more rapidly in the 1979 planting than in the 1977-1978 planting. Neither was there a significant difference in the amount of wilt in Atzimba in plots which had continuous cropping of potatoes and those which had had two rotation crops, although APW values were slightly higher for the former.

The amount of wilt in Atzimba in plots where weeds had been controlled through spraying with paraquat was significantly lower from that of the other treatments, except where resistant tomato had been grown, although in these, average percentage wilt was not significantly different from plots where weeds were allowed to grow, controlled through tillage, or where potatoes had been cropped continuously. Even so, the amount of wilt in plots having previous herbicide applications reached 60%, but the disease developed more slowly. In the case of the plots where weeds were allowed to grow, the disease developed more rapidly than in other plots.

In the 1977-1978 planting, when three varieties were evaluated, Atzimba was the most susceptible and MS35-22 the most tolerant; Rosita was intermediate. Although 50-60% of MS35-22 plants were infected with Pseudomonas in the 1978-1979 planting, with APW values of 12.48 and 16.20 after maize and sweet potato rotations, respectively, equivalent SI_{11} values were only 1.73 and 1.97, and less than 1% of tubers had visible symptoms of the disease at harvest. Yields were the equivalent of 30 t/ha. On the other hand Atzimba had APW's of 41.96 and 38.71, after the same rotations and SI_{11} values of 4.44 and 4.36 respectively, and yielded only approximately 100 gm of healthy tubers per plant, of which a further 15% had rotted after storage for 5 weeks.

It is clear that once the bacterium became established in this inceptisol in Turrialba, the inoculum was maintained despite several different crop rotations. The climate is such that the soil rarely dries out completely, and conditions are obviously favorable for the survival of the bacterium even in the absence of susceptible crops. Although rotations have proven successful against race 3 isolates at higher elevations in Costa Rica they did not reduce the bacterial inoculum in Turrialba. Weed populations, especially of annual weeds, are very high under lowland tropics conditions. In plots where maize and beans had been grown, the weed population was slower developing under dead maize stalks because of shading factors, than in plots from which beans had been harvested, even though the bean stand had suppressed weed growth. Even where sweet potatoes were grown, and the ground quickly covered by stems and leaves, weeds were uncommon, but the bacterial population remained high. Continual tillage of the soil constantly brings weed seeds to the surface where they can germinate. We have observed large populations of Melampodium perfoliatum in recently ploughed soil. Contact herbicides killed these annual weeds before they completed their reproductive cycle.

Obviously in any agricultural system the application of contact herbicides on the scale envisaged from our experimental work is not a commercial feasibility, nor can leaving the land in fallow for a long period be considered. The role of the potato under lowland tropics conditions will eventually become more important as agronomic practices are developed so that it is used as a complementary vegetable produced in back yard kitchen gardens or in commercial production. We are currently studying the weed-soil-pathogen relationship with the help of a graduate student, to determine how and where the bacterium is surviving in the soil, and the level of bacterial inoculum through direct sampling, and we plan to study the effects of mulches and herbicides, and other agronomic factors such as ridge size to provide more efficient drainage, without subsequent earthing-up. The level of tolerance encountered in MS35-22 holds promise for the future, but to ensure complete success of potato cultivation in the lowland tropics an integrated approach to bacterial wilt control would appear to be the most feasible.

Average percentage wilt in three potato varieties after different crop rotations, 1977-1978.

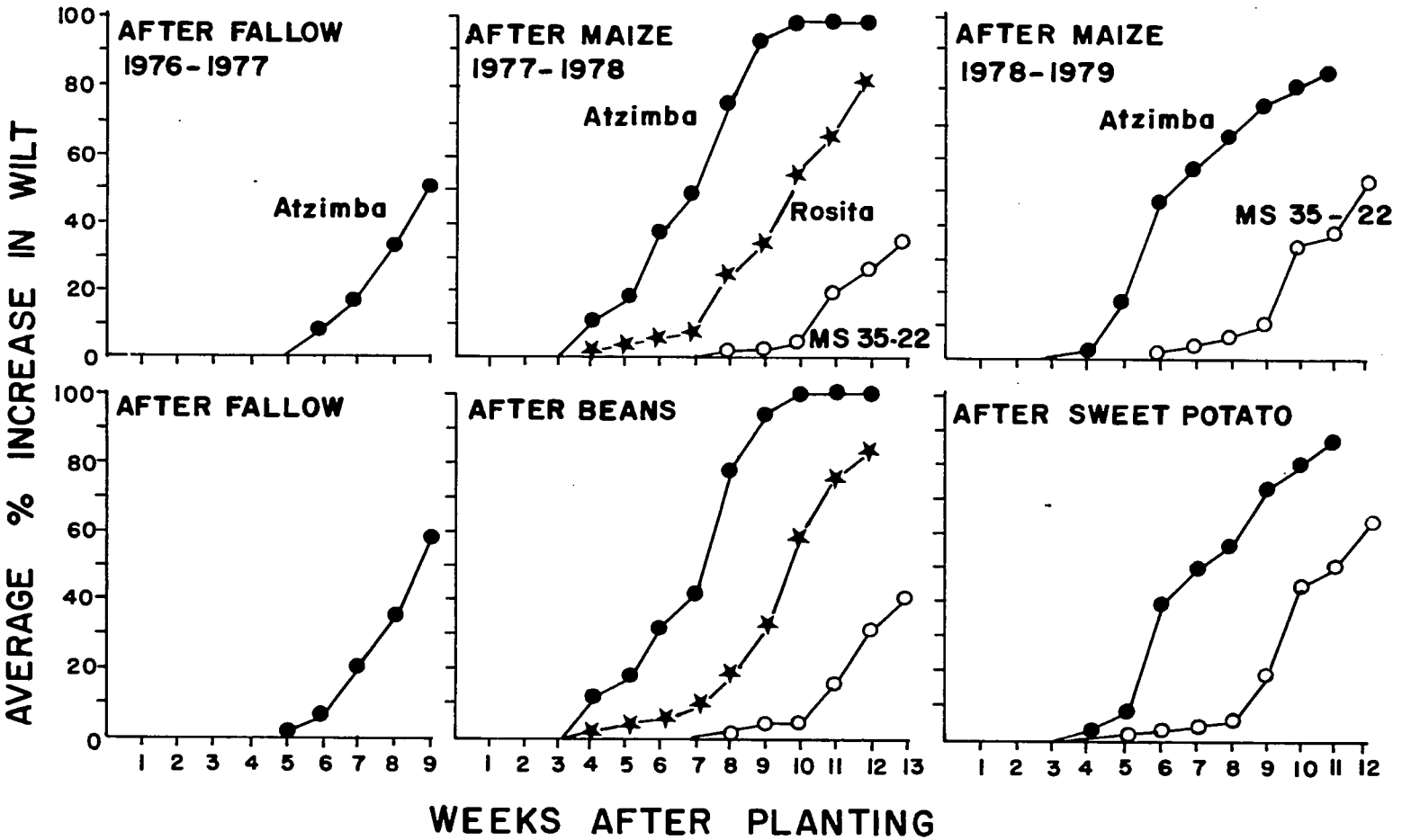
	Atzimba	Rosita	MS 35-22
After maize	53.28	24.73	7.17
After beans	53.13	26.61	8.17

Average percentage wilt (APW) and severity index (SI_{11}) in cv. Atzimba and MS 35-22 after different crop rotations, 1978-1979.

	Atzimba		MS 35-22	
	APW_{11}	SI_{11}	APW_{12}	SI_{11}
After maize	41.96	4.44	12.48	1.73
After sweet potato	38.71	4.36	16.20	1.97

Average percentage wilt in cv. Atzimba after 9 weeks, in plots subsequently planted with different rotation crops, 1976-1977

After fallow Before maize	After fallow Before beans
13.44	15.37


AFTER FALLOW
1976-1977
Atzimba
AFTER MAIZE
1977-1978
Atzimba
Rosita
MS 35-22
AFTER MAIZE
1978-1979
Atzimba
MS 35-22
AFTER FALLOW
AFTER BEANS
AFTER SWEET POTATO
AVERAGE % INCREASE IN WILT
WEEKS AFTER PLANTING

Average percentage wilt (APW_9) in cv. Atzimba before and after rotation with cowpea and weed management.

Intermediate crop/management sequence	Potato planting Dec.'77-March '78	Potato planting Jan.- April '79
Potato-Potato	29.56	49.69 b
Tomato-Tomato	26.13	38.27 a b
Cowpea-Tillage	28.52	43.50 b
Cowpea-Herbicide	23.93	23.81 a
Cowpea-weed growth	27.06	53.79 b

Average percentage wilt (APW_9) in cv. Atzimba before and after different crop rotations.

Intermediate cropping sequence	Potato planting Dec.'77-March '78	Potato planting Jan-April '79
Potato-potato	26.70	54.60
Tomato-Tomato	25.67	51.36
Maize-Cowpea	25.93	47.30
Cowpea-Sweet potato	31.36	46.92
Maize/potato-Maize/ potato	30.52	55.05

PROGRESS IN THE INTEGRATED CONTROL OF BACTERIAL WILT

E. R. French

INTRODUCTION

Bacterial wilt or brown rot disease of potatoes caused by *Pseudomonas solanacearum* is often a limiting factor in potato production in the tropics, subtropics and in the temperate continental climates that have hot summers (9). The potato race (race 3) which is considered to be cold tolerant, occurs generally in the cool upland climates or high latitude regions (10, 11, 15, 26, 33, 38, 40).

The development of *P. solanacearum* immune potatoes would be the ideal solution to control bacterial wilt, but even a good level of resistance would probably suffice if combined with other control measures.

RESISTANCE

Presentations have already been made at this conference on the sources of resistance to *P. solanacearum* and the development of resistance to bacterial wilt derived from *Solanum phureja*. Resistance has been shown not to be general, but rather specific to some pathotypes, regardless of whether these belong to race 1 or 3. However, some of the pathotypes of race 3 seem to have a wide distribution as illustrated by the fact that some cultivars are resistant in Peru, Colombia, Sri Lanka and Nepal, or Costa Rica, Colombia and Peru (E. French, unpublished).

This resistance is conditioned by the environment, it is not immunity (6, 42). Resistance for the lowland tropical rain forest climates where race 1 predominates, seemed harder to accomplish until the new source of resistance mentioned earlier was selected in Turrialba, Costa Rica (see M. Jackson paper); however, initial greenhouse inoculation tests have shown Cruza 148 to be susceptible to some other pathotypes (C. Martin, unpublished data).

An adequate control of the disease when resistance is complemented with other factors that reduce the inoculum potential or that are less conducive to disease development, may be possible for at least race 3, and for race 1 at the cooler reaches of its distribution. Through persistence in breeding, it may in the future be accomplished for the hot humid tropics.

FACTORS COMPLEMENTING RESISTANCE

The principal and best understood factors that can complement resistance (or may be of use without resistance) are the use of knowledge in a given situation about effects of:

Inoculum potential.- Inoculum potential in the field can be maintained in 1) self-sown tubers, 2) debris of a diseased crop, 3) free in the soil, 4) on alternate hosts or harboring plants (host range or virulence of the bacterium).

Factors during cropping are: 1) Planting of diseased seed, 2) Nematode interaction, 3) Temperature, 4) Mechanical damage, 5) Moisture and water flow.

Aggresivity of the *P. solanacearum* strain present.

These factors and their relationship to resistance in causing disease are shown in Fig. 1 and then discussed.

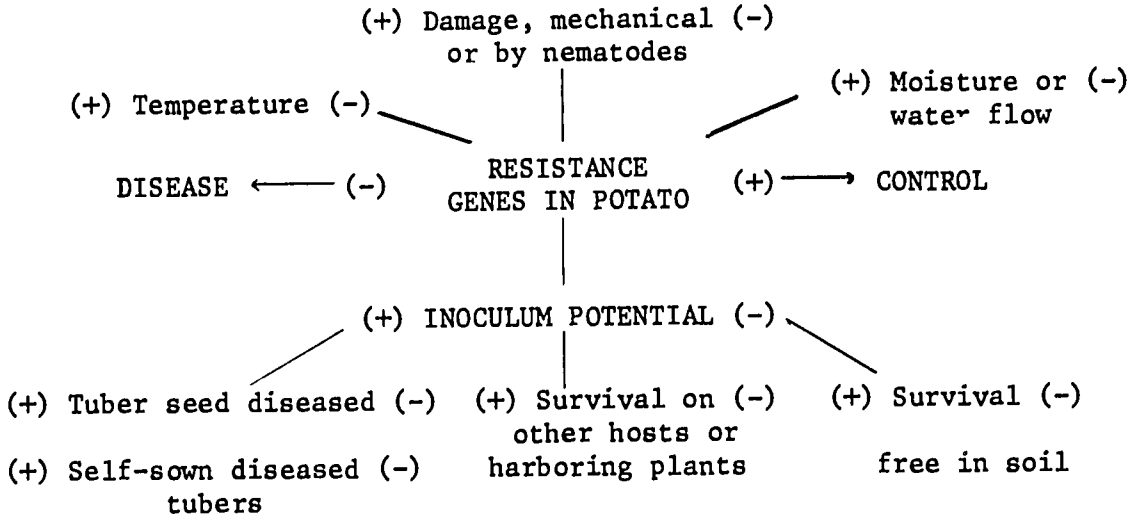


Figure 1. Interrelationship of the various factors that contribute to either bringing about bacterial wilt or to controlling it. (+) = greater, (-) = lesser. The further to the left the position of the stated factor the greater its importance in disease inducement.

INOCULUM POTENTIAL

Self-sown tubers from a previous diseased crop which rot in the soil to liberate P. solanacearum, or give rise to diseased volunteers that exude bacteria from their roots, provide a heavy inoculum potential (27).

Sufficient numbers of bacteria to initiate disease often remain in the debris of a previously diseased plant (potatoes, other hosts or weeds), but the warmer the soil temperature and the greater the microbial activity, the shorter this is (13, 27). Survival of free bacteria in deep layers of the soil where antagonistic microbial activity is low has also been recorded (5, 39). Some soils, however, have a suppressive factor present, which appears to be of a physical nature (31, 36). Some evidence points to salinity (measured by electrical conductivity) as being effective in eliminating P. solanacearum rapidly from soil (19).

Alternate hosts, in cases in which the virulence of P. solanacearum provides it with an ample host range, assures perpetuation in the absence of the potato crop (20, 37). In the case of race 3 the bacterium is primarily restricted to potato, infecting tomato only when the inoculum potential is high after a diseased potato crop. Weeds may also be affected but seldom provide a perennial host for its maintainance, the notable exception being Solanum dulcamara in Sweden (33).

Race 1, however, has an ample host range encompassing many solanaceous crops, and crops and weeds of many other families, but the host range varies with the pathotype. There is also some evidence that race 1 is perpetuated in the rhizosphere of plants that harbor the bacterium in a favorable environment provided on their roots (circumstantial evidence points to sugar cane, for instance, but confirmatory research needs to be done). This may account for the appearance of wilt in potatoes when planted after forest or jungle is cleared (2, 28, 38).

FACTORS DURING CROPPING

Diseased seeds

The major cause of bacterial wilt in potatoes is the planting of diseased seed. Ideally, seed transmission of P. solanacearum should never occur, but it is the principal cause of wilt incidence in the tropics. This is because many potato producers cannot call on reliable indigenous sources of seed, and imported seed, if available, is very costly or its purchase is restricted because of international trade balance factors. This problem can probably be resolved in some regions by utilizing the coolest (highest) sites available for basic seed production, combined with other feasible control measures such as rotation and use of resistant cultivars (8).

Sample test plots or a plant or tuber indexing program can eliminate diseased tuber lots from a program. Tubers can be indexed by incubation at high temperature (25-30°C) followed by detection of those showing symptoms and plating out of vascular tissue from the stem-end of the remaining healthy appearing ones (12). A further improvement in the use of the enrichment culture technique to increase the bacterial numbers to detectable levels (L. Ciampi, personal communication).

Tuber transmission is responsible for the long distance dissemination of bacterial wilt disease. Quarantine measures within a country can help restrict it, and proper importation controls can protect countries from its introduction. Care must also be taken to avoid transmitting the disease from lowland soils in which the bacterium is indigenous by selecting healthy appearing tubers from a diseased crop and planting them in colder (higher) wilt-free soils (10).

Nematode interaction

The root-knot nematode, *Meloidogyne* spp. is a severe problem in potato production in many warm locations, and its interaction with *P. solanacearum* is very marked (22). Wilt-resistant cultivars can become susceptible when attacked by this nematode (23, 43). Since both problems are factors in hot climates, they must be considered together.

Other nematodes such as the potato cyst nematode *Globodera* (=*Heterodera*) spp. interact (21), but since this nematode is favored by cool to cold temperatures and *P. solanacearum* by warm to hot, a joint approach to solving these problems does not seem of high priority. The same may hold true for *Nacobbus* spp. However, *Pratylenchus* sp. may be a greater problem than previously recognized, at warm locations (P. Jatala, personal communication).

Temperature

Temperature plays a key role in the development and epidemiology of the disease. Race 3 is more tolerant to cold, surviving at latitudes of 59°N in the field in Sweden (34); it multiplies in apparently healthy crops at high elevations in the Andes (3000-3500 m), later resulting in severe losses when the seed produced under these conditions is planted in lower warm areas (10). Isolates from potatoes are most often race 3, and when they are race 1, these are usually from the lower elevations in the tropics or from the subtropics (9). High elevation seed farms provide security in the production of wilt-free seed when precautions are taken to avoid contamination of such fields with infected tubers which may result in the disease being latent (10, 16).

Mechanical damage

The underground parts of potato plants suffer rupturing of tissues due to natural growth (24); also damage results from the feeding of insects, and from field practices such as weeding and hilling. I have observed that crops affected early in the growing season show a rapid rise in wilt incidence after hilling. Resistant cultivars may develop some cases of wilt as a result, especially when interplanted with susceptible diseased cultivars or in high inoculum potential soils.

Moisture and water flow

The bacterium cannot withstand dessication, hence regions with defined drought seasons and high dessicating temperatures can probably be freed each year of contaminating bacteria (38). High moisture promotes multiplication and results in faster wilt symptoms; it also facilitates plant-to-plant spread (24).

Excess moisture that results in run-off, and surface irrigation, help to spread the disease along the row. Run-off from one field can result in infection in another field if the disease is severe enough to result in high numbers of bacteria in the run-off water. Irrigation from contaminated streams, as was shown in Sweden where S. dulcamara perpetuated the bacterium in the water, can be a source of infection (33, 34).

When excess water leads to prolonged flooding, or when this is a cultural practice as in the case when paddy rice is grown in rotation with potatoes, P. solanacearum may decrease considerably, but so far no report of eradication is known (4, 37).

AGGRESSIVITY OF STRAINS

Ranges of aggressivity occur in both races 1 and 3 (6). The less aggressive strains are less likely to cause disease and take longer to wilt potato plants. Avirulent mutants develop in any active growing culture, and must be taken into account in inoculation studies, but they probably are of no consequence in nature.

IMPLEMENTATION OF INTEGRATED CONTROL MEASURES

To decide what steps to take to effectively control bacterial wilt of potatoes, it is important to establish the relative importance of the various factors shown in Fig. 1. Crucial to this process is to determine what race or races are involved and the possible host present. The pathotypic characterization of given races may be useful, i.e. the host range. The source of infection should be determined so that if the bacterium is not indigenous it may be eradicated.

In the case of race 3 which has a limited host range and is primarily a problem of dissemination by seed it may suffice to prohibit the planting of other than certified seed and require a two-year rotation to eradicate as has been the case in India (29). In coastal Peru, where soils are antagonistic to the bacterium, certified seed alone has proven an adequate eradication measure (8). In warmer climates such as those of Río Negro (2200 m) in Colombia, a 6 month fallow may suffice to reduce disease incidence to acceptable levels (3). In Kenya a 2-year rotation with pasture is very helpful (15).

Unfortunately, in some tropical countries holdings are small and consequently the task of teaching farmers what to do or enforcing legislated practices is not feasible. Therefore, the development of a resistant variety can be the crucial factor to make eradication possible for the cooler climates (1, 17, 18) and a reduced acceptable level of incidence the result in warm climates. Among the varieties developed for the temperate climates of the higher latitudes, there are some that are more tolerant than others and can contribute to a lower incidence of wilt (3, 11, 32).

With race 1, which survives longer free in soil, at least in Kenya (35), and has a greater host range, the possibility of eradication seems remote. An acceptable level of disease can probably be achieved in most warm to hot areas by a combination of crop rotation, weed control during all crops and fallow periods, planting of healthy seed, low damage tilling and the choice of the least compatible soils for the bacterium. Resistance may be highly useful also, especially if a non-heat labile resistance is found, since race 1 occurs primarily in hot climates which are often beyond the ideal for the potato crop and most favorable for the tropical *P. solanacearum.* It is also probable that as genes for adaptation to heat are added to potato cultivars, the available resistance genes in such an adapted genetic background may be more effective.

The choice of incompatible or wilt suppressive soils can be accomplished by a soil assay employing potted field soil in which indicator plants are sown, either tomatoes in a hot climate where race 1 is present (5), potatoes in cooler situations (7), or especially developed potatoes grown from suberized abscised eyes, seedlings, or sprouts (14).

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PROBLEMS IN THE UTILIZATION OF RESISTANCE BY DEVELOPING COUNTRY POTATO PROGRAMS: THE COLOMBIAN EXAMPLE

G. A. Granada

Introduction

The International Potato Center, in collaboration with the University of Wisconsin, has been providing potato clones which combine resistance to bacterial wilt, late blight and potato virus Y (2). The Potato and Plant Pathology Programs at ICA, Colombia, have evaluated many of these potato clones for more than 10 years (3,5). The general policy that has been established is that each country collaborating in the International Trial for Resistance to Bacterial Wilt is free to select the best clones and release them as varieties or to use them as parents for further breeding. Few participating countries have, so far, made selections that fulfill internal marketing demands and adopted clones as commercial cultivars. Peru and Kenya seem to be the only examples (2).

Programs in Colombia have participated actively not only in the searching for sources of resistance (8) but in the evaluation of hundreds of hybrids produced at the University of Wisconsin (7). Although we have not found clones that satisfy our market demands, there are great expectations because of the good performance of the last set of hybrids that carry resistance to bacterial wilt, late blight, and potato virus Y. Some of these MS clones are very promising.

Main Problems in the Evaluation of Resistance

I want to refer to problems that participating programs in developing countries may run into in the course of evaluation and utilization of wilt-resistant potato clones. Two major problems have affected the current evaluation of potato clones in Colombia: 1) Lack of adequate bacterial inoculum potential in the testing areas; 2) Difficulties in maintaining the selected clones in good sanitary conditions.

1) Adequate bacterial inoculum potential in field plots

Maintenance of adequate bacterial infestation in the test plots from one season to another has been the most important problem in the process of evaluation and selection of clones. This has been partially the result of adverse climatic factors (drought) that affect the inoculum potential

significantly. In addition, unknown soil factors have made it impossible to maintain a high inoculum potential at Popayan, once considered the ideal location to test for wilt resistance.

Because of these problems we decided to test in more than one location where the high incidence of Pseudomonas has been a problem in the past. Discussion. A single test site is not adequate and slows up the process of selection. This was the case in Colombia before we decided to start testing at two localities. Two test sites assure us that data from at least one location will be obtained if something goes wrong in the other. Also, in the process of selection it is very important to determine how the resistant clones will perform when challenged by different strains of the pathogen in different regions. This is urgently needed if we consider that the resistance incorporated in these clones is strain specific and not general (1).

As a general policy, research on biotype or race determination should be included in the evaluation of resistant clones. This is important because it will show the possible variability of the pathogen within an area or country and, what is most important, it will help to interpret the results. In countries where seed potatoes are moved from high to low altitudes and vice versa, there may be great heterogeneity in bacterial populations. Each participating country should be aware of this heterogeneity. One example of this heterogeneity was reported by Martin and French (4) in Peru.

When trained personnel or facilities are not available in a particular country to do biotype or race identification, this could be done by CIP.

2) Maintenance of resistant clones

Most of the clones received for evaluation prior to 1976 have been highly susceptible to most common viruses, especially potato virus X (PVX), potato virus Y (PVY), and potato leaf roll virus (PLRV) (6, P. L. Gómez, personal communication). Although periodical insecticide spraying is practiced, it has been very difficult to keep the clones free of viruses during the process of selection. The virus infection that may take place at one particular time may be such that selection based on probable yield is difficult to make. Also, we do not know how virus infection interacts with Pseudomonas infection, either predisposing to susceptibility or inducing resistance. Based on field observations plants infected with virus may behave as more tolerant to bacterial wilt. Discussion. Plant Protection and Quarantine Agencies in developing countries have become increasingly concerned about new strains of pathogens that may be introduced with new germ plasm and then threaten the production of a particular crop. In the case of potatoes research has shown that vegetative seed may carry bacteria, fungi, and viruses in latent state. without any external symptoms of contamination.

Cloning of each selected clone and sending only the uninoculated material should be a common procedure for all countries involved in the distribution of resistant materials to participating countries. This procedure should be used within each particular country each time resistant clones are moved from one place to another.

Bacterial wilt symptoms are relatively easy to diagnose under field conditions, but viruses are rather difficult. To help determine in a more accurate way the behavior of selected clones for virus susceptibility, antiserum against the most common viruses should be used. Also the use of indicator plants would be of great help. The possibility of providing antiserum to such viruses as well as a complete set of seed of indicator plants to be used at the location(s) where the clones are being tested will be of great help to collaborators.

The designation of a location, where to maintain and increase materials under the best sanitary conditions, constitutes one of the most important steps in the processes of evaluation and selection. No country should depend entirely on disease-free materials received from other collaborators to continue its own research. Each country should have the means to keep materials virus-free until they are tested in the field.

The Need for Speeding up the Evaluation and Selection Process

Much effort is lost when a particular country does not keep on working with clones initially selected in the International Trial for Resistance. There should be a mechanism to speed up, when applicable, the research leading to the release of commercial bacterial wilt-resistant cultivars. The success of the International Trial for Resistance is measured by the number of clones that may become cultivars or serve as parents for further breeding in a particular country. The possibilities of financing well-planned experiments in which selected resistant clones are used in semi-commercial or commercial trials, should be considered. The possibility of maintaining a bank of data on the performance of all selected materials, including those that are tested under semi-commercial or commercial conditions, should be considered as well. This may be of help in the assessment of the project and, more importantly, serve as a guideline for proper budgeting.

The Need for Reports on the Performance of Resistant Potato Clones

Although exchange of information between most researchers and CIP officials has been good, there is a lack of communication among participants of information concerning the performance of potato clones included in the International Trial for Resistance. I think that a "Circular" in

which results are reported would help to keep collaborators more aware of progress. In particular it would make collaborators more enthusiastic about this kind of cooperative research.

Although the methodology as to how to plan the experiments and the forms to report the data obtained are available, a general procedure of how to analyze and present the results in a standard form should be worked out. This will provide some help to those in charge of collecting and analyzing reports from each country.

The Possibility for the Release of a New Variety

In general, the materials that ICA has screened have characteristics which are not acceptable in the Colombian market. Most clones have white color, deep buds, tuber size, and cooking characteristics which are not acceptable (P.L. Gómez, ICA, personal communication). However, the clone 7/10, one of the first clones tested in 1968, has been tested for several years by Ing. Agr. Octavio Pérez, at La Selva, Rionegro, and has shown very good resistance to bacterial wilt and viruses as well as to early blight. If the yields in regional trials in 1979 are good, this clone may be released as a new cultivar, to be planted mostly in areas where bacterial wilt is of major importance in the Department of Antioquia (P.L. Gómez, ICA, personal communication).

SUMMARY

Most of the potato clones evaluated prior to 1976 do not fulfill internal marketing demands, though lately there is great expectation because of the good behavior of the MS type clones.

Evaluation and selection of clones have been directly dependent on adequate inoculum potential in the testing sites as well as on maintenace of good sanitary conditions of them. Several propositions are presented to be considered as a general policy in the International Trial for Resistance:

1. Testing at two localities.

2. Research on biotypes or race differentiation in each participating country.

3. Cloning of each selected clone for shipping to participating countries should be a common procedure for all countries involved in the distribution of resistant materials. When possible this procedure should be extended to movement of materials within each particular country.

4. Provision of antiserum to common potato viruses as well as indicator plants to determine in an accurate way the behavior of selected clones to such viruses is recommended.

5. To speed up the process of releasing of selected clones, the possibility of financing semi-commercial or commercial trials, when applicable, should be considered.

6. Maintenance of a bank of data on the performance of selected clones is suggested.

7. A general report to be distributed to all participating countries concerning the performance of potato clones in the International Trial for Resistance is necessary.

8. A standard procedure of how to analyze data to be presented to CIP should be worked out.

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THE ERWINIAS OF POTATOES IN PERU

E.R. French & L. de Lindo

Isolation of Erwinia spp. were made from potato tubers, stems and roots of plants with symptoms typical of soft rot and blackleg in the three natural regions of Peru: coastal irrigated desert, highlands and tropical rain forest or jungle. Isolations were made from stems of wilting plants showing dark vascular discoloration which resulted in a darkening of stems in non-pigmented cultivars in jungle locations below 1000 m in elevation.

From among hundreds of isolations, 56 representative ones were retained for taxonomic studies. The criteria utilized to separate the two species E. chrysanthemi and E. carotovora and the varieties carotovora and atroseptica of the latter are shown in Table 1.

Table 1. Tests employed in classifying the three erwinias that infect potatoes

Test	Erwinia classification		
	carotovora	atroseptica	chrysanthemi
alpha methyl glucoside	-	+	-
reducing substances from sucrose	-	+	+ or -
growth at 36°C	+	-	+
growth in 5% Na Cl	+	+	-
Phosphatase test	-	-	+

Carotovora was isolated in 12 instances from tubers in all three regions, from 24 stem samples from jungle and highland and from roots in three instances for one highland location. Atroseptica was isolated from seven blackleg stem samples from the highlands. Chrysanthemi was isolated from tubers once in a coastal planting and twice from jungle locations, from stems in 4 jungle specimens and in one from the highlands (3100 m)*, and from roots of one specimen (3 others were carotovora) in another highland site (3300 m). Details are shown in Table 2. Preliminary reports on this subject were published earlier (2, 3).

* Subsequently shown to be a Pseudomonas sp.

TABLE 2. Origin of *Erwinia* spp. isolates in Peru, organ from which isolated and their classification into the species or subspecies *E. carotovora* var. *carotovora* (CAR.), var. *atroseptica* (ATR.) and *E. chrysanthemi* (CHR.)

LOCATION[1] (AND ALTITUDE)	ISOLATE[2]	CAR.	ATR.	CHR.
Satipo, JN (800m)	064t, 065t	X		
San Ramón, JN (800m)	010t, 011t, 012t, 013t, 014t 017t, 018s, 019s, 020s, 033s 034s, 035s, 036s, 037s, 038s 039s, 040s, 062s	X		
San Ramón, JN (800m)	001s, 004t, 005, 006, 032s 061t			X
La Molina, Lima (320m)	053t, 063t	X		
Vitarte, Lima (400m)	080t			X
Comas, JN (3200m)	002, 021s, 026s, 067s		X	
Comas, JN	022s, 023s, 024s, 025s	X		
Concepción, JN (3300m)	047s, 048s, 051s	X		
Concepción, JN (3300m)	049s		X	
La Victoria, JN (3300m)	027r, 028r, 029r	X		
La Victoria, JN (3300m)	030r			X
Palca, JN (3100m)	081s (*Pseudomonas* sp.)			
C. Huaylas, AH (2600m)	003	X		
Yata,(HO) (3400m)	009s	X		
Choquecocha, HO (2400m)	015s, 016s	X		
Chaglla, HO (3200m)	043s		X	
Pumahuasi, HO (2900m)	042	X		
Izcuchaca, HA (3300m)	031t	X		
Salcedo, PUNO (3800m)	007s, 0080s	X		
Azurin, PUNO (3800m)	066	X		

1/ Department abbreviations are: Junín, JN; Ancash, AH; Huánuco, HO; Huancavelica, HA

2/ Letter following number indicates plant part from which isolated (when recorded): r = root, s = stem, t = tuber.

The growth of two isolates of each erwinia in liquid shake culture at approximately one degree stepwise increments in temperature from 5 to 40°C in a temperature gradient incubator, gave the following range for the optimum of each: atroseptica, 27.0 - 27.1; carotovora 28.0 - 30.5 and chrysanthemi, 34.0 - 36.0.

Twenty-six potato varieties, mostly Solanum tuberosum subsp. andigena or crosses of subsp. andigena and subsp. tuberosum, and 324 native varieties (clones) from the germplasm collection selected on the basis of productivity, were screened for tuber rot resistance to a very aggressive chrysanthemi isolate. The infectivity titration method of inoculation, with five levels of inoculum of which the three most useful are shown in Table 3, was utilized. Tubers were inoculated soon after harvest (within 4 weeks) and were still quite turgid. They were injected 5 mm deep into the tuber avoiding the lenticels. After the 0.01 cc inoculum volume (part of which exuded) was fully absorbed, the lesion was sealed with vaseline, the tuber wrapped in wet paper towel and then two layers of saran wrap (polyvinylchloride) which is impervious to oxygen. After 72 h at 25$\pm$ 1°C tubers were cut and the diameter of the cylindrical rot recorded on ten tubers per clone and per variety.

The range of diameter of rot was 0 - 11.7 mm for the clones, and up to 19.7 mm for the varieties at the highest inoculum concentration (4.4 x 10^6). A tentative classification, pending retesting with larger numbers of freshly harvested tubers of the better selections, suggests that four clones are very resistant (VR) or immune and 13 resistant (R). Among the varieties there was a near uniform distribution in degrees of susceptibility, with one, Yungay, warranting a tentative resistance rating and another, Sipeña, moderately resistant (MR), as can be seen in Tables 3 and 4. Note the difference in rating for Revolución in the variety test and as a check for the clones, between the two tests. This probably reflects the greater turgidity of the tubers in the variety test, since these were inoculated sooner after harvest (7 - 10 days).

In greenhouse inoculation tests at two temperature regimes, injecting inoculum in the stem at the soil line, when the temperature high reached approximately 20°C, only atroseptica caused blackleg, but when the high was approximately 30°C it did not while blackleg type symptoms and death occurred with carotovora and chrysanthemi, the latter being the more aggressive. These tests confirm the field observations and agree with a report from Japan on the pathogenicity of E. chrysanthemi to potato (4). This organism was also isolated from potato with blackleg symptoms in Brazil (1) by C. Robbs (M. Pérombelon, personal communication).

TABLE 3. Tuber susceptibility of the 17 best among 324 germplasm clones tested by infectivity titration inoculation to *Erwinia chrysanthemi* (diameter in mm of cylindrical rot 72 h after injecting 0.1 cc inoculum 5 mm deep).

CLONE[1]	Inoculum concentration (cells/cc)			SUSCEPTIBILITY[2]
	4.4×10^6	8.8×10^5	1.76×10^5	
OCH-6022	0	0	0	VR
OCH-5875	0.5	0	0	VR
JAK-119	0	0	0	VR
H/J-560	0	0	0	VR
700546	2.0	1.0	0	R
700614	1.3	0.8	0.8	R
700726	1.0	0.1	0	R
700762	0	0	0.9	R
701020	2.5	0.4	0.1	R
701488	1.5	0.3	0	R
OCH-5248	2.5	0.7	0	R
OCH-5416	4.6	3.5	0.4	R
OCH-5708	2.4	1.6	0.3	R
JAK-072	3.4	0.1	0	R
JAK-109	2.7	1.8	0.7	R
JAK-145	2.9	1.9	0.5	R
JAK-228	1.7	1.2	0.4	R
Revolución (CK)	9.3	6.9	3.6	S

1/ *Solanum tuberosum* subsp. *andigena*, except for OCH-5416, *S. curtilobum* and Revolución, Tbr x Adg.

2/ VR = very resistant; R = resistant; S = susceptible

Table 4. Tuber susceptibility of potato cultivars in infectivity titration inoculations with *Erwinia chrysanthemi*; diameter (mm) of cylindrical rot 72 h after injecting 0.01 cc inoculum 5 mm deep.

CIP No.	CULTIVAR	INOCULUM CONC. (cells/cc)			SUSCEPTIBILITY*
		4.4×10^6	9.9×10^5	1.76×10^5	
720064	Yungay[1]	3.3	2.2	0.7	R
720024	Sipeña[1]	5.2	2.0	0.6	R
720026	Renacimiento[2]	8.8	7.5	5.4	S
	Promesa[1]	9.4	6.9	3.6	S
800222	Molinera[3]	10.0	8.5	4.3	S
720117	Capiro[1]	12.3	7.9	1.0	S
720072	Tomaza Condemayta[1]	10.0	7.3	5.1	S
720065	Huaytapallana[2]	10.5	8.6	5.5	S
	Chata blanca[4]	11.0	8.5	3.9	S
720074	Participación[1]	11.8	8.5	4.5	S
720027	Merpata[1]	11.1	9.1	5.7	S
720063	Antarqui[5]	11.7	8.9	5.1	S
	Collota[1]	13.1	9.8	7.4	S
720028	Renovación[6]	13.0	10.9	9.4	S
720044	Rosita[7]	14.0	11.7	7.9	S
720066	Cuzco[1]	14.6	13.0	9.4	S
720025	Mariva[1]	14.6	11.7	9.9	S
720049	Motsana[7]	15.9	14.3	11.4	S
	N.5784	16.4	15.2	12.2	S
720019	Ticahuasi[1]	16.8	14.3	11.8	S
720043	Revolución[1]	17.9	16.3	11.5	S
720042	Mi Perú[1]	17.4	13.9	11.7	S
720031	Nevada[2]	17.2	14.7	12.5	S
720067	Ranrahirca[1]	17.0	15.7	13.4	S
720047	Anita[7]	16.9	14.8	10.6	S
720073	Huancayo[1]	19.7	16.4	12.3	S

* R = resistant; S = susceptible

1/Tbr x Adg 2/Adg x Adg 3/Phu-Tbr-Dem 4/Adg
5/Tbr-Adg 6/Adg x Cur 7/Tbr x Tbr

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ECOLOGY OF SOFT-ROT ERWINIAS IN RELATION TO POTATOES

M.C.M. Pérombelon

Introduction

The potato is one of the world's major food crops but has been a staple food only in some temperate regions for any length of time. In the tropics and sub tropics it has been used more as a dietary adjunct or as a luxury food. Recently, with the increasing realization of its excellent nutritional qualities and high yield per unit area and time, its potential importance to the diet of people in the warm regions of the world has increased rapidly.

Potato cultivation is specialized and for large scale growth requires expertise not usually available in many tropical countries, especially where cropping tends towards subsistence agriculture. The potential tropical grower is faced with two major problems closely related to the topic of this communication; first, to obtain an adequate supply of inexpensive disease-free seed tubers, and second, access to satisfactory storage facilities. In some tropical countries the cost of seed alone can account for ca. 50% of the total production cost and, therefore, great care should be taken to obtain or to produce high quality seed. Storage losses from diseases are often large and the trend from ambient to cold storage may do no more than change the spectrum of losses from those caused at high temperatures to those more familiar to the potato grower in temperate climates.

This paper reviews the impact of recent progress on the ecology of soft rot erwinias (SRE) on the techniques used in the production of healthy seed aimed at reducing losses in the growing crop and in storage.

The Diseases

Infection of plants by SRE results in maceration and rotting of parenchymatous tissue. In the growing plant SRE cause 'blackleg' which is a field disease affecting the shoots; in store they cause a typical tuber decay from which the term 'soft rot' is derived.

Blackleg is characterized by a soft rot of the basal part of the stem and it is invariably associated with decay of the parent seed tuber. However, whereas blackleg is sporadic it is normal for the seed tuber to rot so that the converse association of rotting tuber and blackleg is not necessarily cause and effect. Sprouts can be infected at or

soon after emergence and they then usually die resulting in blanking in the crop. When older shoots are affected, the leaves in the early stage of disease development tend to become erect and the margins of the younger leaves grow inward mimicking the symptoms caused by the leaf roll virus. However, it is a characteristic of blackleg that frequently only one or two shoots of the plant are affected whereas leaf roll virus commonly affects all the shoots. In dry weather, shoots affected by blackleg dry out and die and the lesion is usually restricted to the underground part of the stem which, when exposed is brown or black, dry and often split. In wet weather, the rot progresses rapidly up the stem and may sometimes extend to the mid-rib of the leaves. Such plants wilt and collapse rapidly without yellowing of the leaves and before much leaf curling develops. In temperate regions the affected stems are usually black which is where the common name of 'blackleg' is derived, but in warmer regions the lesions may also be light brown. Recently, but as yet unconfirmed, it has been reported that the stem symptoms caused by the var atroseptica may be distinguished from those caused by other Erwinia spp.

Symptoms of blackleg can develop at any time during the growing season and affected plants are distributed at random. However, in some fields the disease appears to affect plants in groups, possibly reflecting localized soil conditions particularly favorable to symptom expression. In young diseased plants, in which tuberization has started, the rot frequently extends along the stolon into the heel end of the progeny tuber. There it is usually arrested and the dead tissue dries leaving a characteristic dark sunken area at the stolon end and browning of vascular bundles can be seen to extend for some distance beyond the sunken area. Under very wet conditions or in water-logged areas of soil the rot is not arrested and the whole tuber may decay.

Post-harvest loss of tubers can occur in transit to and from storage as well as in the store. Once rotting of tubers has started, usually in small pockets, it proceeds rapidly to cause a massive breakdown with the tubers liquifying into a molodourous mass. In bulk storage, large numbers of otherwise sound tubers are rendered unmarketable when contaminated by the foul smelling liquid.

The Bacteria

Erwinia carotovora (Jones) Bergey, Harrison, Breed, Hammer and Huntoon 1923, var carotovora Dye 1969, E. carotovora var atroseptica (Hellmers & Dowson) Dye 1969, and E. chrysanthemi Burkholder, McFadden & Dimock 1953, can all cause the symptom called blackleg. The predominant organism in an outbreak will depend on the source of seed and the climatic conditions. Thus, in cool temperate regions the disease is usually caused by the var atroseptica: in warmer areas both the vars carotovora

and atroseptica may be involved. Recent reports have suggested that in hot regions, for example, the lowland jungles of Peru and Brazil, E. chrysanthemi can also cause blackleg.

Whereas only the SRE named above can cause blackleg, a wide range of other pectolytic bacteria have been associated with tuber rots especially in hot countries, inter alia Bacillus subtilis, B. megatherium, Pseudomonas marginalis and pectolytic Clostridium spp. The last named together with the SRE are the organisms most commonly found in rotting tubers in temperate regions.

The SRE have a worldwide distribution and one or more of those three named are present in most potato stocks, but whereas the var carotovora and E. chrysanthemi have a wide host range, the var atroseptica is host specific to the potato; moreover, it is commonly found only in cool regions and its occasional presence in warm areas is probably associated with imported seed.

The SRE, also known as soft rot coliforms, are members of the Enterobacteriaceae and they are fully described in Bergey's Manual (Buchanan & Gibbons, 1974). The main character distinguishing them from other erwinias is their ability to produce large quantities of pectic enzymes and to macerate parenchymatous tissue. They are Gram negative, non-spore forming, facultative anaerobes, most strains not producing large quantities of extra-cellular polysaccharides even on sugar-rich media. Fimbriae or pilli are present on cells of a high proportion of strains of var carotovora and E. chrysanthemi but they are absent from cells of var atroseptica. The bacteria can similarly be differentiated in that strains of the var atroseptica do not produce one or more bacteriocins whereas many strains of the other two bacteria do.

Studies on their serological relationships have produced conflicting results but it appears that although they are serologically related, E. chrysanthemi differs from both var carotovora and var atroseptica. Whereas strains of var atroseptica tend to form a serologically hemogeneous group, those of var carotovora fall into several sub-groups irrespective of source and country of origin. Moreover, antisera prepared against var carotovora and var atroseptica are not always specific since cross reactions occur with a small proportion of strains. Their serological relationships are also reflected in their biochemical characters. They differ in only a few properties which, however, are sufficiently contrasting to allow precise identification of most strains (Table 1).

Their temperature relationships are interesting and could reflect their geographical distribution. Thus, there is an apparent correlation between the contrasting temperature requirements of var atroseptica and E. chrysanthemi (Table 1) and their distribution; the former is restricted to the potato, which is usually grown in cool areas, whereas the latter

is a pathogen of tropical and sub-tropical crops. In contrast, var carotovora has a worldwide distribution and temperature requirements intermediate between the other two.

Methodology

Recent progress in understanding the biology of SRE, in particular of vars carotovora and atroseptica, is largely due, first to the work of Dye (1969) and Graham (1964, 1965) in firmly establishing the taxonomy of the 'carotovora' group and, second, to the development of methods for detecting low numbers of the bacteria. Detection of cryptic infection relies on an enrichment procedure in which tubers are induced to rot under anaerobic conditions in the presence of water (Pérombelon, 1972a, De Boer & Kelman, 1975). Similarly, soil samples are incubated anaerobically in a pectate-basal salt solution (Meneley & Stanghellini, 1976). Following preferential growth of SRE and pectolytic facultative and obligate anaerobes, the presence of the SRE is then detected either by plating on pectate-based selective media (Stewart, 1962, Cuppells & Kelman, 1974) or serologically - including the use of immunofluorescence techniques (Allan & Kelman, 1977, Vruggink & Maas Geesteranus, 1975, Vruggink & De Boer, 1978). Only colonies of SRE will form characteristic cavities in the pectate media within 48 h of aerobic incubation at 27°C. Varietal identification can be done serologically or by testing for acid production from α methyl glucoside, maltose and lactose, and for the production of reducing substances from sucrose (Table 1). The composition of the media for these tests in critical; 1% peptone water with 1% added sugar and bromothymol blue as a pH indicator should be used when testing for acid production, and 4% sucrose followed by a 4:1 ratio of Benedict's reagent to 48 h-old cultures when testing for the production of reducing substances.

Enumeration of the bacteria from plant tissue or in soil can be done by dilution counts on selective media. Alternatively, when very low numbers are suspected, they can be assessed by the more sensitive quantal method (Pérombelon, 1971). When there is a requirement to enumerate vars carotovora and atroseptica separately from the same source, a modified Lederberg's tetrazolium fermentation indicator medium containing α methyl glucoside could be used provided that the numbers of contaminating bacteria are less than the total of SRE (Philips, Pérombelon & Kelman, unpublished).

Etiology

Although many factors affecting the development of blackleg have been identified, the mechanism which triggers the disease is still unknown. In temperate regions, where only var atroseptica causes blackleg, all the seed tubers are often contaminated and the pathogen is present,

Table 1. Main Characteristics of Soft Rot Erwinias Causing Diseases of Potatoes.

	var *carotovora*	var *atroseptica*	*E. chrysanthemi*
Acid from			
lactose	+	+	- or +
maltose	-	+	-
αmethyl glucoside	-	+	-
palatinose	-	+	-
trehalose	-	-	+
Reducing substances from sucrose	-	+	-
Indole	-	-	+
Growth in 5% NaCl	+	+	-
Lecithinase	-	-	+
Phosphatase	-	-	+
Sensitivity of erythromycin	R	R	S
Growth Temperature (°)			
minimum	6	3	6
maximum	37-42	35	>45
optimum	27	27	40
Thermal death point	50-52	48	-

Notation: as in Graham (1972)

albeit in low numbers, in a high proportion of the stems of healthy plants when examined after decay of the mother tuber. Yet the disease incidence is usually low (<1% plants) and only one or two stems of an affected plant develops symptoms.

It has been well established that blackleg incidence may be correlated with the level of seed tuber contamination (Aleck & Harrison, 1978; Pérombelon unpublished). Seed tubers taken from plants affected by blackleg early in the season give rise to more blackleg affected plants than do seed tubers taken at the same time from healthy plants. In contrast, there are no differences in blackleg incidence between plants derived from seed tubers taken at the same time from healthy plants and from plants affected by blackleg late in the season. Experiments have shown that blackleg was associated more with large than with small seed tubers, presumably because breakdown of a large mother tuber takes longer and provides a greater inoculum level than small seed tubers. However, nonemergence or blanking tended to be more extensive when smaller seed tubers were used.

It is common knowledge that crops grown from seed tubers affected either by disease (dry rot, gangrene, scab, late blight and lenticellular hard rot) or by physiological disorders (cracks, freezing injury, over-heating & blackheart) often have blackleg levels that are above average. Moreover, synergistic effects of other diseases (verticillium, fusarium) on blackleg incidence have been recorded by several workers, mostly in warm areas. Lastly, Graham & Harper (1966) reported an association between high blackleg levels and low levels of nitrogen fertilizer applied to the soil.

The role of climatic factors is not readily assessed. The levels of blackleg may be very low or high irrespective of whether other conditions favoring it prevail or not (Table 7). Experience suggests that in temperate regions high disease levels occur in years with rainfall above and temperature lower than average. Preliminary results in Scotland indicate that symptoms tend to appear after a period of 10-14 days, especially during the first half of the growing season, when soil water deficit (evaporation-rainfall) is nil, i.e. when there is excess water in the top 20 cm of the soil. Similarly, blackleg tends to occur more often where the soil has been waterlogged, e.g. at the bottom of a field or following excessive irrigation, particularly surface irrigation. In warm regions, blackleg incidence is greater than in cool regions, especially in crops grown from seed tubers imported from cold regions. Such tubers are frequently contaminated by SRE whereas locally produced seed tubers tend to be cleaner (see below). In addition, high ambient temperatures are more favorable for symptom expression.

Finally it appears that factors which lead to early decay of the mother tuber are associated with high levels of both blanking and blackleg.

In addition, conditions which are not optimum for the pathogen tend to result in high blackleg expression but low blanking incidence, whereas the reverse is true when conditions favor the pathogen. The mother tuber rots readily in wet soils as the conditions become anaerobic. If the initial inoculum load is large, high numbers of the pathogen will develop rapidly in the rotting mother tuber and may move in the shoots thus extending the decay above ground. It is interesting to note that SRE have been shown to be primarily vascular pathogens when inoculated into stems (Hellmers & Dowson, 1953). Progressive decay of parenchymatous stem tissue is subsequent to movement up the stem in the vascular system.

The mechanism of factors affecting decay in tubers is better understood. Initiation of rotting occurs only when free water is available and anaerobic conditions prevail. The role of water is two-fold. First, it leads to an increase in the turgidity of tuber tissue which is usually under water stress and will therefore absorb water at a rate primarily dependent on the degree of suberization of the periderm. Second, the presence of an uninterrupted water film on the tuber surface rapidly causes anaerobiosis within the tuber through O_2 depletion by respiration e.g. in 2.5 h at 22°C (Burton & Wigginton, 1970).

Pérombelon & Lowe (1975) suggested on the basis of their experiments, the following sequence of events which could lead to the initiation of decay. As water is absorbed and tissue turgidity increases the suberized layer of a lenticels is broken. Oxygen deficiency affects cell membrane integrity and there is leakage of water and of solutes from turgid cells which establishes between the cortex and the lenticels a continuous liquid phase in which organisms within the lenticels are able to penetrate deeper into the tuber. The leaked cell contents, together with the apparent reduction of tuber resistance under anaerobic conditions, allow rapid growth of the bacteria. If these include pectolytic forms - anaerobic or facultative anaerobic - a soft rot lesion will then be established.

Soft rot erwinias and occasionally some pectolytic clostridia are usually associated with tuber decay in temperate regions and not several other pectolytic soil bacteria usually present on most tubers. This differentiation can be attributed to ecological factors preferentially favoring their growth and pectic enzyme production (Pérombelon et al., 1979b). Thus, SRE may be viewed as opportunist pathogens able to invade their hosts and cause decay only when conditions other than host susceptibility *per se* favor their growth and hence the production of large quantities of pectic enzymes. Thus, if ventilation is defective within a stack of stored potatoes, both temperature and humidity increase and should the temperature fluctuate by only one or two degrees, condensation occurs. The resultant wetting of tubers, coupled with a high respiration rate at the higher temperatures, would then lead to locally anaerobic conditions and eventually to rotting by the chain of events outlined above. Similarly,

rotting could be initiated when sound tubers are wetted and 'sealed' by liquid which is squeezed from diseased tubers (e.g. blight, pink rot, soft rot) by pressure of the stack above them. Liquid from rotting tubers would progressively wet and cause more rot in more tubers below resulting in the characteristic cone shape of a soft rot pocket.

Pathogenicity/temperature Interactions

There is one overriding environmental factor - temperature - which appears to affect the relative pathogenicity of the SRE. Its effects can be summarized as follows:

Pathogenicity Expressed in Terms of Frequency of Isolation from Lesions

Climatic Regions	Blackleg		Tuber Decay	
	carotovora	atroseptica	carotovora	atroseptica
Cool	-	++	+	++
Warm	++	+	++	(+)

This summary is somewhat indeterminate, especially with regard to warm regions, because of a paucity of information and for this reason E. chrysanthemi has been omitted. Moreover, it refers only to indigenous material and not to potatoes grown from seed imported from other regions. Nevertheless, it is interesting to note that the pattern is similar to that obtained when pathogenicity of the bacteria is tested in vitro at different temperatures (Pérombelon, unpublished). Whereas the pathogenicity of SRE to potato tubers (or the degree of tuber resistance to SRE) can accurately be assessed (De Boer & Kelman, 1975), the ability to cause blackleg (or stem susceptibility to blackleg) is still scored qualitatively or, at best, semiquantitatively. Lesion development on stems is recorded following either stab inoculating them with ca. 10^8 cells or, in an attempt to simulate natural infection, by inoculating the seed tuber by vacuum infiltration in a suspension of the bacteria (Pérombelon, 1979) or inoculating the mother tuber of a young plant (Munzert, 1975).

Relative Pathogenicity of SRE

Incubation Temperature	Stems		Tubers	
	carotovora	atroseptica	carotovora	atroseptica
15°C	+	++	+	++
30°C	++	+	++++	+++

[1] stem inoculated
[2] based on the ED50 for induction of visible decay in injected tubers incubated anaerobically

Further proof of the differential effect of temperature on the pathogenicity of SRE was obtained by noting which organism predominates in soft rot lesions when equal numbers of var carotovora and var atroseptica were jointly inoculated into potatoes and incubated at 15° and 30°C.

Relative Proportions of var carotovora and var atroseptica in Lesions when Inoculated Together in a 1:1 Ratio

Incubation Temperature	Stems		Tubers	
	carotovora	atroseptica	carotovora	atroseptica
15°C	+	++	+	+++
30°C	++	(+)	+++	(+)

Although these results do not precisely fit the observed disease patterns in warm and cool regions, they are sufficiently close to suggest that the predominance (pathogenicity) of a given organism in any one region and not in another can be attributed to temperature. Preliminary results (Pérombelon & Ghanekar, unpublished) indicate that the explanation could be found in (a) the level of production of one pectic enzyme -endopolygalacturonic acid trans-eliminase (PATE) by var atroseptica, high at 15°C and nil at 30°C, whereas enzyme production by var carotovora was similar at both temperatures, and, (b) the relative rates of growth of the two organisms at low and high temperatures. It is of interest to note that Chatterjee & Starr (1977) found the strains of E. chrysanthemi which have lost the ability to produce large quantities of PATE were no longer virulent but that their virulence was restored by transferring this character from a wild virulent strain.

Epidemiology

Survival in soil

Longevity of the bacteria in soil has been a controversial subject since it was first examined by Morse (1909). The difficulty of explaining how seed tubers collected from blackleg free plants often produce infected crops led many workers to believe that the pathogen was soil borne. Support for this belief was given by Leach (1931) who showed that the bacteria could contaminate and survive on the larvae of certain flies in soil. However, many workers believed that the pathogen was not endemic in soil and that its widespread distribution was due to its recurrent introduction with infected planting material.

Pérombelon (1973b) studied the longevity of both var carotovora and atroseptica in soil and found that it was more related to soil temperature than moisture content. In summer, under Scottish conditions, both

varieties survive in soil for only a few weeks (3-6) and the survival was ephemeral when temperatures exceeded 25°C although strains of var carotovora tended to persist longer than those of var atroseptica. However, at temperatures below 0°C, not only was the longevity of both organisms reduced, but the varietal reaction was reversed. The limiting factor at temperatures above freezing is probably not temperature per se but it is more likely to be of microbial origin. Thus, in experiments on the effects of temperature on survival in the soil, significant differences in longevity were found between samples of the same soil taken from the field in summer and in winter.

In summary, although longevity in soil during the summer in temperate regions is short, when the soil becomes colder later in the year it increases and it is possible that under favorable conditions the bacteria could overwinter provided the temperature stays above freezing. Nevertheless, survival in the field long enough to enable SRE to span a period of 3-5 years from one potato crop to the next is most unlikely.

There are reasons to believe that longevity of SRE in soil in warm regions could be different. Thus, Meneley & Stanghellini (1976) reported the persistence of the bacteria in fallow soil in Arizona, USA, where soil temperatures often exceed 40°C.

Survival of var carotovora and E. chrysanthemi could be extended through alternative hosts, especially in tropical regions where plant growth is often continuous and the vegetation is more diverse. In the absence of host plants, SRE may still persist in soil in the rhizosphere of many plants including weeds (Kikumoto & Sakamoto, 1969; Burr & Schroth, 1977) and their longevity may also be prolonged when they are protected in infected debris and in association with groundkeepers or volunteers.

Now that techniques of great sensitivity are available to detect low numbers of SRE, their survival in soil should be re-examined, especially in tropical regions. It is important to stress that soil temperature should be recorded as number of hours per day, at a given depth, that the temperature exceeds a critical level; meteorological records often comprise only single readings, usually taken only once a day in the morning, well before maxima occur. Laboratory studies of the temperature characteristics of strains from temperate and tropical regions might provide a useful guide for field studies on the longevity of SRE in the soil.

Role of the Mother Tuber

Until recently, it was commonly accepted that the transmission of the blackleg pathogen from one potato generation to the next was either by means of the blackleg affected plant or by the bacteria surviving freely in soil. To those who did not believe that the pathogen was soil-borne,

only the progeny of blackleg plants and of neighboring healthy plants were latently infected at harvest time and only such tubers gave rise to blackleg plants in the following season. Since the incidence of blackleg in most crops in temperate regions is usually low, the number of tubers successfully carrying latent infection would also be low.

Several workers, however, have demonstrated that the amount of blackleg in a crop has no bearing on the incidence of the disease in plants grown from its progeny. Thus, whereas tubers taken from healthy plants and planted the following year may give rise to blackleg plants, tubers obtained from blackleg plants do not necessarily produce diseased plants.

Work done in Scotland since 1966 not only supported the view that SRE do not survive long in the soil, but also resulted in a new concept of the epidemiology of the disease in which the mother tuber plays a key role.

Large scale surveys have shown that a high proportion of tubers in commercial seed stocks, irrespective of whether the tubers were obtained from blackleg infected or blackleg free crops, were contaminated with E. carotovora in the proportions, 80% var atroseptica to 20% var carotovora (Pérombelon, 1972b). Similar results were obtained by De Boer & Kelman (1975) and Nielsen (1978) in the USA and by Ficke et al. (1973) in Germany. The bacteria were located on the tuber surface and in the lenticels but they were rare in the vascular ring. The level of contamination of lenticels varied widely from stock to stock and it was only slightly affected after 6-7 months bulk storage which, however, substantially reduced surface contamination (Pérombelon, 1973a). There is now little doubt that in most temperate areas a high proportion of seed tubers at planting time could be contaminated by both var carotovora and var atroseptica and this is the main source of contamination for the growing crop.

Further evidence to support the importance of the mother tuber as a source of contamination was obtained when Pérombelon (1974) demonstrated that extensive contamination of progeny tubers occurs only after decay of the mother tubers. Under the climatic conditions prevailing and the growing practices in use in Scotland, most mother tubers are extensively rotted early in August in wet years and later in dry years. Both var carotovora and var atroseptica can be detected in the decayed mother tubers, in the soil in the tuber zone, and on the progeny tubers. By the end of August or early in September most of the progeny tubers were contaminated as they were at harvest time. The absence of contamination in progeny tubers of pot and field grown plants, when mother tubers had been removed at an early stage of the plant growth, contrasts with its presence when mother tubers were present and lends further credence to the view that the seed is an important source of the pathogens (Table 2).

Indirect evidence (Pérombelon, 1976) suggests that soil water is probably the main factor affecting not only movement of the bacteria from the decayed mother tubers into the soil and to the daughter tubers, but also the levels of progeny tuber contamination during the growing season. The levels of lenticel and soil contamination do not gradually increase but markedly fluctuate; they tend to be low during dry spells but to rise after heavy rain. The variations were tentatively explained in terms of (a) evapo-transpiration forces which redistribute in the potato hill soil water containing the bacteria leached from the mother tubers after rain, and (b) the condition of the progeny tuber lenticels - when the soil is wet (water deficit of the tuber low) they tend to open (proliferate) and the bacteria in the continuous water film joining soil particles to the complementary cells of the lenticels can enter; or, when the soil is dry (water deficit of the tuber high) they close (tuberize) and the bacteria present are 'lost' when the complementary cells are sloughed off. Although the implied precise and uniform synchronization of rainfall, soil water movement, lenticel condition, and eventually soil and lenticel contamination would be rare, this hypothesis nevertheless provides a plausible explanation of variation in the levels of tuber (lenticel) contamination found during the growing season and at harvest.

Effect of Climate on Tuber Contamination

The level of contamination of progeny tubers at harvest could be less than that of their parent seed tubers (Table 3). For example, when rainfall during the growing season is below average and soil moisture deficit remained high, decay of the mother tuber is retarded and few will rot and movement of the bacteria in the soil is impeded by discontinuity of the water film around soil particles. Alternatively, progeny tuber contamination would also be low if the seed crop is harvested early in the season (and before the mother tubers have rotted) as means of avoiding aphid disseminated virus infection. Similarly, an initial high level of tuber contamination, in particular by var atroseptica, in seed stocks exported from a cool to a warm region tends to fall rapidly within only a few generations. Although the prevailing high ambient temperature favors blackleg development, it is inimical to the survival of the pathogen in the soil and if, in addition, soil moisture deficit tends to be high, the progeny tubers will progressively escape contamination.

Other Sources of Contamination

Several sources of SRE which could be implicated in the contamination of potatoes have been identified and still are being investigated. In addition to contaminated farm machinery and groundkeepters, two hitherto little known sources have been identified as being potentially important: they are air-borne insects and wind dispersed aerosols.

Table 2. Contamination by SRE of Daughter Tubers of Field Grown Potato Plants with Mother Tubers Present or Removed Soon After Emergence.

Mother Tubers	Replicate	Tuber Numbers: Contaminated Daughters	Tuber Numbers: Rotting Mothers
Present	1	12[1]	8[2]
	2	14	4
	3	12	6
	4	16	7
Absent	1	0	-
	2	0	-
	3	1	-
	4	0	-

[1] n = 16, 2 per plant
[2] n = 8 plants

Table 3. Contamination by SRE of Stocks During Their Multiplication.

Stock	Age (yr)	Number of Tubers Examined	% Tubers Contaminated
King Edward	2	36	0
	3	19	26
	4	30	3
	5	48	23
King Edward	3	28	0
	4	30	27
	5	38	8
Maris Page	1	24	83
	2	30	0
Maris Piper	4	50	33
	5	23	2

In Scotland several insect species trapped near potato and vegetable refuse dumps have been found to be contaminated by var carotovora and to a lesser extent by var atroseptica (Harrison et al. 1977). There is little information on the numbers of bacteria per insect, but preliminary results (Pérombelon unpublished) suggests that while there are usually <50, there could be as many as 10^3. Using marker strains of E. carotovora Graham et al. (1976) and Philips & Kelman (personal communication) have shown that some bacteria isolated from potato dumps were also found on contaminated insects and on potato plants growing nearby. However, in the absence of quantitative data it is difficult to assess the importance of insects as sources of contamination.

E. carotovora is invariably present in aerosols generated by haulm pulverization prior to harvest, even in the absence of blackleg (Pérombelon et al. 1979a), and by rain splash from blackleg stems in the field (Graham et al. 1977) both systems releasing an estimated 10^8 bacterial cells per ha into the air. When air was sampled in rural areas in the east of Scotland, even with no potato crop visible, cells of var carotovora and to a lesser extent var atroseptica were readily detected on rainy, but not on dry days and not before August (Graham & Quinn, personal communication).

Laboratory experiments in controlled environments and in the open air have shown that although survival of the bacteria in aerosols is poor, nevertheless 50% remain viable for 5-10 min which is long enough for them to be blown several hundred meters (Graham et al. 1979, Pérombelon et al 1979a). The results of calculations using different diffusion and deposition models and data from field experiments suggest that under optimal atmospheric conditions 10^3 cells per m^2 or less would be deposited 100 m downwind from a ground level point source of 10^8 cells (Pérombelon et al. 1979a, Table 4). It is therefore possible that small numbers of E. carotovora, either insect-borne or in aerosols, contaminate some potato crops throughout the growing season. The practical significance of this contamination will depend on the survival, growth and subsequent spread of the bacteria to the daughter tubers.

The results of field and controlled environment experiments using antibiotic-resistant strains and bacteriocin-typing methods suggest that survival and growth of both var carotovora and var atroseptica inoculated to potato leaves occurs only when the leaf surface is wet. Thus, when 10^3 cells per cm^2 were inoculated and allowed to dry at 80% RH the bacteria could no longer be enumerated after 3 days, but they could still be detected up to 7 weeks later by an enrichment procedure (wetting the leaf surface with 0.1% peptone water and incubating anaerobically for 24 h). In contrast, when inoculated leaves were kept at ca. 100% RH and remained wet, bacteria were found in large numbers after 10 days. More bacteria survived on senescent than on young leaves and along the mid rib and lateral veins than elsewhere. The bacteria (more var carotovora than

var atroseptica) also were detected frequently in large numbers on leaves of non-experimental potato crops especially in September and October when many leaves were senescent and frequently wet with rain or dew.

Tubers of plants whose leaves were inoculated with marker strains in September, but not earlier, were found to be contaminated in October as were the leaves and progeny tubers of neighboring plants 1 to 2 m along and across the ridges. Furthermore, tuber and leaf strains reacted differently to bacteriocin-typing and although some leaf types were detected on tubers the reverse rarely occurred suggesting that contamination of progeny tubers by bacteria that had come from the leaves and, by implication, outside the crop, may not be uncommon.

To summarize, it appears that small numbers of SRE carried by insects or in aerosols are probably deposited on potato crops randomly throughout the growing season. During wet spells the bacteria may grow on the leaves, especially if senescent, and may also be spread by rain from foci throughout the crop to the leaves of neighboring plants. From the leaves, they can be washed by rain into the soil to contaminate the progeny tubers. Although the phenomenon has yet to be quantified, tuber contamination by airborne SRE is clearly a hazard additional to that posed by bacteria from the seed tubers.

Finally, the importance of contaminated farm machinery cannot be over emphasized. The bacteria can be transmitted from tuber to tuber when the seed is cut before planting, or spread within a crop by the wheels of tractors during the growing season and by harvest machinery and implements. However, the most common and important method of spread, both between and within stocks, is the grader (Table 5). Graders are rarely adequately clean and even less often disinfected between stocks and the problem is often made worse by the wounding of tubers and sprouts when graders are badly adjusted.

Control

Assuming that the pathogens are not soil-borne, the main source of contamination is the seed tuber. The role of air-borne bacteria probably becomes significant only to crops grown from erwinia-free seed. Some degree of disease control can therefore be achieved by reducing the incidence of tuber contamination. Success is likely to be only partial and temporary because of the rapid build-up of residual contamination and of re-contamination.

Chemotherapeutic treatment of seed has been tried repeatedly with little consistent success because the bacteria are often located in the lenticels where they are well protected from liquid disinfectants. An alternative solution, which has been and still is in force in many countries, consists of removing the presumed source of the pathogen - the blackleg

Table 4[1]. Numbers of SRE Deposited per m^2 Downwind From a Ground Level Point Source Strength of 10^8 Bacteria.

	Atmospheric Conditions	Distance from Source (m)			
		50	100	1000	10,000
A. Diffusion models					
Pasquill	stable	-	300	5	0.1
	unstable	-	30	4	0.02
Chamberlain	stable	2000	70	1	0.2
B. Experimental data					
(i)	stable	300	60	-	-
(ii)	intermediate	60	7	-	-
(iii)	unstable	1	0	-	-

[1]From Pérombelon et ae. (1979)

Table 5[1]. Percentage Blackleg in Crops Grown from Seed of Stem Cutting Origin (Erwinia-free) After Two Grading Treatments.

Type of Grader	Treatment	% Blackleg 1	2
Spool		16	2
Riddle		15	11

treatment 1: grader contaminated with E. atroseptica;
treatment 2: after treatment 1, 250 kg of erwinia-free seed graded followed by the seed of treatment 2.

[1] D.C. Graham, personal communication

affected plants - coupled with planting seed obtained from crops certified as either free of blackleg or in which blackleg does not exceed some arbitrary low level. Experience has shown that these measures are not very effective since blackleg can occur, and at times in epidemic proportion, in crops grown from such certified seed. Moreover, this procedure has no effect on those SRE which do not cause blackleg but which nevertheless are involved in tuber decay. The revised concepts of the epidemiology of blackleg in which the mother tuber is the main source of contamination, and not as previously believed the blackleg plant, makes it clear why this approach has failed. They have also led us to a different approach using erwinia-free stocks raised from stem cuttings which, if taken from plants before the onset of decay of their mother tuber, are unlikely to carry even cryptic contamination. The progeny tubers are free of erwinia and the stocks remain clean unless recontaminated from the outside sources indicated above.

Large scale production of stocks from stem cuttings was first practiced in Scotland (Graham & Hardie, 1971) and was followed by schemes in Australia (Victoria, Tasmania) and the USA (Colorado, Idaho and Wisconsin). In Scotland, the stem cuttings are raised under hygienic conditions on an isolated farm where potatoes have never been grown commercially. The tubers obtained therefrom, are distributed to selected growers who multiply them for 4-5 years. The progeny are then marketed as certified elite stocks from which all other seed grades are derived. A continuous supply of clean elite stocks is the main feature of the Scottish Certification Scheme which limits the life of higher grades in order to reduce the risks of build-up of contamination.

Monitoring elite stocks during the first five years of their multiplication from 1973 to 1978, showed that few remained uncontaminated for long (Pérombelon, 1976). The contamination pattern appears to be affected seasonally and locally by summer weather and, though less quantifiable, by the amount of care given by individual growers. However, the average contamination levels are less now than before the introduction of the stem cutting scheme and stocks are also now cleaner than in the earlier years of the scheme (Table 6). The proportion of var carotovora to var atroseptica in stocks derived from stem cuttings, especially in their early years of multiplication, is much greater than that found in stocks not so derived and it is relevant that var carotovora is more frequently detected on insects, in aerosols and on potato leaves.

In temperate regions, in the past, stocks were extensively contaminated with SRE, but because of the climate blackleg levels were usually very low other than locally, under wet conditions, or seasonally if the weather were unusually wet and hot. Similarly, given good harvest conditions, tubers went into the store dry and with little soil, and good storage conditions, tubers remained dry and cool, then extensively contaminated crops could store well. In contrast, in the tropics, heavily contaminated

Table 6. Contamination by SRE of Tubers from Stocks of Different Ages Grown on 4 Different Farms from 1973 to 1978.

Surveyed in	Stock Age (years)	Number of Stocks	Number of Tubers	% Tubers Contaminated
1973	1	3	25	8
	2	2	71	45
	3	4	192	70
	4	8	304	29
	5	5	154	48
1974	1	4	87	0
	2	5	141	11
	3	4	104	21
	4	4	87	39
	5	3	77	40
1975	1	4	66	6
	2	9	186	11
	3	5	155	16
	4	6	156	18
	5	2	57	53
1976	1	7	63	2
	2	4	123	3
	3	6	194	9
	4	3	124	27
	5	4	148	19
1977	1	5	79	39
	2	7	160	3
	3	4	130	2
	4	8	293	16
	5	4	170	57
1978	1	5	71	18
	2	7	195	12
	3	7	248	8
	4	4	105	21
	5	4	134	34

stocks tend to develop high levels of blackleg and extensive losses in storage are not uncommon. Intermittent high soil moisture from either tropical rain or irrigation, together with high temperatures, enhance seed tuber rot and blackleg development; persistent high ambient storage temperatures will, of course, enhance most storage rots as well as those due to SRE and other pectolytic bacteria. Because of the environmental hazards in warm regions, seed production programs based on the 'clean seed' concept alone may prove inadequate and attention must therefore be focused on breeding.

Although resistance to bacterial diseases has been improved by breeding, few attempts have been made to breed potatoes resistant to SRE. The generally low incidence of blackleg in temperate regions, the tendency to blame storage losses on poor management, and past erroneous concepts of epidemiology (which led to the belief that control could be achieved by rogueing and seed certification) probably all militated against a determined effort by plant breeders.

Some resistance to SRE is present even in the narrow genetic base of modern European cultivars (Pérombelon, unpublished, Table 7). Preliminary work on the relationships between inoculum density disease level, and cultivar reaction to blackleg suggest that two distinct resistance/susceptibility mechanisms are involved: first, the susceptibility of the mother tuber to decay, and second, the susceptibility of the haulm to rotting. The results also indicate that in a given cultivar these two reactions are not necessarily correlated; tubers of some cultivars apparently highly resistant to blackleg are more susceptible to decay than those of some very susceptible cultivars. However, the results are difficult to interpret because of variations within and between seasons.

A major difficulty in breeding for resistance to SRE is the lack of screening methods. Resistance to tuber decay can be assessed accurately by the tuber injection method of De Boer & Kelman (1975), but it is elaborate and time consuming. One method of screening for resistance to blackleg is to vacuum infiltrate the seed tubers in a suspension of the test pathogen before planting. This technique cannot yet be used for large scale field testing in temperate regions because disease development is, inter alia, weather dependent, and disease development is accordingly erratic; more information is needed on factors affecting the disease before the test can be further developed - probably for use in large glasshouses under partly controlled conditions.

Summary and Future Research Requirements in the Tropics

Work carried out during the past decade has led to a new concept of the epidemiology of blackleg and, in addition, the etiology of the disease in the field and of soft rotting in store has been placed on a sound basis.

Table 7. Blackleg Incidence in Crops Grown from Seed of Different Cultivars Inoculated with var atroseptica in Different Years.

Cultivar	Year	% Blanks	% Blackleg
Aran Consul	1971	2	14
	2	0.7	6
	3	-	0.3
	4	10	0.1
	6	7	0
	7	5	8
Bintje	1976	5	2
	7	12	11
	8	7	26
Golden Wonder	1971	38	4
	2	5	0.3
	3	-	1
	4	4	0.1
	6	2	0
	7	11	2
	8	6	22
Majestic	1971	11	32
	2	28	6
	3	-	2
	4	6	0
	6	3	0.2
	7	11	5
	8	8	22
King Edward	1971	7	5
	2	4	3
	3	-	0.2
	4	8	0
	6	5	0.2
	7	10	5
	8	5	8
Pentland Crown	1971	11	5
	2	2	0.7
	3	-	0.1
	4	22	0
	6	3	0
	7	7	2
	8	4	4

Table 7. (contd.)

Cultivar	Year	% Blanks	% Blackleg
Record	1971	14	5
	2	11	3
	3	-	0.3
	4	16	0.2
	6	5	0.2
	7	6	8
	8	6	28
Redskin	1971	2	17
	2	23	4
	3	-	1
	4	5	0
	6	5	0
	7	12	7
	8	8	8
Up-to-Date	1971	4	14
	2	4	3
	3	-	0.5
	4	11	0
	6	3	0.2
	7	7	4
	8	16	25

Most of this work has been done in temperate regions and it is clear that the results cannot directly be applied in the tropics or sub-tropics or even regions with mediterranean-type climate because of the dominant influence of rainfall and temperature. Moreover, it is reasonable to assume that the relative importance of the three coliforms, E. chrysanthemi, var carotovora and var atroseptica may be different, as may their importance in relation to other pectolytic bacteria.

In warmer regions research needs to be done on a climatological basis - tropic, sub-tropic, temperate (x lowland, upland), montane (x high, moderate rainfall), and arid, but ignoring combinations not suited to potato growing (e.g. tropical, lowland, arid - unless irrigated). Within each climate combination evidence should be sought for: the dominant micro-organism(s) causing blackleg and storage soft rot and then for their survival in soil in relation to the importance of the seed tuber as the infection carrier. The greater diversity and number of insects in many warmer areas and of other potential vectors of the macro- and micro-fauna, together with areas of intense heavy rain clearly require that the role of vectors and aerosols be examined. There is no reason to doubt that the route air : leaf : multiplication on leaf : rain : soil : progeny tubers, may be of the same, or more, potential importance as has been suggested for temperate regions, especially in areas of high temperature/ high rainfall. The importance of volunteer potatoes (groundkeepers) may be greater than in temperate regions where winter-kill is an aid to their control. In addition, the greater diversity of plant species means that there are more potential alternative hosts to help bridge the gap between one potato crop and the next.

The projects here briefly outlined would clearly call for large resources of manpower, doubtless greater than those that are available and it would seem desirable to form a working party to plan and coordinate work and to set priorities.

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THE IMPORTANCE OF BACTERIA IN POTATO STORAGE

R. H. Booth

I must first remind participants that, although originally trained as a plant pathologist, I am not a bacteriologist and have had no working experience with bacteria on potatoes either in or out of storage. Secondly, I would like to point out that had I simply been invited to discuss the topic of "The Importance of Bacteria in Potato Storage" in front of this audience I undoubtedly would have declined. However, CIP in common with many other such institutions is not a true democracy and so here I am. Because of the concentration of experienced bacteriologists in this group, I have attempted to approach this subject from a practical storage point of view and have left the more basic bacteriological aspects to be discussed by you.

From the point of view of a storage specialist, it is generally true to say that there are no true storage diseases of potatoes and that all diseases in storage are inherited problems, either directly from field infections or from wound infections which occur during harvesting and pre-storage handling. An exception to this generalization is the bacterial soft rot complex where tuber infection and spread of rotting from infected to sound tubers can occur, under certain conditions, within the storage.

Examples of bacterial storage diseases inherited directly from the field include brown rot (*Pseudomonas solanacearum*) ring rot (*Corynebacterium sepedonicum*) and bacterial soft rot resulting from blackleg field infections (*Erwinia* spp.). The importance of these diseases in the store, therefore, depends almost entirely on pre-storage factors such as incidence and control of the diseases in the field and degree of pre-storage selection. For successful storage, therefore, it is highly desirable to have a knowledge of the field history of the crop to be stored. Thus, for example, if the crop is known to come from fields infected with brown rot or blackleg the crop can be rejected for storage purposes and recommended for immediate sale. If for any reason such a crop has to be stored then even greater care than normal should be taken during pre-storage handling and selection operations and during management of the storage environment.

Bacterial soft rot, potentially the most important bacterial disease in storage, can as mentioned above result from infection of tubers in the field and which, if not discarded prior to storage, may continue to develop and possibly spread during storage. Also, as will be discussed

below, infection of tubers may under certain specific conditions occur within a store. However, bacterial soft rot storage problems can also result from wound infections occuring during harvesting and pre-storage handling and thus provide an example of the second group of inherited storage disease problems.

It is probably true to say that all potato tubers carry on their surface a population/inoculum of soft rotting or pectolytic bacteria, predominantly Erwinia spp. but also including Pseudomonas spp., Bacillus spp., Clostridum spp., Aerobacter spp., and Flavobacterium spp. While such bacterial populations may survive in almost undiminished or in certain conditions increased numbers on the tuber surface, tuber rotting does not occur unless they are provided with a means of entry into the tuber. This means of entry may be provided by mechanical damage inflicted during harvesting and handling operations. Thus, once again the importance of this type of soft-rotting during storage is dependent largely on pre-storage factors, i.e. the care with which tubers are treated. From the storage point of view such infections may be reduced by again carefully selecting tubers prior to storage and then by subjecting all tubers to a wound healing or curing process. Because of the relatively high temperature and relative humidity conditions (15°C and 95% RH) required for successful curing, it is commonly stated that tubers are most susceptible to bacterial decay during this period. This may be true when discussing bacterial decay resulting from already present field infections in which case if the tubers have to be stored, which is not recommended, the curing process should be omitted and the temperature of the tubers reduced as soon as possible. However, in all other situations tubers should be cured and then remain undisturbed until finally removed from storage.

Within the store itself modes of entry for soft rotting bacteria may be provided by unhealed wounds, as discussed above, by open lenticels or occasionally by small wounds which develop around eyes during the sprouting process. Under recommended storage conditions, tuber lenticels are normally closed and suberized. However, under conditions of very high humidity and especially if tubers have not been long in storage the lenticels will proliferate during which process they progressively open and rupture the suberized layer which thus provides a point of entry into the tuber for the ever present bacteria. There is some discussion in the literature as to relative importance of a water film and anaerobic conditions required for bacterial infection to occur once the opening into the tuber has been provided. It would, however, appear safe to conclude that both a water film and some degree of anaerobiosis is required for successful infection. Unless tubers are wet when placed into storage, water films develop as a result of condensation within the stores. Condensation of moisture and anaerobic conditions in potato stores result from poor control of the storage environment usually associated with poor or inadequate ventilation and insulation which may be

general or in restricted areas of the store. Where ventilation is defective in a potato stack, both temperature, humidity and CO_2 concentration of the air increase and the O_2 concentration declines. Under conditions of very high relative humidity, which stimulates opening of lenticels as described above, condensation can easily occur if the temperature fluctuates by only one or two degrees which is generally difficult to avoid. Such conditions of very high storage relative humidities are more commonly encountered in large bulk stores which rely to a high degree on a closed internal recirculating ventilation system to control storage temperatures than in small simpler stores using open natural ventilating systems. As such large sophisticated storages are more common in the highly developed potato growing regions of Europe and North America, it is probably true to say that soft rot storage problems are of greater importance in those regions than in the less developed production areas where simple open ventilated stores are most common.

This viewpoint is possibly contrary to the generally held belief that because of generally higher ambient temperatures and relative humidities in many of the less developed and so-called "tropical" or "subtropical" potato production areas that the risk of storage losses due to bacterial soft rotting is greater in such areas.

As mentioned above, an alternative source to condensation of water films is where wet potatoes are placed into storage. This practice will always increase the likelihood of soft rot problems. Wet potatoes resulting from harvesting from very wet soils or as a result of inadequate protection from rain should be excluded from potato stores. If such wet tubers are placed into storage the very real importance of soft rotting bacteria will be rapidly realized if the tubers are not rapidly dried by the use of high levels of ventilation, either natural in the field or forced draft in the storage.

Thus, the control of this type of storage infection and spread of soft rots is primarily dependent on crop and storage management, particularly through control of the storage environment by adequate ventilation. Nevertheless, it would be of assistance to storage managers to have additional means at their disposal to help reduce the risk of soft rot development and spread within stores. Thus, the studies recently initiated by the CIP plant pathology program, on the evaluation of tuber resistance to soft rot organisms within existing genetic material and the effect of tuber greening during the seed storage in diffuse light on disease resistance, are of real interest to storage workers. Neither natural nor artificially induced increased levels of tuber resistance to soft rot organisms will, in any case, replace adequate storage design and management but they could help reduce the risk of losses especially in those stores where for other reasons it is desirable to maintain high relative humidities.

An alternative mode of entry into tubers by soft rotting organisms, both prior to and during the storage period, is provided by other tuber diseases or insect damage in which case the soft rot is referred to as a secondary infection. However, from a storage point of view the importance of such secondary infections should not be overlooked as in practice these are the most common. Thus, while the primary or initial cause of loss or damage in a storage may be traceable to a minor incidence of another disease or pest, the very considerable losses which can result are likely to be the result of secondary infection and then spread of bacterial soft rotting. Such secondary infections are commonly most severe in connection with other fungal diseases which themselves result in tuber soft rotting and the exudation of liquid from tubers as for instance is the case with pink rot (Phytophthora erythroseptica) and leak (Pythium spp.). While major emphasis on the control of such spreading secondary infections should be placed on reducing sources of the primary disease or insect damage, attention could also usefully be paid to means of increasing tuber resistance and so reducing the spread of soft rotting.

In recent years a further example of the potential importance of soft rotting bacteria has become apparent. As a result of consumer and marketing pressures within some of the more sophisticated market situations, potatoes are offered for sale in a washed condition and soft rotting problems in this post-storage marketing phase have consequently become increasingly common. This situation has been particularly marked in those regions where the washed tubers have been packed, distributed and marketed in polythene bags.

A yet further consideration in the importance of bacteria in potato storage is that of the survival and carry-over of inoculum on stored seed tubers. For all bacterial diseases of potatoes the tuber-borne inoculum is of primary importance. Thus, in the case of seed storage it is possibly incomplete to think only in terms of preventing gross rotting losses; should we not, in addition, be also searching for means of reducing inoculum carry-over. It has, for example, been shown repeatedly that the incidence of blackleg (Erwinia carotovora var atroseptica) in a growing crop is more influenced by the treatment and handling of tubers during the post-harvest period than by the incidence of the disease in the previous crop. Thus, again we seem to return to the importance of relatively simple effective managerial practices.

It is clear that it is very difficult to generalize about the importance of bacteria in potato storage. It is, however, true that because of the ever present inoculum of soft rotting bacteria that a potential danger is present and the expression of this danger in the form of extensive rotting is determined by many pre-storage factors. In addition, control over the storage environment through appropriate and well managed storage designs and ventilation systems is important. Similarly, where bacterial

diseases such as brown rot and blackleg are known to exist in the field, these can lead to important storage problems, if not correctly managed.

Thus, in conclusion I would venture to suggest that the expression of the importance of bacteria in potato storage is more dependent on general crop and storage management factors than on strict bacteriological considerations. It is, however, accepted that basic bacteriological knowledge is required so as to assist in the development of the most appropriate and effective management systems.

THE ROLE OF CLOSTRIDIA IN BACTERIAL SOFT-ROT OF POTATO

A. Kelman

Certain bacteria of the genus Clostridium are of significant economic importance because of their capacity to degrade the pectic components of plant tissue (Raynaud, 1949; Lanigan, 1959). This capacity to macerate plant tissues is the basis of the ancient process of retting that is essential for the production of major textile fibers such as flax, jute, and hemp. Notwithstanding the importance of pectolytic clostridia as a group, little attention has been given to the taxonomy or physiology of the pectolytic clostridia frequently isolated from soil and decaying plant material.

The possibility that certain anaerobic bacteria such as clostridia can cause the decay of stored potatoes was perhaps first recognized by Van Tieghem as early as 1884. However, a search of the literature on tuber decays from 1884 to 1970 revealed only a few additional reports indicating that clostridia can cause potato tuber tissue to decay (Wehmer, 1898; McCoy et al., 1926; Allen, 1944; Rudd-Jones and Dowson, 1950; Prevot, et al, 1967).

Rudd-Jones and Dowson were perhaps the first to consider the possibility that clostridia-like bacteria obtained from decayed tubers in storage could be important primary agents in decay of stored potatoes. The strains or species of Clostridium that they obtained were not identified; however, one strain which produced a pink color in decaying tubers was considered to be similar to Clostridium roseum. When intact tubers were inoculated and placed in moist chambers, the strains of Clostridium tested did not cause the tubers to decay. However, when mixtures of Erwinia carotovora and clostridia were tested under the same experimental conditions, the inoculations caused more extensive decay than when inoculations were made solely with Erwinia carotovora. The type of decay that resulted was characterized by the foul odor, gas bubbles, and extensive slime that was also produced in the decay of potato tuber tissue slants in test tubes following inoculation with a pure culture of one of their strains of Clostridium. Rudd-Jones and Dowson concluded that the clostridia were secondary invaders that grew extensively in tissue only after colonization of tubers by Erwinia and if temperatures in the storage bin had also increased as a result of the infection by Erwinia.

Over two decades were to elapse before the role of clostridia in decay of potatoes was to be examined again. In studies to determine the effect of low oxygen levels on decay of tubers by Erwinia carotovora, Lund and Nichols (1970) observed the development of decay in certain control tubers that had neither been surface-sterilized nor inoculated with Erwinia carotovora. From these decayed tubers, pure cultures of pectolytic clostridia were isolated that decayed potato tubers under anaerobic conditions. These observations were amplified by Lund in a subsequent paper in which techniques for isolation of pectolytic clostridia from potatoes were reported (Lund, 1972). Independent studies by Perombelon and Lowe (1971) that were completed during this period also implicated clostridia in the decay of potatoes in storage.

In an evaluation of the mist chamber method of determining bacterial soft rot potential (Lund and Kelman, 1977), clostridia were isolated consistently from decaying tubers after a 4-day incubation period at 20°C (see Table 1). This data was considered to indicate the wide prevalence of soft-rotting clostridia on tubers (1) harvested from the field and damaged in the harvesting or storage procedures or (2) obtained from storage bins in central Wisconsin. It was not clear, however, whether clostridia were primary agents of decay at normal storage temperatures of 4-5°C.

Subsequently, control of temperature was found to be a very critical factor in the mist chamber incubation procedure for assay of soft rot potential in potatoes. In those instances in which temperatures rose above 25°C, there was a marked increase in the percentage of tubers with the characteristics of clostridial infections. These include production of a slimy ooze, gas bubbles and foul odor.

Temperature proved to be important in determining the number of clostridia obtained in isolations from lenticels of infested tubers (Perombelon, Gullings-Handley, and Kelman, 1979). Sebago and Russet Burbank tubers were incubated anaerobically at two temperatures: 16°C for 8 days and 22°C for 4 days. In isolations from tubers held at 16°C the number of lenticels from which clostridia were obtained was significantly lower than the number with Erwinia (Table 2). In contrast, at 22°C, the numbers were approximately equal.

As one adjunct to these studies, inoculations with pure cultures of clostridia produced rapid decay in Erwinia-free tubers that were incubated anaerobically at 20°C or above. In inoculations with mixtures of cells of E. carotovora and clostridia, at 16°C populations of E. carotovora usually increased more rapidly than those of clostridia; the reverse usually occurred above 20°C. When both organisms are present at the onset of decay, the increase of clostridia at temperatures above 20°C reduces the prospect of obtaining E. carotovora in isolations from rotting tissue.

Table 1. Percentage of tubers from which pectolytic clostridia were isolated after incubation in a mist chamber. (Lund and Kelman, 1977)

Type of tuber sample	Percentage of tubers with clostridia
Unwashed tubers after 2 months in storage	100
Unwashed tubers after 3 months in storage	100
Washed tubers after 9 months in storage	80
Washed tubers after 10 months in storage	100

Table 2. Percentage of wounded lenticels[a/] in Russet Burbank and Sebago potato tubers incubated under anaerobic conditions from which *Erwinia carotovora* and clostridia (types L and S) were isolated (Perombelon, Gullings-Handley, and Kelman, 1979)

Temperature of incubation (C)	Variety	Lenticels decayed[b/] (%)	*E. carotovora* (%)	Clostridia (%)
16	Russet Burbank	75	68	13
	Sebago	72	70	5
22	Russet Burbank	73	56	44
	Sebago	83	52	58

[a/] Based on 100 wounded lenticels (10 lenticels/tuber; a total of 10 tubers/stock for each incubation temperature). Incubated at 16°C for 8 days for *E. carotovora* and at 22°C for 4 days for clostridia.

[b/] Both *E. carotovora* and clostridia were isolated from some lenticels.

In a recently completed survey (Campos and Kelman, unpublished data), relatively low percentages of pectolytic clostridia were present in isolations from decaying tubers taken at weekly intervals from a large storage facility maintained at about 5°C (see Table 3). The soft-rotting Erwinia were obtained more frequently than clostridia (48% in Bin A and 46% in Bin B). In about 10% of the tubers, clostridia were present, with Erwinia or other pectolytic bacteria. Neither Erwinia nor clostridia were recovered from 7% (Bin A) or 15% (Bin B) of the tubers from these two samples. On the basis of colony type and appearance on the crystal violet pectate medium, it was concluded that a majority of the other pectolytic bacteria were pseudomonads.

Studies on characterization and identification of the species of Clostridium involved in the decay of potatoes in England and the U.S.A. are in progress. A number of strains or species are undoubtedly involved (Lund, 1979). On the basis of studies by Lund and Brockelhurst (1978) on the pectic enzymes of pigmented clostridia, the pigmented pectolytic clostridia isolated from rotting potatoes may not be strains of Clostridium felsineum. This is a highly pectolytic species associated with retting of flax. Clostridium roseum and C. felsineum are considered to belong to the same or closely related species. Pure cultures of C. felsineum will rot potato tubers in the absence of other bacteria, but differ from the clostridia isolated from potato in the types of pectic enzymes formed as well as in several other tests (Table 4). Initial studies on the identification of isolates of clostridia obtained in Wisconsin have been completed with the assistance of Dr. L. Holdeman of the Anaerobe Laboratory at VPI. The results of these investigations indicate that the clostridia from potatoes are species that have not been described previously.

When the prevalence of pectolytic clostridia on potatoes was recognized in recent years, it was natural to ask whether these bacteria produce the potent toxins attributed to other members of this genus. Recent data indicate that these bacteria can be distinguished from the known toxigenic species by a number of physiological tests, including the ability to degrade pectin.

In view of the prospect for increased production of potatoes in the lowland tropics it is likely that high temperatures and moisture during harvest may enhance the importance of clostridia as a cause of post-harvest decay problems. A knowledge of the nature and ecology of these organisms will be needed in the development of rational storage and handling procedures. Presumably those procedures effective in reducing losses from soft-rotting Erwinia will minimize damage from the clostridia and other opportunistic pathogens such as Bacillus polymyxa, Pseudomonas marginalis, and Flavobacterium spp. which can also cause tubers to decay.

Table 3. Types of bacteria isolated from decaying potatoes[a] from commercial storage bins. (Campos, unpublished data)

Types of bacteria obtained	Percentage of isolations Bin series A[b]	B[b]
Clostridia only	6	6
Clostridia and *Erwinia*	8	8
Clostridia and other pectolytic bacteria	3	2
Erwinia only[c]	40	38
Aerobic pectolytic bacteria (not identified)	7	15

[a] Approximately 25% of the tubers in each series were infected with fungi. Major fungi involved were *Fusarium* spp. No pectolytic bacteria were isolated from 7% of the tubers in Bin A and 1% in Bin B, respectively.

[b] Based on isolations from 96 Russet Burbank tubers from Bin A and 101 tubers from Bin B, respectively.

Table 4. Pectic enzymes formed by clostridia on potato tissue. (Lund and Brockelhurst, 1978)

Group	Endopectate lyase	Pectic[a] hydrolase	Pectin esterase
Group I			
Pectolytic clostridia from potatoes	+	-	+
C. aurantibutyricum	+	-	+
Group II			
Clostridium felsineum	+	+	-
C. roseum	+	+	-

[a] Both endopolygalacturonase and exopolygalacturonase formed.

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THE IMPORTANCE OF *CORYNEBACTERIUM SEPEDONICUM* TO POTATO PRODUCTION IN THE DEVELOPING WORLD

M. Velupillai

The disease of potato known as bacterial ring rot is one of the most destructive diseases of this crop and always is a threat to successful production. This disease was first described from Germany in 1904 and later it became a disease of major importance in North America, USSR, and Japan (12). The disease is less important in Western Europe and is not known to occur in Great Britain (8), but there are doubtful records of occurrence in Asia.

The causal organism, *Corynebacterium sepedonicum* is a slow growing, gram positive, anaerobic, non-motile, rod-shaped bacterium and varies from small coccoids through rod shapes to large spherical globules. Symptoms appear in growing plants and in tubers. Tuber infection may include either infection of the vascular ring that leads to color change from yellow to brown and cream or appearance of spottiness under the skin on the surface of the tuber (6). *C. sepedonicum* is not well adapted to a saprophytic existence in soil and quickly loose viability in warm moist soil; persistence of bacteria in the soil is inversely proportional to the soil moisture and temperature (2, 15).

Dissemination of the bacterium occurs largely through infected tubers at harvesting, in storage, or in seeding operations. Longevity of this bacterium on various surfaces like wool, blue demin, boot leather, rubber belting, burlap, concrete, kraft paper, plastic, and plywood was shown to be up to 7 and 10 months or longer, but on steel surfaces it did not survive four months, as shown on Table 1 (14).

Assays from the apical, middle, and basal region of the stem of infected plants revealed absence of bacteria from apical cuttings for two months after inoculation (Table 2), whereas the bacterium was present in the other two regions within four weeks. This information lead Nelson (13) to conclude that apical cuttings could yield plants free of ring rot and serve as a method to eradicate the bacteria from valuable stocks. The organism was found to attack the maturing plants more readily than young plants.

Diagnosis of bacterial ring rot usually is based on symptomatology in the field and in storage, but field symptoms may vary according to climate, cultural, and biological factors such as drought stress, frost injury, and other diseases.

Methods that have been used to detect the bacteria in the laboratory include the gram stain technique of Racicut et al. (17) the Immunoflourescence test of Iverson & Kelly (5) and the ooze test method (1). Diagnosis of ring rot infection based on grown positive rods in smears from tubers can be misleading because nonpathogenic gram positive endophytic bacteria may be present in tuber tissue (4). Also it is not a good method to detect a low population of bacteria in infected tissue (7). The eggplant test has been used in Sweden since 1968; it can distinguish the ring rot bacterium from other gram positive bacteria (16).

Table 1. Survival of ring rot bacteria on contaminated surfaces stored in an unheated machine shed and a root cellar (14)

Storage area	Surface	Time in storage (months)			
		0	4	7	10
Machine shed	Wool	100	100	80	90
	Blue denim	100	20	30	0
	Boot leather	100	100	60	10
	Rubber belting	100	100	80	50
	Steel	100	0	0	0
	Burlap	100	60	30	80
	Concrete	100	80	80	90
	Kraft paper	100	100	70	60
	Plastic	100	90	60	10
	Plywood	100	90	30	20
Root cellar	Burlap	100	80	50	60
	Concrete	100	90	90	0
	Kraft paper	100	100	80	0
	Plastic	100	80	10	0
	Plywood	100	30	20	0

The ideal diagnostic procedure for bacterial ring rot should be rapid, sensitive for limited numbers of bacterial cells, specific for C. sepedonicum and easy to perform. Of the techniques available at present, a serological procedure offers the best possibility. In earlier studies antiserum prepared against C. sepedonicum was nonspecific and cross reacted with C. flaccumfaciens and C. poinsetiae. It was reported that reactivity of antisera to Corynebacterium species was not the same when nucleoproteins rather than polysaccharides were used as antigens, and divided the genus Corynebacterium into five distinct groups, one being C. sepedonicum. Clafflin and Shepard (3) obtained agglutination with

antiserum prepared against C. sepedonicum only with tubers affected by ring rot and not from tubers affected by Erwinia sp. (soft rot), Fusarium sp. (dry rot), Phytophthora erythroseptica (water rot) nor Pythium debaryanum (leak) (Table 3). They also found that the antiserum prepared against C. sepedonicum was serologically distinct from its antigens prepared against six other species of Corynebacterium (Table 4).

Table 2. Ring rot development on potato plants originating from stem cuttings taken from different positions on plants exhibiting mild ring rot symptoms (13)

Position of cuttings	No. of cuttings	No. of cuttings diseased	Average disease severity of plants from cuttings
Apical	21	0	0
Central	19	17	3.84
Basal	18	16	4.44

Table 3. Reaction of potato extracts with antiserum prepared to Corynebacterium sepedonicum (3).

Antigen	Source	Reaction
Tuber, C. sepedonicum	Montana	++++
Tuber, C. sepedonicum	Maine	++++
Tuber, C. sepedonicum	Washington	++++
Tuber, C. sepedonicum	Wisconsin	++++
Stem, infected (C. sepedonicum)	Montana	++++
	Washington	+++
Tuber, blackleg (Erwinia carotovora var. atroseptica)	Montana	-
Tuber, Fusarium dry rot	Montana	-
Tuber, leak (Pythium debaryanum)	Montana	-
Tuber, Rhizoctonia solani	Montana	-
Tuber, net necrosis	Montana	-
Tuber, water rot (Phytophthora erythroseptica)	Montana	-
Control, healthy tuber	Montana	-
Control, healthy stem	Montana	-
Control, C. sepedonicum cells	Montana	++++

Table 4. Serological reactions of Corynebacterium spp. with antiserum prepared to C. sepedonicum (3)

Antigen	Agglutination
C. flaccumfaciens	-
C. flaccumfaciens var. aurantiacum	-
C. flaccumfaciens var. violaceaum	-
C. insidiosum	-
C. michiganense	-
C. poinsettiae	-
C. sepedonicum (Isolate MS-2)	++++
Control, sterile saline	-

= -no agglutination; +discernible; ++moderate; +++strong; ++++complete.

Many chemicals have been recommended for disinfesting cutting knives, storage surfaces, machinery and equipment. Disinfesting jute bags, wood, and metal surfaces with a quartenary ammonium compound was an early recommendation (10, 11). Other methods that have been recommended for disinfesting jute bags are 1) immersing the jute bags in formalin for 24 hours and 2) passing a single layer of bags through an infrared oven (about 960 bags can be disinfested by the latter method in one hour). Mercuric chloride and Semesan Bel were found to be the most efficient disinfestants for cutting knives. Recent findings by Letal (9) have shown that total control of blackleg and ring rot bacteria from contaminated surfaces could be obtained with mercuric chloride and formaldehyde, but problems may arise with mercury residue, therefore, formaldehyde would be preferred (Table 5). Because of zero tolerance of bacterial ring rot in seed potato production, any material used in controlling ring rot in the seedpiece should consistently provide absolute control. The following measures may be recommended:

1. Use foundation or certified seed only.
2. Disinfest all machinery viz planting harvesting.
3. Proper sanitation of storage facilities and equipment, disinfest cellars and sacks.
4. Clonal selection (if seeds cannot be discarded for some reason) and multiplication by stem cutting and tissue culture techniques.

Table 5. Growth of *Corynebacterium sepedonicum* from infested wood, metal, and burlap after treatment with various disinfestants (9)

Disinfestant	Treatment time (min)	Metal	Wood	Burlap
Control (H_2O)	5	+	+++	++
	10	+	+++	++
	30	+	+++	++
	60	+	+++	++
10% Bleach	5	-	-	-
	10	-	-	-
	30	-	-	-
	60	-	-	-
0.1% Mercuric Chloride	5	-	-	-
	10	-	-	-
	30	-	-	-
	60	-	-	-
Quaternary ammonium solution	5	-	++	++
	10	-	++	-
	30	-	-	-
	60	-	-	-
10% Dettol	5	-	+(-)	-
	10	-	-	-
	30	-	-	-
	60	-	-	-
2% Formaldehyde	5	-	+++	+
	10	-	++	-
	30	-	-	-
	60	-	-	-
5% Formaldehyde	5	-	-	-
	10	-	-	-
	30	-	-	-
	60	-	-	-
Soapy H_2O	5	+	+++	++
	10	+	+++	++
	30	+	+++	++
	60	+	+++	++
0.13% Zephiran	5	+	++	+
	10	-	+	+
	30	-	+	+
	60	-	-	-
Hibitane	5	+	+++	+
	10	-	+++	-
	30	-	++	-
	60	-	-	-

- no growth; + slight growth; ++ moderate growth; +++ heavy growth

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