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Bacterial Wilt Disease in Asia and the South Pacific

Proceedings of an international workshop held at PCARRD, Los Baños, Philippines 8–10 October 1985

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Contents

Foreword

A WORKSHOP on bacterial wilt was held in Los Baños, Philippines from 8 to 10 October 1985. The meeting was sponsored by the Philippine Council for Agriculture and Resources Research and Development (PCARRD), the International Potato Centre (CIP), the Southeast Asian Program for Potato Research and Development (SAPPRAD), and the Australian Centre for International Agricultural Research (ACIAR).

The workshop was attended by some 40 participants from 12 countries, including the member countries of SAPPRAD, a regional research and development network on potatoes, sponsored by the Australian Development Assistance Bureau (ADAB).

The purposes of the workshop were to identify the present distribution and importance of bacterial wilt, to assess the current 'state of the art' of research on bacterial wilt in the Asia/Pacific region and to identify future research which would result in more effective control.

The papers presented at the workshop represent an important record of research on bacterial wilt and include a critical analysis of gaps in the present research coverage, which may profitably be explored. ACIAR hopes that the volume will provide a useful guide to the present status of bacterial wilt in Asia and the Pacific and stimulate more work on this damaging plant disease.

ACIAR would like to thank PCARRD and its Executive Director, Dr Ramon Valmayor, for hosting the workshop. The excellent arrangements made by Dr Dely Gapasin, Mrs L. Nallana and their colleagues at PCARRD are much appreciated. ACIAR is also pleased to have been able to cooperate with CIP through its regional representative, Dr Peter Vander Zaag, and SAPPRAD through its coordinator, Dr Pons Batugal, in sponsoring the workshop. We thank Mr Reg MacIntyre, Mrs Janet Lawrence and Mr Paul Ferrar of ACIAR for their valuable work in the preparation of the proceedings.

J. R. McWilliam
Director
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Summary of Discussion and Recommendations

G. J. Persley, P. Batugal, D. Gapasin, P. Vander Zaag

ONE of the most destructive bacterial diseases of plants, and the most widespread in tropical, subtropical and warm temperate regions is bacterial wilt caused by *Pseudomonas solanacearum*. In potato, loss of about 75% of the crop has been reported. In tomato, the disease can bring about almost total destruction during the rainy season. It also affects other crops of economic importance in the Asia/Pacific region, such as tobacco, banana and peanut.

The destructiveness of the disease is compounded by the wide host range of the causal organism. Thus, in areas where intercropping and continuous cropping are practiced, the severity of the problem increases each year.

In recognition of the importance of bacterial wilt on potato and other crops in Asia and the Pacific, the Philippine Council for Agriculture and Resources Research and Development (PCARRD), the Southeast Asian Program for Potato Research and Development (SAPPRAD), the Centro Internacional De La Papa (CIP) and the Australian Centre for International Agricultural Research (ACIAR) jointly sponsored a workshop on bacterial wilt, held in Los Baños, Philippines, from 8 to 10 October, 1985. Over 40 participants attended, from 12 countries.

The objectives of the workshop were:

(1) To assess the extent of problems caused by *Pseudomonas solanacearum*, by crop and by country in the Asia/Pacific region;
(2) To assess progress in research on *P. solanacearum* particularly in relation to control;
(3) To identify the most important constraints in developing prevention and control strategies for *P. solanacearum;*
(4) To identify research needs;
(5) To identify scientists who may wish to participate in collaborative research on bacterial wilt;

The workshop was concentrated on four themes.

(1) Distribution and importance of bacterial wilt in Asia and the South Pacific;
(2) Ecology of the disease and variation in the pathogen;
(3) Cultural and biological control; and
(4) Breeding for disease resistance.

Papers reviewing the current status of knowledge and research in progress in these four areas were presented. The workshop then discussed research needs on each of these topics. A summary of the discussions and the recommendations of the workshop are given below.

Distribution and Importance

Country reports indicated that bacterial wilt occurred on a wide range of crops in China, Fiji, India, Indonesia, Papua New Guinea, the Philippines, Sri Lanka and Thailand. It is also known to occur in other Asian/Pacific countries not represented at the workshop such as Malaysia and Western Samoa.

	India	Vietnam	Fiji	PNG	China	Sri Lanka	Thailand	Indonesia	Philippines
Potato	+	+	+	+		+	+	+	+
Tomato	+	+	+	+	+	+	+	+	+
Banana	-		-	-			-	+ ?	
Eggplant	+	+	+	+	+	+	+		+
Capsicum				+					
Peanut	+	-	-	-	+	-	-	+	-
Cassava		-						+	
Clove								+ ?	
Ginger	+			-	+		+	+	+
Teak									+
Casuarina					+ ?				
Pepper		+	+		+			+	+
Bitter gourd									+
Squash									+
Winged Bean									+
Cowpea									+
Tobacco	-	+	+	-	+	+	+	+	+
Castor bean									+
Marigold									+
Snapbean									+
Stringbean									+
Pechay									+
Chinese cabbage									+
Fennel	+								+
Sesame					+	+	+		
Chilli	+	+	+				+		
Olive					+				
Sweet potato					+				
Symphytum					+				
Mulberry					+				
Jute	+	+							
Soybean		+							
Cotton	+								
Cumin	+								
Ajwain	+								
Broad bean	+								

Bacterial wilt has been regarded as a problem primarily of solanaceous crops. However, it is also causing losses in non-solanaceous crops such as cassava in Indonesia and mulberry and olive in China. Thus the disease appears to be increasing in importance, or is at least being more widely recognised as a problem. Many more weed hosts have been determined, emphasising the difficulty in control through cultural practices.

Losses due to bacterial wilt in the region have not been quantified and not even an approximate estimate is possible from the reports presented at the workshop, due to the lack of suitable data. The country reports emphasised bacterial wilt as a serious disease, often the most serious, on several crops.

Surveys to determine losses are needed. Losses are increasing because of the expansion of agriculture into areas where susceptible crops have not previously been grown. Avoidance has proven possible for certain crops, by selecting areas free of *Pseudomonas solanacearum*, or soils which are apparently suppressive and thus wilt is minimised even when accidental introduction of the bacterium takes place as with potato seed tubers.

Breeding for disease resistance is the most appropriate solution. The use of tolerant cultivars of peanut in Indonesia has limited the bacterial wilt problem on that crop. Resistance to bacterial wilt in tomato has been moderately successful. On potato, cultivars with resistance have been identified in Fiji, Papua New Guinea and Sri Lanka.

Breeding and selection programs for resistance to bacterial wilt in several crops are under way at the University of the Philippines at Los Baños, and Fiji. In Papua New Guinea and Sri Lanka resistance selection work is only on potato.

Successful breeding programs require knowledge of the biology of the pathogen, and this is inadequate. The race/biovar distribution picture is unknown in many countries. Further research is required to clarify the occurrence of different races and biovars on crop and weed hosts. To accomplish this objective in different countries some programs require better facilities, supplies and training. Alternatively, strain identification could be done through collaboration with a central facility able to provide such a service. Quarantine regulations must be taken into account in this context.

Two issues that arose from the increased efforts under way are related to classification of *P. solanacearum*. Firstly, the determination of biovars needs to be carried out utilising all the tests proposed by Hayward (see proceedings of the 1976 North Carolina State University workshop) when a new incidence is being reported, whereas a limited number of tests may be acceptable in other instances. Secondly, the naming of pathovars, when our knowledge of host range and how to determine it is limited, may be premature and could lead to even greater confusion in the literature.

Ecology of the Disease and Variation in the Pathogen

Strains of *P. solanacearum* differ substantially in their ecology and it is a mistake to extrapolate from results obtained with one pathogen population in one environment to generalisations on the ecology of the disease in all environments.

There are now five races described, which differ in host ranges, geographic distribution and ability to survive under different environmental conditions:

- Solanaceous strain (Race 1): wide host range, distributed throughout the lowlands of the tropics and subtropics;
- Musaceous strain (Race 2): restricted to *Musa* and a few perennial hosts, initially limited to American tropics, now spreading to Asia;
- Potato strain (Race 3): restricted to potato and a few alternative hosts in the tropics and subtropics;
- Ginger strain (Race 4) from the Philippines; and
- Mulberry strain (Race 5) from China.

Several environmental factors affecting the survival of the pathogen are:

- High soil moisture content, favouring survival and increase in pathogen production;
- High soil organic matter content leading to decline in pathogen population;
- High temperatures decreasing pathogen population;
- Presence of alternate hosts favouring survival, as the pathogen is able to survive in association with non-host plants.

The major means of dispersal are: vegetative seed pieces of crops such as potato and ginger; root-to-root spread in tomato and tobacco; contaminated implements; flood/irrigation water.

The importance of true seed in pathogen dispersal is not considered important, but there have been few critical studies to examine if the pathogen can be dispersed in this way.

Information on the ecology of the disease and variation in the pathogen is important in establishing national disease management strategies, including the development of resistant varieties and appropriate cultural management.

Ecological studies have relied on selective media or biological assay systems to study population changes over time. Techniques using monoclonal antibodies offer considerable potential for following population changes in a particular strain in a mixed bacterial population over time.

Research Needs on the Ecology of the Disease

The areas requiring further research are:

- The importance of insect transmission;
- True seed transmission;
- Relationships between environment conditions such as soil type, salinity, temperature and soil moisture and the occurrence of bacterial wilt. Preliminary observations suggest bacterial wilt may not occur in soil flooded for paddy rice, and this observation needs to be confirmed;
- Role of alternative hosts and non-hosts in survival.

Ecological studies in different agroecosystems should consider:

- The strain composition of the pathogen population;
- Host range of the strain(s) present;
- Ability of the strain(s) to survive in the soil or in association with alternative hosts and non-host plants;
- Effects of environmental factors on survival ability;
- Sources of inoculum for newly planted crops.

Cultural and Biological Control

The ecology of bacterial wilt is poorly understood. However, the reality of development demands that useful information, even if imperfect, be made available to farmers whenever possible. Thus, inferences are being drawn from observations and experiments that do not precisely explain why and how such results are observed. Indeed the generalisations deduced do not necessarily hold true in all cases. Such inferences are useful for two reasons. Firstly, they provide a basis for initial recommendations on how to reduce disease occurrence, and secondly, the observations can serve as a basis for more rigorous studies on the disease.

It has been observed in the Philippines that under high rainfall conditions, increased planting depth of potatoes increased bacterial wilt incidence. This was attributed to higher moisture conditions in deeper soil layers. Despite increased bacterial infection, however, deeper planting has a slight positive effect on total tuber yield.

Under low bacterial wilt pressure, hilling does not affect disease incidence, while under high bacterial wilt pressure hilling results in an increased infection.

No interaction between hilling and planting depth was observed. Experiments on multiple cropping and intercropping showed that there was lower bacterial wilt incidence in intercropped experiments.

For potatoes, factors such as planting depth, plant population, hilling, time of planting and physiological age of the seed are considered important for the control of bacterial wilt. Their role in disease control needs to be verified under local conditions.

Improved cultural practices and the use of clean planting material is an important factor in reducing the incidence of bacterial wilt. Recommended practices include the burning of infected plants, use of clean equipment, removal of other solanaceous crops or weeds and drying out of the soil for 3–5 weeks.

Avoidance of the disease is possible through the use of bacterial wilt-free planting materials, rotation with non-host plants and growing after flooded crops in some instances. In the Philippines bacterial wilt appears not to be a problem in flooded rice fields, river flood plains and sugarcane land. It was pointed out that this observation does not hold true in all countries, and the basis of the lower disease incidence after rice or in sugarcane land in some environments merits investigation.

The field observations in the Philippines have provided useful information. They also highlight the need to understand the pathogen and its ecology if control recommendations are to be made with confidence.

Any attempt to lower the incidence of the disease must combine the use of resistant varieties, cultural practices which lower susceptibility of plants to the disease and use of clean planting materials.

The possibilities for biological control were discussed, with experimental systems based on antagonistic bacteria such as *Bacillus polymyxa* and *Pseudomonas fluorescens* being described.

The possible long-term approach of developing a biological control system based on avirulent mutants of *P. solanacearum* was also discussed. The range of genetic variation in *P. solanacearum* and the basis of this variation is poorly understood. Modern genetic studies on the organism are required to have a better understanding of the genetic basis of pathogenicity. These would include studies to identify virulence gene products, their mode of action and regulation mechanisms of their expression. Such genetic studies may provide the basis of a biological control strategy. Once mechanisms of virulence are understood, plant inoculation with avirulent bacterial mutants may be possible, to prevent entry of normal virulent bacteria.

Breeding for Disease Resistance

Bacterial wilt is still a serious disease on crops such as potato, tomato, eggplant, and tobacco where breeding for resistance has been conducted for many years. Some useful resistance exists, but not enough to have reduced bacterial wilt to a minor disease.

The impression gained at the workshop was that there are few breeding programs in countries in Asia and the Pacific actively seeking resistance to bacterial wilt in crops in which it is considered to be a major limiting factor. This may reflect the lack of published work by breeders, especially commercial breeders, and the regrettable lack of interaction between pathologists and breeders in many countries.

Individual countries should conduct their own crossing and selection programs using broadly based genetic material. For example, CIP in Lima will not be able to breed potatoes for east Java suited to that particular ecosystem. There should be much more emphasis on local breeding programs to identify material suited for particular ecosystems.

The potential of molecular genetic studies to elucidate the genetic basis of pathogenicity in *P. solanacearum* was noted. Such studies may play a much more important role in the future in guiding plant breeders in their search for resistance to particular strains of *P. solanacearum*.

The key questions to consider are:

- What breeding strategies could be considered that would identify material which, when combined with appropriate crop management practices, would reduce bacterial wilt to a minor disease?
- Which countries have breeding programs for potato, tomato, other vegetable crops? How important do breeders consider bacterial wilt?

- How much resistant material is available but not widely distributed? Are there any varieties of tomato and other vegetables available which could be more widely distributed in the tropics for use by farmers?

There is a need for a review of the presently available sources of varieties of various crops with putative resistance and its distribution in many countries.

There are many reports of varieties resistant in one area and susceptible in another. This may be due to differences in environment or different strains of *P. solanacearum*, or interaction between the two.

It would be useful to assemble sets of the reportedly resistant lines of the major hosts (potato/tomato/eggplant) and distribute them to several countries—especially where different strains are reported. These could serve as sets of differential varieties to give more information on the likely stability of resistance in different countries.

Sources of resistance have been identified in wild species related to potato, tomato and eggplant. There is a need for a wider search for sources of resistance for other hosts especially in areas where the cultivated crops originated. For example, wild species related to peanut may also contain sources of resistance.

Potato

Potato Bacterial Wilts

The differences between bacterial wilt of potato caused by Race 3 (the potato race with strains largely restricted to potato) and Race 1 (with its wider host range and much greater genetic diversity) were noted. The narrowness of Race 3 should make it an easier breeding target than resistance to race 1 combined with heat adaptation in potatoes for the lowland humid tropics. Some programs should concentrate on resistance to specific strains of Race 3. For example, there may be some countries where a specific strain of Race 3 has recently been introduced. Resistance to a single strain would be a relatively simple breeding target and the problem of bacterial wilt on potatoes could be solved in such countries. Combined resistance to both races is obviously attractive, if it can be obtained.

In China, Race 3 is the major problem on potatoes and breeding efforts are concentrating on resistance to Race 3.

Genotype and Environmental Interaction

The differing behaviour of the same lines in different environments was noted from several countries. There is a continuing need to evaluate material in different locations and in a number of seasons in order to obtain a reliable indication of its likely behaviour in the field as a commercial line. Lines susceptible in one environment can show useful resistance in another. Genotype × environment interactions should be the guide to parental selection for further recurrent selection breeding. It should also provide the ammunition for research on pathovar delineation.

Narrow Genetics Base of Resistance

The narrow genetic base of early resistant lines of potato was noted. The workshop commended the existing approach of CIP in expanding the genetic base in resistance breeding.

Peanut

Bacterial wilt is a more restricted problem on peanut than on potato. The disease is considered important in China, Indonesia, Malaysia and Uganda. However, it may be more widely distributed than this.

Why bacterial wilt of peanut is not more important is a puzzling question, since the crop is widely grown in tropical environments where bacterial wilt occurs on other crops.

Even in Indonesia and China, bacterial wilt is restricted to certain regions, and is not universally distributed on peanut. In Indonesia, it occurs in the wet tropical

climate of West Java but not in the drier areas of East Java.

Resistance to bacterial wilt in peanut exists in Indonesia and China. There may be other useful resistance in the ICRISAT collection which could be combined with high yield and other desirable traits.

It would be useful to compare isolates from peanut in different countries at a single location to see if they have common pathogenic features. Monoclonal antibodies may be a useful technique for such comparative studies.

Tomato

Some resistant varieties of tomato are available from breeding programs in the Philippines, AVRDC (Asian Vegetable Research and Development Centre) and the commercial breeding company Peto Seed Co.

More breeding for resistance with a wider genetic base needs to be done for tomato. Resistance to bacterial wilt needs to be combined with other characteristics, such as high yield, nematode resistance and fruit quality.

Other Crops

There is a need for a more critical analysis of how serious bacterial wilt is on other crops, and what efforts are being made to breed for disease resistance in other crops. National programs, commercial seed companies and international institutions may have more information on breeding for disease resistance in other crops than is available in the literature.

Resistance to bacterial wilt in several other vegetable crops such as eggplant occurs and some resistant lines have been reported which merit wider evaluation, and use as parents in local breeding programs. Resistance to bacterial wilt in sweet potatoes is being sought in China.

Conclusion

The workshop recommended the establishment of a Working Group on Bacterial Wilt in Asia and the Pacific. Dr A. C. Hayward of the University of Queensland, Australia, agreed to act as chairperson of the working group. The working group will undertake the following activities:

- Sponsor 'state of the art' studies for particular countries, which provide a critical analysis of published literature, describing the distribution and importance of bacterial wilt, the strain composition of the population, sources of resistance and current research programs;
- Publish a newsletter to facilitate greater exchange of information;
- Identify common research needs, and foster collaborative research, with scientists in different countries studying different aspects of bacterial wilt.

Bacterial Wilt Caused by *Pseudomonas solanacearum* in Asia and Australia: an Overview

A. C. Hayward*

BACTERIAL wilt caused by *Pseudomonas solanacearum* is one of the most important and widespread bacterial diseases of plants in the tropics, subtropics and warm temperate regions of the world. The major economic hosts include potato, tomato, eggplant, pepper, ginger, peanut and banana; there are also many weed hosts. Representatives of more than 35 families of plants are affected by the disease (Kelman 1953). However, it is less well known that *P. solanacearum* is a significant pathogen on some economically important trees and shrubs, several recognised recently (Table 1).

The purpose of this paper is to review current progress in understanding the biology, ecology and control of bacterial wilt with particular attention to work in Asia and Australia in the last 20 years.

Definition of Strains

Understanding of the potential host range of strains of *P. solanacearum* endemic in any area, and recognition of the existence of strains specialised to particular hosts, is necessary in the effort to devise rational control strategies based on crop rotation. There have been two principal approaches to the definition of strains of the bacterial wilt pathogen, the first based on hosts of origin and host range of isolates, the second on the basis of differences in cultural, biochemical, serological or other properties (Buddenhagen and Kelman 1964; Hayward 1964). The term 'race' has been used to designate isolates of the pathogen of a particular origin and host range on inoculation. However, this use of the term race is different from that familiar to mycologists who work with the rust fungus of wheat, for example. The term 'biovar', which is considered preferable to 'biotype' for reasons considered elsewhere (Hayward 1976) has been used for isolates of the bacterial wilt pathogen differing in biochemical properties.

The race classification for isolates of *P. solanacearum* originally comprised race 1 for isolates affecting solanaceous and other plants, race 2 for isolates affecting bananas and Heliconias, and race 3 for isolates primarily affecting potato (Buddenhagen and Kelman 1964). Four biovars have been designated according to ability to oxidise certain hexose alcohols and disaccharides (Hayward 1976). Both systems have to be extended in the light of

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Table 1. Some tree and shrub hosts of bacterial wilt caused by *Pseudomonas solanacearum*.

Host	Family	Countries	Reference
Teak *(Tectona grandis)*	Verbenaceae	Malaysia, Indonesia, ? Philippines	Mitchell (1962)
Mulberry *(Morus alba)*	Moraceae	China	He et al. (1983)
Australian-Pine	Casuarinaceae	Mauritius, China,	Orian (1952)
Horsetail Beefwood *(Casuarina equisetifolia)*		Kerala (India)	He et al. (1983) Ali (Pers. Comm.)
Olive *(Olea europaea)*	Oleaceae	China	He et al. (1983)
Cassava *(Manihot esculenta)*	Euphorbiaceae	Indonesia	Nishiyama et al. (1980)
Custard Apple *(Annona* spp.)	Annonaceae	Queensland (Australia)	Mayers and Hutton (Pers. Comm.)

recent work showing that there are other strains specialised to particular hosts and a new biovar possessing a new combination of biochemical properties.

New Hosts of Bacterial Wilt

Mulberry (*Morus alba*)

A new race of the bacterial wilt pathogen has been described in China which is not known to occur elsewhere (He et al. 1983). The new race, designated race 4, differs from all other strains of the pathogen in having low virulence for potato (*Solanum tuberosum*) (cultivar Russet Burbank) and eggplant (*Solanum melongena*) (cultivar Black Beauty), whereas most other strains are highly virulent on these hosts. The isolates from mulberry were also biochemically distinct comprising a new biovar, designated biovar 5, which differed from established biovars in producing acid from lactose, maltose, cellobiose and mannitol but not from dulcitol and sorbitol (He et al. 1983).

Winged Bean (*Psophocarpus tetragonolobus*)

Bacterial wilt of winged bean was described by Abdullah (1980b) in Malaysia and shown to be caused by a biovar 3 strain. The isolates affecting winged bean are specialised in pathogenicity to that host. All isolates from ginger, french bean and groundnut were non-pathogenic to winged bean, whereas isolates from solanaceous hosts were variable in their pathogenicity to this host. Two weed hosts, *Croton hirtus* and *Euphorbia prunifolia* (Table 2), infesting cultivated areas were shown to harbour strains of the bacterial wilt pathogen which were as virulent on inoculation to winged bean as isolates from that host. Isolates from solanaceous hosts pathogenic for winged bean were less virulent than the winged bean and weed host isolates, indicating the existence of a strain of the pathogen specialised to winged bean (Abdullah 1982). There has been no formal proposal to designate a new race for the winged bean pathogen; however, the recognition of pathogenic specialisation in this case and of weed hosts which harbour the pathogen is clearly of great importance in understanding of the biology of the disease and attempts to control the disease by crop rotation.

Custard Apple (*Annona spp.*)

One of the principal factors limiting expansion and affecting the stability of the industry in Queensland (Australia) has been an unidentified soil-borne disease affecting trees of bearing age known since 1918 as base and root rot. The disease manifests in two forms: (1) a rapid wilting followed by tree death occurring in the first 3 years after planting out in the field, or (2) a slow decline and death of mature trees. Mayers and Hutton (1986 in preparation) of the Queensland Department of Primary Industries have made an important advance in understanding the etiology of the disease. They isolated *Pseudomonas solanacearum* from internal discoloured root and trunk tissue from a 3-year-old tree and subsequent pathogenicity tests established that this bacterium was the causal agent of bacterial wilt of custard apple. Isolates were identified as biovar III. Cross inoculation tests using cultures of *P. solanacearum* from custard apple and tomato on to *Annona* spp., *Capsicum annuum,* tomato cultivars and potato failed to distinguish differences in virulence among these isolates. Accordingly in this case there is no evidence of pathogenic specialisation, and bacterial wilt of custard apple is caused by the same strain (race 1) endemic on tomato in coastal areas of Queensland.

Some other recently described economic and weed hosts are listed in Tables 1 and 2.

Table 2. Some recent new host records of bacterial wilt in Asia and Australia.

Host	Family	Countries	Reference
Ipomoea setosa	Convolvulaceae	Malaysia	Abdullah (1983)
Hyptis capitata (weed host)	Labiatae	Sarawak	Abdullah (1983)
Cosmos caudatus	Compositae	Sarawak	Abdullah (1983)
Jute *(Corchorus capsularis* and *C. olitorius)*	Tiliaceae	West Bengal	Sharma and Mukherji (1970)
Euphorbia prunifolia (weed host)	Euphorbiaceae	Malaysia	Abdullah (1980a)
Croton hirtus (weed host)	Euphorbiaceae	Malaysia	Abdullah (1980a)
Solanum cinereum (weed host)	Solanaceae	N.S.W., (Australia)	Graham and Lloyd (1978)
Anthurium andreanum	Araceae	Sri Lanka	Gunawardena (pers. comm.)
Winged bean *(Psophocarpus tetragonolobus)*	Leguminosae	Malaysia Philippines	Abdullah (1980b, 1982) Valdez and Almodovar (1980)
Stylosanthes humilis	Leguminosae	Northern Territory (Australia)	Aldrick (1971)

Present Status on Established Hosts

Peanut (*Arachis hypogaea*)

The two countries in which bacterial wilt has been reported to be a major problem are China (Sun Darong et al. 1981) and Indonesia (Kelman 1953). Kelman and Cook (1977) gained the impression during their survey of plant pathology in the People's Republic of China that bacterial wilt of peanut was no longer the cause of loss: they commented that the system of alternating peanuts and rice had apparently resulted in successful control of the pathogen in certain areas. However, Sun Darong et al. (1981) give a different picture of the present importance of this disease. They reported that in Southern China the disease is particularly severe in wet soils and commonly affects 10% of the planted area. Losses of up to 30% of the crop are sometimes experienced. Crop rotation which is known to reduce the incidence of this soil-borne disease has been neglected in parts of Southern China: continuous cropping in some of the most concentrated production areas has greatly accentuated the risk of damage from bacterial wilt. Chinese plant breeders and plant pathologists have noted a relationship between environmental conditions and resistance to bacterial wilt, with all the resistant material originating in lower latitudes where selection pressures would be greatest.

In Indonesia during the 1920s bacterial wilt was considered to be the most important peanut disease in Java as well as Sumatra and other parts of the country. In seasons favourable for disease development it was estimated that 25% of the total peanut crop was lost. Heavy losses continued for many years (Kelman 1953). Attempts to develop a resistant variety were intensified and culminated in the development of cultivar Schwarz 21. This cultivar is now grown in all parts of Java, and has made peanut cultivation possible in areas where previously the native cultivars had almost disappeared as a result of infection by *P. solanacearum*. During the early 1940s Schwarz 21 became mixed with other cultivars and after 1945 selection work had to be started again. Bolhuis (1955) stated that the main problem in peanut cultivation in Java was still bacterial wilt, and that no hybrid resistant to bacterial wilt could be obtained without using a line of Schwarz 21 as parent. Recent surveys in Indonesia have shown that bacterial wilt is still widely distributed on peanuts, but is more severe in South Sumatra, West Java and South Sulawesi than in Central Java, East Java, Bali and Northern Sulawesi (Machmud, these Proceedings).

Bacterial wilt of peanut has been reported to occur in several other countries but rarely as the cause of significant loss of crop. Rothwell (1963) stated that the disease was found on peanuts occasionally in Southern Rhodesia (Zimbabwe). Severe losses due to bacterial wilt have been recorded on rare occasions in Uganda (Simbwa-Bunnya 1972). In Mauritius peanut was stated to be less susceptible than potato to bacterial wilt; however, sporadic severe infections occurred during the wet season. Various cultivars were tested for resistance during 1970–71; the most susceptible were Virginia Bunch, Florigiant and Shulamit, whereas GA 119/20 and Cabri were resistant (Felix 1971). These results are comparable to those obtained in Georgia, USA, where, although bacterial wilt is widespread on other hosts, peanut wilt has been of relatively minor importance (Jenkins et al. 1966).

In Australia, bacterial wilt of peanut has not been reported in Queensland (Purss 1962). However, Pegg and Moffett (1971) isolated *P. solanacearum* from the root systems of 3 of 35 harvested Red Spanish peanut plants in a disease nursery established inland near Nambour (Queensland) in which a ginger planting had been completely destroyed by bacterial wilt in the previous season. Bacterial wilt of peanuts has been reported in the Northern Territory (Aldrick 1971) but no information is given on the incidence or importance of the disease. It is noteworthy that bacterial wilt of peanut has not been reported in the Philippines. Zehr (1969a) made an extensive survey of the common susceptible hosts but found no evidence of bacterial wilt on peanut. In inoculation experiments 9 of 35 isolates tested induced mild wilt symptoms but none of these were virulent to peanuts. The remaining 26 isolates induced limited vascular browning without external wilt symptoms in inoculated peanut plants.

Ginger (*Zingiber officinale*)

This disease has been reported from several countries (Table 3). Evidence indicates that ginger is affected by at least two strains of the pathogen of different pathogenic specialisation. With the exception of reports of biovar I from ginger in Malaysia (Abdullah 1982), all isolates from naturally infected ginger have proved to be either biovar III or IV (Table 3); however, the relationship to virulence and pathogenicity, if any, is not clear. In Queensland (Australia), biovar IV was the cause of a severe and rapid wilt distinct from the slow wilt caused by biovar III, which was also less commonly encountered on ginger (Hayward et al. 1967; Pegg and Moffett 1971).

Table 3. Biotypes of *Pseudomonas solanacearum* associated with bacterial wilt of ginger (*Zingiber officinale*).

Country	Biovars isolated	Reference
Australia	3 and 4	Hayward et al. (1967) Pegg and Moffett (1971)
China	3 and 4	Ren et al. (1981) He et al. (1983)
Hawaii (USA)	4	Quinon et al. (1964)
India (Kerala)	3	Indrasenan et al. (1981)
Indonesia	Not known	Machmud (pers. comm.)
Malaysia	3 and 4	Lum (1973) Abdullah (1982)
Mauritius	3	Orian (1953) Hayward (1964)
Philippines	4	Zehr (1969, 1970a)

Zehr (1969b, 1970b) showed that isolates from ginger in the Philippines were more virulent to ginger but less virulent to tomato than were isolates obtained from other hosts. Isolates of high virulence to ginger were obtained only from diseased ginger plants. Ginger isolates were, however, highly virulent to potatoes and eggplants. It is not clear whether the Philippine ginger strain attacks only ginger in nature, or its absence in other hosts is due to its limited distribution.

Lum (1973) found that bacterial wilt of ginger in Malaysia did not occur with the rapidity characterising wilt on tomato and other solanaceous crops. Infected ginger plants became stunted, yellowed, and the lower leaves dried out over an extended period of time before the plant was finally killed. Complete wilting of ginger plants inoculated with ginger isolates occurred within 3 weeks of inoculation. The ginger isolate, however, caused only limited infection of tomato, tobacco and peanuts, whereas tomato isolates caused typical wilting of tomato, tobacco, peanut as well as ginger. Reaction of the four test species to the isolates indicated that the Malaysian isolates were a weakly virulent form of *P. solanacearum*. Somewhat similar results were obtained in Hawaii (Quinon et al. 1964) where hosts infected by a ginger strain of *P. solanacearum* differed from those infested by isolates from tomato. The Hawaiian ginger strain failed to bring about a wilt in tomato, tobacco and peanut, whereas the tomato isolates wilted tomato and peanut but not ginger and tobacco. The similarity of the results of cross-inoculation studies in Hawaii and Malaysia indicate that the ginger strains occurring there are similar.

Banana and Abaca (*Musa spp.*)

Until recently bacterial wilt of bananas (Moko disease) had the most limited distribution of any major banana disease (Stover 1972) being restricted to parts of Central and South America and the West Indies. However, recent investigations by Rillo (1981) have shown clearly that both race 1 strain affecting banana and abaca, and race 2, the cause of Moko disease, occur in the Philippines. A non-banana strain of race 1 of *P. solanacearum* has been known in the Philippines since the 1930s, which is reported to be a weak pathogen on *Musa* spp., naturally infecting both bananas and abaca (Zehr 1970a). Symptoms are only mild and apparently only plants growing under unfavourable conditions are invaded. In the late 1960s, however, the much more serious race 2 infection was introduced and by the early 1970s had reached disturbing proportions in Davao del Norte and Davao City banana plantations (Rillo 1981). Rillo showed that the isolates of the bacterial wilt pathogen from abaca and commercial banana cv. Giant Cavendish were clearly differentiable in terms of host range, colony characteristics, melanin pigment formation, carbon utilisation, hypersensitive reaction on tobacco leaves, and phage typing. The abaca and banana isolates were shown to belong to race 1 and race 2, respectively.

Other Hosts

There are many other economically important hosts of bacterial wilt in Asia and Australia. Potato, the most important of these, is not considered here because this host will be dealt with in detail elsewhere in this workshop.

Loss Assessment and Economic Importance

Although a great deal of work has been directed towards an understanding of bacterial wilt, there have been few systematic studies of loss assessment and economic importance. There is no doubt, however, that interference with land usage is widespread in Asia and Australia, where the planting of susceptible crops such as tomato in infested soils under conditions of high temperature and rainfall cannot be contemplated. Earlier literature on loss assessment, much of it of a fragmentary rather than a systematic nature, has been comprehensively reviewed by Kelman (1953).

Zehr (1969a) made a systematic study of the distribution and economic importance of bacterial wilt in certain crops in the Philippines. The disease was widespread in the Philippines from 1966 to 1968 and losses averaging 15, 10, 10 and 2–5% for

tomato (*Lycopersicon esculentum*), eggplant (*Solanum melongena*), pepper (*Capsicum annuum*) and tobacco (*Nicotiana tabacum*), respectively, were observed in many widely scattered localities. Bacterial wilt in susceptible crops was not observed in mountain elevations above 1540 m, and in certain localities near to the coast.

Damage was also severe in new agricultural land, up to 50% loss having been observed in pepper planted for the first time in virgin forest soil. Economic loss due to bacterial wilt was greater for tomatoes than in any other crop; almost total loss frequently occurred, and only during the dry season were plantings likely to be found which were free of disease. Bacterial wilt of potatoes was sometimes severe at lower elevations, one field having 50% wilted plants. However, 75% of the potato crop is grown in mountainous areas above 1540 m where the disease was not found. This contrasts with the situation in Sri Lanka, for example, where the low temperature strain of *P. solanacearum* (race 3, biovar II) occurs which is primarily a pathogen of potato (Thurston 1963; Seneviratne 1969). Evidently at the time of the survey the potato race did not occur in the Philippines, a finding of considerable quarantine significance. However, more recent reports suggest that race 3 (biovar II), the potato race may occur at mid to high elevations in the Philippines (Valdez, these Proceedings). The occurrence of races 1 and 3 on potatoes in the Philippines needs to be clarified. Strains virulent to peanuts were also absent in the Philippines and exclusion of these strains should be enforced (Zehr 1969). Bacterial wilt is the most serious factor limiting commercial tomato production in the lowlands of Malaysia (Graham et al. 1977; Graham and Yap 1976) to the extent that tomato cultivation is now mainly confined to areas in the Cameron Highlands.

In recent years, bacterial wilt of cassava in Indonesia has become one of the important problems in parts of Southern Sumatra and Central Java; Kuning, one of the high-yielding and high-quality varieties, is very susceptible to the disease (Nishiyama et al. 1980).

Bacterial wilt is one of the most important and widespread bacterial diseases of crops in China especially in the southern provinces (He et al. 1983), and substantial losses have occurred on mulberry, olive and casuarina.

Control of Bacterial Wilt

The approach to integrated control will depend on the nature of the crop, strains of the pathogen present, and knowledge of their survival sites and modes of transmission. Methods for the integrated control of bacterial wilt (Moko disease) of banana developed 20 years ago were based upon blocking of the multiple means of transmission of the pathogen. Essential features of control included disinfection of machetes used in pruning operations, and fallowing of infested soil for periods of 6–18 months depending on the longevity in soil of the endemic strain. Since insects can carry some strains of the Moko disease bacteria to the male flower bud, this should be removed as soon as the last female hands have emerged on varieties with dehiscent bracts (Stover 1972). In the case of bacterial wilt of potato, French (1979) has stated that the use of resistant varieties is complemented by understanding of the following factors which contribute to inoculum potential: self-sown tubers; debris from a diseased crop; populations free in the soil and associated with alternate hosts and in the rhizosphere of non-hosts. Factors during cropping which are important include planting of diseased seed, nematode interaction, soil temperature, mechanical damage, moisture and water flow. The aggressiveness of the endemic strains of *P. solanacearum* also has to be taken into consideration (Table 4).

Table 4. Factors to consider in integrated control of bacterial wilt (based on French 1979).

Host Range and Aggressiveness of Endemic Strains
Are there latent weed hosts, or is there maintenance in the rhizosphere of a non-host?
Resistant Cultivars
Temperature of Soil During Cultivation
Inoculum Potential
What is the previous cropping history of the land?
What capacity have endemic strains for survival in soil?
Are there 'protected sites' such as self-sown tubers, plant debris, deeper soil layers?
What is the effect of soil factors (pH, salinity, clay versus sand content, etc)?
Prevailing Moisture and Water Flow
Nematode Interaction
Avoidance of Injury during Weeding, Tillage, Hilling and at Transplantation
Dissemination of Latently Infected Planting Material (e.g. of potato tubers, ginger or banana rhizomes, etc.)

Resistant cultivars

Early progress in the breeding or selection of cultivars of various crops for resistance to bacterial wilt has been described by Thurston (1976). There is

substantial evidence that the expression of resistance is influenced by soil temperature, soil moisture and rainfall, light intensity and photoperiod. In both tomato and potato, tolerant but not immune varieties have been developed.

Resistant varieties of potato have been developed from resistant lines of *Solanum phureja* originating in Colombia (French 1979; Rowe et al. 1972; Sequeira and Rowe 1969; Thurston 1976). In the southeastern United States a sandblasting technique (Gitaitis et al. 1983) has been used to screen selections of potato to bacterial wilt caused by race 1 (biovar I). Bacterial wilt-tolerant selections of *Solanum sucrense* and *S. tuberosum* have been released for further evaluation (Jaworski et al. 1984).

Breeding for resistance in tomato has been directed towards development of varieties which combine bacterial wilt resistance with heat tolerance and desirable agronomic features, with partial success (Graham et al. 1977; Mew and Ho 1976; Thurston 1976); however, varieties bred for resistance in one area frequently do not sustain that resistance when transferred elsewhere (Rao et al. 1975; Sonoda and Augustine 1978).

Several controlled-environment studies have shown that high soil temperature is the most important factor determining the breakdown of resistance in potato and tomato (Ciampi and Sequeira 1980a; French and de Lindo 1982; Krauz and Thurston 1975; Mew and Ho 1977). Latent infections developed at cool temperatures in potato cultivars inoculated by stem puncture, but not by soil infestation, and at warm temperatures with soil infestation. It was concluded that inoculation by infesting the soil is a more useful procedure to evaluate for resistance because it more closely correlates with field observations (French and de Lindo 1982). These results are in agreement with those of Ciampi and Sequeira (1980a). They found that all strains of race 1 and race 3 caused a complete wilt of potato in 15 days at 28 °C and no differences in virulence were apparent, whereas at 16, 20 and 24 °C only the race 3 isolates killed all the inoculated plants, thus confirming the existence of a low-temperature strain of the pathogen.

In potato resistance was expressed more frequently at high rather than low light intensities at 24 and 28 °C (Sequeira and Rowe 1969). Reduced light intensity and photoperiod reduced resistance in some tomato lines (Krauz and Thurston 1975).

Bacterial wilt is usually more serious under wet conditions and in moist soils (Abdullah et al. 1983). High soil moisture increases survival of the bacterium in the soil, increases infection, inoculum release from host plants and spread through the soil, and the rate of development after infection (Buddenhagen and Kelman 1964).

Latent Infections of Potato

It is now well established that there is a low temperature strain *P. solanacearum* (race 3, biovar II) which occurs at high altitude in the tropics (Ciampi and Sequeira 1980a; Katayama and Kimura 1984; Thurston 1963), and which has the potential for distribution on latently infected tubers to latitudes distant from the accepted geographical distribution of the disease. The pathogen was introduced into Sweden probably from an infection source in the Mediterranean, and was shown to be able to overwinter in *Solanum dulcamara,* a perennial weed host, at 59 °N (Olsson 1976). The same low temperature strain can also be distributed from seed production sites at high elevations in the tropics to uninfested soils at lower elevations as has been shown in Kenya (Nyangeri et al. 1984). Infected tubers are important sheltered sites for the long-term survival of *P. solanacearum* in the field; about 10% of tubers infected with race 3 grown in non-infested soil gave rise to diseased plants (Graham et al. 1979).

With the increasing demand for seed of resistant cultivars, latent infections in seed tubers enhance the possibility of long-distance movement of virulent strains of the pathogen (Ciampi and Sequeira 1980b). Both race 1 and race 3 isolates of the pathogen can multiply to high population levels in resistant clones of *Solanum phureja.* Although multiplication in the resistant plants was substantially less than in susceptible potato cultivar Russet Burbank, large numbers of bacteria reached the base of the stem of plants maintained at 28 °C for 20 days after stem inoculation; from there bacteria moved rapidly into roots and developing tubers. Strains of the pathogen differed in their ability to establish latent tuber infections. One resistant clone never yielded infected tubers, suggesting that there appear to be genetic factors which may be useful in breeding programs aimed at eliminating latent tuber infection (Ciampi and Sequeira 1980b).

Survival in Soil

There is some disagreement in the literature on the longevity of *P. solanacearum* strains in soil in the absence of protected sites (Graham and Lloyd 1979) and on resistance to desiccation. Different strains of race 2 causing Moko disease of banana differ markedly in survival in soil (Stover 1972). The survival of race 3 (biovar II) is less than that of race 1 (biovar III) (Granada and Sequeira 1983; Moffett

et al. 1983; Ramos 1976; Shamsuddin et al. 1979). Moffett et al. (1983) compared the survival of race 1 and race 3 in clay loam, sandy loam and clay soils at three pressure potentials of -0.003, -0.05 and -0.15 kPa.

Race 3 declined more rapidly in all soil types. The rate of population decline of both races was greater in clay loam than in sandy loam, and at all pressure potentials it was greater in clay loam and sandy loam than in clay. The longevity of both races was substantially greater in one study (Moffett et al. 1983) using an antibiotic-resistant mutant than in another relying upon a selective medium for the recovery of *P. solanacearum* from soil (Granada and Sequeira 1983).

It is frequently stated in the literature that *P. solanacearum* cannot survive dry conditions (French 1979, Sequeira 1962). In Sri Lanka areas with a hot dry season are those where bacterial wilt is least damaging (Seneviratne 1979). However, Moffett et al.(1983) showed that although initially numbers of the pathogen declined most in the driest soil treatment, at the end the driest soil contained generally higher numbers of viable pathogens than did the wetter treatments. The pathogen was not sensitive to dry soil conditions provided that the pressure potential remained constant (Moffett et al. 1983). Okabe (1971) found that *P. solanacearum* grew more actively in dry (15-20% water content) than in moist soil (40-50% water content).

Latent Weed Hosts

There is disagreement in the literature on the significance of weed species in increasing the inoculum potential of soils (Abdullah 1980a, b, 1983; Granada and Sequeira 1983; Moffett and Hayward 1980; Quimio and Chan 1979). Greenhouse studies showed that *P. solanacearum* declined in the rhizosphere of several resistant hosts and presumed non-hosts; when high populations were detected in the rhizosphere they were associated with localised or systemic infection of the roots of plants which did not show wilt symptoms (Granada and Sequeira 1983). However in other studies a positive rhizosphere effect was demonstrated in several weed species (Quimio and Chan 1979). The rate of population decline in the rhizospheres of weeds and mungbean was much more gradual than in non-rhizosphere soils, indicating that the rhizospheres of these plants are favourable for the survival of the pathogen in the soil. By contrast planting with rice or corn caused significant reductions in incidence of bacterial wilt compared with bare fallow soil or weed species, providing a rationale for the use of these crops in rotations in the Philippines (Quimio and Chan 1979). Common purslane (*Portulaca oleracea*) was the only weed species examined shown to be latently infected resulting in increased rhizosphere populations.

In another study the same weed species was the only one of many examined in tomato cropping land to show symptoms of wilt and from which the pathogen could be isolated from the root system (Moffett and Hayward 1980). *Solanum cinereum* has been shown to be a secondary weed host of race 3 (biovar II) in a cool, temperate region of Australia (Graham and Lloyd 1978), and *Solanum dulcamara* a latent overwintering host in Sweden (Olsson 1976).

Crop Rotations

There is an extensive literature on the use of crop rotations in the control of bacterial wilt with inconsistent results (Graham and Lloyd 1979; Granada and Sequeira 1983; Kelman 1953; Sequeira 1962). In Costa Rica in a naturally infested soil none of several crop rotations involving maize, sweet potatoes, kudzu, cowpeas or wilt-resistant tomatoes significantly reduced the inoculum potential of soils. The severity of the disease on potato was significantly reduced only in plots in which weeds had been eliminated by a contact herbicide (Jackson and Gonzalez 1981). Several new weed hosts of race 1 have been described in Malaysia (Abdullah 1980a, 1983) which can serve as a reservoir of inoculum for tomato and winged bean (Abdullah 1980b; 1982), thus making crop rotation ineffective in reducing the population in the soil.

Other Methods of Control

In various parts of the lowland tropics grafting on to resistant rootstocks has been used with success particularly with susceptible tomato cultivars (Kelman 1953). In Malaysia tomato scions have been grafted onto selected resistant eggplant (*Solanum melongena*) rootstocks and the incidence of bacterial wilt consistently reduced to below 10% in fields where ungrafted tomato cultivars suffer total loss (Lum and Wong 1976). Grafting of tomatoes on resistant eggplant has been used on a large scale in Thailand and Panama, whereas in Java *Solanum torvum* was found to be most promising as a rootstock (Thurston 1976).

In the long term, biological control is an approach which holds promise. Biological control of crown gall has been achieved using bacteriocin-producing strains of *Agrobacterium radiobacter* strain 84. Chen and Echandi (1984) have studied the effect of avirulent bacteriocin-producing strains of

P. solanacearum on the control of bacterial wilt of tobacco. Root systems of tobacco were dipped in suspensions of the avirulent strain and planted in infested soils. Although efficient control of bacterial wilt was achieved by this method, it was not an effective protectant of new root growth because the avirulent strain did not readily move over the root system. Only 2% of the total avirulent population was found on the new roots 20 days after transplantation. However, roots of plants grown in soil infested with the virulent strain were readily colonised by the pathogen. They concluded that although the avirulent bacteriocin-producing strains available at present were unsatisfactory for control of bacterial wilt, there was potential for selection of strains which were better adapted to movement and colonisation of the rhizosphere and rhizoplane of plants.

Conclusion

Bacterial wilt occurs on a wide variety of economic hosts, including several newly described tree and shrub hosts, resulting in severe losses throughout Asia and Australia, on solanaceous and other hosts, particularly under conditions of high temperature and rainfall. Susceptible crops cannot be grown in many infested soils. Some strains of *Pseudomonas solanacearum* affect a wide range of hosts, others show specialisation for such hosts as potato, banana, mulberry and ginger. Control of bacterial wilt in the lowland tropics presents a challenge of considerable scale and complexity, because of the many possible interactions between strains of the pathogen, hosts and environments. Progress depends upon a comprehensive knowledge which is often lacking; there are conflicting reports in the literature on soil survival and relationship of strains to possible weed hosts and other vegetation. There are instances at high elevation in the tropics, or at extremes of latitude, where bacterial wilt of potato caused by race 3 (biovar II) can be controlled by the use of resistant varieties or by eradication during fallowing or crop rotation, and subsequently by exclusion using certified disease-free planting material. However, in the lowland tropics where race 1 (biovars I, III and IV) predominates on diverse hosts, integrated control combining adequate levels of resistance with improved cultural practices presents the best prospect.

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Bacterial Wilt in Fiji

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FIJI lies in the South Pacific between the longitudes of 177 °E and 178 °W and extends from 15 ° to 22 °S. Of the total 194 000 km^2 the islands have 18 200 km^2 or 10% area as dry land. There are about 350 islands of which about 100 are inhabited. The islands are mostly of volcanic origin and although the land is mostly below 1300 m they are extremely rugged. The two major islands Viti Levu and Vanua Levu comprise 10 400 km^2 and 5500 km^2 respectively. Cultivation is largely restricted to the coastal areas and river valleys.

Fiji has diverse vegetation and climatic conditions and grows a wide range of crops for local consumption (e.g. root crops, bananas, vegetables) and export (e.g. sugar, ginger). However, inadequate supply of most commodities leads to large imports and a drain on Fiji's foreign reserves. Despite a concerted effort to bring about self sufficiency, imports (food imports in 1983 were 15.8% of all imports) still have to be made every year to fill the demand.

Amongst the many factors responsible for food imports, pests and disease play a significant role in reducing the potential yield and value of the existing crop plants. One of the most important diseases is bacterial wilt caused by *Pseudomonas solanacearum*. The organism causes serious yield losses in potato, tomato, eggplant and chilli. The losses are as high as 90% but generally range between 40 and 60%. In some areas, peanuts are also severely attacked but in general the loss is lower compared to other crops.

Bacterial wilt is considered to be responsible for the F$3 million imports of potato and F$230 000 of fresh tomato and tomato products during 1984 (Bureau of Statistics 1985), which in effect constituted a loss of F$3.23 million in foreign reserve (US $1 = F$1.10). If this disease was not present in Fiji, more foreign exchange could be derived through the growing of crops such as capsicum and eggplant for export.

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Occurrence and Distribution

In Fiji, the bacterial wilt organism was first reported on tomato (Parham 1934). Subsequently other reports were made on potato, tomato, eggplant, tobacco and peanut (Parham 1936; Johnston 1965; Graham 1971; Firman 1972). The disease is now also present on chilli and sweet pepper.

It is common and lethal in most vegetable growing areas in Fiji. The disease is most serious in areas which have a continuous cropping pattern, particularly of solanaceous crops. According to Erline (1976) and Harris (1976) the situation in Africa is further aggravated in areas with such weed hosts as *Aegeratum conyzoides, Commelina* sp., *Vigna* sp., *Portulaca oleracea, Solanum nigrum,* and *Datura stramonium*. A similar situation is likely in Fiji where these weeds are also present in the cropping areas.

Though the various races of *P. solanacearum* present in Fiji have not been identified as yet, it is quite likely that the strain affecting solanaceous crops belongs to race 1. The pathogen inhabits the soil and is usually a wound parasite, entering plants through wounds in the roots during transplanting or during cultivation. The foliage drops from ends of branches and collapse of the plant follows. The bacterial wilt is characterised by the slimy ooze exuding from the vascular system when cut across.

The major source of infection of bacterial wilt pathogen in Fiji is bacteria surviving from previous disease outbreaks. In the case of potatoes, contaminated seeds also pose a severe threat as sources of the pathogen.

Soil Type

Bacterial wilt occurs throughout the world in soils of great diversity (Kelman 1953). In Fiji, the disease has been observed both on light and heavy soils. Simmonds and Parham (1934) mentioned that the disease is most prevalent on black dust soils, but it has been noticed on all the arable soils used for vegetable production (Cumulic Haplustoll, Typic Hapludoll, Fluventic Hapludoll, Mollic Ustifluvent).

The disease is found in Sigatoka soils with a pH of 5.5–7.0. However, no investigation has been carried out to determine the soil pH favoured by the wilt pathogen.

Temperature

The mean maximum air temperature during the main vegetable season in Fiji ranges from 25.4 to 32.2 °C and the mean minimum temperature from 10.4 to 18.7 °C. Between November and April (i.e. during the wet, hot off-season), the mean maximum temperature falls between 27.1 and 33.8 °C while the mean minimum temperature ranges between 12.2 and 21.4 °C. Though it is known that temperature plays an important role in the geographic distribution of *P. solanacearum,* since it is rarely found in areas where the mean temperature falls below 10 °C in mid winter months (Kelman 1953), the temperatures in Fiji are generally not as low and the disease has been noted in highland areas (1000 m above sea level) as well as in the lowlands (10 m above sea level).

Rainfall

The rainfall in Fiji is seasonal with most of it falling between November and April (average 142–692 mm/month), while from May to October a somewhat drier period is experienced (average 49–254 mm/month). High soil moisture favours the development of the disease and this has been noticed particularly in areas where waterlogging has been experienced after a heavy downpour. A high percentage of wilt occurs in localised areas even in a well drained soil such as the Sigatoka Valley alluvial sandy loams (Mollic Ustifluvent). Though bacterial wilt is severe during the wet season, it has also caused high mortality in the dry and cool period during the year. Following infection, losses were more severe if plants were subjected to dry weather or other physiological stresses. It was also noticed that during the periods of high humidity (70–95% RH), the disease incidence was higher.

Control

Resistant varieties offer the best means of control. Resistant varieties which are performing reasonably well have been released for a number of crops in Fiji. These are Domini variety of potato, Sadabahar and Alton varieties of tomato, Chahat and Sigatoka Beauty eggplants and Red Fire and Hot Rod chillies.

There is a lower incidence of wilt in areas where a previous crop of rice or maize has been grown. On the other hand, it has also been noticed that a bare or pasture fallow followed by a crop of tomatoes has led to severe wilting in tomatoes.

Removal and burning of diseased material, avoidance of susceptible weed hosts, wilt-free seed potatoes, and clean bed for seed and seedling planting will ensure reduced disease incidence and prevent inoculum buildup.

Bacterial wilt symptoms have not appeared following flooding of fields in the Sigatoka Valley. Growing tomatoes as an intercrop with sugarcane has produced the lowest incidence of bacterial wilt and has potential for potato production under a similar cropping system.

Future Needs

Bacterial wilt is a serious disease limiting production of a number of crops in Fiji and though the organism has been known for some 50 years, little research has been carried out in Fiji. Emphasis needs to be directed towards identification of the various races and their host range, and edaphic conditions favouring the multiplication and spread of the pathogen. Only a thorough study of these various factors will ensure adequate and economic control measures.

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Bacterial Wilt in India

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BACTERIAL wilt disease caused by *Pseudomonas solanacearum* affects more than 200 species of plants belonging to 33 families with the largest number of hosts in Solanaceae. In India, the economically important hosts include potato, tomato, brinjal, chillies, groundnut, fennel, cumin, ajwain, cotton, jute, ginger and broad bean. In potato, in addition to wilt, rotting of vascular elements in tubers is a characteristic symptom from which the disease derives its names like brown-rot, bengle-blight, bangdi and ring diseases.

Races

Three races of the bacterium have been recognised based on host range studies (Buddenhagen and Kelman 1964). In India races 1 and 2 and biovars II, III and IV (Hayward 1976), are prevalent. Race 2 and biovar I have not yet been recorded in India. In the cool humid hilly areas only the race 3 and the biovar II are prevalent whereas on the plains and plateau areas race 1 and biovar III are predominant. Biovar IV is prevalent only at a few locations.

Distribution of the Disease

In plains and plateaus the disease is widespread throughout the west coast from Trivandrum in Kerala to Khera in Gujarat, in Deccan and central plateau of Karnataka, Western Maharashtra and Madhya Pradesh, in the eastern plains of Assam, West Bengal and Orissa and in Chhotanagpur plateau area of Bihar. The crop plants affected are potato, tomato, brinjal, chillies and wild *Datura metel*. *Pseudomonas solanacearum* has not been observed in banana and tobacco in India.

The disease is endemic in the northwestern hill country of Himachal Pradesh and Uttar Pradesh up to 2200 m; the eastern hills of Bengal, Meghalaya, Manipur and Tripura, Mahableshwar, and Panchgani hills in Maharashtra, Mahadeo hills in Madhya Pradesh and Nilgiris and Anamalai and Palni hills in Tamil Nadu. In hilly areas the disease affects only the potato.

The disease is not endemic in the northwestern high hills (about 2200 m) of Bundelkhand and northern Malva plateau and northern plains including Bihar, Uttar Pradesh, Delhi, Haryana, Punjab and Rajasthan and in the south in Andhra Pradesh.

Losses

Bacterial wilt is one of the most lethal diseases of plants. In potato, the disease causes premature wilting of the plants in the field, and rotting of the tubers in the field and also in transit and storage. In India, losses due to wilting in potato range from 30 to 70% in Kumaon and Nilgiri hills. In Bengal 62–65% loss of brinjal crop has been reported.

Symptoms

The disease affects both the above-ground and below-ground parts in all the hosts causing wilt in the former case and partial or complete rot in the latter. The characteristic symptoms on potato in India are described below.

In potato, leaves and succulent top portion of the plants infected prematurely, become flaccid and droop. In the beginning this symptom is seen only during clear sunny and warm days and the plants recover during evening, but later the wilting becomes permanent and is followed by drying of the leaves. Yellowing of lower leaves may occur before wilting in plants about to mature. It is not uncommon to find plants with only a single branch wilted. The bacterium invades the vascular system of stem and tuber and the vascular strands may turn brown in advanced stages of disease development depending on the strain involved. There are certain strains which neither produce brown pigment in culture nor cause browning of vascular strands.

The infected tubers show external symptoms only in advanced stages of infection, and these include greyish-brown discoloration of the flesh visible through the skin, and accumulation of whitish slimy bacterial exudate glued with soil on the eyes and stolon end. Such tubers when cut open show

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localised decay in the vascular ring. If left in the soil or at warm temperature after harvest, extensive tuber rot follows. All the tubers of a wilted plant may not necessarily be infected but occasional infected tubers may be found in plants showing no wilting.

In Simla, Meghalaya and Darjeeling hills and in the central plateau lenticel infection of potato tubers is observed occasionally. Watersoaked lesions develop at the site of lenticels. The lesions enlarge 0.5–1 cm in diameter and form 0.5 cm deep pits. The tuber skin remains intact but becomes shrivelled. When the tuber is cut, a dirty white bacterial mass is detected below the skin. The vascular tissues of such tubers are healthy. The symptoms are observed mostly in tubers from plants showing no wilt.

The bacterium may cause leaf spots which appear as circular to irregular greasy spots. The older spots are tan-coloured surrounded by darker margins or chlorotic haloes.

Disease Cycle

The probable sources of primary inoculum for the disease are: (i) infested soil, (ii) infected plant debris, (iii) infected seed, and (iv) weed hosts.

In India the pathogen perpetuates in soil in northern and southern hills (up to an altitude of 2200 m) in Deccan, central and eastern plateau and in eastern plains at least for 2 years in the absence of host plants and in many soil types having acidic (pH 3.5–6.5) and alkaline (pH 7.6–8.4) reaction. In the high hills (above 2200 m) and northwestern plains it does not perpetuate in soil.

The infected or surface-contaminated potato tubers carry the pathogen and may provide primary inoculum for disease development. In the hills bacterial wilt does not develop in the autumn potato crop (September-January) but the apparently healthy tubers carry the pathogen. The pathogen is not considered to be transmitted through true seed.

If the diseased potato tubers are planted, infection proceeds directly from tuber to the vascular system of the sprout. From soil, the invasion occurs through wounds on the roots created during cultural practices and at the point of emergence of secondary roots. Root knot and other nematodes aid the bacteria in root penetration. Following entry the organism migrates primarily into xylem vessels and later may invade phloem. The bacterium produces a polysaccharide which increases viscosity of vascular stream and gradually causes a mechanical occlusion of the vessel thereby hindering the uptake of water (Hussain and Kelman 1958). In young succulent stem the bacteria may invade parenchymatous tissues intercellularly causing lysogenous cavities in pith and cortex. Middle lamellae in vascular tissues are degraded. In potato tubers the organism may produce an internal decay and breakdown of bud tissues of the eyes. The secondary spread of the disease from plant to plant occurs through egress of large numbers of bacteria into soil from roots of infected plants. The irrigation and drainage water is believed to assist the rapid spread of the bacterium within a field and also from one field to another.

In general high temperatures (28–36 °C) and high soil moisture (50–100% water-holding capacity) favour rapid development of the disease. Infection can occur below 21 °C but wilt symptoms do not develop.

The disease occurs in most of the soil types prevalent in India. It is more widespread in the heavy and acidic (pH 3.5–6.5) soils compared to light and neutral or alkaline (pH 6.5–8.4) soils.

Varietal Resistance

The resistance reported in *S. phureja* in the United States has not proved useful under Indian conditions. Resistance has been detected recently in clones of *S. microdontum* at the Central Potato Research Institute and breeding for resistant varieties is in progress.

Control

The inoculum coming from potato tubers can be effectively checked by growing seed potatoes in brown, rot-free zones (viz. northwestern high hills (above 2200 m), northwestern plains and north central plains). Northwestern high hills are already well known for healthy potato seed production. In northwestern and north-central plains equally good seed potato can be produced by adopting seed plot techniques. The other areas where the disease is endemic and the pathogen perpetuates in soil should be declared non-seed areas.

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Bacterial Wilt in Indonesia

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SINCE bacterial wilt caused by *Pseudomonas solanacearum* was first reported from Indonesia in the 1890s, it has caused serious losses of many crops. Kelman (1953) considered the importance of bacterial wilt in Indonesia ranked second in the world after the United States, based on the number of available reports and economically important hosts affected by the disease. Intensive research has been done in Indonesia and various control measures have been developed to combat the disease, but it is still a major problem.

The first major period of research on bacterial wilt in Indonesia started with the first reported occurrence and continued until the early 1950s when most of the Dutch scientists left Indonesia. Most of the work on bacterial wilt was done during this period. The second period of research started in the early 1970s and continues to the present.

This paper presents a brief review of the history, distribution and economic importance of bacterial wilt, its current status and research progress on the disease in Indonesia.

History, Distribution and Economic Importance

Although we refer to the original papers which are mostly written in the Dutch language, most of the citations in this section are taken from the reviews of Kelman (1953) and Haryono Semangun (1971).

The history of bacterial wilt in Indonesia started with the first reports on the occurrence of the disease on tobacco made by Janse in 1892 and van Breda de Haan in 1897. However, losses due to the disease had occurred prior to that time, since Honing mentioned the case of a Deli tobacco grower who had lost an entire field due to 'slime disease' in 1864. The disease, however, did not cause great concern until 1880, when tobacco culture was intensified in northern Sumatera. Since then, it has been the major pathological problem in the Deli region, the previously most important tobacco-growing area of the island. Many tobacco growers suffered heavy losses, not only due to reduction in yield of tobacco, but also because it was frequently necessary to abandon large numbers of tobacco seedbeds. For example, in 1931 about 11% of tobacco seedbeds on 65 estates in the Deli region (North Sumatera) were discarded, and a number of years thereafter approximately 50 000 tobacco seedbeds were ploughed up annually because of bacterial wilt. The disease has also been identified as the major limiting factor on tobacco production in Java.

Bacterial wilt on peanut was first observed in Cirebon area by van Breda de Haan in 1905. The disease had become a major threat to peanut production in Java, when the crop was widely cultivated in the area to meet the lucrative peanut marketing trade in China. Since then the disease has been reported in several other localities in Indonesia, including Sumatera, Java, Bali, Lombok, and Sulawesi. In seasons favourable for disease development it was estimated that 25% of the total peanut crop was lost and heavy losses continued for several years. Bolhuis (1955) reported that after 1920 all soils in Java were strongly contaminated with the bacterium which caused crop reductions approaching 90%. He also stated that bacterial wilt will continue to be the main problem in peanut crops in this area. Attempts to develop resistant varieties culminated in the release of variety Schwarz 21 in 1925. After the release of Schwarz 21 and the consequent increase of area planted with this variety in Indonesia, losses due to bacterial wilt were markedly reduced. After 1945, the reaction of Schwarz 21 became heterogeneous. After 1951

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Gadjah variety, which has a more stable resistance and high-yielding ability, was recommended to replace Schwarz 21. This variety was the result of selection done by Bolhuis in 1936 (Sauer 1953).

Bacterial wilt of potato was first observed by van Hall (1912) in Karo Highland, North Sumatera and subsequently in other parts of Indonesia. On a susceptible potato variety yield losses can reach 40%. In 1924 losses of potato were so heavy that cultivation was discontinued in one locality. In 1926 heavy damage to the potato crop occurred in West Sumatera, and plantings of tomato also had to be abandoned due to bacterial wilt damage. In the early 1920s bacterial wilt caused only minor losses of potato in Java. Since then the disease has become a more important problem, and it ranks with late blight as the major potato diseases.

The wilt disease of tomato was first reported by Hunger in 1901. Heavy losses occurred in the west coast of Sumatera, and cultivation of tomatoes in certain areas of West Java (Jakarta and Bogor) had to be discontinued after 1924. The disease also causes serious damage to tomatoes in other parts of Java.

Current Status and Research Progress

Bacterial Wilt of Solanaceous Plants

Most of the recent work on bacterial wilt of potato, tomato and peppers was done by Hutagalung (1983, 1984). Field observations in Java from 1979 to 1983 indicated that bacterial wilt is still an important disease in vegetable production, particularly on potato, tomato and peppers. The range of disease incidence varied with the crops and localities, from 1 to 32% (Table 1). The disease incidence in upland areas is higher than in areas rotated with lowland (flooded) rice (Table 1).

Hutagalung (1984) estimated the economic yield losses on the three vegetable crops, based on data collected by Bos and Kartapradja (1977) where the disease intensity on potato, tomato and pepper was 18, 16 and 8% respectively, and the price of 1 kg of potatoes was Rp. 122. Tomato and potato suffered more losses (Rp. 360 000 and 485 000 respectively) than pepper (Rp. 146 000; 750 Rp = $1A).

Table 1. Incidence of bacterial wilt on potato, tomato and peppers in production centres in Java.[a]

Crop	Disease intensity (%)[b]	
	Upland	Lowland
Potato	4–32	4–12
Tomato	2–30	2–15
Tobacco	1–15	1–6

[a] Hutagalung (1984).
[b] Disease intensity is based on the percentage of wilted plants; Lowland = sawah, in rotation with lowland rice.

Following the success of potato cultivation in Jambegede, 335 m above sea level, the government plans to increase potato production and extend tomato cultivation in medium elevation areas between 500 and 750 m. There are 250 000–400 000 ha of land available for this program. Another important problem is the production of seed potato tubers free of bacterial wilt, since at least 5.5–6.6% of seed-potato tubers produced locally have been infected with the disease (Hutagalung 1984). A practical method needs to be developed to detect the bacterium on the tubers, so that selection or sorting of healthy seed potato tubers may be done prior to planting.

The presence of synergism between *P. solanacearum* and nematodes, particularly *Meloidogyne* sp. also has been studied (Hutagalung and Widjaya 1976). It has been shown that wilt intensity was increased with the increased number of nematodes in the field. Several nematicides also have been tested for their effectiveness against the nematodes, and at the same time their effectiveness in reducing the incidence of wilt. The effects of nematicides were not significant (Hutagalung 1984).

A study on a strain of the bacterial wilt of potato in East Java has been reported (Agus Yuniar and Tjuk Suwartiah Basuki 1983). Disease samples were collected from five different potato production areas in East Java, namely Batu, Pujon, Tosari, Gubuk Klakah and Sumberbrantas, all over 1600 m elevation. Ten of the isolates were identified as biovar III of *Pseudomonas solanacearum*. These isolates were capable of producing wilt symptoms on potato, tomato, pepper and eggplant, but not on tobacco. The bacterium also caused stunting on red pepper. Hutagalung (1984) considered that race 1 and race 3 of *P. solanacearum* were found in Indonesia.

Screening for resistance has been done for potato and tomato (Hutagalung 1984). Some potato varieties showed resistance to bacterial wilt, although most were susceptible. Most of the tomato varieties tested were susceptible to bacterial wilt although a few varieties, including the Indonesian varieties Ratna and Intan, were resistant.

Antibiotics for the control of bacterial wilt on tomato and potato have been used (Ati Setiawati 1977; Hutagalung 1984). Antibiotics, such as agrimycin and streptomycin, did not give a complete control of the disease, and their economic value was

questionable, particularly when they were applied to crops grown at low or medium altitudes (Machmud, unpublished data).

Bacterial wilt is still an important disease of tobacco. Recent reports mentioned that losses of tobacco due to bacterial wilt in the Deli region reached an average of 8.3% in 1983. In tobacco seedbeds the losses ranged from 8.3% in 1980 to 11.6% in 1981 (Anonymous 1980, 1981, 1983).

An attempt was made to control bacterial wilt on tobacco seedbeds by flooding the seedbeds for different periods of time, and the tobacco seeds were sown 1 month after drying the flooded seedbeds. It was shown that a 6-month flooding could reduce the incidence of bacterial wilt in the seedbeds from 44.7% in the artificially inoculated control plots, to practically 0%, in the plots flooded for 6 months (Zainal Abidin et al. 1983).

A *Pseudomonas* sp. causing wilt on *Solanum khasianum* has been isolated recently (Supriadi 1983). Based on the disease symptoms and physiological and biochemical characteristics of the bacterium, it is suggested that the disease is caused by *Pseudomonas solanacearum*. Different degrees of resistance to the disease were observed among cultivars of *S. khasianum*.

Bacterial Wilt of Cassava

Although bacterial wilt of cassava (*Manihot esculenta*) has been reported in Indonesia since 1922 (Palm 1922), there has been no significant report on the identification of the causal bacterium. In the early reports (Palm 1922; Schwarz 1926) *P. solanacearum* was inadequately described. More recently, Nishiyama et al. (1980) have described bacterial diseases on cassava in Indonesia. They reported that symptoms of bacterial wilt of cassava could be distinguished in three types: drooping of plants, severe defoliation, and die-back. The first two symptoms were usually accompanied with discoloration of the below-ground parts of the plant, which was not observed on the third type. Isolation from different types of symptoms has resulted in two distinct colony types of bacteria: the white fluidal and the white mucoid colonies. Following a detailed identification study they concluded that the white fluidal colonies commonly isolated from plants showing drooping and leaf defoliation were *P. solanacearum*, the bacterial wilt pathogen, while the white mucoid colonies isolated from plants showing die-back were *Xanthomonas campestris* pv. *manihotis* (Berthet and Bondar 1915) Dye 1978, causal agent of cassava bacterial blight.

Field surveys indicated that bacterial wilt caused by *P. solanacearum* occurred in Lampung and Java. Inoculation studies with inoculum from isolates collected from different localities showed that the *P. solanacearum* isolates causing wilt on cassava were either biovar III or IV.

An earlier study on bacterial wilt of cassava by Nakagawa (1978) showed that bacterial wilt is the major limiting factor on cassava production in the Lampung area. A survey in three cassava production companies in the Lampung area indicated that the high incidence of bacterial wilt of cassava related to the high incidence of the disease in weeds commonly found in the area (Nakagawa 1978). Six species of weeds (*Croton hirtus, Ageratum conyzoides, Spigelia anthelmia, Fagopyrum sagittatum, Sesbania exaltata)* and another unidentified species have been observed to serve as hosts for the bacterial wilt pathogen. *Croton hirtus* which is common in the area showed the highest incidence of bacterial wilt, in three areas, ranging from 2.6 to 20% with an average of 9.0% of samples collected being infected.

Cross inoculation of cassava with isolates from weed hosts produced wilt symptoms on cassava. The weeds may serve as the primary source of inoculum of the pathogen and a bridge for the disease during the off-season.

Field screening has also been done in the Lampung area to evaluate the resistance of cassava lines against bacterial wilt. The results indicated that cultivar Kuning was the most susceptible to bacterial wilt followed by SPP Pandesi and Genjah, while other lines and clones were resistant (Tahum, Keton Merah, SPP Kretek, Singkong Putih, W-528, Ketan Putih, Genjah Hitam, Baserat, No. 802 and No. 547). During the test, disease incidence on *Croton hirtus* growing in the neighbouring field was 8.8%.

Laboratory and field trials at Bogor, however, have not identified cassava lines resistant to bacterial wilt (Nunung H. A. Yahya, personal communication).

Bacterial Wilt of Peanut

There has been little research progress on bacterial wilt of peanut. Field surveys in 1983 from various parts of Southern Sumatera, Java, Bali and Sulawesi indicated that bacterial wilt was present in these areas. Intensity of the disease varied with localities.

Generally the disease is more severe in South Sumatera, West Java and South Sulawesi than other areas such as Central Java, East Java, Bali and Northern Sulawesi (Machmud, unpublished data).

The disease incidence ranged from 0.8 to 10.1% with an average of 3.5% regardless of the varieties and age of the plants. The low disease incidence in some areas was probably because most of the peanut varieties grown by farmers are Gadjah and other Indonesian improved varieties, such as Macan, Tupai, Pelanduk, Kidang and Anoa which have some resistance to the bacterium. A realistic estimate of recent yield losses is not available. In favourable conditions, however, the wilt incidence is very high and economic losses occur.

Bacterial Wilt of Ginger

In 1976 there was an outbreak of bacterial wilt on ginger (*Zingiber officinale*) in Bogor and in some other areas of West Java. However, there have been no studies of the disease in these crops. In the Bogor area, bacterial wilt has been a problem on ginger (Machmud, unpublished data). In 1984 another outbreak of bacterial wilt on ginger was recorded from Central and North Sulawesi (Johanna Paath, personal communication). More research is needed on this crop, since ginger is an important export commodity for Indonesia.

Sumatera Disease of Clove Trees

The name 'Sumatera Disease' was first used by Waller and Sitepu (1975) to describe a clove tree disease in West Sumatera characterised by leaf fall progressing from the upper to lower branches. It was often associated with sudden wilting, leading to tree death in 6–18 months and, internally, by the presence of discoloured streaks in the wood of roots, trunk and some branches. There have been reports of similar and recurrent disease outbreaks in western coastal regions of Sumatera dating back at least 60 years, which is generally referred to as 'mass decline' (*mati massal*) (Kalshoven 1936). Another disease of clove trees described by Toyib Hadiwidjaya (1956) under the name 'mati bujang', for which a physiological cause was proposed, is probably the same as Sumatera disease. Recently the disease was identified as important on clove plantations. During the period 1968–76 more than 70% of clove plants at their productive stage, covering over 10 000 ha in West Sumatera, were wiped out by the disease (Ariful Asman 1983).

Extensive research has been done to identify the causal agents of the disease. Some reports mention that *P. solanacearum* was always associated with the disease (Hidir Sastraatmadja 1971, 1973; Ati Wasiati, et al. 1977; Hunt 1983). Isolations of the possible microorganisms involved in Sumatera disease included *Phytophthora cinnamomi* Rand (Djafaruddin et al. 1979), *Pseudomonas solanacearum* (Hidir Sastraatmadja 1971; Hunt 1983) and Rickettsia-like bacterium (RLB) (Hunt 1983).

Inoculation experiments have been done on young clove plants, and the results indicated that these pathogens were able to reproduce similar symptoms to the Sumatera disease individually. Hunt (1983), however, was doubtful about the direct involvement of *P. solanacearum* in Sumatera disease and believed that RLB is the main causal pathogen. Thus the causal agent(s) of Sumatera disease are not clear.

Some research has also been done on controlling the Sumatera disease, particularly the possible use of antibiotics (Ariful Asman, 1983). However, there have been no conclusive reports. Preliminary study of the disease resistance indicated no difference in susceptibility of the clove cultivars tested (Hunt 1983). Therefore, more work is needed to solve the problem.

Banana Diseases

Two banana diseases caused by bacteria have been reported in Indonesia, 'penyakit darah' or 'blood disease' and 'penyakit pembuluh Jawa' or 'Java vascular disease'. The blood disease was first observed in 1910 on Selayar island, southern Sulawesi and known to cause serious damage to bananas (Haryono Semangoen 1971). Prior to 1912, approximately 900 000 bunches of banana were sent to Makasar (now Ujungpandang) annually. This was completely stopped due to the disease. The disease has caused not only loss of income, but also losses in the main staple food of the people. In 1923 the disease spread over South Sulawesi. Gaumann (1921, cited in Haryono Semangoen 1971) reported that the disease was caused by a bacterium called *Pseudomonas celebensis*. However, this identification has not been confirmed nor have isolates from banana in Indonesia been compared with isolates from banana elsewhere (race 2), or with *P. solanacearum* isolates from other crops in Indonesia.

The Java vascular disease was first observed by Gaumann in 1915 (Haryono Semangoen 1971). Nearly all banana plants of cultivar Java were infected with the disease, although 90% of them did not show visible symptoms. Therefore, at that time the disease was not considered as economically important. Gaumann (1921, as cited by Haryono Semangoen 1971) proved that the disease was caused by a bacterium which he named *Pseudomonas musae* Gaumann. Regarding the two diseases, Wardlaw (cited by Haryono Semangoen 1971) believed that the blood disease was identical to Moko disease

caused by *Pseudomonas solanacearum* and the Java vascular disease was identical to Panama disease caused by *Fusarium oxysporum* var. *cubense*, or a mixed infection of Panama disease with Moko disease. However, there has been no further research on these diseases. Recent surveys in Java and Sulawesi indicated that both diseases are present in all of the areas, although the intensity was not high (Iswoto et al. 1983). There is a need to clarify the causal agents of these banana diseases of unknown etiology in Indonesia.

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Bacterial Wilt in Papua New Guinea

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PAPUA New Guinea comprises the eastern half of the island of New Guinea together with the adjacent islands of New Britain, New Ireland, North Solomons and Manus off the northeastern coast (Fig. 1). The western part of the island, Irian Jaya, a province of Indonesia, is separated from Papua New Guinea by a poorly defined border some 800 km in length.

Papua New Guinea occupies an area of approximately 462 840 km^2 and lies between latitudes 1 and 12 °S and longitudes 141 and 160 °E. It is characterised by mountainous terrain with peaks rising to 4600 m separated by fertile, highly populated valleys lying between 1500 and 1800 m. Mean daily maximum and minimum temperatures of the highlands regions are 25 and 13 °C, respectively, with occasional frosts above 2000 m. Mean annual rainfall varies with locality between 2000 and 3000 m with little seasonality.

The lowland areas of the country comprise extensive coastal plains, alluvial plains and swamplands. Mean maximum and minimum temperatures are close to 32 and 21 °C, respectively, and mean annual rainfall is in excess of 2400 mm with the exception of a small area around the capital, Port Moresby, which receives approximately 1000 mm of rainfall per year.

The indigenous population is just 3 million people speaking over 700 different languages. There is also a sizeable expatriate population numbering between 80 000 and 90 000. The major subsistence crops are sweet potato (*Ipomoea batatas*), taro (*Colocasia esculenta* and *Xanthosoma* sp.), bananas (*Musa* spp.) yam (*Dioscorea* spp.), sago (*Metroxylon* spp.), aibika (*Abelmoschus manihot*), and cassava (*Manihot esculenta*).

Introduced food crops, currently of minor importance, are generally grown to service the preferences of the expatriate community. They include potato (*Solanum tuberosum)*, tomato (*Lycopersicon esculenta*), capsicum (*Capsicum annum*), cabbage (*Brassica oleracea* var. *capitata*), cauliflower (*B. oleracea* var. *botrytis*), chinese cabbage (*Brassica chinensis*), eggplant (*Solanum melongena*), peanut (*Arachis hypogoea*), carrot (*Daucus carota*), onion (*Allium cepa*) as well as a number of other species of minor importance.

Status of Bacterial Wilt

Bacterial wilt caused by *Pseudomonas solanacearum* is of minor economic importance in the context of Papua New Guinea agriculture. This disease is limited to a few introduced crops which include English potato, tomato, eggplant and capsicum. Moko disease of banana caused by race 2 of *P. solanacearum* (French 1979), and considered the most important disease of banana in some Central and South American countries (Stover 1972), is not recorded in PNG. Moko disease does occur in the southern Philippines (Rillo 1979) and must be considered a quarantine hazard for Papua New Guinea. Similarly the strain of *P. solanacearum* causing bacterial wilt of peanut is absent from the country and the disease has not yet been recorded on tobacco, a minor cash crop, or ginger, a spice often grown in subsistence gardens (Tomlinson 1985).

Race 1 of the pathogen (Buddenhagen and Kelman 1964) occurs principally as biovar III (Hayward 1964, 1976) in lowland areas on tomato, eggplant and capsicum. Biovar IV has been recorded infrequently on tomato (Shaw 1984). In the highland areas race 3/biovar II of the pathogen predominates in English potato although race 1/biovar IV has also been recorded on this host.

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Biochemical Studies

Isolation of *P. solanacearum* from diseased plants was carried out by the method described by Tomlinson (1985). Biovar determinations were made by methods used by Hayward (1964). Antibiotic sensitivity was assessed using Oxoid An-Ident discs (Oxoid Ltd, Basingstoke Hamps., UK) and acid production from an additional range of carbohydrates and carbohydrate derivatives was assessed using API CH diagnostic Kits (API System A.S., La Balme-les-Grottes 38390, Montalieu-Vercien France). Results of biochemical tests are presented in Table 1. Representative cultures of biovar II, III and IV strains were sent to the Commonwealth Mycological Institute, Kew, Surrey, U.K. (CMI) for confirmation, and samples of most isolates have been stored under refrigeration as a suspension in sterile distilled water.

The biochemical studies show that Papua New Guinea isolates of *P. solanacearum* conform well to the biovar classification (Hayward 1964). Some isolates of biovar II, however, consistently differ from the type strain in producing acid from trehalose. Differences in fluidity and rate of mutation to the rough colony form have been observed in cultures of biovar II. The presence of a number of strains within biovar II is suspected but not yet confirmed.

Bacterial Wilt in Tomato

In the tropics tomatoes have traditionally been grown at higher elevations or in the lowlands only in the cooler months. Successful production has been hampered in the lowlands by poor fruit set caused by high temperatures, heavy rainfall and the prevalence of leaf spotting diseases and bacterial wilt (Villareal 1980). Nevertheless, good markets exist for tomatoes in lowland urban centres. Such demand in Papua New Guinea led, in 1974, to the introduction of over 300 tomato varieties for screening and selection at Laloki Plant Quarantine and Horticultural Research Station situated 25 km from Port Moresby (Bull et al. 1985). Initially, introductions came from Australia, but because of the susceptibility of these cultivars to bacterial wilt (Blackburn 1976), further introductions were made from the Philippines, Taiwan, New Zealand, South Africa, Thailand and Nigeria. The primary aim of the program was to identify cultivars with heat tolerance and bacterial wilt resistance which could also produce good yields of acceptable quality under Papua New Guinea conditions. Indeterminate types in which the main stem continues growing all season were found to be difficult to manage and more prone to disease than determinate cultivars in which the stem terminates in a blossom cluster. More emphasis was therefore placed on the latter cultivars in the screening program.

Bacterial wilt is by far the most consistently serious disease of tomato in lowland Papua New Guinea. Other diseases, particularly early blight (*Alternaria solani*), leaf moulds (*Pseudocercospora*), (*Fulvia fulvum*), collar rot (*Sclerotium rolfsii*), root knot nematode (*Meloidogyne arenaria, M. incognita, M. javanica*) and bacterial spot (*Xanthomonas campestris* pv *vesicatoria*) are damaging in some areas. Spraying with fungicides to control fungal leaf diseases (Mancozeb 0.5 kg/ha) is necessary. Additional minor diseases also occur from time to time (Shaw 1984).

Table 1. Physiological properties of some isolates of *P. solanacearum* from Papua New Guinea.

Property	Biovar II	Biovar III	Biovar IV
Acid production from:			
Glucose, fructose, galactose, glycerol, inositol, saccharose	+	+	+
Mannitol, sorbitol, dulcitol, trehalose	−	+	+
Lactose, maltose, cellobiose, D-fucose	+	−	−
Raffinose, salicin, palatinose, erythritol, melezitose, rhamnose, sorbose, turanose	−	−	−
Hydrolysis of aesculin, starch	−	−	−
Nitrate to nitrite, oxidase	+	+	+
Gas from nitrate	−	+	+
Arginine dihydrolase, indole	−	−	−
Antibiotic susceptibility			
Kanamycin (1000 μg)	S	S	S
Erythromycin (60 μg)	S	S	S
Rifampicin (15 μg)	S	S	S
Colistin (10 μg)	R	R	R
Penicillin (2 units)	R	R	R
Vancomycin (5 μg)	R	R	R

* Reading taken after 4 days at 30°C.

Biovar III is the most common biovar affecting tomato in the Port Moresby region, being the only one isolated since 1980. Biovar IV has, however, previously been identified in tomatoes in this locality (Shaw 1984). In addition, only biovar III has been recorded from other lowland sites since 1980, although the limited number of samples examined precludes any definite conclusions on biovar distribution in tomato.

Early in the screening program Blackburn (1976) selected one line NG 7536 originating from the Asian Vegetable Research and Development Centre (AVRDC), Taiwan (CV C555F5-A) as superior to other varieties. This variety is heat-tolerant, large-fruited and resistant to bacterial wilt although fruit quality is not good. In more recent trials the variety Vuavina has performed as well as NG 7536 and produces better fruit than the latter variety.

Details on bacterial wilt susceptibility of a number of tomato varieties screened at Laloki between 1974 and 1982 are given in Table 2. The currently recommended varieties for lowlands Papua New Guinea where bacterial wilt is a problem are NG 7536 and Vuavina. In localities where bacterial wilt does not occur the commercial variety Walter Improved is recommended for its high yields and good quality fruit.

Bacterial Wilt in Potato

Potato, like many other crops in Papua New Guinea, is free from some of the more serious diseases which limit production in other parts of the world (Shaw 1984). Thus late blight *(Phytophthora infestans)* and black wart *(Synchytrium endobioticum)* and cyst nematode *(Globodera rostochiarsis* and *G. pallida)* are absent from the country. Early blight *(Alternaria solani)* is a serious disease under some circumstances, particularly when plants are in a weakened condition caused by nutritional or environmental stress, or disease.

Tuber rots caused by *Erwinia chrysanthemi* and *E. caratova* pv. *caratovora* can cause serious localised losses to crops and stem rot caused by the latter bacterium can pose as great a threat to potato production under wet conditions as bacterial wilt. Nevertheless, bacterial wilt caused by *Pseudomonas solanacearum* is the most widespread and consistently destructive disease of potato in highlands Papua New Guinea, in both subsistence gardens where it is intercropped with sweet potato and taro, and in commercial fields.

Approximately 3000 t of ware potato are produced annually in Papua New Guinea. This competes directly with a similar tonnage imported directly from Australia. The major market for pota-

Table 2. Percent bacterial wilt (angular transformation) recorded in some tomato and potato varieties screened in Papua New Guinea.

Variety	Source	Percent	Wilt (SE)
Tomato			
NG 7536*	AVRDC	1.9	1.6 (19)
Vuavina	Yates Research New Zealand	4.2	1.4 (9)
Scorpio	D.P.I. Queensland	13.7	8.1 (4)
Floradale	Commercial Line	57.0	9.6 (5)
Walter Improved	"	61.9	6.8 (8)
Floradel	"	72.0	(1)
Tropic	"	89.0	(1)
Grosse Lisse	"	47.0	11.7 (3)
Red Cloud	"	63.0	24.6 (3)
Sutana	"	90.0	(1)
Rodade L52/299	S. Africa	16.5	(1)
Potato			
Sequoia*	Plant Research Institute Burnley, Victoria	23.5	5.5 (3)
Ontario	"	2.6	1.8 (3)
Snowchip	"	27.2	15.8 (3)
800224 BR-63.75	CIP	3.0	1.7 (2)
800226 BR-69.84	CIP	28.0	39.6 (2)
800926 MS-35.22	CIP	16.7	9.8 (2)
800929 MS-35.4	CIP	48.9	3.1 (2)
800934 MS-35.9	CIP	35.3	9.7 (2)
800935 MS-10.2	CIP	31.0	9.1 (2)
800942 BR-63.15	CIP	30.5	30.1 (2)

* Current recommended varieties; no. of trials in parentheses.

toes in Papua New Guinea is Port Moresby. No road links exist between Port Moresby and the main potato-producing areas. Thus locally produced potatoes destined for Port Moresby have either to be trucked to Lae and then transported by boat, or flown directly to the capital. Locally produced potatoes are often more expensive than imports from Australia. Thus a serious economic constraint on potato production exists even in the absence of increased production costs caused by bacterial wilt.

To overcome the disease problem two strategies are being simultaneously followed. Firstly, certified seed potatoes (var. Sequoia) are imported directly from Victoria, Australia, by the Department of Primary Industry. These undergo a preliminary multiplication at Taluma, a remote area in Enga Province which until recently has been free from bacterial wilt. Part of the progeny is distributed locally and part is further multiplied at the High Altitude Research Station at Tambul (2240 m) which is also isolated from the main potato-growing area, and progeny distributed to local growers as disease-free seed.

Secondly, plant material tested and found to be resistant to bacterial wilt elsewhere, is imported and tested under local conditions. So far preliminary trials have been conducted using nine potato clones from the International Potato Centre (CIP), Peru and two from the Victorian Plant Research Institute, Burnley, Australia. Details of these clones together with an indication of wilt resistance are presented in Table 2. Trials were carried out using fully randomised blocks with Sequoia as a control. Between six and ten replicates were used per block with ten plants per plot. Tubers were planted in wilt-infested ground and inoculated 6–8 weeks after planting by pouring 50 ml of a concentrated suspension (10^8 cells/ml) of *P. solanacearum* on the area of emergence of each plant. Estimation of wilt development was made at regular intervals. Complications due to the development of stem rots *(E. caratovora* pv. *caratovora)* commonly occurred and wilted plants were often also affected by the fungus *Leptosphaerulina trifolii* a common weak pathogen of potato in the Papua New Guinea Highlands. Preliminary indications suggest that Ontario (PRI,

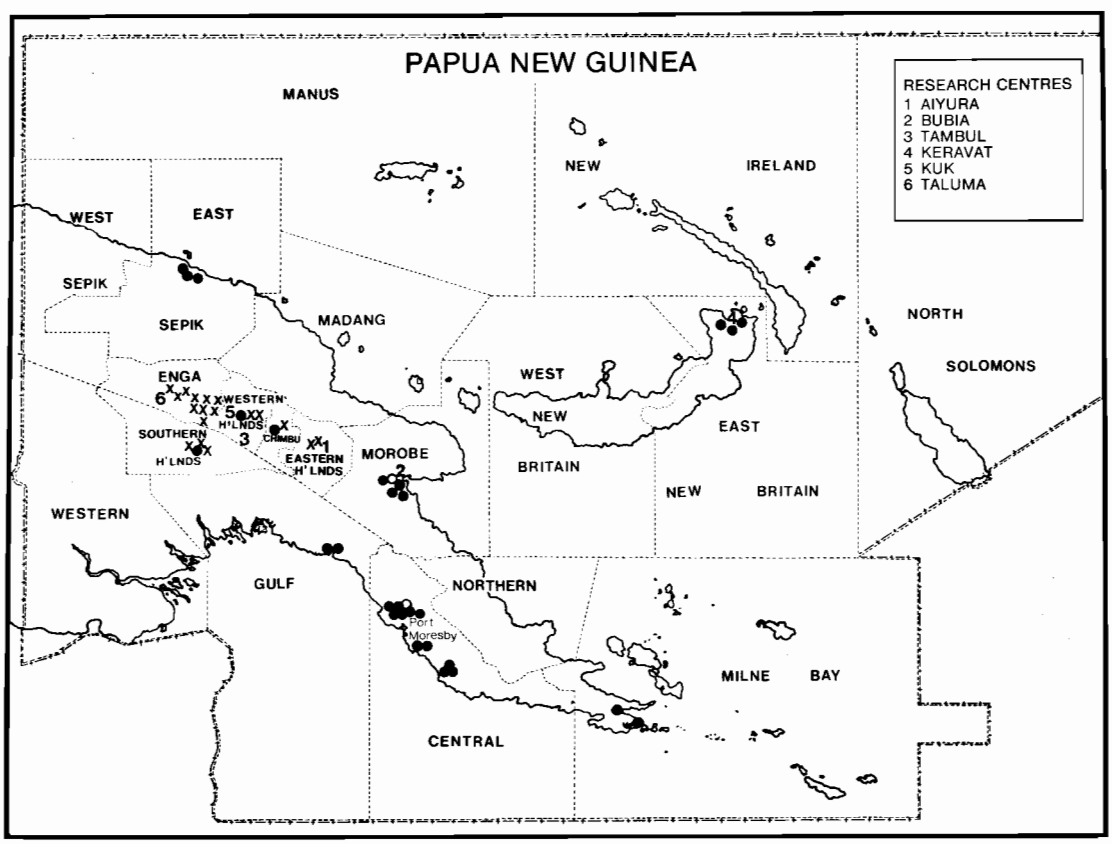

Fig. 1. Distribution of biovars of *P. solanacearum* in Papua New Guinea: X—biovar 2; ●—biovar; ○—biovar 4.

Burnley, Australia) and CIP clone 800224 show some resistance to local strains of *P. solanacearum*.

Distribution, Maintenance and Spread

No information exists on the maintenance of *P. solanacearum* in lowland soils although work in Australia has identified a total of 35 hosts for the bacterium (Pegg and Moffett 1971; Hayward 1975; Pitkethley 1981). Many of these hosts are common weeds, some of which also occur in Papua New Guinea. It is likely, therefore, that such weeds could provide a reservoir of infection in the absence of crop plants, particularly for race 1 of the pathogen.

The disease in potato is maintained and spread principally through infected tubers. Absence of restrictions on the import of seed (both certified and non-certified) from Australia may also be a factor in disease spread. Biovar II of *P. solanacearum* has been isolated from the weed *Solanum nigrum* commonly found in potato fields. This plant along with other as yet unidentified weed carriers may play a part in the maintenance of the disease in Highlands soils, despite the reputed limited host range of this biovar (French 1979). No work has yet been done in Papua New Guinea on the survival of *P. solanacearum* in soil in the absence of potential hosts or in the rhizosphere of weeds, from which the bacterium had previously been recorded in Australia. Current information on the distribution of biovars of *P. solanacearum* in Papua New Guinea, based on over 200 determinations, is given in Fig. 1.

Future Work

Multiplication of selected potato clones with potential resistance to bacterial wilt is being achieved using stem cuttings taken from mother plants grown from certified tubers. These are planted in a mixture of sand and vermiculite in seed trays at a density of 100 cuttings/tray. Cuttings are planted in the field after 2-3 weeks when root development has become established. It is intended to expose such cuttings to locally isolated strains of *P. solanacearum* in an attempt to identify promising wilt-resistant clones quickly. Such clones can then be further evaluated under field conditions. The problems of bacterial concentration to be used and the timing and method of inoculation have yet to be decided.

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Bacterial Wilt in the People's Republic of China

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BACTERIAL wilt due to *Pseudomonas solanacearum* is a widespread and severe disease of many crops of economic importance in China. Since the late 1970s the infected area has greatly expanded and some new host plants have been observed (Hayward 1964; Lai et al. 1982; Liang and Chen 1982; Liao et al. 1982; Ren and Fang 1981; Sichuan Institute of Forestry (1977)). For instance, the sweet potato wilt (or blast disease) of sweet potato (*Ipomoea batatas*) was reported only in a few counties of Guandong and Guangxi provinces before the 1960s but now it can be observed everywhere in Fujian and Zhejiang provinces (He and Hua 1985; Zhen and Fan 1982). Bacterial wilt of potato (*Solanum tuberosum*) was first reported in the late 1970s and was limited to small areas of Hunan and Sichuan provinces, but now it has spread to 40° north latitude into Changbei County of Hebei province (He and Hua 1985). In China, this disease attacks not only solanaceous and other herbaceous plants, but also some woody plants, including horsetail beefwood (*Casuarina equisetifolia*), common olive (*Olea europaea*) and mulberry (*Morus alba*) and causes substantial losses (Guangdong Academy 1975; He and Hua 1983; Lai et al. 1982; Liang and Chen 1982; Ren and Fang 1981; Sichuan Institute of Forestry 1977). In recent years this pathogen has been reported to affect some cultivated medicinal plants, such as *Pogostemon cablin* and *Kaempferia galanta* and an important forage crop *Symphytum* sp. (Liao et al. 1982; Wen and Zheng 1984). These hosts have not been reported from other countries.

Despite the wide host range of *P. solanacearum*, marked differences have been observed in its pathogenicity to various hosts and in epidemic characters in different regions. For instance, bacterial wilt is reported to severely attack tobacco in Fuqing County of Guizhou, but is not observed in Luiliang County of Yunnan, where tobacco is grown surrounded by severely infected potato fields. Bacterial wilt of sesame (*Sesamum indicum*) has been reported to be an important disease in Jiangxi provinces, but is not observed in Huongang County of Hubei Province, where farmers usually use sesame as a substitution crop in wilted peanut fields (He and Hua 1983). Moreover, according to Zhen and Fan (1962) the strains from sweet potato were not virulent to peanut and the strains from peanut were not virulent to sweet potato. It is evident there may exist different strains with host specialisation within this species of pathogen, and some of them may be indigenous to local regions.

Some work on strains of *P. solanacearum* was conducted in China in recent years (Liang and Chen 1982; Liao et al. 1982; Ren et al. 1981), but a limited number of cultures were used in these studies. Sometimes only a few isolates from one or two host plants in one province or region were examined. On the basis of previous work in 1981 (He et al. 1983) I have made some comparative studies on a larger number of isolates of this bacterium collected from a wide range of host plants and localities, with the purpose of increasing our knowledge about strain composition and distribution in China.

Materials and Methods

Cultures of 110 isolates of *P. solanacearum* were collected from different host plants and locations in China (Table 1). One isolate of *P. solanacearum* was obtained from the bacterial collection maintained at the Department of Plant Pathology, University of Wisconsin (Table 1). The virulent, wild colony type of *P. solanacearum* cultures was selected on 2, 3, 5-tetrazolium chloride medium (TZC) and used in a series of studies (Kelman 1954).

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Table 1. Sources of isolates of *Pseudomonas solanacearum* used in tests.

Isolate and host	Plant	Location
M2–M9	(8 isolates of Mulberry (*Morus alba*))	Guangzhou; Guangdong; Guangzhou, Guandong; Guangzhou, Guangdong; Guangzhou, Guangdong; Shunde, Guangdong; Guangzhou, Guangdong; Huazhou, Guangdong; Shunde, Guangdong;
PO1–PO46	(46 isolates of Potato (*Solanum tuberosum*))	Taian, Shandong; Fuzhou, Fujian; Luliang, Yunnan; Xinhuang, Hunan; Nanhai, Guangdong; Sichuan; Fuzhou, Fujian, Fuzhou, Fujian; Nanhai, Guangdong; Guangzhou, Guangdong; Wuhuan, Hubei; Nanhai, Guangdong; Fuzhou, Fujian, Xinhuang, Hunan; Beijing; Beijing; Beijing; Beijing; Huaihua, Hunan; Huaihua, Hunan; Huaihua, Hunan; Huaihua, Hunan; Huaihua, Hunan; Suining, Hunan; Suining, Hunan; Xinhuang, Hunan; Xinhuang, Hunan; Xinhuang, Hunan; Xinhuang, Hunan; Changsha, Hunan; Shimen, Hunan; Shimen, Hunan; Longshan, Hunan; Longshan, Hunan; Emei, Sichuan; Emei, Sichuan; Pengxian, Sichuan; Pengxian, Sichuan; Pengxian, Sichuan; Beijing; Hunan; Beijing; Enshi, Hubei; Bashang, Hebei;
B1–B6	(6 isolates of Sweet potato (*Ipomoea batatas*))	Fuqing, Fujian; Fengting, Fujian; Shaoan, Fujian; Shaoan, Fujian; Wuping, Fujian; Fuding, Fujian;
TM1–TM8	Tomato (*Lycopersicum esculentum*)	Guangzhou, Guangdong; Sanmenjian, Guandxi; Quanzhou, Fujian; Xiamen, Fujian; Fuzhou, Fujian; Wuhuan, Hubei; Nanhai, Guangdong; Chengdu, Sichuan;
PE1–PE2	Pepper (*Capsicum annum*)	Sanmenjiang, Guangxi; Quanzhou, Fujian;
E1–E2	Eggplant (*Solanum melongena*)	Fujian; Wuhuan, Hubei;
U1	*Urtica nivea*	Tiantai, Zhejiang;
Z1–Z5	Ginger (*Zingiber officinale*)	Zaozhuang, Shandong; Zaozhuang, Shandong;Fujian; Chengdu, Sichuan; Sishui, Shangdong;
C1–C4	Horsetail beefwood (*Casuarina equisetifolia*)	Gungzhou, Guangdong; Dianbai, Guangdong; Guangzhou, Guangdong; Dianbai, Guangdong;
S1	Sesame (*Sesamum indicum*)	Luzhai, Guangxi;
TB2–TB3	Tobacco (*Nicotiana tabacum*)	Fuqing, Guizhou; Xinhui, Guangdong;
01–03	Common olive (*Olea europeae*)	Wuhuan, Hubei; Nanning, Guangxi; Sanmenjiang, Guangxi;
P1–P22	Peanut (*Arachis hypogaea*)	Hongan, Hubei; Hongan, Hubei; Hongan, Hubei; Hongan, Hubei; Guangzhou, Guangdong; Nanning, Guangxi; Sanmenjiang, Guangxi; Nanqung, Guangxi; Baihe, Guangxi; Nanning, Guangxi; Jingxi, Guangxi; Huanjiang, Guangxi; Quanzhou, Guangxi; Quanzhou, Fujian; Longxi, Fujian; Shaoan, Fujian; Shaoan, Fujian; Linyi, Shandong; Hongan, Hubei; Gguangzhou Guangdong; Macheng, Hubei;
SN1	Black nightshade (*Solanum nigrum*)	Baihe, Fujian;
82	Potato	(Collection at Dept. of Plant Pathology of University of Wisconsin).

Table 2. Pathogenicity of 38 Isolates of *P. solanacearum* to 13 host plants and classification of them.[a]

Isolate	Pathogenicity on													Race
	Black nightshade	Eggplant	Tomato	Potato	Heart-shaped tobacco	Pepper	Common tobacco	Sesame	Peanut	*Urtica nivea*	Sweet potato	Ginger	Mulberry	
03	H[b]	H	H	H	H	M	M	M	M	O	O	O	O	1
Po7	H	H	H	H	H	H	L	M	M	O	O	O	O	1
Tb2	H	H	H	H	H	H	H	L	L	O	O	O	O	1
P7	H	H	H	H	H	M	M	L	H	O	O	O	O	1
C4	H	H	H	H			M	L	H	O	O	O	O	1
Tm4	H	H	H	H	M	H	L	L	L	O	O	O	O	1
Tm6	H	H	H	H			L		L	O	O	O	O	1
E2	H	H	H	H	H		L	M	O	O	O	O	O	1
Po1	H	H	H	H	H	H	L	L	O	O	O	O	L	1
C2	H	H	H	H	H	H	O	L	H	O	O	O	O	1
C3	H	H	H	H	M	M	O	M	L	O	O	O	O	1
C1	H	H	H	H	M	M	O	L	L	O	O	O	O	1
Pe2	H	H	H	H	H	H	M	O	M	L	O	O	O	1
E1	H	H	M	H	H	M	O	O	H	O	O	O	O	1
Tm1	H	H	H	H	H	M	O	O	H	O	O	O	O	1
02	H	H	H	H			O	O	M	O	O	O	O	1
01	H	M	M	M	H	M	O	O	L	O	O	O	O	1
P16	H	H	H		H	M	O	O	H	O	O	O	O	1
Pe1	H	H	H	H	H	H	O	O	O	O	O	O	O	1
P1	H	H	M	H	M	L	O	O	H	O	O	O	O	1
P6	H	H	H	H	M	O	L	O	H	O	O	O	O	1
P19	H	M	M		M	O	O	O	H	O	O	O	O	1
P20	H	H	H	H			O	O	H	O	O	O	O	1
P9	H	H	H	H	M	O	O	L	H	O	O	O	O	1
S1	H	H	H	H	H	M	L	H	O	O	O	O	O	1
Tm2	M	M	M	M	L	L	O	O	O	O	O	O	O	ND
Sn1	M	L-M	M	M	L	O	O	O	O	O	O	O	O	ND
B2	H	H	H	M	M	M	O	O	O	O	H	O	O	1
B4	H	H	M	H	M		O	O	O	O	H	O	O	1
Z2	H	H	M	H	H	M	L	O	O	O	O	H	O	1
Z3	H	H	H	H	M	H	L	L	L	O	O	H	O	1
Bn1	H	H	H	H	H	M	L	M	O	H	O	O	O	1
Tm7	M	M	M	H		L-M	O	O	O	O	O	O	O	3
Po3	M	M	M	H	M	L	O	O	O	O	O	O	O	3
Po2	M	L-M	L	H	L	O	O	O	O	O	O	O	O	3
Po10	M	L	M	M		O	O	O	O	O	O	O	O	3
M4	O	O-L	O	O		O	O	O	O	O	O	O	M	4
M8	O-L	L	L	L		L	O	O	O	O	O	O	H	4

[a] Results based on average disease indices of more than 5 plants 15–21 days after inoculation.
[b] H = high (4.1–5.0), M = moderate (2.6–4.0), L = low (1.0–2.5) O = none (1.0), ND = not determined.

Test Plants

Plants used in cross inoculation tests were tomato (*Lycopersicum esculentum*), eggplant (*Solanum melongena*), potato (*Solanum tuberosum*), pepper (*Capsicum annum*), heart-shaped tobacco (*Nicotiana glutinosa*), common tobacco (*N. tabacum*), black nightshade (*Solanum nigrum*), peanut (*Arachis hypogaea*), sesame (*Sesamum indicum*), ginger (*Zingiber officinale*), sweet potato (*Ipomea batatas*), mulberry (*Morus alba*), and *Urtica nivea*.

Inoculation Methods

The stem puncture technique described by Winstead and Kelman (1952) was used in most tests. Lum and Kelman's (1981) micropipette titration method also was adopted for some experiments. Ginger was inoculated by inserting a cotton ball immersed in bacterial suspension into the base of the stem. The details about inoculation procedures were similar to descriptions of He et al. (1983) and French and Sequeira (1970).

Physiological Tests

To examine oxidation of carbohydrates, the methods described by Hayward (1964) were used. Determination of optimum growth, temperature and reaction of litmus milk were conducted according to the Institute of Microbiology's (1978) Manual.

Studies Using Monoclonal Antibodies

Glycoprotein extracts from nine isolates (TM1, TM2, PO3, 82, P14, 03, B2, M5 and Z2) were used as antigens for immunising BALB/C mice. The myeloma cell line SP1/0-AG 14 was fused with spleen cells of immunised mice (Cai et al. 1985; Kohler and Milstein 1975; Yao et al. 1985). The ELISA technique was adopted to screen hybridoma cells, which were then cloned by continuous dilutions (Yao et al. 1985).

Results

Pathogenicity

Results of cross inoculation of 38 isolates on 13 different host plants demonstrated that all isolates except those from mulberry could highly or moderately infect black nightshade, eggplant, tomato, potato and heart-shaped tobacco (Table 2). On common tobacco, pepper, peanut and sesame a number of isolates appeared to be avirulent. To sweet potato, ginger and *Urtica nivea* only those isolates obtained from the respective host plants were virulent. Two mulberry isolates (M4, M8) showed high virulence on mulberry, low virulence

Table 3. Pathogenicity of isolation from potato to potato and tomato.

Isolate	Pathogenicity on		Isolate	Pathogenicity on	
	Potato	Tomato		Potato	Tomato
Po2	H	L	Po23	M	L
Po3	H	M	Po29	H	H
Po4	M	L	Po30	H	L
Po5	M	L	Po31	M	M
Po6	M	L	Po32	H	H
Po8	H	M	Po33	M	H
Po9	M	L	Po34	M	L
Po10	M	L	Po35	H	M
Po11	M	L	Po36	M	L
Po12	M	L	Po38	M	M
Po15	M	L	Po39	H	L
Po16	M	L	Po40	M	L
Po17	H	L	Po41	H	H
Po18	H	L	Po42	H	M
Po19	M	L	Po43	H	M
Po20	H	L	Po45	H	–
Po21	M	L	Po7[a]	M	M
Po22	H	M	Po13[a]	M	L
Po23	M	L	Po14[b]	M	L
Po24	H	H	Po37[a]	M	L
Po25	M	L	CK	O	O
Po26	H	H			
Po27	M	L			

[a]Belonging to biotype 3
[b]Belonging to biotype 4
H, high, M, medium, and L, low pathogenicity.

Table 4. Oxidation of carbohydrates by isolates of *P. solanacearum*.

Host	Isolate	Lactose	Maltose	Cellobiose	Dulcitol	Mannitol	Sorbitol	Biovar
Tomato	Tm7	+[a]	+	+	–	–	–	II
	Tm2.Tm3.Tm4.Tm5.Tm8.	+	+	+	+	+	+	III
	Tm1.Tm6.	–	–	–	+	+	+	IV
Pepper	Pe1.Pe2.	+	+	+	+	+	+	III
Eggplant	E1.E2.	–	–	–	+	+	+	IV
Potato	Po2.Po3.Po4.Po5.Po6.Po8.Po9.Po11. Po12.Po15.Po16.Po17.Po18.Po19.Po20. Po21.Po22.Po23.Po24.Po25.Po26.Po27 Po28.Po29.Po30.Po31.Po32.Po33.Po34. Po35.Po36.Po38.Po39.Po40.Po41.Po42. Po43.Po44.Po45.Po46.	+	+	+	–	–	–	II
Potato	Po10.	+	+	+	+	–	–	II-I
	Po1.Po7.Po13.Po37.	+	+	+	+	+	+	III
	Po14.	–	–	–	+	+	+	IV
Tobacco	Tb2.Tb3.	+	+	+	+	+	+	III
Black nightshade	Sn1.	+	+	+	+	+	+	III
Peanut	P7.P11.P12.P13.P16.P17.	+	+	+	+	+	+	
	P1.P2.P3.P4.P6.P8.P9.P10.P14.P15. P18.P19.P20.P21.P22.	–	–	–	+	+	+	IV
Ginger	Z3.	+	+	+	+	+	+	III
	Z1.Z2.Z4.Z5.	–	–	–	+	+	+	IV
Sesame	S1.	+	+	+	+	+	+	III
Sweet potato	B1.B2.B3.B4.B5.B6.	–	–	–	+	+	+	IV
Urtica nivea	Bn1.	+	+	+	+	+	+	III
Horsetail beefwood	C1.C3.C4.	+	+	+	+	+	+	III
	C2.	–	–	–	+	+	+	IV
Common olive	O2.O3.	+	+	+	+	+	+	III
	O1.	–	–	–	+	+	+	IV
Mulberry	M6.	+	+	+	+	+	+	III
	M2.M3.M4.M7.M5.M8.M9.	+	+	+	–	+	–	V

[a] + positive and – negative reaction.

on eggplant, black nightshade and other solanaceous plants. All non-mulberry isolates failed to cause wilting symptoms on mulberry. All of the 43 isolates from potato were somewhat virulent on potato, as well as on tomato (Table 3).

Oxidation of Carbohydrates

Marked differences amongst isolates were observed in oxidation abilities to three disaccharides and three hexose alcohols (Table 4). In accordance with the Hayward and He et al. schemes the 110 isolates tested were identified as biovars II, III, IV and V; 46 isolates from potato belonged to biovar II (and II-1), biovar III and biovar IV, respectively. The potato isolate P010 differed from others in its ability to oxidise three disaccharides and dulcitol and in its lack of ability to oxidise mannitol and sorbitol. Since this isolate was similar to those of biovar II in all other characteristics, it was considered as a subtype in biovar II and, therefore, designated biovar II-1. Seven of eight mulberry isolates were classified as biovar V and one of them was identified as biovar III, which was characterised by intensively oxidising three disaccharides and three hexose alcohols.

Optimum Growth Temperature

Results of experiments to determine the optimum growth temperature of 13 isolates representing different races and biovars indicated that isolates of race 3, an isolate in race 1 (SN1) and some isolates from mulberry (M4, M8) grew better at 27 °C than at 37 °C (Table 5). Two sweet potato isolates grew as well at 27 °C as at 37 °C. Both nutrient broth cultures and glucose agar cultures showed similar results.

Reactions of Litmus Milk

In litmus milk without cream, all 44 isolates tested caused an alkaline reaction, changing the medium from light purple to a blue colour. After incubation for 17 days in litmus milk containing cream, isolates of biovars III and II caused a red acidic reaction with coagulation in the lower layer of the medium. Under the same conditions biovar IV isolates caused an alkaline reaction, and biovar V isolates caused a slightly acidic to neutral reaction at the first stage of growth and an alkaline reaction after 4 weeks. Isolates P010 and TM2, which caused indefinite reactions were an exception to these results (Table 6).

Monoclonal Antibodies

Six hybrid myeloma cell lines specific to strains P14, 03, TM1, TM2, B2, M5 and B2 were successfully obtained (Table 7).

Discussion

Races and Pathovars

Based upon reactions of inoculated plants 81 isolates from China could be classified as race 1, 3 and 4 (Buddenhagen et al. 1962; Hua et al. 1984). Race 1 included 37 isolates from a wide range of hosts. Most race 1 isolates showed high virulence on solanaceous plants examined, especially on eggplant and black nightshade. Isolates of race 3 were mainly from potato, and showed moderate or low virulence on eggplant, black nightshade, heart-shaped tobacco and other plants. Race 4 consisted of mulberry isolates (M4, M8), which weakly infected the solanaceous plants tested, including eggplant and black nightshade.

Table 5. Growth of some isolates of *P. solanacearum* at 37 and 27 °C.

Isolate	Biovar	Turbidity of broth medium		Length of yellowed glucose agar slant after 5 days incubation (mm)		Race
		37 °C	27 °C	37.5 °C (+0.5)	27 °C	
Po2	II	++$^{+}$	++$^{+}$	21.7	28	3
Po3	II	++$^{+}$	+++	25	30.7	3
Po10	II-I	+	++	0	19.7	3
Tm7	II	+	++	25.7	32.7	3
Po7	III	++++	+++	29.3	25.3	1
Sn1	III	+	+++	0	23.3	1
Bn1	III	++++	+++	25	22.3	1
Tm1	IV	++++	+++	25	22	1
E2	IV	+++	++$^{+}$	29.3	22.3	1
B2	IV	+++	+++	23	23	1
B4	IV	+++	++$^{+}$	25.3	24	1
M4	V	++$^{+}$	+++	19.7	23.7	4
M8	V	+	++	22	24	4

Table 6. Effect of isolates of *P. solanacearum* on litmus milk containing cream.

Biovar	Number of isolates	Upper layer				Lower Layer			
		1st day	10th day	17th day	30th day	1st day	10th day	17th day	30th day
II	3	white	red	red	red	purple	red	red coagulation	reddish pale
III	18	white	red	red	red	purple	red	red coagulation	reddish pale
IV	17	white	red, reddish purple	purple	purple	purple	light blue, purple	dark blue	dark blue
V	4	white	red, reddish purple	red	purple	purple	purple	light blue	dark blue
II-I	1 (Po10)	white	red	reddish purple	purple	purple	purple	light blue	purple
III	1 (Tm2)	white	red	red	red	purple	light blue	purple	purplish pale

Table 7. Hybridoma cell lines secreting monoclonal antibodies specific to strains of *P. solanacearum*.

Hybridoma cell line	Strain of *P. solanacearum*								
	Tm1	Tm2	O3	Po3	B2	M5	82	Z2	P14
BW1	+++[a]	-	+	-	-	-	-	-	+++
BW3	+++	-	+++	-	+++	+	-	+	+
BW4	++	++	++	-	++	++	-	++	++
BW5	+	-	+	-	+++	-	-	+	+
BW8	+	+	+++	-	+++	-	-	-	+++
BW15	-	+++	-	-	+++	-	-	+++	+++

[a] +++ strong reaction, ++ moderate reaction, + weak reaction, - no reaction.

Marked differences in pathogenicity were observed amongst isolates of race 1. Some showed specificity to their host of origin. For instance, only those isolates that were isolated from sweet potato, ginger and *Urtica nivea* appeared virulent to those respective hosts. We probably have sufficient reasons to designate these strains as pathovars, namely pv. *batatae,* pv. *zingiberi* and pv. *urtici,* respectively. For the same reasons we propose to designate the mulberry isolates as pv. *mori* and the low-temperature isolates from potato and tomato as pv. *potatus*. As for those isolates that infect solanaceous plants the designation of pathovar *solanacearum* is acceptable. This pathovar may be divided into pathotypes based on difference in the pathogenicity of a large collection of isolates to common tobacco, peanut, sesame, common olive and other plants.

Biovars

The biovars occurring in China were shown to be biotypes II, III, IV and V, as has been demonstrated before (He et al. 1983). It was unexpected that no biotype I strains would be observed among the numerous isolates tested.

The majority of potato isolates belonged to biovar II and were characterised by growing at a relatively low optimum temperature (27 °C). It should be noted that this strain is not only spreading in the areas of higher altitude in Southern China, but also is moving over the 40° north latitude line into the main potato-growing regions of Northern China.

Many isolates in our tests belonged to biovar III, which was found on 12 of 14 natural host plants we investigated. This biovar was reported to exist on eggplant, *Symphytum* sp. and *Pogostemon cablin* (Ren et al. 1981; Wen and Zheng 1984). It appears this is a predominant biovar in China. Biotype IV was reported only on ginger and tomato in other countries, but it has already been observed on peanut, potato, eggplant, horsetail beefwood, common olive, sweet potato and *Kaempferia galanga* as well as ginger and tomato in China. Obviously, this is an economically important strain in China.

Seven of eight mulberry isolates belonged to biovar V. These isolates showed a delayed oxidation of carbohydrides. A marked acidic reaction was observed but only after about 2 weeks of incubation, or sometimes even later. Ren et al. (1981) reported that the mulberry strains they tested belonged to biovar I, although an acidic reaction of medium containing mannitol was observed after 4 weeks of incubation. In a recent report, Lai et al. (1982) indicated that mulberry strains showed acid production from maltose and mannitol. One of eight mulberry isolates in the present study showed the ability to oxidise three disaccharides and three hexose alcohols, and was classified as biovar III. This suggests that there might exist other biovars within the mulberry strains other than biovar V.

The isolates of *P. solanacearum* tested showed clear differences while growing in litmus milk containing cream. Most isolates of biovars III and II produced stronger acid reactions than isolates of biovars IV and V, which, in fact, caused an alkaline reaction of cream milk medium (in the lower layer) after 17 days incubation. It is well known that biovar III is able to strongly oxidise lactose, which is found in high concentrations in milk. This biotype is a common strain widely distributed in most Asian countries (Buddenhagen and Kelman 1964). This may be the reason why E. F. Smith designated the strain of *P. solanacearum* in Asia as var. *asiaticum* (Sequeira and Kelman 1976).

Antigenicity of Glycoprotein

Glycoprotein extracts of bacterial cell walls were used as antigens in monoclonal antibody studies. Results demonstrated that glycoproteins of various strains of *P. solanacearum* consisted of both common and specific antigenic determinants, which

will be the basis of future studies of the differences among *P. solanacearum* strains.

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Bacterial Wilt in the Philippines

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BACTERIAL wilt caused by *Pseudomonas solanacearum* is one of the most important plant diseases in the Philippines, particularly on solanaceous vegetable crops grown at low and medium elevations. The pathogen is widespread in the country, has many host plants and has different biovars/pathovars that may cause the breakdown of some resistant varieties. This disease was first reported in the Philippines by Reinking in 1918 on tomato, eggplant, pepper, white potato and tobacco. Welles and Roldan (1922) observed bacterial wilt to be serious on solanaceous crops in Southern Luzon and estimated annual losses of 10–20%. In 1923, Welles and Roldan reported *Chrysanthemum coronarium* as another host of the causal bacterium. Agati (1949) estimated that losses due to bacterial wilt on tomato, eggplant, pepper and tobacco in Central Luzon to be 80–95, 30–80, 10–40 and 10–50% respectively. Zehr (1969) reported that bacterial wilt was widespread in the Philippines. He estimated that the average crop losses in tomato, eggplant, pepper and tobacco were 15, 10, 10 and 2–5% respectively. He also reported *Solanum jamaicense,* a common weed in newly cleared areas, to be a resistant host of *P. solanacearum*.

The other plant species reported as hosts of *P. solanacearum* in the Philippines are abaca, banana, peanut, ginger, castor bean, cowpea, bush lima bean, bitter gourd, winged bean, marigold and teak (Zehr 1969b; Quimio 1976; Valdez and Almodovar 1980).

Strain Identification

Earlier studies have shown that there is considerable variability amongst Philippines isolates of *P. solanacearum* in both host range and biochemical characteristics, although the two are not necessarily correlated (Tabei and Quimio 1978; Zehr 1970a, b).

Bacterial wilt-infected vegetable crops were collected from 23 provinces in the Philippines and pure culture isolates of *P. solanacearum* were made using Kelman's (1954) triphenyl tetrazolium chloride medium. A total of 264 tomato isolates from 47 sampling areas in 19 provinces, 111 white potato isolates from 13 sampling areas in 8 provinces, 41 eggplant isolates from 22 sampling areas in 13 provinces and 26 pepper isolates from 12 sampling areas in 8 provinces were obtained. In addition, 48 isolates of *P. solanacearum* were obtained from wilted non-solanaceous vegetable crops, namely, bitter gourd, squash, snapbean, winged bean, string bean, Chinese cabbage, pechay and fennel grown at the Central Experiment Station in College, Laguna. The provinces are: Albay, Benguet, Bukidnon, Bulacan, Camarines Sur, Capiz, Cavite, Iloilo, Isabela, Laguna, Leyte, Misamis Oriental, Negros Occidental, Negros Oriental, Nueva Ecija, Pangasinan, Quezon, South Cotabato, Parlac, Camarines Norte, Nueva Vizcaya and Pampanga.

All the isolates were tested for pathogenicity on 4-week-old susceptible tomato cv. Yellow Plum seedlings grown in sterilised soil in seedboxes. All the pathogenic isolates were maintained in sterile distilled water stock cultures in screw cap test tubes.

The pathogenic isolates were classified into biovars using Hayward's (1964, 1978) biochemical methods. The results showed that of the 264 tomato isolates, 1.8% belonged to biovar I, 89.7% were of biovar III and 8.3% were of biovar IV. Of all 111 white potato isolates, 5.4% were of biovar II, 70.3% were of biovar III and 24.3% were of biovar IV. Of the 41 eggplant isolates 4.9% were of biovar I, 80.5% were of biovar III and 14.6% were of biovar IV. Of the 26 pepper isolates, 65.4% were of

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biovar III and 34.6% were of biovar IV. Of the 48 isolates from eight non-solanaceous vegetable crops one isolate from bitter gourd was of biovar I, two isolates each from bitter gourd and string beans were of biovar II, the single isolate from fennel was of biovar IV, and 42 of the 48 isolates were of biovar IV including 75% of the isolates from bitter gourd and 100% each of the isolates from squash, snapbean and winged bean.

The percentage occurrence of the different biovars based on a total of 490 isolates from various vegetable crops was 1.6, 2.0, 83.0, and 13.3 for biovars I, II, III and IV, respectively (Table 1). It is interesting to note that none of the 264 tomato isolates, 41 eggplant isolates and 26 pepper isolates were of biovar II in spite of the fact that many were obtained from high elevations in Baguio, La Trinidad, and Buguias in Benguet province and also in the medium elevations of Sankanan, Bukidnon and Claveria, Misamis Oriental. Similarly, none of the white potato isolates from the medium and high elevation areas were of biovar II but were predominantly of biovar III, with some biovar IV. The six biovar II isolates from white potato were obtained from low elevation areas in Piat, Cagayan and in the Central Experiment Station, UPLB, College, Laguna. Two isolates each from bitter gourd and string beans were also classified as biovar II.

On the distribution of the different biovar isolates in the Luzon, Visayas and Mindanao and Min-

Table 1. Biovar classification of *P. solanacearum* isolates from naturally infected vegetable crops in 23 provinces of the Philippines.

Host crop	No. of isolates	Occurrence (%) and No. of isolates in biovar[a]			
		I	II	III	IV
Tomato	264	1.9 (5)	0	89.8 (237)	8.3 (22)
White potato	111	0	5.4 (6)	70.3 (78)	24.3 (27)
Eggplant	41	4.9 (2)	0	80.5 (33)	14.6 (6)
Pepper	26	0	0	65.4 (17)	34.6 (9)
Bitter gourd	12	8.3 (1)	16.7 (2)	75 (9)	0
Snapbean	12	0	0	100 (12)	0
Squash	11	0	0	100 (11)	0
Winged bean	8	0	0	100 (8)	0
String bean	2	0	100 (2)	0	0
Chinese cabbage	1	0	0	100 (1)	0
Pechay	1	0	0	100 (1)	0
Fennel	1	0	0	0	100 (1)
Occurrence of biovars (%)		1.6	2.0	83.1	13.3

[a] Figures in parentheses refer to the number of isolates.

Table 2. Distribution of biovar isolates of *Pseudomonas solanacearum* in Luzon, Visayas and Mindanao.

Host crop	Number of biovar isolates in							
	Luzon				Visayas		Mindanao	
	BI	BII	BIII	BIV	BIII	BIV	BIII	BIV
Tomato	5	0	135	8	16	6	87	7
White potato	0	6	67	17	4	1	7	9
Eggplant	2	0	29	5	1	1	3	0
Pepper	0	0	11	6	0	0	6	3
Bitter gourd	1	2	9	0	0	0	0	0
Snapbean	0	0	12	0	0	0	0	0
Squash	0	0	11	0	0	0	0	0
Winged bean	0	0	8	0	0	0	0	0
String bean	0	2	0	0	0	0	0	0
Chinese cabbage	0	0	1	0	0	0	0	0
Pechay	0	0	1	0	0	0	0	0
Fennel	0	0	0	1	0	0	0	0
Occurrence (%)	1.63	2.04	57.96	7.55	4.29	1.63	21.02	3.88

danao island groups, biovars I and II were found only in Luzon while biovars III and IV occurred in all the three island groups (Table 2). Based on a total of 408 biovar III isolates 69.6, 5.1 and 25.2% occurred in Luzon, Visayas and Mindanao, respectively. Similarly, 57.8, 12.5 and 29.6% of the 64 biovar IV isolates occurred in the three island groups, respectively.

The results of studies on the occurrence of different biovar isolates in one field lot are shown in Table 3. Included in this table are the sampling batches where two or more biovars were found in one sampling area only. Out of the 13 sampling areas grown to five different vegetable crops, namely, tomato, white potato, eggplant, pepper and bitter gourd, only one showed the occurrence of biovars I, III and IV. This lot, located in the UPLB Central Experiment Station, College, Laguna, has been used as a bacterial wilt screening nursery for about 10 years and the disease was observed to be more severe than elsewhere. Based on the 222 isolates from the 13 sampling areas with two or more biovars, biovar III gave the highest percentage occurrence with 72.9 compared with 24.3, 1.8 and 0.9 for biovars IV, I and II, respectively.

Virulence Tests of Tomato Biovar Isolates of *Pseudomonas solanacearum*

The results of tests to compare the virulence of tomato isolates belonging to biovars I, III and IV are given in Table 4. Five isolates each of biovars I and IV and 10 isolates of biovar III were inoculated on two resistant tomato selections, 507 and 508, and the susceptible cultivar, Yellow Plum. Based on the average percentage of plants that survived in the resistant test plants, the biovar I isolates appeared slightly more virulent than those of biovar III and the biovar IV isolates were the least virulent. However, among the isolates in each biovar group, the most virulent were T122, T23 and T6 of biovars I, III and IV, respectively. Isolate T23 of biovar III was slightly more virulent than T122 of biovar I with average percent survival in the resistant cultivars of 46.8 and 50.5, respectively, as against 68.6 in the isolate T6 of biovar IV. These three isolates together with other virulent ones that were identified later are used in varietal screening for resistance.

Resistant Varieties

The development of varieties of tomato and other vegetable crops resistant to bacterial wilt is

Table 3. Occurrence of different biovars of *Pseudomonas solanacearum* in one field lot.

Host crop/locality	No. of isolates in biovar			
	I	II	III	IV
Tomato				
RRC, UPLB, College, Laguna	3	0	19	2
Lot A2, CES, College, Laguna	1	0	19	0
Claveria, Mis. Oriental (BPI)	0	0	46	3
Claveria, Mis. Oriental (Bliss II)	0	0	25	3
Sankanan, Bukidnon	0	0	10	2
La Granja, Negros Occidental	0	0	6	4
White potato				
BPI, Baguio City	0	0	15	16
Claveria, Mis. Oriental (BPI)	0	0	6	8
Lot B8, CES, College, Laguna	0	0	3	7
Eggplant				
Lot 2, CES, College, Laguna	0	0	4	1
Pepper				
Mamala, Sariaya, Quezon	0	0	3	6
Claveria, Mis. Oriental (BPI)	0	0	2	2
Bitter gourd				
Lot A10, CES, College, Laguna	0	2	4	0
Total	4	2	162	54
Occurrence (%)	1.8	0.9	72.9	24.3

being attempted at the College of Agriculture, U.P. at Los Baños. These efforts were accelerated with the creation of the Institute of Plant Breeding (IPB) that provided better facilities and a team approach between the plant breeders and plant pathologists.

On the search for resistant germplasm, numerous problems have been met, among them the need to standardise the screening techniques, the availability and maintenance of germplasm materials, and the occurrence of different biovars of the pathogen. The last factor appears to cause the breakdown of resistance in the tomato VC-11 series. To date, we have screened for resistance 1133 accessions of tomatoes, 233 tuber families and 402 clones of white potatoes, 267 accessions of eggplant, 72 of hot pepper, 34 of sweet pepper, 19 of bitter gourd and 15 of snapbean.

The scalpel-leaf-clip method was used in screening for resistance. This involves the use of bacterial suspension prepared from 24-hour-old cultures of virulent isolates representing different biovars of *P. solanacearum* that have been standardised separately at 50% transmittance in the Spectronic 20 spectrophotometer, and 4-week-old seedlings of test plants, such as tomatoes, grown in baked soil in seedboxes or pots placed on greenhouse benches. In inoculation, a No. 3 scalpel was dipped into the bacterial suspension and the lowest two true leaves were cut at the axil. Disease reaction was taken after 3, 7 and 14 days from inoculation using the scale: Resistant (R) = 80–100% survival, Moderately resistant (MR) = 60–79% survival, Moderately susceptible (MS) = 30–59% survival, and Susceptible (S) = 10–29% survival. Yellow Plum tomato, Red Pontiac white potato, Dumaguete Long Purple eggplant and California Wonder sweet pepper were used as susceptible checks in each screening batch of these crops. Accession 508 of tomato was used as the resistant control. At least two screening trials of 50–100 plants per accession per trial were con-

Table 4. Virulence test of tomato biovar isolates of *Pseudomonas solanacearum* on resistant (R) and susceptible (S) tomato selections[a].

Biovar/isolate no.	Survival of selection (%)		
	507 (R)	508 (R)	Yellow plum (S)
Biovar I			
Isolate T5	97.9	100.0	2.0
T116	63.8	68.0	0
T122[b]	51.0 (50.5)	50.0	0
T123	44.9	85.4	0
T139	74.0	77.6	0
Mean	66.3 (71.2)	76.2	0.4
Biovar III			
Isolate T7	79.6	77.7	0
T11	72.3	66.0	0
T12	79.6	92.0	0
T20	62.0	49.0	0
T22	98.0	66.7	0
T23[b]	44.7 (46.8)	48.9	0
T24	90.0	74.0	2.0
T26	68.0	100.0	0
T31	68.0	96.0	0
T43	84.0	86.0	0
Mean	74.6 (75.1)	75.6	0.2
Biovar IV			
Isolate T2	78.0	78.0	0
T3	100.0	67.4	2.0
T6[b]	74.0 (68.6)	63.3	0
T41	93.6	65.3	4.0
T46	96.0	96.0	0
Mean	88.3 (81.1)	74.0	1.2

[a] Mean of 2 trials.

[b] Selected virulent isolates.

() Mean of resistant selections.

Table 5. Tomato lines resistant to virulent biovar isolates of *Pseudomonas solanacearum* (greenhouse tests; means of at least two trials with 50–100 plants each per trial).

Description	Survival to biovar isolate (%)		
	BI (T116)	BIII (T21)	BIV (T6)
A. Resistant to 3 biovar isolates			
Tm-L46-N-12-6	100	100	96
Tm-L114-45-8-N-1 V.P.	98	100	96
Tm-L114-42-1-N-4 P. early H.T.	100	100	88
Tm-L114-42-1-N-7 P. early H.T.	94	94	98
Tm-L114-48-3-N-N H.T.	97	94	95
Tm-L114-41-N H.T. oblate	100	96	89
Tm-L114-48-5-N-N H.T.	98	87	100
Tm-L114-42-1-N-6 P. early H.T.	100	94	88
Tm-L114-N-8	97	91	94
Tm-L114-N-28	97	91	94
Tm-L114-35-4-N-3	98	93	91
Tm-L114-N-2 V.P. round	95	97	89
Tm-L114-56-16	100	98	83
Tm-L114-N-4	100	98	82
Tm-L114-48-3-N-1 H.T.	98	94	88
Tm-L114-41-N-11 H.T. oblate	90	97	89
Tm-L114-42-1-N P. early H.T.	100	81	94
Tm-L114-N-20	89	93	93
Tm-L114-48-5-N-3 H.T.	93	95	86
Tm-L114-48-3-N-7 H.T.	98	91	85
Tm-L114-56-7	89	94	90
Tm-L114-41-N H.T.	86	95	89
Tm-L114-42-1-N-N P. early H.T.	84	90	95
Tm-L114-41-N-6 H.T. oblate	81	95	93
Tm-L114-56-9	82	82	100
Tm-L114-48-6	88	90	85
Tm-L114-48-N H.T. spreading	80	98	85
Tm-L114-N-29 round	89	86	83
Tm-L114-N-10	86	87	84
CL 5915-553 D4-3-0	100	100	100
CL 5915-396 D3-2-1-0	95	100	100
CL 5915-93 D4-1-0	93	88	100
CL 5915-206 D4-2-4-0	95	88	97
CL 5915-39 D4-1-4-0	88	93	96
CL 5915-214 D4-1-3-0	87	94	87
CL 1131-0-0-13-0-6	88	85	89
F7-80-457-9 oblate	96	97	87
F7-80-378-N	95	97	83
F7-80-465-5	94	90	90
F7-80-465-10 Pink	87	94	89
F7-80-516-10	87	88	82
B. Resistant to 2 biovar isolates			
Tm-L114-48-N-8 H.T.	73	100	94
Tm-L114-42-N H.T. P. early	79	96	91
Tm-L46-N-12-N early H.T.	77	94	83
Tm-L114-42-1-N-9 P. early H.T.	67	98	89
Tm-L114-48-5-N-6 H.T.	84	90	79
Tm-L114-42-1-N-11 P. early H.T.	67	88	92
Tm L216-N-15-2-N crres	86	96	56
Tm L114-42-1-N-1 P. early	63	88	85
Tm-L114-48-5-N-9 H.T.	67	85	82
Tm-L114-48-3-N H.T.	64	96	83
Tm-L114-42-1-N P. early	61	90	86
Tm-L216-M-15-2-N-UG	60	85	80
Tm-L114-35-4-N	66	95	88
Tm-L114-56-20	58	87	94
CL 5915-223 D3-3-1-0	89	76	97

Table 5. Continued.

Description	Survival to biovar isolate (%)		
	BI (T116)	BIII (T21)	BIV (T6)
CL 5915-206 D4-2-1-0	100	80	76
CL 5915-223 D3-2-0	84	77	95
CL 5915-136 D4-1-0	75	81	92
CL 5915-206 D4-2-5-0	84	66	95
CL 5913-223 D3-2-2-0	85	70	89
CL 1131-0-0-43-8-1	80	80	65
F8-80-378-5-N	72	91	81
F7-80-465-19	100	100	70
F8-80-378-5-24-N	93	88	54
F8-80-378-5-24	80	94	57
C. Resistant to 1 biovar isolate			
Tm-L114-45-8-N-11 V.P.	67	79	87
Tm-L46-N-12-N	60	78	92
Tm-L46-1-32 V.P. N	53	80	75
Tm-L114-45-8-N V.P.	70	96	60
Tm-L114-42-1 early H.T.	75	86	66
Tm-L46-1-33-5 st. det	66	88	66
Tm-L114-56-12	64	79	96
Tm-L114-N-5	77	88	59
Tm-L114-N-6	77	97	56
CL 5916-214 D4-1-6-0	90	75	74
CL 5915-22 D4-0-4-0	82	78	65
F6-80-452-7 round	95	77	67
F8-80-378-5-37-N	86	68	71
F7-80-452-10 Plum shape	77	80	70
F7-80-470-3 V.P.	70	100	52
2159-21-VC 11-1	79	92	57
Marilag	39	92	61
VC 11-1	83	58	33
D. Checks			
508 (Resistant)	81	85	75
Yellow Plum (Susceptible)	3	2	0

ducted in greenhouse tests. The survivors were planted in pots for seed multiplication and further screening. The promising accessions were grown in replicated tests in the disease nursery for final evaluation before passing on the materials to the plant breeders.

Tomato

The best tomato selections that possessed resistance to the three tomato biovars of *P. solanacearum* are shown in Table 5. Forty-one selections showed resistance to virulent tomato isolates representing biovars I, III and IV of *P. solanacearum*, 25 selections were resistant against two of the three biovar isolates, and 18 selections showed resistance to only one of the three isolates. The average percentage survival of the 41 resistant selections to biovar isolates I, III and IV were 93, 95 and 91% respectively, showing that the degree of virulence of the three isolates was similar.

It is clear in the results that some tomato varieties are resistant to all the biovars of *P. solanacearum* tested while others are resistant to two biovars or to only one biovar. For example, tomato varieties VC 11-1 and Marilag showed resistance only to biovars I and II, respectively.

Potato

In potato, 15 clonal selections from CIP, Cornell, New Zealand, and PPC were rated resistant and some as moderately resistant in greenhouse screening tests (Table 6). The results, however, were based on a limited number of test plants. Hence, they were screened using a mixture of biovar isolates II, III and IV instead of against single biovar isolates. These results need further confirmation.

Table 6. White potato clones and tuber families with resistance to mixture of white potato biovar isolates II, III, IV of *Pseudomonas solanacearum* (greenhouse tests).

No. of clone/tuber family	Source	Survival (%)
1282-19	PPC	
AT 100-67	Cornell	
AT 100-71	Cornell	
1282-19-40	PPC	
S 726014-6	New Zealand	100
CIP No. 376019-8-21	CIP	
CIP No. 376019-8-22	CIP	
CIP No. 376019-8-41	CIP	
1282-19-14	PPC	
1282-19-62	PPC	
1282-19-68	PPC	92
S 726014-14	New Zealand	83
1282-19-17	PPC	83
AT 110-24	Cornell	80
CIP No. 376019-8-31	CIP	80
S 811011-10-a	New Zealand	79
Philco - 17	CIP	75
CIP No. 376019-8-23	CIP	75
CIP No. 376019-8-36	CIP	75
CIP No. 379426	CIP	71
1282-19-5	PPC	71

Table 7. Hot and sweet pepper cultivars and accessions with resistance to pepper biovar isolates III, IV and mixture of *Pseudomonas solanacearum* (greenhouse tests).

Description	Survival to biovar (%)		
	BIII (P15)	BIV (P10)	Mixture
A. Hot Pepper			
(F-5) BPI Ligao Albay-3	100	100	-
(F-5) BPI Ligao Albay-5	80	100	-
PI No. 163189-N-1-3	83	88	-
Kawit Strain	100	78	-
Kawit Strain-9	98	75	-
Kawit Strain-10	95	67	-
(F-5) BPI Ligao Albay-1	88	71	-
PI No. 133166-N-3	88	59	-
PI No. 183439-6	82	39	-
PI No. 244670-N	60	65	-
Red Santaka	68	14	-
87	13	83	-
PI No. 173877-N			
B. Sweet Pepper			
22-6-1	-	-	96
74-61-6-1-5-1	-	-	95
74-56-3-4-5-2	-	-	95
22-4-2	-	-	86
22-4-1	-	-	82
22-10-2	-	-	80
74-56-3-4-5-1 UP early	-	-	75
22-10-1	-	-	67
C. Checks			
Jalapeño (hot)	0	0	0
California Wonder (sweet)	0	0	0

Pepper

In hot pepper, only three varieties were resistant to both biovar isolates III and IV, two of which were local selections. The rest were either resistant to the biovar III isolate only and moderately resistant or susceptible to the biovar IV isolate only or vice versa (Table 7). Six accessions of sweet pepper were resistant to a mixture of biovar isolates III and IV and two were moderately resistant. The lack of test materials did not allow screening against single biovar isolates.

Eggplant

Of the 267 entries of eggplant that were screened for resistance using single and mixture of eggplant biovar isolates I, III and IV, only accession PI 362727 was resistant to the biovar isolates and to the mixture of the isolates. Accession PI 358311 was resistant to the mixture of the three biovar isolates. Both accessions, however, were of the wild type. Accession PI 285422 was moderately resistant to biovars I, IV and the mixture but was very susceptible to the biovar III isolate. Similarly, CA cluster and accessions PI 320507 and 40-N showed moderate resistance to biovar IV but were susceptible to the other biovars and the mixture.

Bitter Gourd

In bitter gourd, accessions 83-006, 83-003 and 9-32 were resistant to biovar isolates II and III with 87-100% survival in greenhouse tests.

All the 15 snapbean cultivars screened in greenhouse tests for resistance to a virulent isolate of biovar III were susceptible with 0-16% survival.

Chinese Cabbage

The commercial Chinese cabbage varieties, Corazon and Esperanza, were resistant to biovar II isolates from white potato and biovar III isolates from tomato, Chinese cabbage, pechay and bitter gourd. However, both varieties were susceptible to biovar IV isolates from pepper, moderately susceptible to biovar II isolates from bitter gourd, biovar III isolates from eggplant and biovar IV isolates from tomato. A high percentage of the infected plants in both varieties still survived. Corazon appears to have a slightly broader degree of resistance than Esperanza.

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Bacterial Wilt in Sri Lanka

Malarmagal Velupillai*

Sri Lanka has a total area of 6.6 million ha and is divided into three climatic zones: a wet zone of 1.54 million ha in the southwest, a dry zone of 4.17 million ha in the north, northeast, northwest and southeast, and an intermediate zone of 0.85 million ha sandwiched between the two.

The three climatic zones are subdivided into eight major agroecological regions by altitude and landform. Both the wet and intermediate zones range from low country (0–300 m), mid country (300–1000 m), to up-country (> 1000 m). The dry zone is in the low country. Land in the low country varies from flat to undulating, while the mid and up-country vary from undulating, rolling, hilly, steeply dissected to mountainous. These eight zones are further subdivided into 24 well-defined agroecological regions, each with its unique combination of rainfall pattern, elevation, land form, temperature range and soil types.

Rainfall in Sri Lanka is determined both by regional and local phenomena. The monsoonal rains occur as a regional phenomenon while the convectional inter-monsoon rains are a local phenomenon. The mean annual rainfall ranges from 5000 mm in wet regions to 1250 mm in semi-humid regions, and it follows a distinctive bimodal pattern. Consequently there are two cultivation seasons: the *Maha* season from October to January, which is influenced by convectional rains and the Northeast Monsoon, and the *Yala* season from May to September when the Southwest Monsoon is in force.

Rainfall in the wet zone is adequate for year-round crop growth. In the intermediate and dry zones the rainfall is adequate only during the Maha season (Oct.–Jan.). Temperature within the country shows a marginal variation, the maximum and minimum air temperature being 20 and 10 °C in the up-country wet zone, and 32 and 25 °C in the low-country dry zone. Sri Lanka's wide variation in precipitation, topography, and soil makes it possible to grow a wide range of crops, including solanaceous crops like chilli (*Capsicum annuum*), eggplant (brinjal) (*Solanum melongena*), tomato (*Lycopersicon esculentum*), and potato (*Solanum tuberosum*), which are susceptible to the bacterial wilt disease caused by *Pseudomonas solanacearum*.

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Distribution and Importance

Nuwara Eliya is the coldest region in Sri Lanka, but a high incidence of bacterial wilt is sometimes observed in crops grown in this region.

A survey carried out by Abeygunawardena and Siriwardena (1961) on disease of potato revealed that bacterial wilt was a limiting factor to the extension of this crop to elevations below 1540 m. Elevations above 1890 m were believed to be wilt-free areas for the production of seed potato. However, eventually wilt was found even at altitudes between 1820 and 2200 m.

Bacterial wilt is also a problem with tomato and eggplant in the wet zone where it was first detected in the 1930s (Park and Fernando 1938).

Screening for Resistance

Eggplant

Park and Fernando (1940) detected a tolerant Matale variety of eggplant from Raitalawela in the Matale district. This had enlarged purple skin and white flesh fruits and tolerance to bacterial wilt.

Udugama and Seneviratne (1983) found that a *Solanum* species referred to as 'Thiththathibbatu' and *Solanum torvum* had high levels of resistance to bacterial wilt. The possibility of using these as resistant root stocks on which susceptible eggplant varieties of desired fruit quality could be grafted was considered.

Tomato

Park (1941) as reported in Abeygunawardena and Siriwardena (1963), found the local tomato 'Talatuoya' to be tolerant to bacterial wilt when compared to introductions such as Marglobe. The bacterial wilt percentage of Marglobe and Talatuoya were found to be 81.5 and 47.0, respectively.

Peiris (1962), as reported in Abeygunawardena and Siriwardena (1963), compared two North Carolina commercial tomato varieties with Marglobe and Ponderosa and demonstrated Marglobe as superior in resistance to the two commercial varieties. Abeygunawardena and Siriwardena (1963) compared the resistance of local tomato selections with introductions such as Roma and Moscow and demonstrated that the Rahangala selection II (*Lycopersicon esculentum* var. *cerasiforme*) had greater resistance. He also tested the two North Carolina selections 1960–2a and 1960–8 with the local variety grown in these areas and found the N.C. selections to be more resistant.

Seneviratne (1979 unpublished data) selected two varieties of tomato, Saturn and Venus, as being more tolerant to bacterial wilt than Marglobe. He also selected cultivar 78-B-21 as having a high degree of tolerance.

Potato

Abeygunawardena and Siriwardena (1961) selected the Prisca variety of potato received from North Carolina State College which had a higher tolerance level than the variety Dekama (which was susceptible).

Strain Characterisation

Seneviratne (1969) classified the isolates of the pathogen in Sri Lanka into three biovars using Hayward's (1964) biochemical classification. Biovar II was obtained from potato cultivated above 1890 m and in virgin lands in the hill country wet zone; biovar III was obtained from tomato and potato in several locations previously cropped with cultivated species in the warmer dry zone; and biovar II and III in the transitional regions between the dry and wet zones. Biovar IV was found in potatoes grown at Gorandiyatenna (up-country dry zone).

A striking correlation was found between zonal agroclimatic and soil characteristics and the biovars. However, the rapid development of potato production combined with the Department of Agriculture's inability to meet the demand for seed potatoes, prompted farmers in the Jaffna district who were unable to obtain their requirements of certified seed from official sources, to purchase uncertified seed from private traders in Nuwara Eliya and Badulla. This has led to the spread of the different strains of the organism to areas where they are ecologically unrelated. The cool temperature biovar II has been detected (Velupillai and Seneviratne 1983) from isolates collected from potato plants during the outbreaks of bacterial wilt in Jaffna, in Maha season 1982–83.

Survival

Seneviratne (1976) found that wilt was not completely controlled even in paddy fields which had been flooded for several years. It was also observed that during the dry season in paddy fields a higher incidence of wilt occurred close to the waterholes and irrigation drains.

Seneviratne (1978) studied the survival of *P. solanacearum* biovars II and III. Biovar III survived at Sita Eliya for 24 months when the soil was kept free from other hosts between subsequent plantings of potatoes. Temperature was the main factor responsible for the survival of the organism. The rise in soil temperature to over 40 °C in the Jaffna district probably explains why bacterial wilt is not a serious problem in this region.

Studies made by Seneviratne (1979 unpublished data) on the survival of *P. solanacearum* using four soils with different characters from Katugastota, Bombuwela, Thirunelvelly and Horton Plains showed that the organism was not able to survive in any of these places when subjected to four successive days of 6-hour periods at 43 °C alternating with 18-hour low-temperature periods. At 41 °C it survived up to 18 days with similar time periods in the Katugastota, Bombuwela and Thirunelvelly soils, although the organism in the acidic peaty soil of Horton Plains could not tolerate exposure to temperatures above 40 °C. Seneviratne was able to recover *P. solanacearum* at a site in Horton Plains 15 years after the first detection there, although it had not been cultivated with potatoes during the intervening period. Gunawardena (1976 unpublished data) isolated a bacterium from irrigation water at Getambe, identified as *Chromobacterium violaceum* which had a strong antagonistic effect on *P. solanacearum*.

Host Range

Gunawardena et al. (1980) detected *P. solanacearum* in several hosts hitherto unrecorded in Sri Lanka. They included *Anthurium andraeanum* from Handessa (mid country), *Curcuma domestica* (tumeric) and *Zingiber officinale* (ginger) from Gampaha (low country), *Phaseolus vulgaris* (bush bean) and *Psophocarpus tetragonolobus* (winged bean) from Gannoruwa (mid country), *Croton hir-*

tus, Hyptis suaveolens and *Sesamum idicum* (gingelly) from coconut-growing regions in Kurunegala (mid country). More recently, *P. solanacearum* has been detected in *Momordica charantia* (bitter gourd) from Marassana (mid country). This appears to be the first record of *P. solanacearum* on *A. andraeanum, C. hirtus, P. tetragonolobus, C. domestica,* and *M. charantia.*

Resistance Screening

Recent Work on Potato

A collaborative program with the International Potato Centre (CIP) was initiated in 1980. Clones derived from the Colombian diploid *Solanum phureja* and hybrids derived from crosses made between lines derived from *S. phureja* and lines adapted for lowland tropics were screened in the infested field at Rahangala (up-country dry zone), Sita Eliya (up-country wet zone) and Getambe (mid-country wet zone) with the overall objective to detect lines with tolerance to the disease in these three types of climate.

Hybrids having combined resistance to bacterial wilt and adaptability to lowland tropics (379 series) were received from CIP as tuber families in two sets. Each tuber in a family was numbered and multiplied at the Agriculture Research Station, Sita Eliya, before screening in the infested field.

The first set was screened in Maha season 1981–82 and Maha 1982–83, the second set was screened in Yala season 1982, Maha 1983–84 and Yala 1984. Of the first set of screenings four lines (Table 1A and B) 25/40, 27/15, 27/40 and 28/75 maintained resistance without showing any symptoms of wilt in both screenings (Velupillai et al. 1983). With the second set of screenings lines 77/16 and 86/20 were selected (Table 2: A and B–1).

Lines 25/40 and 28/75 were derived from a parent of LT series which was adapted to hot tropics. Tuberisation of these two lines when planted at the

Table 1. Lines selected from the first set of tuber families.

A. CIP Family number and pedigrees

Family number	Line number	Pedigrees
379418	25/40	Lt. 7.48 × Bulk *Pseudomonas**
379420	27/15	Lt. 18.50 × Bulk *Pseudomonas**
379420	27/40	Lt. 18.50 × Bulk *Pseudomonas**
379421	28/75	Lt. 18.40 × Bulk *Pseudomonas**

* Pollen collected from lines resistant to bacterial wilt.

B. Performance in the field for resistance to bacterial wilt

	Maha* 1981–82		Maha 1982–83	
Line number	Percent diseased	Yield per plant (g)	Percent diseased	Yield per plant (g)
25/40	00	222	00	136
27/15	00	86	00	53
27/40	00	40	00	138
28/75	00	215	00	139
Check	100 (Diamont)	—	100 (Vekaro)	139

C. Performance for adaptability to low land tropics at A.R.S. Thirunelvelly, Jaffna

Line number	Number of tubers per plant	Yield per plant (g)	Number of tubers per plant	Yield per plant (g)
25/40	3.80	136	6	142
27/15	NT		NT	
27/40	–*	–	–	–
28/75	8.00	476	3	79
NT—Not tested	*not tuberised			
Date planted:	23.12.81		07.01.82	
Date harvested:	10.03.82		25.03.82	

Seasons: Maha, Oct.–Jan.; Yala, May–Sept.

Table 2. Lines selected from the second set of tuber families.

A. Family number and pedigrees

Family number	Line number	Pedigrees
379687	77/16	377831.5 × CGN 69-1
379696	86/20	377847.3 × CGN 69-1

B. Performance in the field at ARS Rahangala for resistance to bacterial wilt and at ARS Sita Eliya for resistance to late blight

I. Screening at ARS Rahangala

	Yala 1982		Maha 1983–84		Yala 1984	
Line number	Percent diseased	Yield per plant (g)	Percent diseased	Yield per plant (g)	Percent diseased	Yield per plant (g)
77/16	00	104	00	146	00	317
86/20	00	385	00	84	00	227
Check	100 (Vekaro)	-	100 (Isna)	106	100 (Desiree)	92

II. Screening at ARS Sita Eliya

Line number	Percent diseased
77/16	21.67
86/20	2.50

Date planted: 25.10.83
Date harvested: 09.02.84

Table 3. Performance of the selected lines as tolerant to bacterial wilt at three locations.

CIP number	Pedigrees	Rahangala	Sita Eliya	Getambe
377830.3	BR63.65 × N574.1	S	R	S
377831.1	BR63.65 × Katahdin	RR	S	S
377835.12	BR63.65 × Atlantic	R	S	S
377838.2	BR63.65 × N 522.22	S	S	RR
377847.4	BR63.74 × Anita	R	RR	S
377847.5	BR63.74 × Anita	R	R	S
377850.1	BR63.74 × DTO 28	RR	S	S
377850.2	BR63.74 × DTO 28	R	S	S
377852.2	BR63.74 × 1923.1	R	R	S
377863.2	URF 1919.2 × BR63.65	RR	R	S
800223	BR63.74	RR	RR	S
800224	BR63.76	R	R	R
800226	BR69.84	R	RR	S
800928	MS42.3	S	RR	S
800935	MS IC.2	S	RR	S
380576.4	A52.2 × PI/PS Bulk	S	R	S
380576.6	A52.2 × PI/PS Bulk	S	R	S
380576.11	A52.2 × PI/PS Bulk	R	R	S
380577.7	A49.2 × PI/PS Bulk	R	S	R

RR = Tolerance level above 80%, R = Tolerance level ranging from 60 to 80%.
S = Susceptible.

Agriculture Research Station, Thirunelvelly, Jaffna, during the time when commercial varieties do not usually tuberise because of the high temperature, showed its adaptability and tolerance to bacterial wilt (Table 1C).

Lines 77/16 and 86/20, in addition to tolerance to bacterial wilt, showed a high level of resistance to late blight when tested at the Agricultural Research Station, Sita Eliya (Table 2-B-II). These two lines have been derived from parents having combined resistance to late blight and bacterial wilt with adaptability to cool and warm tropics.

Lines 3778, 3796, 3805 and 800 having resistance to bacterial wilt alone and/or in combination with resistance to late blight were also screened at Rahangala, Sita Eliya and Getambe for many seasons. Performance of the selected lines is given in Table 3.

Tolerance level of line 800223 (BR63.74) was high at Rahangala and Sita Eliya. This is a line selected by CIP as having resistance to late blight bacterial wilt with moderate resistance to leaf roll virus and adaptability to cool and warm tropics.

At Rahangala lines 377831.1, 377850.1 and 377863.2 have shown a high degree of tolerance to bacterial wilt. Lines 377831.1 and 377863.2 have been derived from parent BR63.65. Line 377850.1 is a hybrid of the parents having adaptability to cool and warm tropics (BR63.74) and adaptability to hot tropics (DTO 28).

Lines 377847.4, 800226, 800928 and 800935 have shown a high degree of wilt tolerance at Sita Eliya. CIP has selected these lines for their resistance to late blight bacterial wilt and adaptability to cool and warm tropics.

Performance of the line 800224 (BR 63.76) indicates its adaptability to different weather conditions as it has performed well at all three locations. Lines 377847.5 (BR 63.74 × Anita), 377852.2 (BR 63.74 × 1923.1) and 380576.11 < A 52.2 × PI/PS Bulk) have shown moderate levels of tolerance at Rahangala and Sita Eliya. It is very interesting to note that the first two lines have had one parent in common, BR 63.74, and the line 380576.11 has been derived from the parent 377852.2 (BR 63.74 × 1923.1).

Line 377838.2 (BR 63.65 × N 522.22) is the only line which has shown a high degree of tolerance at Getambe over many seasons. Could it be due to the presence of the N series which is supposed to be adapted to the hot tropics?

Selected lines were screened in Yala 1984 in a farmer's paddy field to learn the farmer's view regarding the performance of these lines. The trial was planted and harvested on the same dates as the farmer's crops, and harvested 71 days after planting. Farmers did not favour lines that grew tall and lodged because they interfered with irrigation. Long-duration varieties like 77/16 and 86/20 were

Table 4. Results of the performance of selected lines in the screening carried out in farmer's paddy field.

Clone number	Local number	Number of plants emerged	Number of plants harvested	Height of plants (cm)	Percent diseased	Number of tubers per plant	Yield per plant (g)
379418	25/40	16	14	65	6	5.3 (1)*	260
379420	27/15	08	06	73	25	4.1 (1)	140
379420	27/40	10	08	103	0	6.0 (3)	193
379421	28/75	12	12	100	7	6.5 (2)	310
379687	77/16	19	17	100	6	8.0 (8)	235
379691	81/8	20	17	107	35	8.0 (1)	335
379693	83/8	20	14	120	10	5.0 (3)	151
379696	86/20	17	17	89	11	6.0 (2)	224
800223	D223	17	17	100	7	10.0 (11)	235
800224	D224	05	04	120	58	1.0 (2)	43
800226	D226	09	03	80	0	8.0 (2)	415
377850.1	A50.1	06	05	50	42	5.0 (2)	238
377850.2	A50.2	09	09	30	0	4.0 (2)	162
377847.5	A47.5	17	17	98	10	7.0 (3)	402
377849.2	A49.2	12	11	78	30	7.0 (6)	195
377835.12	A35.12	09	04	70	20	5.0 (3)	298
377852.2	A52.2	08	07	60	0	10.0 (3)	420
Desiree		20	09	65	23	7.4 (0)	341

Planted on 04.08.84; harvested on 14.10.84.

* Within parentheses is number of unmarketable tubers.

also considered unfavourable as harvesting is generally done early. Lines 377847.5 and 377852.2 were the preferred ones (Table 4) with 25/40 and 28/75 second best.

Information gathered from the performance of lines resistant to bacterial wilt and adapted to lowland tropics showed : (1) of the lines CGN 69-1 and Maria Tropical used for adaptability to lowland tropics, those derived from CGN 69-1 were better than those derived from M tropical (Table 5); CGN 69-1 is selected by CIP as having combined resistance to late blight bacterial wilt and adaptability to cool and warm tropics; (2) hybridisation of BR series, especially 63.55, 63.74 and 63.76 with N series, CGN 69-1 or DTO series and 1923.1 series, would be suitable for Sri Lankan conditions.

Variability in Disease Reaction

Sequeira (1979) found the variability in the expressions of resistance by the resistant clones derived from *S. phureja* due to environmental fluctuations such as temperature and light intensity. Schmiediche (1984) referred to the challenges the breeders face owing to the complexity of *P. solanacearum* and its interaction with the host and environment. A detailed study was made on the variability in the performance of some of the lines at the Agriculture Research Station, Rahangala, where the same field was used season after season. Rainfall and sunshine hours were considered for the study.

A striking difference could be seen in the distribution of the rainfall during the three cropping periods. In Maha 1983 the monthly average rainfall ranged from 6.94 to 14.46 mm, in Yala 1984 from 0.84 to 3.73 mm and in Maha 1984 from 4.37 to 8.84 mm. Similarly a variation was seen with the length of sunshine hours. During the cropping period in Maha 1983 the longest sunshine hours of 2.68 was in the month of January, which was much lower when compared to the length of sunshine hours in the other two cropping seasons, where it ranged from 3.44 to 4.83 in Yala 1984 and 2.71 to 4.69 in Maha 1984.

Performance of lines 77/16, 85/22, 80/6, 85/7, 88/6 and H77.7 was studied (Fig. 1). The lines 77/16 and 85/22 were screened in Maha 1983, Yala 1984 and Maha 1984. Lines 80/6 and 85/7 were screened in Maha 1983 and Yala 1984 and the lines 88/6 and H77.7 were screened in Yala 1984 and Maha 1984. Line 77/16 did not show any wilt in Maha 1983 and Yala 1984 but succumbed in Maha 1984. Line 85/22 showed a low level of resistance in the first two seasons which deteriorated still further in Maha 1984. The line 85/7 which showed no incidence in Maha 1983 showed a high level of disease incidence in Yala 1984. Lines H77.7 and 88/6 which showed either no incidence or a low level of disease incidence in Yala 1984 showed a very high level in Maha 1984. Line 80/6 does not seem to have any gene for resistance as the level of the disease incidence was very high. Thus it would appear that

Table 5. Comparison of the performance of lines derived from *Solanum phureja* for resistance to bacterial wilt with hybrids derived from the same line crossed with a parent having adaptability to lowland tropics.

Clone number	Local number	Pedigrees	Percent diseased							
			Yala 81	Maha 81/82	Yala 82	Maha 82/83	Yala 83	Maha 83/84	Yala 84	Maha 84/85
377831.5	(A31.5)	BR63.65 × Katahdin	100	–	–	–	–	–	–	–
379687	(77/16)	A31.5 × CGN 69-1	–	–	00	–	–	00	00	65
379687	(77/19)	A31.5 × CGN 69-1	–	–	00	–	–	–	20	65
379687	(77/55)	A31.5 × CGN 69-1	–	–	33.3	–	–	20	–	–
379687	(77/67)	A31.5 × CGN 69-1	–	–	00	–	–	33.3	–	–
377835.12	(A35.12)	BR63.65 × Atlantic	20	–	–	–	00	–	–	31.2
379693	(83/8)	A35.12 × CGN 69-1	–	–	00	–	–	13.3	–	15.0
379693	(83/2)	A35.12 × CGN 69-1	–	–	00	–	–	15.4	–	–
377847.4	(A47.4)	BR63.64 × Anita	40	–	–	–	–	–	–	–
379697	(87/1)	A47.4 × CGN 69-1	–	–	–	–	–	–	00	30
379697	(87/2)	A47.4 × CGN 69-1	–	–	00	–	–	23.2	62.55	–
379673	(68/21)	A47.4 × M Tropical	–	–	00	–	–	50.0	–	–
379673	(68/29)	A47.4 × M Tropical	–	–	00	–	–	36.4	–	–
379673	(68/16)	A47.4 × M Tropical	–	–	00	–	–	31.2	–	–

the incidence of wilt was higher during Maha 1984.

Unlike in Maha 1983 where continuous rain had occurred throughout the cropping period with low sunshine hours, in Yala 1984 from the 6th week after planting drought had prevailed till the 8th week with long sunshine hours and the rainfall was moderate before and after the drought period. In Maha 1984 drought prevailed during the 5th and 6th week with heavy rainfall and long sunshine hours before and after the drought.

Disease progress curves of line 85/22 (Fig. 1) shows the breakdown of resistance by the 8th week after planting in Maha 1983. However, in Yala 1984 lines 85/7 and 85/22 showed the breaking down of resistance by the 6th week after planting and it increased slowly till the 12th week. Performance of the lines 77/16, 85/22, 88/6 and H77/7 in Maha 1984 indicates that the disease incidence became severe about the 7th week after planting and it rapidly increased by the 9th to the 10th week.

The high degree of tolerance showed in Maha 1983 was affected during Yala and Maha 1984. The two seasons differed markedly in the rainfall pattern and length of sunshine hours. It seems probable that these factors influence the multiplication of the pathogen and its aggressiveness. It is also possible that the influence of climatic conditions (long sunshine hours) on transpiration also aggravates the disease. Plant characters related to loss of resistance with high temperature may also be a contributing factor. These aspects need to be studied further.

The results show that it is necessary to test varieties over a number of seasons and in association with control lines of known disease reaction before deciding on their resistance/susceptibility to bacterial wilt.

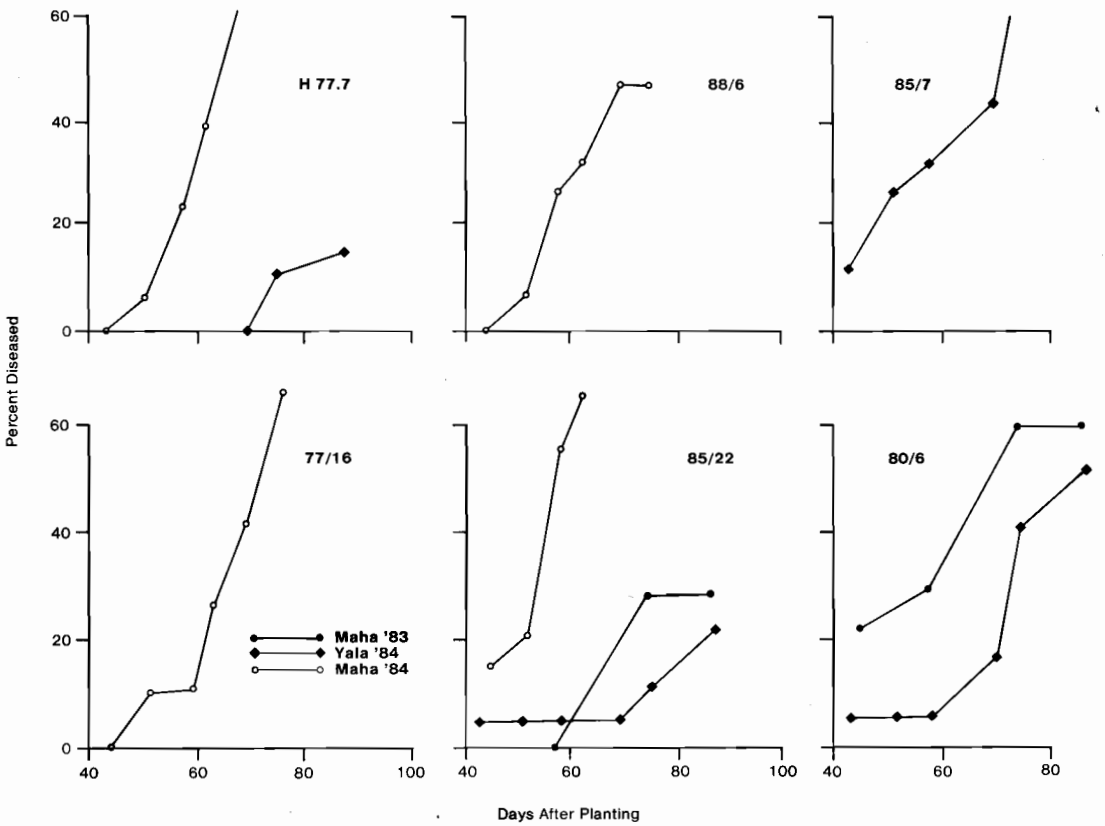

Figure 1. Disease progress curves of the lines screened for resistance to bacterial wilt.

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Bacterial Wilt in Thailand

V. Titatarn*

IN Thailand, bacterial wilt caused by *Pseudomonas solanacearum* is an important disease and widely distributed throughout the country. The disease was first recorded in 1957 in tomato (Chandrasrikul 1957), and later in tobacco (Kaewkumnerd 1965), eggplant (Anonymous 1957), and chilli (Chandrasrikul and Wannapee 1972). Severe losses were reported in ginger planted in the south (Titatarn and Tananuson 1981) and in sesame grown in Nakorn Sawan and Mahasarakam Provinces (Uematsu et al. 1981). Titatarn et al. (1983) made intensive surveys on bacterial diseases in Chiang Mai Province and indicated that not only bacterial wilt, which widely spreads in these areas, occurred on potatoes but also bacterial soft rot and blackleg (*Erwinia* spp.) cause serious damage to potato both in the fields and in storage. No evidence of ring rot (*Corynebacterium sepedidonium*) was found in the surveys.

The bacterium *Pseudomonas solanacearum* has wide host ranges including many weeds. Pegg et al. (1974) reported some weed hosts of the disease: e.g. *Solanum nigrum* (black berry nightshade), *Crassocephalum crepidioides* (thick head), *Physalis minima* (cape gooseberry) and *Solanum mauritianum* (wild tobacco tree). Weeds are regarded as a source of inoculum from which the disease spreads to nearby crops during growing seasons. All these weeds are commonly found in cultivated lands in Thailand.

Presently, bacterial wilt is an endemic disease which is found in almost all areas under cultivation in Thailand. The disease greatly reduces yields in ginger, tomato and potato. The occurrence of bacterial wilt on potato, ginger, tomato, sesame, eggplant, chilli and tobacco has been recognised in Thailand. Titatarn and Tananuson (1981) identified the pathogen of rhizome rot disease of ginger as *P. solanacearum*. This isolate utilised maltose, lactose and arabinose.

Uematsu et al. (1983) reported the results of pathogenicity tests and some bacteriological properties of six strains of *P. solanacearum* from tomato, chilli, eggplant, sesame, tobacco and ginger studies. These and related studies are summarised below.

Host Range

The pathogenicity of six strains was tested on their host of origin (Uematsu et al. 1983). All strains were pathogenic on tomato and most were pathogenic on eggplant except for one isolate from eggplant. Ginger plants were infected and wilted only when inoculated with isolates from ginger indicating some host specialisation in ginger strain. Chilli, sesame and tobacco plants showed wilt symptoms when inoculated with some isolates from the six strains.

Preliminary studies by Uematsu et al. (1983) on the host range reaction of 10 selected isolates (2 tomato strains, 2 chilli, 1 sesame, 1 eggplant, 1 tobacco and 3 ginger) were made by inoculating crops such as angle loofah, banana, cassava, castorbean, garden pea, jute, kenaf, mulberry, mungbean, peanut, pineapple, rice, soybean, sweet corn and yard long bean. Only three crops, mungbean, castorbean and garden pea, showed symptoms after inoculation with some of the strains. The other crops showed no symptoms.

From the host range study of *P. solanacearum* biovar III on weed plants, 10 types of weed plant (*Eclipta prostrata, Jussiaea linifolia, Ageratum conyzoides, Vernonia cinerea, Hyptis suaveolens, Portulaca oleracea, Eupatorium odoratum, Solanum nigrum, Crassocephalum erepidioides,* and *Bidens pilosa* (Titatarn and Tongjeam 1985)) were found to serve as a primary source of inoculum for

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the bacterial wilt pathogen to the following season's crop.

FORMAZAN FORMATION AND COLONY TYPE

The colonies of isolates were all slimy but differences were seen in size, shape, fluidity, texture of formazan pigmentation and slime deposition. The isolates from each of the six hosts were grouped in five categories based on colony texture and formazan formation. No correlation was observed between strains (i.e. host of origin) and bacterial colony form and melanin formation (Uematsu et al. 1983).

MELANIN FORMATION

Only three isolates from tomato produced high amounts of brown pigment, others were very low or intermediate (Uematsu et al. 1983).

PHAGE SENSITIVITY TESTS

Nine bacteriophages were isolated from the samples of ginger wilt disease. The phages were classified into three groups by using the host range reaction on 43 ginger isolates and their morphology. The sensitivity of selected isolates of tomato, chilli, ginger, eggplant, sesame and tobacco strains to three bacteriophages were determined by the host range reaction. The isolates were classified into four categories by three phages (No. 1, 5 and 8) from a ginger strain. All ginger isolates were sensitive to No. 5 phage. Phage No. 1 was specific to ginger isolates only. Some ginger, tomato and tobacco isolates were sensitive to No. 5 and No. 8 phage and sesame isolates were not sensitive to all of the three phages. From the results of host range reaction, phage No. 5 was suitable for the detection of the ginger strain (Uematsu et al. 1983).

BIOVAR CLASSIFICATION

Almost all isolates belonged to biovar III with the exception of one isolate of the ginger strain which belonged to biovar IV and two isolates of the tomato strain which could not be classified into any of biovar I–IV of Hayward (1976).

Serology

Serological affinities between the isolates of six strains and five antisera prepared from five selected strains were observed from the agar double diffusion tests. They formed clear homologous precipitin bands against large numbers of isolates of six strains except for the combination of sesame strain and antisera from ginger strain. No strain-specific band, however, was recognised throughout this study. Similarly there was no definite correlation between the six strains and phenotypic natures of colony form, phage group, biovar and serological properties.

Chayachavalit (1985) investigated the serological relationship of pathogenic isolates isolated from tomato, tobacco, potato, sesame, eggplant, chilli and ginger. Antiserum prepared with tomato strain of *P. solanacearum* was tested against the seven isolates employing the tube agglutination and enzyme-linked immunosorbent assay (ELISA) techniques. The results showed that the seven isolates of *P. solanacearum* are serologically closely related, although strain-specific antigenic reactions are evident.

ANTIBIOTIC SENSITIVITY

Ginger isolates showed sensitivity to oleandomycin, but sesame isolates were resistant or weakly sensitive. They were resistant to penicillin, lincomycin, nitrofurantoin, polymyxin B, sulfisoxazol, fusidic acid, bacitracin and colistin, and sensitive to streptomycin, tetracycline, erythromycin, nolidixic acid and kanamycin. All isolates showed resistant to weak sensitivity to chloramphenical, spiramycin, novobiocin, leucomycin and mikamycin.

Titatarn et al. (1983) reported that bacterial wilt of potato was spread over potato-growing areas and biovar classification of *P. solanacearum* isolates were biovar III and IV. Collateral hosts of this pathogen were found to be two native weed varieties *Vernonia cinerea* and *Ageratum conyzoides* commonly present in the area. In 1984, Titatarn and Tongjeam reported the survival of *P. solanacearum* race 3 (biovar III) in infested clay loam soil for 360 days.

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Bacterial Wilt in Vietnam

Pham Xuan Tung*

BACTERIAL wilt is commonly known in Vietnam as 'Green wilt' or 'die young' by farmers. It was considered to be only a minor production constraint, however, with the intensification of production of crops such as potato, tomato, tobacco, peanut and soybean in certain areas, the prevalence of the disease has increaed.

Vietnam is located between 8°30' and 23°22'N latitude. It is characterised by a diverse set of ecological zones which are influenced by latitude and elevation (Fig. 1). The northern region has a subtropical climate with the Red River Delta (RRD) being the major population area around Hanoi. Temperatures and rainfall vary substantially throughout the year (total rainfall 1644 mm). The RRD has typically annual flooding of the land and grows two rice crops followed by a dryland winter crop such as potatoes (50 000 ha) or sweet potatoes (90 000 ha). Regions 2 and 3 increase in altitude with some flooded rice in region 2. Sweet potatoes and maize are the two other major crops besides rice.

Regions 4, 5 and 7 have both paddy rice and upland crops dominated by sweet potatoes, maize and cassava. Region 6 is highland country; Dalat (1500 m) has 1500 mm rainfall and Duc Trong (900 m) has 1633 mm, with temperature ranges of 11.3°C in January to 25°C in March—May for Dalat and 13.4°C and 29.5°C for Duc Trong. This region is still heavily forested with increasing population pressure as homeless families have been moved here to settle. Crops such as potatoes, temperate vegetables, peanut, maize and sweet potatoes are grown; research is under way on wheat and barley. Region 8, the Mekong River Delta (MRD), is the rice bowl of the south with two or three rice crops/year. The climate is isohyperthermic.

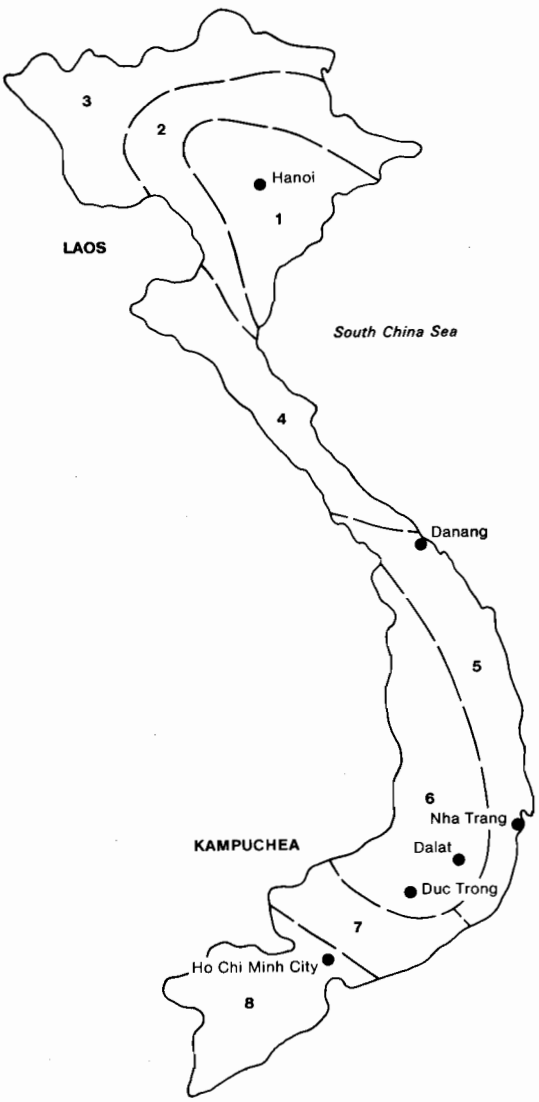

Fig. 1. Regions of Vietnam based on geography and climate: 1—Red River Delta; 2—Northern mid elevation; 3—Northern highlands; 4—North Central; 5—South Central; 6—Southern highlands; 7—Southern mid elevation; 8—Mekong River Delta.

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Vietnam depends heavily on four major food crops: rice, maize, sweet potatoes and cassava which are not known to be affected by bacterial wilt (Table 1). The area grown with bacterial wilt-susceptible crops is given in Table 2.

Table 1. Production area of some main crops.

Crop	Hectarage (million ha)	Per cent of total
Rice	4.7	66.2
Maize	0.4	5.9
Sweet potato	0.4	6.7
Cassava	0.4	6.6

Table 2. Production area of more important bacterial wilt-susceptible crops.

Crop	Hectarage (thousand ha)	Per cent of total
Potato	52	0.07
Peanut	300	4.2
Sesame	200	2.8
Tobacco	61	0.09
Jute	50	0.07
Soybean and Bean	253	3.6
Tomato	25	0.03

The government has instituted a policy of developing 'green belts' or vegetable belts around the major cities of Hanoi, Ho Chi Minh City (HCMC), Nha Trang and Danang. With the exception of Hanoi, a major portion of the land is not flooded and grown to vegetables for a large part of the year.

Distribution of Bacterial Wilt

Bacterial wilt is known to exist in all eight regions of Vietnam to varying degrees. The flooded RRD and MRD, which are the two regions with the greatest population and the most productive and intensive cropping, has virtually no bacterial wilt problem in growing any of the crops listed in Table 2 during the drier months (November–March) of the year. The notable exception would be in the green belt of HCMC. Bacterial wilt is now becoming more prevalent as land is being taken out of rice, not flooded and growing more and more vegetables such as tomatoes, eggplant, pepper, tobacco and peanuts. No exact estimate of losses due to bacterial wilt is available, however a reassessment of which vegetable to grow in the green belt is needed.

Bacterial wilt is most severe during the wet season (April–October) and substantially less destructive during drier months (November–March).

Regions 2, 3, 4, 5, 6 and 7 are the ones noted for bacterial wilt on non-flooded soils. The six regions cover a large portion of the country. The population which is still low is rapidly expanding into the marginal areas with upland soils. This is resulting in more intensive cropping and in the case of bacterial wilt-susceptible crops the disease is spreading.

In Dalat (1500 m) potato production has rapidly expanded in the past 4 years as the crop has become more profitable due to improved cultivars. This has resulted in a more intensive cropping of potatoes with a similar increase in bacterial wilt levels. Up to 20% loss has been observed in some fields. The provincial government is trying to impose a strict rotation of potatoes once every 2 years.

The Duc Trong plateau (800–1000 m) is a large area of flat land within Region 6. Here the expansion of all those crops listed in Table 2 is possible. Efforts with potatoes have met with some success, however, already an average of 15% of the potato crop is affected by bacterial wilt (Khuong, personal communication). Here rotation with cereals such as wheat and barley is planned as a way to control the disease.

Bacterial wilt is most severe during the wet season (April–October) and substantially less destructive during drier months (November–March).

Research on Bacterial Wilt

No significant research has yet been done on bacterial wilt. It is only in the last few years that the disease has been recognised as a notable problem particularly around HCMC (green belt), Duc Trong and Dalat as intensification of vegetable production has taken place.

The breeding and selection for resistant varieties has just started with some crops. For potatoes this work is in the Dalat and Duc Trong areas with the evaluation of genetic materials from the International Potato Centre.

The races of bacterial wilt have not been analysed as yet in Vietnam. It is assumed that Race 1 is the predominant race in most of the country with the possible exception of the high elevation areas in Regions 2, 3 and 6, where race 3 may occur.

For the immediate control of the disease, farmers are instructed to do a proper crop rotation, provide good drainage for upland crops and to eliminate infected plants.

Conclusion

The intensification of vegetable production in the green belts of the major cities and the expansion of farming into numerous mid-elevation zones has resulted in increasing levels of bacterial wilt. Farmers are presently being taught to control the disease through crop management. Research is only now commencing to look at developing improved varieties with resistance to bacterial wilt. The flooded RRD and MRD fortunately do not have bacterial wilt disease as a problem.

Ecology of *Pseudomonas solanacearum*, the Causal Agent of Bacterial Wilt

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THE critical questions to consider in the ecology of a plant pathogen are:

1 What are the sources of inoculum for the affected cultivated crop?
2 How does the pathogen survive the absence of the susceptible crop?
3 How is the pathogen disseminated from place to place and season to season?

An ecologically competent pathogen must be able to survive the absence of its host, and be able to over-winter in temperate zones and survive the dry season in tropical areas. The world-wide distribution and damaging nature of bacterial wilt on many crops suggest that *Pseudomonas solanacearum* is an ecologically competent pathogen. Its ecology is, however, poorly understood.

This paper discusses the information required to understand the ecology of the pathogen in particular environments; assesses some of the presently available data; describes a few detailed ecological studies from Australia; and identifies some future research opportunities in the Asia/Pacific region.

Bacterial wilt caused by *P. solanacearum* has been recognised as a major bacterial disease since at least the late nineteenth century. Kelman (1953) considered the earliest known record of bacterial wilt to be on tobacco in Indonesia in 1864, where entire fields were lost to bacterial wilt. Bacterial wilt was recorded on potatoes in the United States in 1890 (Kelman 1953) and in Australia in 1894 (Tryon 1895).

Since these early reports, bacterial wilt caused by *P. solanacearum* has been described on a wide range of hosts in many tropical, subtropical and warm temperate regions. There is extensive literature on the disease, particularly in terms of host range, geographic distribution, occurrence of various biovars, races and strains, and ability to survive in soil and in association with plant debris and weed hosts. There are surprisingly few detailed ecological studies on the disease designed to elucidate the life cycle of the pathogen in particular ecological niches, and to identify the points in the cycle where the pathogen is most vulnerable and hence may be most amenable to control.

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Geographic Distribution

Bacterial wilt is predominately a disease of the tropical, subtropical and warm temperate regions. A few instances of its occurrence on potato in cool temperate regions are known. The optimum temperature range for the pathogen is 25–35 °C. The disease rarely occurs in areas where the mean monthly temperature is less than 10 °C. In the few instances where bacterial wilt has been recorded in cooler climates, the outbreak of the disease has usually been associated with the introduction of infected planting material, such as potato tubers carrying latent infection of *P. solanacearum*, such as has occurred in Sweden (Olsson 1976a, b).

In Asia, bacterial wilt is a serious pathogen in the lowland humid tropics and the cooler tropical highlands. It is also found on several (but not all) Pacific islands. Bacterial wilt is not considered to be as damaging in the semi-arid tropics, even when crops are grown under irrigation. The ecological basis of this observation merits examination.

Dissemination

Infected planting material is the major means of dispersal of *P. solanacearum* from place to place and season to season, particularly in vegetative seed pieces of crops such as potato and ginger. The introduction of infected potato seed pieces is one of the most common means of dispersal of the pathogen on potatoes. Latent infection of potato seed pieces has been demonstrated, with strains differing in their ability to establish latent infection in resist-

ant and susceptible potato clones (Ciampi and Sequeira 1980; Ciampi et al. 1980). In Australia, the introduction of infected potato seed pieces was considered to be the major reason for the occurrence of the disease in cool temperate areas in New South Wales (Lloyd 1976; Graham et al. 1979), Victoria (Harrison 1976), and more recently, South Australia (Akiew 1985).

Insect transmission has been demonstrated for several strains of *P. solanacearum*, but its importance in the dissemination of the disease in affected crops is poorly understood. Perhaps the best documented instances of the important role insects can play in the dissemination of *P. solanacearum* are the epidemics of Moko disease of bananas in Central and South America caused by an insect-transmitted strain of race 2 of *P. solanacearum* (Buddenhagen and Elsasser, 1962; French and Sequeira 1970; Buddenhagen, this volume).

Few studies have considered the possibility of dissemination of *P. solanacearum* on true seed (Devi and Menon 1980). Moffett et al. (1981) showed that artificially infested seed of tomato and capsicum could act as a source of inoculum for the emerging cotyledons. True seed may be a more important source of inoculum for the long distance dispersal of *P. solanacearum* in vegetable crops such as tomato and capsicum than is presently recognised. Tomato seedling transplants have been shown to be responsible for the long distance dispersal of the pathogen from south Georgia, USA to Ontario, Canada (Layne and McKeen 1967).

Localised dispersal of the pathogen can occur by root-to-root spread in tomato and tobacco (Kelman and Sequeira 1965). Mechanical transmission by the use of contaminated implements can also be important (Buddenhagen and Kelman 1964). There are a few reports of transmission by flood and irrigation water, but these are not considered common methods for the dispersal of *P. solanacearum*.

Survival

The survival of strains of *P. solanacearum* in soil and plant debris is poorly understood. There are many reports of the soil-borne nature of bacterial wilt disease but there are widely differing views regarding the longevity of the pathogen in soil. There are a few detailed studies which examine the ability of particular strains to survive in the soil and the influence of environmental factors such as temperature and moisture on survival.

The ecology of bacterial wilt on potatoes is of interest. The potato race (*Pseudomonas solanacearum* race 3) is a low-temperature adapted pathovar which occurs mainly at the extremes of altitude and latitude in the distribution of *P. solanacearum*. The distribution of race 3 in cool temperate areas is due to its close association with potato, and its ability to survive under cool temperatures in the areas most suitable for potato cultivation. *Pseudomonas solanacearum* race 3 may have a limited distribution in warm temperate or tropical regions because of its restricted host range, its close affinity with potatoes and the probable rapid decline of pathogen populations in warm, moist soil where host debris is degraded rapidly. Thus where race 3 occurs in tropical regions, it may have been introduced with potatoes rather than be a natural inhabitant of that environment.

In contrast, *P. solanacearum* race 1 which is also a pathogen of potatoes, is commonly found in tropical and subtropical regions, where it infects a wide range of solanaceous crops including potatoes, and weeds, and these are present over a long growing season. In warmer climates, plant debris decomposes more rapidly and infected debris would provide only temporary sheltered sites for *P. solanacearum*. Thus although both *P. solanacearum* race 1 and race 3 are pathogens of potato, they differ in their host ranges, geographic distribution, and ability to survive under different environmental conditions.

It is therefore misleading to think of bacterial wilt of potatoes as a single disease. Control measures developed on the basis of the behaviour of race 1 may be inappropriate for control of race 3 and vice versa. It is important to consider the local situation and particularly the behaviour of the local strain(s) in order to devise effective control methods for bacterial wilt of potatoes.

For instance, some interesting studies on the survival of *P. solanacearum* in soil have been conducted by Lloyd and his colleagues on the northern tablelands of New South Wales, a cool, temperate region where bacterial wilt of potatoes has occurred in each potato season since 1955. The disease is caused by a strain of the potato race (race 3) and corresponds to a biovar II in the classification of Hayward (1964).

The strain of *P. solanacearum* in northern New South Wales has a limited host range, being restricted to potato and two solanaceous weed hosts, *Solanum nigrum* (Hayward 1975) and *Solanum cinereum* (Graham and Lloyd 1978a). Neither of these weed hosts over-winters vegetatively in the cold winters on the northern tableland. Thus, the bacterium either over-winters in the soil, or is reintroduced in latently infected tubers introduced from warmer areas of Australia.

Shamsuddin et al. (1978), examined the survival in soil of *P. solanacearum* race 3 in northern New South Wales and the value of rotating infected potato crops with pastures as a disease control measure, comparing the survival time under conditions of bare-fallow, weed-fallow and sown pastures (rye grass, cocksfoot and white clover). They showed that in bare-fallow and weed-fallowed soil, the bacterium survived up to 2 years. The population of *P. solanacearum* declined markedly in infested soil rotated with pastures. After 2.5 years rotation with pasture, no infected potato plants were observed when the field was replanted to pasture. Their conclusion was that this strain of *P. solanacearum* does not survive indefinitely in soil in cool temperate conditions and that effective disease management can be achieved by fallowing or pasture rotation in fields for 3 years before resowing to potato. It is important that new potato seed pieces should come from a bacterial wilt-free area if the pathogen is not to be reintroduced into the area.

Other studies by Graham and Lloyd (1979) showed that a strain of *P. solanacearum* (race 3, biotype II), was able to survive better (up to 82 days) in the deep soil layers (55–65 cm) than in the topsoil (10–15 cm) (10 days). They suggested that in addition to survival in sheltered sites such as alternative weed hosts and infected debris, pockets of infestation in the deeper soil layers may serve as an additional site for the long-term survival of the bacterium.

These findings agreed with those of Okabe (1971) in Japan, who detected *P. solanacearum* at depths of 80–100 cm in naturally infested tobacco fields in Japan 4 months after harvest of the crop. The persistence of pockets of infestation in the deeper soil layers could explain reports of the failure of soil disinfectants in the control of bacterial wilt (Kelman 1953).

Graham et al. (1979) also studied the survival of *P. solanacearum* race 3 in plant debris and latently infected potato tubers. They showed that plant debris remained infested with *P. solanacearum* 33 weeks after a potato crop had been abandoned in a field. Soil from a field infested with bacterial wilt also retained *P. solanacearum* for 33 weeks. Tubers from the infested crop harboured latent infections by *P. solanacearum* for up to 26 weeks. It was concluded that partially decomposed plant debris from a previous crop can be an important source of inoculum for new potato crops in the cool temperate regions of New South Wales since the pathogen can survive in decomposing debris from crop harvest in January to the next season's planting in September. Symptomless tubers left in the field are also an important long-term survival site, as these can produce heavily infected plants in the summer which act as foci of infection in a subsequently planted crop.

Effects of Environmental Factors on Survival

There are conflicting reports as to the influence of soil moisture on the survival of *P. solanacearum*. Most reports suggest that *P. solanacearum* is better able to survive under conditions of high soil moisture. This correlates with the field observation that bacterial wilt is more serious in the wet, humid tropics than in desert areas, even under irrigation (Buddenhagen and Kelman 1964). However, there are reports of better survival of some strains under conditions of low soil moisture (Okabe 1971).

Detailed studies by Moffett (1981) and Moffett et al. (1983) in Australia showed that while *P. solanacearum* biovars II and III were sensitive to drying, the pathogen survived under dry conditions provided the pressure potential of the soil remained constant. Populations of biovar II declined more rapidly than those of biovar III. Soil type influenced the rate of population decline, with more rapid decline in soils of higher organic matter, which may be related to a higher level of microbial activity (Moffett et al. 1983).

Studies by Akiew (1985) also showed that the presence of high organic matter in soil caused a more rapid decline in the population of both biovars II and III. Akiew (1985) also suggested that *P. solanacearum* strains did not survive well in South Australia, which has dry summers with high desiccating temperatures.

Alternative Hosts

The wide host range of *P. solanacearum* is well known and there are numerous reports of many different crop plants and weeds that are able to be infected with strains of *P. solanacearum* with or without symptom expression. Within the species there are considerable differences in the host range of individual strains. In general, the solanaceous race (race 1) has a wide host range, the musaceous strain (race 2) has a host range restricted to *Musa* and a few related perrenial hosts, and the potato strain (race 3) is restricted to potato and a few other alternative hosts.

Alternative weed hosts and non-host plants play an important role in the survival of *P. solanacearum* strains in the absence of a susceptible crop. Recent studies by Granada and Sequeira (1983) suggested that the ability of strains of *P. solanacearum*

to invade the roots of resistant cultivars of host species such as tomato, and presumed non-hosts such as bean and corn, may be critical to the survival of *P. solanacearum*. These studies also showed that *P. solanacearum* was a poor soil survivor in its free state. Strains of *P. solanacearum* race 1 survived in soil for up to 20 weeks, while strains of race 2 and 3 survived less than 20 weeks. The same strains were able to invade the roots of non-host plants such as corn and bean, and this extended their ability to survive in the absence of a susceptible host for at least 33 weeks.

Quimio and Chan (1979) showed in the Philippines that the population of a strain of *P. solanacearum* declined in the rhizosphere of crops such as corn, rice and mungbean, but increased in the rhizosphere of the weed, common purseglove (*Portulaca oleracea).*

Alternative hosts are significant in the life cycle of *P. solanacearum* in that they allow the pathogen to survive for extended periods in the absence of a susceptible crop. The ability of *P. solanacearum* strains to survive as a symptomless infection in the roots of alternative weed hosts, or in presumed non-host plants, makes disease control by crop rotations difficult. This may explain the variable results reported for the use of crop rotations as a means of disease control. Reports from Australia (Graham and Lloyd 1979), Sri Lanka (Seneviratne 1976) and Costa Rica (Jackson and Gonzalez 1981), differ in the recommended rotation crops and suggested host-free period, and in the degree of success reported for crop rotation as a means of disease control.

Methodologies for Ecological Studies

Useful ecological studies depend on a sensitive and reliable method to follow changes in a population of *P. solanacearum* over time, often in the presence of competing microorganisms.

Most recent studies rely on the use of selective media. Several useful media have been described (e.g. Karganilla and Buddenhagen 1972; Graham and Lloyd 1979; Granada and Sequeira 1981; Chen and Echandi 1982; Akiew 1985). In most cases, at least one of these or other media can be modified to suit the particular microbial composition of the soil in which the studies are being constructed.

While selective media are useful in monitoring population changes, the development of new selective media can be time-consuming if modifications to existing media do not give satisfactory results. Useful ecological studies have also been conducted using biological assays based on planting susceptible indicator plants in test soils (Graham and Lloyd 1978b; Shamsuddin et al. 1978).

A new possibility for a more sensitive and specific technique to follow population changes of a bacterial strain in soil over time is the rapidly developing serological technique of monoclonal antibodies, which is now being used with plant pathogenic bacteria (Alvarez et al. 1985). This merits investigation for use in ecological studies with *P. solanacearum*. Monoclonal antibodies can be identified which are highly strain-specific. Suitable monoclonal antibodies could be used to compare the survival of indigenous strains on a soil, and strains introduced in infected planting material, to determine when and how much inoculum is being introduced into an area in infected seed pieces and how well the introduced strain spreads and survives in comparison with strains already present in the field. Monoclonal antibodies may also be useful in determining if *P. solanacearum* can be disseminated in true seed.

There is a need for a reliable method to detect latent infections of *P. solanacearum* in potato seed pieces, where there are likely to be low populations of *P. solanacearum*. Serological techniques such as monoclonal antibodies or ELISA techniques are most likely to be useful here.

Conclusion

This brief synopsis of the ecology of *P. solanacearum* shows that while there have been some interesting and useful ecological studies conducted in various locations, many critical questions on the ecology of the pathogen in different environments remain unanswered.

Much of the literature comments on the apparently conflicting results obtained by various workers in ecological studies in different locations (e.g. longevity of the pathogen in soil and infected plant debris; importance of alternative or symptomless hosts; crop rotation sequences and rotation times required for effective disease management).

Many of these discussions in the literature have been based on the premise that bacterial wilt is one disease caused by one pathogen and that results obtained in one environment can be extrapolated to another. Given the extraordinary range of variability within the species *P. solanacearum*, it is to be expected that such an approach is unproductive. In Australia, for example, bacterial wilt of potatoes in the cool temperate areas of southern Australia and bacterial wilt of custard apple (*Annona* spp.) in the subtropical areas of northern Australia are different diseases and different approaches to their control need to be considered.

Similarly, in Asia, there are substantially different diseases commonly referred to as 'bacterial wilt'. These range from bacterial wilt of groundnuts and cassava in Indonesia, to bacterial wilt of potatoes in the tropical highlands of the Philippines and Sri Lanka. It is unlikely that ecological studies conducted elsewhere will provide more than a guide to the understanding of the ecology of *P. solanacearum* in Asia. If effective means of control are to be found, more ecological studies are necessary in the different agroecosystems where bacterial wilt is damaging, to establish the ecology of the disease caused by a particular strain in a particular environment.

For example, bacterial wilt is recorded as a damaging disease of groundnuts and cassava in Indonesia, hosts which are not commonly attacked elsewhere. Both hosts are widely grown throughout the tropics in areas where bacterial wilt occurs on other hosts. These diseases merit more detailed study, due to this anomaly and since both are on economically important crops in Indonesia. Little is known of the strain(s) affecting groundnuts and cassava, their range of alternative or symptomless hosts, or the ability of the strain(s) to survive in soil, plant debris or in association with the roots of host or non-host plants.

With regard to bacterial wilt of potatoes in Asia, there is a need for more information on the distribution of *P. solanacearum* strains on potatoes growing at different altitudes. Does the same race or strain of *P. solanacearum* cause bacterial wilt of potatoes in the highlands of the Philippines and the lowlands of Indonesia? Do the strains on potatoes have the same host range, and ability to survive in soil, plant debris, and in association with weeds on non-host plants? Can strains of *P. solanacearum* be transferred from one region to another as latent infection in potato tubers? Can such strains establish themselves in new environments? As new potato cultivars are being developed for resistance to bacterial wilt, against which strains should they be screened?

Answers to such important ecological questions are essential for the development of a long-term control strategies for bacterial wilt caused by strains of *P. solanacearum* common in the target environments. Disease management by agronomic practices such as crop rotation needs to be based on information on the ability of the prevalent strain of *P. solanacearum* to survive in the absence of its economic hosts, and the influence of cultural practices on its survival.

In summary, the key ecological questions to consider when studying bacterial wilt on a particular crop in a particular environment are:

1 What strain(s) of *P. solanacearum* are present in the population causing the disease?
2 What is the host range of the strain(s), in terms of species on which the pathogen is able to cause symptoms in the field.
3 On which plant species is the pathogen able to cause latent (symptomless) infection in non-host plants and weeds?
4 How long can the pathogen survive in the soil or in association with alternative hosts and non-host plants?
5 How do environmental factors such as temperature, moisture and soil type affect the pathogen's ability to survive in soil or plant debris.?
6 What are the sources of inoculum for a newly planted host? Soil? Alternative hosts? Infested true seed or vegetative planting material?

Answers to such questions would provide a sound ecological basis from which to devise disease control measures, whether these are based on the development of resistant varieties or agronomic management practices. Few such studies have been conducted for bacterial wilt in the Asia/Pacific region. The field is open for some innovative research on the ecology of this important disease.

Acknowledgement

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Influence of Soil Moisture and Temperature on the Persistence of *Pseudomonas solanacearum*

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BACTERIAL wilt caused by *Pseudomonas solanacearum* is an important disease affecting a number of economic crops such as potato, tobacco, tomato and bananas. The host range includes more than 30 families including Solanaceae, Musaceae, Asteraceae, and Fabaceae (Kelman 1953). Buddenhagen and Kelman (1964) described three races based on host ranges. *Pseudomonas solanacearum* is widespread in tropical, subtropical and warm-temperate parts of Asia, Africa, Australia, Europe, North America, Central America and the West Indies (Commonwealth Mycological Institute Map 138 Ed.2).

In naturally infested soils, *P. solanacearum* can survive on infected host-plant debris (Lloyd 1978), alternative wild hosts (French 1983) in the rhizosphere of some weeds and economic crops (Quimio and Chan 1978), and in the roots of presumed non-host crops (Granada and Sequeira 1983). The bacterium can survive for a long time in deep soil layers (McCarter et al. 1969). The effects of differing soil factors on the survival of *P. solanacearum* in soil have seldom been studied by direct techniques, probably due to lack of suitable selective media. Nesmith and Jenkens (1979) found that most media developed to detect *P. solanacearum* from soil were unsatisfactory because they allow growth of too many background bacteria, or they are appropriate only for certain strains of the bacterium. Hence, they developed a new selective medium derived from a modification of the standard triphenyl tetrazolium chloride (TZC) medium. A useful bacteriocin technique was developed by Chen and Echandi (1982). Granada and Sequeira (1983) developed a modified TZC medium with good plating efficiency and high selectivity.

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In studies conducted in South Australia, these three media were evaluated and two such media have been satisfactorily used, with some modification, to investigate the influence of soil moisture and temperature on persistence of *P. solanacearum* in artificially infested field-soil. A summary of the results is presented below. Full details are found in Akiew (1985).

Materials and Methods

Changes in populations of *P. solanacearum* (biovars II and III of Hayward 1964) were monitored over time in artificially infested soil. Soil samples were taken from a permanent wheatfield with characteristics as follows: pH 5.4; total C 1.48%; total N 0.15%; total P 316 ppm; clay 19.4%; silt 31.3%; fine sand 43.8%; coarse sand 2.0% and moisture content at field capacity 24%.

Cultures of *P. solanacearum* were obtained from Dr A. C. Hayward, University of Queensland, Australia. Strains 025 (biovar II), and 170 (biovar III), potato and tomato isolates, respectively, were used to inoculate soils in plastic containers (12 cm diameter × 12 cm deep) at an inoculum density of 10^7 cells per gram oven-dry soil. Soil moisture was adjusted to 20–25, 45–50 and 95–100% of field capacity and was maintained at approximately the same level by constantly weighing the soil and adding sterile distilled water when necessary. The inoculated soil samples were kept in growth cabinets set at 20 and 30 °C.

Bacterial populations in the soil were determined during the first, second, third and fourth day after inoculation, and every 14 days thereafter. A 10 g (oven-dry weight) sample of soil was placed in a flask and distilled water added to the 100 ml mark and shaken for 30 min. Aliquots of 0.1 ml from 10-fold dilutions were spread with a bent pasteur pipette, on plates containing selective medium. The selective medium (Granada and Sequeira 1983),

modified by not including the antibiotic, Thiomersal, contained the following (in mg/l of distilled water): crystal violet 50.0, Polymyxin B 100.0, Tyrothricin 20, Chloramycetin 5.0 (antimicrobial compounds were sterilised in 70% ethanol, diluted to desired concentration); crystal violet was sterilised for 7 min at 121 °C; TZC agar was used as basal medium and each antimicrobial compound added just before dispensing the medium).

The bacteriocin technique developed by Chen and Echandi (1982) was used to check the colonies on the selective medium. Inoculated plates were usually incubated at 30 °C for 2–3 days. The experimental design was a randomised complete block with 12 treatment combinations (2 strains × 2 temperatures × 3 moisture levels), replicated three times.

Results and Discussion

The results presented in Fig. 1 show that the population of *P. solanacearum* decreased sharply with increase in temperature, and with decrease in soil moisture. In general, the biovar III strain survived longer and its density declined at a lower rate than the biovar II strain. At 20 °C and at field capacity, the biovar II strain survived for a period of about 24 weeks whereas the other biovar was still detectable in soil dilution of 10^{-1}–10^{-2} 28 weeks after inoculation (populations of the pathogen were not monitored after that date). With the same moisture level but at 30 °C, the biovar III strain persisted in the soil 12 weeks longer than the other strain, indicating that it is adapted to a wider range of environmental conditions.

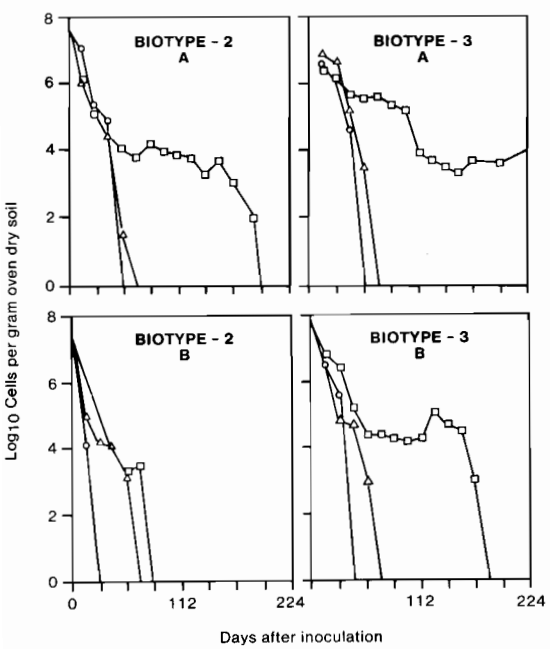

Fig. 1 Population density trends of *P. solanacearum* in soils at 20°C (A), 30°C (B), and at soil moisture of 25% (○), 50% (Δ) and 100% (□) of field capacity. Soil moisture content at field capacity was 24%.

High soil moisture and low temperature appear to favour long-term survival of the bacterium in the soil, whereas the effect of dry soil on bacterial viability appears to be a major factor in the absence of the bacterium or its failure to increase in hot, dry areas. The biovar III (race 1) isolate survived longer and was more tolerant to desiccation than the other isolate (biovar II, race 3). Also, the ability of both types to survive in soil devoid of host-plant debris, but with high moisture and at lower temperature, suggests that *P. solanacearum* can persist in deep soil layers where those conditions are likely to occur. McCarter et al. (1969) investigated the vertical distribution of the bacterium in several soil layers, and found that a high infestation of the pathogen occurred in the top 30 cm, and a low infestation at deeper levels (60–75 cm), and suggested that the absence of the bacterium in the top 15 cm of one soil type was due to the dryness of the soil. The results obtained in this study support his findings.

With the use of selective media, such as those used in this study, more investigations on the ecology of the pathogen can be conducted to provide information that is relevant to the control of bacterial wilt, including resistance breeding programs for crops such as potatoes.

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Effect of Planting Depth and Hilling on Bacterial Wilt in Potato

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BACTERIAL wilt caused by *Pseudomonas solanacearum* is considered the second most important disease on potatoes in the tropics (after late blight caused by *Phytophthora infestans*) and also is an important problem for other solanaceous crops (Buddenhagen and Kelman 1964). Race 1 of *P. solanacearum* as defined by Buddenhagen et al. (1962) is most common in the Philippines, while race 3 only occurs in the highlands of Benguet and Mountain provinces and some isolated areas on Mindanao at higher elevations (Vander Zaag et al. 1985).

Large parts of Mindanao are between 500 and 1500 m above sea level and climate and soils are favourable for potato production. However only 110 ha were planted in 1983 with average yields of 7–8 t/ha in Bukidnon province, the major potato-growing province of the island. Bacterial wilt is the most important disease accounting for the small area planted and the low yields (Kloos and Fernandez 1985). The pathogen occurs in virgin soils and has an exceptionally wide host range (Hayward 1978). High rainfall, its uniform distribution and non-flooding of the soils in Mindanao permits bacterial wilt to survive, even if non-susceptible hosts are grown (Vander Zaag et al. 1985). Also contaminated seeds are an important source of latent infection (Ciampi et al. 1980). Resistant varieties in combination with improved agricultural practices are needed to control the disease and have economically acceptable yields.

Root damage facilitates entry of bacteria into plant tissue, and this can be a result of the hilling operation. In this paper results are presented of six experiments evaluating the effect of planting depth and hilling on the incidence of bacterial wilt.

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Materials and Methods

All experiments except No. 3 were grown at Dalwangan, Bukidnon (800 m elevation) in natural bacterial wilt-infested soils. For all experiments 210 kg/ha N, P_2O_5 and K_2O along with 7 t/ha chicken manure were applied in the row and mixed with the soil prior to planting. Chemical control of pests and diseases was applied during the growing season and Furadan 3G was applied in the planting hole to control RKN. Bacterial wilt incidences were recorded at weekly intervals.

Experiment 1

Cultivar Cruza 148 was planted with three seed sizes (medium 35 g, small 17 g and pebble 7 g per tuber) and three planting depths (2, 5 and 10 cm of soil on top of the tuber). Per treatment 12 tubers were planted in a double row bed 1.2 m wide at a spacing of 4.4 hills/m^2. Hilling was done 3 weeks after emergence. The experiment was planted 21 June in three replications and harvested 3 October 1984.

Experiment 2

Cultivar MS-35-22 was planted with two seed sizes (medium 39 g and small 17 g per tuber) and two planting depths (2 and 5 cm of soil on top of the tuber). Per treatment 12 tubers were planted in a double row bed 1.2 m wide at a spacing of 4.4 hills/m^2. No hilling was done. The experiment was planted 9 July in three replications and harvested 3 October 1984.

Experiment 3

Cultivars Cosima and Isola were planted with two planting depths (2 and 10 cm of soil on top of the tuber), combined with hilling and no hilling. The experiment was planted in four locations in farmers field: two in Intavas, Impasogong and two in Cawayan, Lantapan. Per treatment 3 double row beds per cultivar 1 m wide each and 7 m long were

planted at a spacing of 4.0 hills/m^2. Hilling, in the hilling treatments, was applied 2–3 weeks after emergence. The experiment was planted in the second week of August with one plot Cosima and one plot Isola per location and harvested the last week of October 1984.

Experiment 4

Cultivar Cosima was planted with three planting depths (2, 5 and 10 cm of soil on top of the tuber) combined with hilling and no hilling. Per treatment three double row beds 1 m wide each and 4 m long were planted at a spacing of 4.0 hills/m^2. Hilling, in the hilling treatments, was applied 2 weeks after emergence. The experiment was planted in four replications 13 November 1984 and harvested 17 January 1985.

Experiment 5

Cultivar Isola was planted with three seed sizes (large 58 g, medium 40 g and small 21 g per tuber) and three planting depths (2, 5 and 10 cm of soil on top of the tuber). Per treatment one double row bed 1 m wide and 4 m long was planted at a spacing of 4.0 hills/m^2. No hilling was done. The experiment was planted in four replications 4 June and harvested 23 August 1985.

Experiment 6

Cultivar Isola was planted with three planting depths (2, 5 and 10 cm of soil on top of the tuber) combined with hilling at two stages (3 and 5 weeks after planting) and no hilling. Per treatment one double row bed 1 m wide and 4 m long was planted at a spacing of 4.0 hills/m^2. The experiment was planted in four replications 6 June and harvested 23 August 1985.

Results and Discussion

Planting Depth

Deeper planting increased bacterial wilt infection. In all experiments, except in experiment 1, tubers planted 2 cm deep resulted in fewer incidences of wilt compared with tubers planted 5 and 10 cm deep (Table 1). In experiment 1 hilling might have caused a higher infection in 2 cm compared with 5 cm planting depth. The shallow root system developed by the 2-cm-deep planted tubers was more injured as a result of the hilling operation. Tubers planted 10 cm deep didn't always have the highest bacterial wilt infection, while differences in infection between 5 and 10 cm deep planted tubers were small.

Table 1. The last bacterial wilt incidence (%) recorded in each experiment as affected by the planting depth.

Planting depth (cm)	Experiment					
	1	2	3	4	5	6
2	18	51	15	50	12	15
5	11	63	—	67	22	19
10	31	—	23	62	16	25
cv %	61	29	47	26	74	63

The number of lenticels per unit of tuber surface is influenced by moisture conditions in the soil, particularly during the time of tuber formation (Meinl 1966). Under dry conditions a suberised layer forms below the complementary cells of the lenticels while increased soil moisture leads to swelling of the cortical cells and eventually rupture of the suberised layer, resulting in proliferating open lenticels (Perombélon and Lowe 1975). This probably facilitates entry of bacteria in plant tissue. With increased planting depth roots and tubers are formed under increased moisture conditions, resulting in more open lenticels and easy entry of bacteria in the plant tissue. Particularly under high rainfall conditions, like in Mindanao, increased planting depth resulted in a higher bacterial wilt infection.

Despite increased bacterial wilt infection, deeper planting had a slight positive effect on the total tuber yield, except in experiment 2 (Table 2). In experiment 2 infection occurred in an earlier stage of plant development in tubers planted 5 cm deep. Also bacterial wilt incidence differed between 2 and 5 cm planting depths, and was more marked in an early stage of plant growth than in a later stage, resulting in a higher yield for shallow-planted tubers.

Table 2. The total tuber yield (t/ha) in each experiment as affected by the planting depth.

Planting depth (cm)	Experiment					
	1	2	3	4	5	6
2	12.9	13.0	19.5	5.2	11.3	11.9
5	15.8	9.6	–	5.2	11.7	13.3
10	16.3	–	20.9	6.1	12.1	12.5
cv %	37	25	17	24	18	13

In experiment 1, deeper planting had a substantial positive effect on total tuber yield compared with shallow planting: 15.8 t/ha and 16.3 t/ha versus 12.9 t/ha for 5, 8 and 2 cm deep planting respectively. In experiments 3, 4, 5 and 6 differences were small but deep planting had a slightly higher yield. More affected by the planting depth was the marketable yield. Shallow planting resulted in a higher greening incidence (Table 3).

Table 3. Greened tuber weight (%) in each experiment as affected by the planting depth.

Planting depth (cm)	Experiment					
	1	2	3	4	5	6
2	-	-	17	14	26	17
5	-	-	-	7	15	9
10	-	-	7	3	4	3
cv %			36	40	40	67

Under the high rainfall conditions in Mindanao, no effect of planting depth on emergence was observed in most experiments. Only in experiment 3 deep-planted tubers emerged earlier and had a faster initial growth due to a relatively dry period at planting time. In deeper soil layers moisture is better preserved and temperature is more favourable for plant growth under hot conditions (Beukema and Vander Zaag 1979).

Hilling

The effect of hilling on the incidence of bacterial wilt is shown in Table 4. The hilling treatments 3 and 5 weeks after planting of experiment 6 were pooled, and no differences in infection as a result of timing of hilling was observed. Hilling results in root injury and through the wounds bacteria entry is facilitated. In experiments 3 and 6 hilling did not increase bacterial wilt infection, while in experiment 4 hilling resulted in 10% more infection. In experiment 4 bacterial wilt incidences occurred in an early stage of plant development. The bacterial wilt pressure was high and bacteria could easily enter plant tissue through the wounded roots. In experiments 3 and 6 bacterial wilt pressure was low and incidences occurred in a later stage of plant development. Wounds as a result of the hilling operation had ample time to cure, not facilitating an easy entry of bacteria in plant tissue.

Table 4. The last bacterial wilt incidence (%) recorded in each experiment as affected by hilling applied.

	Experiment		
	3	4	6
No hilling	18	55	22
Hilling	19	65	18
cv %	17	31	45

In these experiments no interaction between hilling and planting depth on bacterial wilt infection was observed. Shallow formed roots of tubers planted 2 cm deep were expected to be more injured by hilling compared with deep-planted tubers, resulting in a higher infection. In experiments 3 and 6 wounds had ample time to cure before bacterial wilt incidences occurred. In experiment 4 no interaction was observed although infection occurred in an early stage of plant growth. In experiment 1, tubers planted 2 cm deep had a higher infection compared with tubers planted 5 cm deep. This could be attributed to more root damage in the shallow planting. This was not confirmed, however, by results of experiments 3, 4 and 6.

Hilling had a positive effect on yield in experiments 3 and 6 (Table 5), while in experiment 4 it was the reverse. Under low bacterial wilt pressure (experiments 3 and 6), incidences were not affected by hilling, resulting in an increased yield for hilling treatments. Under high bacterial wilt pressure (experiment 4), yields were generally low and negatively affected by hilling, as a result of increased bacterial wilt infection after the hilling operation. The marketable yield was positively affected by hilling, because less greening occurred (Table 6). Particularly under high rainfall conditions in Mindanao, raised beds are easily eroded, exposing tubers.

Table 5. The total tuber yield (t/ha) in each experiment as affected by hilling applied.

	Experiment		
	3	4	6
No hilling	19.2	6.0	10.7
Hilling	21.2	5.0	13.5
cv %	15	33	19

Table 6. Greened tuber weight (%) in each experiment as affected by hilling applied.

	Experiment		
	3	4	6
No hilling	18	12	18
Hilling	6	4	5
cv %	43	33	58

Conclusion

Under high rainfall conditions increased planting depth increases bacterial wilt incidences. More open lenticels formed facilitate entry of bacteria in plant tissue.

Deeper planting has a positive effect on marketable yield since less greening occurs, while total tuber yield is slightly higher.

Under low bacterial wilt pressure hilling doesn't affect the incidences, since wounded roots have ample time to cure, while under high bacterial wilt pressure hilling results in an increased bacterial wilt infection.

Hilling has a positive effect on total tuber yield under low bacterial wilt pressure, and greening is positively affected under high rainfall conditions.

No interaction between hilling and planting depth as a result of different levels of root injury on bacterial wilt incidences was observed.

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Potato Production Under *Pseudomonas solanacearum* Conditions: Sources and Management of Planting Material

P. Vander Zaag*

PSEUDOMONAS SOLANACEARUM is the causal organism of bacterial wilt or brown rot, major diseases of potatoes in the lower elevation tropics. The problem of bacterial wilt has been observed from sea level to over 2500 m. The general characteristics of those environments are: a udic moisture regime which has less than 90 days of moisture deficit (Soil Taxonomy 1975). Temperatures can vary from mesic (average of <15 °C at 50 cm soil depth) to isohyperthermic, however soil temperatures rarely exceed 30 °C. Personal observations in Southeast Asia suggest that flooded soils, sugarcane lands having a pronounced dry season (ustic moisture regime) and river flood plains (flooded annually and experience extreme heat when dry) are free of bacterial wilt. Results show that introduced bacterial wilt in a sugarcane field (clay loam soil having an ustic moisture) and isohyperthermic temperatures with soil temperature approaching 40 °C when dry and exposed has disappeared after 1 year (unpublished data). Most potato production in India (Punjab), West Bengal, Bangladesh and Vietnam is in lowlands having a pronounced dry season after flooded rice or wheat.

Potato production in areas with bacterial wilt is presently limited to those with low levels of incidence of wilt, and farmers have been successful to a certain degree in growing potatoes. The area in the tropics when the disease is a problem is generally typified by resource-poor subsistence farmers particularly those living between 200 and 1000 m elevation. They do low-input farming and often do limited slash and burn agriculture. These farmers also have limited education and no source of credit and are thus unable to make major investments and cannot afford to take risks. Using a series of case studies, I want to show how farmers and scientists have been successful and in other cases unsuccessful in growing potatoes as a result of the availability or non availability of good planting material. The basis for this is personal observations and experience. Based on those examples, I wish to suggest some strategies and improved practices.

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Case Studies

Rwanda

Over 40 000 ha of potatoes are grown at elevations from 1500 to 3000 m. Bacterial wilt is observed up to 2300 m. There has been a general movement of seed down from the higher elevations. Mixed cropping with beans, peas, sorghum and maize has reduced planting populations and this has reduced the rate of spread. In 1979 a seed farm was established at an elevation of 2300 m, and the disease was present. Through the use of positive selection (extremely healthy plants and not neighbour to a virus or bacterial wilt plant) and negative selection (removal of all virus and bacterial wilt plants) seed production was established (Fig. 1, Tegera and Vander Zaag 1981). Even after 6 years, using a 2-year rotation, seed production has been successful even with the presence of the disease. Results did show that plants bordering on a bacterial wilt-infected plant often did carry latent bacterial wilt and this was manifested when it was replanted particularly at lower elevations (CIP 1984). A similar result was observed in Kenya when seed produced at 2480 m had some latent tuber infection when placed in warmer temperatures (Nyangeri et al. 1984). In Rwanda neighbouring plants to bacterial wilt-infected plants are also rogued, reducing latent infection to much lower levels. The seed program in Rwanda has provided a flush-out system whereby farmers replace seed once every 5–10 years and by the seed moving down hill, potato production has been very successful even as production expands with the presence of bacterial wilt.

Burundi

Potatoes are grown at elevations of 1700 to 2500 m with most of them being above 2000 m. In contrast to Rwanda, potato production has decreased to 10 000 ha because of bacterial wilt. The yields are low due to the disease and *P. infestans* problems. Burundi experiences a more distinct cool dry season forcing farmers to plant potatoes in the valleys on organic soils during the dry season. Here they have enough moisture but also bacterial wilt and *Meloidogyne* spp. nematodes which infect roots and render them completely susceptible to bacterial wilt. Little or no *P. infestans* is present. Potatoes from these valleys are replanted on the hills. The movement of seed up and later down has resulted in the spread of bacterial wilt with the seed (latent infection) and caused severe reductions in yield. This along with high levels of *Meloidogyne* spp. nematodes and the dependence on one variety, Arka, has left Burundi with very poor potato production levels as of 1982. Concerted efforts at seed potato production of cultivars with combined resistance to bacterial wilt, *P. infestans* and nematodes is in progress which will lead to a gradual improvement of potato production (Potts, personal communication). The lack of elevation areas >2400 m and the presence of chemically poor highly weathered soils make this a great challenge.

Philippines

Potatoes have been grown on various mountains in the Davao, Misamis Oriental, Bukidnon and Zamboanga areas of Mindanao at elevations of 800–1300 m. Initially after clearing the forest, potatoes have grown well, although cases of bacterial wilt have been encountered. In 2 or 3 seasons, disease levels have increased due to latent infection of tuber seed and a spread of the wilt in the soil. The remoteness of Mindanao from the highlands of Benguet (2000–2400 m) has left the farmers using local small tubers for seed purposes. Due to this isolation, significant potato production only remains on the high slopes of Mt Kitanglad in Bukidnon with slightly more than 100 ha grown annually (Kloos and Fernandez 1985). The topography on this mountain has permitted farmers to grow potatoes on higher, newly cleared areas. The potential for potato production in Mindanao is well over 5000 ha if a proper seed production scheme coupled with appropriate cultivars is established. Presently, cuttings and true potato seed (TPS) are being used to produce many tuberlets in small 'clean' areas. These tuberlets are used as the planting material for commercial crops. Preliminary results have been good, however government support is needed to successfully carry out such a program on a large scale.

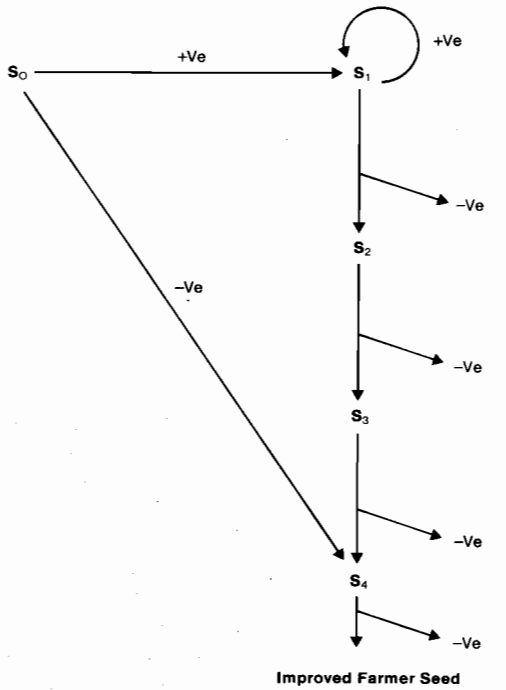

Fig. 1. Simple selection program used in Rwanda for improved tuber seed production. S_0 is locally available tubers starting the program. Best plants selected become basic seed S_1. In the S_1 through positive (+ ve) selection the best plants remain S_1. Through negative (– ve) selection (roguing out all virus, off types, bacterial wilt and neighbouring plants to bacterial wilt plants) you arrive at S_2 and repeat this to S_3 and S_4 and improved seed for farmers.

Indonesia

South Sulawesi is proximate and similar to Mindanao in topography and climate. The potato production status is similar with severe bacterial wilt resulting in low yields of 3–7 t/ha. The inability to get clean seed from the highlands of Java has resulted in a continued limited production of less than 1000 ha in a few high locations (>1000 m) namely: Bantaeng, Malino and Enreking.

Central Highlands, Vietnam

The paper by Tung (these Proceedings) gives the background information on the situation. Basically there is no potato production at elevations between 10 and 1100 m due to bacterial wilt. In Dalat (1100–1500 m), the disease is managed through the use of cuttings which provide clean

seed stocks in an informal seed program. The replacement of the seed is every 2-3 years using a flush-out system. A strict crop rotation which has limited potato production to about 400 ha on 1200 ha of cultivated land has also been very helpful in keeping bacterial wilt at a manageable level (Uyen and Vander Zaag 1985). The potential for expanded production in areas of 800–1300 m is great once a more resistant cultivar is identified for the use of cuttings. Government authorities are focusing on the use of TPS as a better source of clean planting material in these lower elevations.

Fiji

The paper by Iqbal and Kumar (these Proceedings) gives the background information on the present situation. Bacterial wilt is a serious constraint both on the hills (800 m) and in the Sigatoka Valley (100 m). The highlands have only a limited cool dry season. The valley is growing many vegetables, has irrigation, and thus bacterial wilt is maintained continuously. Farmers indicated that they could reduce bacterial wilt levels by drying out the soil during the hot dry months and ploughing the soil once or twice. There is no seed program and no adequately resistant cultivar which has acceptable tuber qualities and wide adaptation. The cultivar Domoni has continued to show good resistance but has limited adaptation. Fiji continues to import over 10 000 t of table potatoes annually. The main strategies being developed are again the use of TPS, selection of resistant widely adapted cultivars and identifying areas free of bacterial wilt, and particularly intercropping with sugarcane.

Recommended Strategies and Practices

Simple Selection

At the national level, a seed production program must be operational in the area or island where the potatoes are grown. This program should not be a certified seed program in the traditional form. The only objective should be to produce seed of superior quality to that of the farmer. This was the case with the program in Rwanda (CIP 1982). The Rwanda program had a four-step program (Fig. 1). S_1 seed was always selected on the basis of the best plants in the field true to type for the variety. The S_1 seed would be planted to produce S_2, which when replanted became S_3 similarly S_4.At each step only negative selection was done. Negative selection included removal of virus as well as bacterial wilt plants from the field and the removal of neighbouring plants to the bacterial wilt infected plants for table potato purposes. Later it became apparent that neighbouring plants should also be removed immediately (CIP 1984). Such a program is simple, not requiring a virology lab but simply good eyesight and a commitment to roguing on a regular basis. Such a program should be conducted on land at the highest elevation possible in the potato-growing area.

Farmers should also have a simple selection program. In Rwanda this did not meet with much success as results did not show much benefit from selection. Under moderate to high bacterial wilt pressure, selection of the best plants for seed purposes is suggested. The roguing of infected plants simply goes against the values of most poor farmers. In Rwanda, farmers became upset if too many wilted plants were rogued. The work with the farmers should be followed by the national level seed program staff located nearby on the seed farm.

True Potato Seed

The problem of latent tuber infection can be overcome through the use of TPS. The suggested methodologies for the use of TPS would be one of the following. A national level seed production program would operate a small farm or nursery to produce bacterial wilt-free tuberlets. This would involve the use of simply constructed nurseries in which TPS is sown directly or pricked as outlined by Wiersema (1985). These nurseries should use fumigated or sterilised local media or subsoil (free of bacterial wilt) to assure 100% freedom from the disease. The harvested tuberlets would then be further multiplied to produce S_2, S_3 and possibly S_4 as outlined above using negative selection (Fig. 1). The number of steps is dependent on the seed requirements (demand and quality). The other option would be to provide tuberlets from TPS directly to the farmers and assist them in properly multiplying them using negative selection for bacterial wilt plants. The general reluctance of farmers to do negative selection (removal of plants) may require the use of positive selection of the best plants or areas of the field for seed purposes.

The second alternative would be for farmers to establish their own nurseries. TPS seed would be provided by the national agency or preferably the farmer could produce his own hybrid or open pollinated seed of the appropriate cultivar. His nursery would have to use local subsoil (generally free of bacterial wilt) and apply lime and NPK as necessary. The tuberlets produced would provide his planting material for his commercial crop. From his commercial crop he should make positive selection of the best plants for seed purposes.

As farmers in such areas are generally poor, they do not have controlled irrigation facilities, therefore I see little possibility for the use of TPS as a means of producing transplants for field production.

Rapid Multiplication Using Cuttings

This could be utilised in a similar fashion as outlined under the two alternatives for the use of TPS. In vitro plants would have to be maintained by the national seed program for use in producing mother plants and cuttings at the seed farm in producing tuberlets. This methodology has proven successful in the Philippines in a mid elevation (800 m) location (Graza and Vander Zaag 1985). The resultant tuberlets could be replanted at the national seed farm or given to the farmers for multiplication as outlined under TPS.

The second alternative would be the production of tuberlets from in vitro material by farmers. This would only appear possible in cases where the national seed program would provide at least the initial mother plants to the farmers. The farmer could produce more mother plants and establish cuttings as transplants in high-density beds. The rest of the procedure would be similar to those outlined for TPS.

The ability of farmers in Vietnam to successfully produce seed and commercial potatoes is probably an exceptional case (Uyen and Vander Zaag 1983, 1985). These farmers are not resource-poor, they do intensive vegetable farming and are able to irrigate their land. The use of cuttings has been used by farmers to produce commercial (large) and seed (small) potatoes. It was observed that about 5% of the land needs to grow cuttings to provide enough small tubers to keep seed for three generations to cover the remaining 95% of the area (Uyen and Vander Zaag 1985). After three generations problems of bacterial wilt and viruses had risen to such levels that seed needed to be replaced.

Farmers preferred to plant tubers compared to transplants (cuttings) as it required less labour and produced superior yields particularly under short day conditions from November to March.

Reduction and Avoidance of Bacterial Wilt

In the areas where bacterial wilt is a problem much could be done to reduce it. The most important is sanitation. The burning or burial of all bacterial wilt-infected plants and tubers in deep pits at the lower end of fields is probably the simplest. At harvest bacterial wilt-infected tubers are invariably left in the field, spreading the disease. Sterilisation of hoes, feet and any equipment used is essential; proper grading of seed at the storage and proper burial of infected tubers is mandatory. The removal of volunteer plants and the control of alternate hosts such as other solanaceous crops and weeds is important. During a dry part of the year allow the soil to bake through drying and ploughing every 4–5 days. These few simple steps will do much to reduce bacterial wilt levels.

Avoiding bacterial wilt is difficult but possible. Frequently fields will not have initial infection after clearing or before introducing the potato. The use of clean planting material is the most obvious method of avoiding the disease. Rotating potatoes with non-host plants such as maize, sugarcane or flooded paddy rice has proven beneficial in many cases. The failure in other cases is attributed to contaminated water coming from higher elevation areas.

Conclusion

Bacterial wilt is a major constraint to expanding potato production at elevations of 500 to 1000 m where the climate is favourable for both potatoes and the disease. A combination of bacterial wilt-resistant and heat-adapted cultivars, good agronomy and a carefully planned planting material production program are essential in expanding the crop in bacterial wilt areas. The placement of government priority, financial and manpower support are essential to establish and maintain an effective planting material program.

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Potential Biological Control of Bacterial Wilt in Tomato and Potato With *Bacillus polymyxa* FU6 and *Pseudomonas fluorescens*

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THE bacterial wilt disease caused by *Pseudomonas solanacearum* is a serious disease in the Philippines, except in areas of high elevations and areas near the seashores. An estimated loss of 10–15% is attributed to the effects of this disease (Zehr 1969).

Bacterial wilt disease cannot be controlled economically or effectively by soil fumigants or systemic fungicides (Parel 1958). Control measures commonly employed include resistant cultivars, crop sanitation, crop rotation and other cultural practices, selection of disease-free planting material, and more recently, the use of microbial antagonists. The use of resistant varieties is the most popular way of controlling the disease. Development of resistance has been hampered by the linkage of some resistance genes to small fruit size, and the high degree of variability of the pathogen.

The use of microbial antagonists has been noted as a promising control strategy. In 1953, Kelman reported that various fungi, actinomycetes, and bacteria exhibited antibiotic effects against *P. solanacearum*. They include *Bacillus mesentericus, B. megaterium, B. subtilis, B. prodigisus, B. mycoides, B. proteus, Azotobacter chroococcum, Erwinia aroideae, Aspergillus oryzae, Actinomyces californicus,* and *A. violaceus-ruber.* Direct evidence of the potential use of antagonists was reported by Celino and Gottlieb (1952). They found that by treating the infested soil with the broth cultures of *Bacillus polymyxa* B_3A, only 33% of the total population of tomato succumbed to bacterial wilt as against 70% on non-treated infested soil. More recently, Ling (1977) tested a total of 106 fungi, actinomycetes, and bacteria for their antagonistic activities against *P. solanacearum*. Only 13 isolates were antagonistic to the pathogen in vitro. Of these, three fungi and one bacterium were selected and tested for effectiveness when applied to tomato seedlings transplanted to infested soil. The three fungal isolates retarded seedling growth and failed to reduce wilt while the bacterial isolate identified as *Bacillus* sp. reduced wilting incidence by 46–70% using the 'seedling-watering' method.

Bacteria can be important in biological control of plant pathogens. They may exceed the number and weight of any group of microorganisms in soil, and their rapid growth and ability to utilise varied substrates under different conditions is surpassed by no other group (Baker and Cook 1974). Some species such as the *Bacillus* spp. and *Clostridium* spp. have the added advantage of being able to produce heat-resistant spores while others are motile. While some bacteria are antibiotic producers, others are effective fast-growing colonisers. Bacilli and pseudomonads are abundant in the rhizosphere and they could be important in competition with the root pathogens (Baker and Cook 1974). The experiment described below was designed to determine the extent by which *Bacillus polymyxa* FU6 and *Pseudomonas fluorescens* could effectively protect tomato and potato from bacterial wilt by pre-emptive colonisation of the plant roots.

Materials and Methods

Plant Preparation

Plant varieties used were Yellow Plum (susceptible) and VC 11-1 (medium-susceptible) cultivars of tomato and the Red Pontiac (susceptible) cultivar of potato were used in this experiment.

Tomato seeds were sown directly into moist sterile soil contained in plastic trays, and grown 8 days before bacterial treatment.

Potato plants were surface-sterilised with disinfectants. The potato nutrient solution (potato me-

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dium) was prepared, dispensed in tubes and then sterilised by autoclaving in a pressure cooker at 121 °C for 15 minutes. Single-node cuttings were prepared under the laminar flow hood and each cutting was inserted into a tube with the node resting on the filter paper bridge. Incubation of potato plants under illumination was carried out for 2 weeks. Further propagation was carried out using the same procedure until the required 80 plants were obtained.

Preparation of Bacterial Cultures

Bacillus polymyxa FU6 was isolated from the roots of *Ficus ulmifolia* obtained from Taal Volcano (Aspiras et al. 1985). It is a fast-growing, gram-positive rod and showed positive test for in vitro nitrogenase activity (acetylene reduction assay).

Pseudomonas fluorescens was isolated from a water sample taken from Laguna de Bay. It is a fast-growing, gram-negative rod. Both bacteria are mobile.

Bacillus polymyxa, Pseudomonas fluorescens and *Pseudomonas solanacearum* were grown on nutrient agar. The plates were incubated at 30 °C for 24 hours. Cell suspension was prepared by washing the bacterial growth into a beaker and the volume was adjusted to obtain O.D. at 540 nm of 0.30 (equivalent to 50% transmittance) using a Spectronic 20.

Bacterial Treatment

Before bacterial treatment, a sharp blade was used to cut deep into the soil at approximately 2.5 cm away from the base of the tomato seedlings Cultivar (VC 11-1) in order to damage a portion of their roots. This was followed by pouring at the base of the plant *Bacillus polymyxa* or *Pseudomonas fluorescens*. One hundred millilitres of the culture suspension was applied per tray or 50 ml per row, this being sufficient to moisten the base of the plant.

One day later, the *Pseudomonas solanacearum* cultures were applied the same way to those that received either *B. polymyxa* FU6 or *P. fluorescens*. In addition, a new set of two trays was given the *P. solanacearum* treatment alone while an equal number of trays remained untreated (control). There were approximately 30 plants per row or a total of 60 plants/tray.

In the case of the wilt-susceptible cultivar of tomato (Yellow Plum) the seedlings were removed from the tray and a small portion of the fine roots was cut using a pair of scissors. Two seedlings were transplanted into a small clay pot (size 4) containing sterile soil. There were five pots per treatment. Fifteen millilitres of either *B. polymyxa* FU6 or *P. fluorescens* cell suspension was poured at the base of the plants per pot. Five hours later an equal volume of the cell suspension of *P. solanacearum* was added to the plants.

The rooted potato cuttings were removed from the test tubes and placed in sterile distilled water, a small portion of the fine roots cut, and soaked in *P. fluorescens* and *B. polymyxa* FU6 culture suspension for 1 hour. The plants were planted in small plastic bags containing sterile potting medium composed of pig manure and husk straw in a 1:1 mixture by volume. Each bag was then moistened with 15 ml of the *P. solanacearum* cell suspension. Twenty bags were maintained per treatment.

The tomato plants were maintained in the greenhouse. The potato plants were kept in a growth chamber provided with artificial light and maintained at 22 °C.

Confirmatory Test for the Presence of *P. fluorescens*

Two weeks after incubation, potato plants treated with *P. fluorescens* plus *P. solanacearum* were dug out from plastic bags and 1 g of fresh roots was placed in a 250 ml Erlenmeyer flask containing 100 ml water. The roots were shaken for 20 min in a water bath shaker maintained at 28 °C. Serial dilutions to 10^{-5} were prepared. Aliquots (0.1 ml) from each dilution were transferred to King's medium Bagar (King et al. 1954). The plates were incubated overnight at room temperature, then placed under UV lamp to detect the presence of fluorescent colonies. The fluorescent pseudomonad is distinguishable from other organisms because it produces a yellow-green pigment (fluorescein).

Test for Antagonistic Effect of *B. polymyxa* FU6 against *P. solanacearum*

In vitro determination of the antagonistic effect of *B. polymyxa* FU6 against *P. solanacearum* was made by the agar plug method. First, *B. polymyxa* FU6 was surface-planted on yeast-malt extract agar. After incubation at room temperature for 3 days a sterile cork borer, 1 cm diameter, was used to obtain agar plugs of *B. polymyxa* FU6. These agar plugs were planted aseptically on antibiotic assay plates prepared previously by inoculating 4 ml of top agar with a loopful of *P. solanacearum* and overlaying it onto the antibiotic medium 1 plates. The assay plates were incubated at 32 °C for 24 hours and then examined for the presence of inhibition zones around the agar plugs.

Results and Discussion

The wilt-susceptible tomato cultivar Yellow Plum was devastated by bacterial wilt 4 days after treatment with the wilt organism (Table 1). However, the wilt incidence was reduced markedly by treating the seedlings 5 hours earlier with *Bacillus polymyxa* FU6 and *Pseudomonas fluorescens* prior to *Pseudomonas solanacearum* treatment. Survival rates against bacterial wilt were maintained at 60 and 90% for 10 days from treatment with *B. polymyxa* FU6 and *P. fluorescens,* respectively.

Table 1. Percent survival of young tomato (cv. Yellow Plum) plants under different bacterial treatments (average of replicates).

Treatment	Days after treatment	
	4	10
Control (untreated plants)	100	100
P. solanacearum	0	0
B. polymyxa FU6 + *P. solanacearum*	60	60
P. fluorescens + *P. solanacearum*	90	90

Using a medium-susceptible tomato cultivar, VC 11-1, the trends of results obtained were identical (Table 2). The plants were destroyed by wilt 5 days after treatment with *P. solanacearum*.

Table 2. Percent survival of tomato (cv. 11-1) seedlings under different bacterial treatments.

Treatment	Days after bacterial treatment		
	5	10	15
Control (untreated plants)	88	87	87
P. solanacearum	4	0	0
B. polymyxa FU6 + *P. solanacearum*	90	85	85
P. fluorescens + *P. solanacearum*	97	94	93

However, when *B. polymyxa* FU6 and *P. fluorescens* were inoculated 1 day earlier than *P. solanacearum,* the wilt incidence was controlled completely.

In the case of potato, only *P. fluorescens* proved effective in reducing the wilt incidence (Table 3). The *B. polymyxa* FU6 culture used may have been contaminated and this may explain its poor performance in the control of bacterial wilt.

While both *B. polymyxa* FU6 and *P. fluorescens* have been shown effective in controlling bacterial wilt experimentally it is not known if these bacteria would be able to sustain their effectiveness over long periods of time. In tomatoes, the trends of results were consistent for at least 10 days for Yellow Plum and for 15 days in the case of VC 11-1.

Table 3. Percent survival of the potato (Red Pontiac) rooted cuttings subjected to different bacterial treatments.[a]

Treatment	Time (weeks) from treatment	
	1	2
Control (untreated plants)	94	89
P. solanacearum	94	39
B. polymyxa FU6 + *P. solanacearum*	86	57
P. fluorescens + *P. solanacearum*	94	80

[a] These data form part of the undergraduate thesis of Mr Emmanuel S. Lantican for a BS Biology degree at UPLB, 1985.

Roots of potato cultivar Red Pontiac obtained 2 weeks after treatment with *P. fluorescens* and *P. solanacearum*, were dominated by the fluorescent bacterium (up to 10^5cells/g fresh root). Root samples were not examined similarly for *B. polymyxa* FU6. Direct microscopic examinations of root revealed the presence of bacterial spores on the surface.

The beneficial attribute of these bacteria in disease control derives mainly from their ability to grow rapidly and colonise root surfaces. In vitro determination of the antagonistic effect of *B. polymyxa* FU6 against *P. solanacearum* failed to establish the production of antibiotic by *B. polymyxa* FU6 against *P. solanacearum*. However, Broadbent et al. (1971) have shown that *Bacillus* isolate WW 27 could exert antagonistic effect on *Pythium* damping-off of snapdragon seedlings.

Bacillus spp. are highly suitable for use in biological control because of the following characteristics: omnipresence in soils; high thermal tolerance; rapid growth in broth culture; and ready formation of resistant spores. *Bacillus polymyxa* FU6 is particularly interesting because of its ability to fix atmospheric nitrogen. The potential of this organism to contribute nitrogen to crops will be looked into in the near future.

Pseudomonas spp., on the other hand, are metabolically active, have a high growth rate and aggressively colonise root systems (Burr et al. 1978). Some of these, specifically those belonging to the *P. fluorescens* and *P. putida* groups, caused substantial increases in plant growth and yield. They would then fall under the category of plant growth-

promoting bacteria (PGPB) to describe these and other bacteria well-adapted as epiphytes on plant roots and to differentiate them from soil bacteria that do not colonise roots or cannot do so aggressively.

By treatment of potato seed pieces with specific strains of *Pseudomonas fluorescens* and *P. putida* yields increased 5–33% in field plots in California and Idaho (Burr et al. 1978; Kloepper et al. 1980a). The treatment of sugar beet seeds with selected strains of fluorescent *Pseudomonas* spp. resulted in yield increases of 4–8 t/ha in 6 of 8 trials with increases in sugar ranging from 955 to 1227 kg/ha (Suslow and Schroth 1982).

In considering the cost effectiveness of the development of this potential biological control method, we compared the growth of the root-colonising bacteria in coconut water and nutrient broth (Table 4).

Table 4. Comparative cell yields of *B. polymyxa* FU6 and *P. fluorescens* in coconut water and nutrient broth (average of four replicates). The cultures were grown in 50 ml volumes and incubated with shaking for 5 days under room temperature.

	Cell yield (mg/ml)		
	Coconut water from		
Organism	Young nut 6 mths	Mature nut 10 mths	Nutrient broth
B. polymyxa FU6	26.8	7.6	.9
P. fluorescens	21.0	9.0	11.4

Concentrated bacterial inocula could be produced cheaply using coconut water. There is, however, need to determine the shelf-life of this inoculant and study more carefully the characteristics that allow these bacteria to proliferate effectively on roots.

Conclusion

Rapidly growing bacteria such as *B. polymyxa* FU6 and *P. fluorescens* have demonstrated great effectiveness in reducing the incidence of bacterial wilt in tomato and potato in an experimental situation. These organisms were able to colonise aggresively the roots of young plants and pre-empt the entry of *P. solanacearum*. These results could be useful in developing a biological control method to control this important pathogen. *Bacillus polymyxa* FU6, for example, may be applied very conveniently as a spore preparation during seeding time. Additionally, the bacterial inocula could be produced using coconut water as growth medium to make the product cost-effective and adapted to fit the technology of modern agriculture. However, more extensive field testing of the potential biological control agents is required to verify the promising results to date.

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Genetics of *Pseudomonas solanacearum* and Prospects for Biological Control

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THE phytopathogenic pseudomonads possess virulence abilities which enable them to parasitise most major plant groups (Schroth et al. 1981). The mechanisms of pathogenesis have not yet been determined fully for a pseudomonad, but once elucidated, this knowledge should facilitate development of disease control strategies. To this end, there is a need to identify those gene products which are concerned in the first instance with recognition and invasion and secondly, with changes in the host tissues which result in the disease (see Gross and Cody 1985).

One of the success stories of modern biology is the way in which genetics has been used in the analysis of complex interactions. In simple terms this is because there are now techniques by which individual genes can be studied away from the background of the whole genome, and thus the importance or otherwise of a particular biochemical component of the biological system can be determined more readily.

There are two main approaches to the genetic study of bacteria. One is the more traditional, if something only 40 years old can be called traditional, and that is the analysis of bacterial genes using plasmid mediated conjugation, phage mediated transduction or transformation. These methods are highly effective but as yet available in comparatively few bacteria. Fortunately, the pseudomonads are well served by this approach (Holloway 1984).

The second approach is more recent, using recombinant DNA technology. By these means, the bacterial genome can be dissected into short regions, sometimes even individual genes, transferred to other bacteria, the gene products readily identified and the fine structure of the genes determined (Cohen 1975; Maniatis et al. 1982).

Two discoveries were essential for the establishment of recombinant DNA technology: the identification of plasmids and the characterisation of restriction endonucleases.

The study of resistance to antibiotics in bacteria in Japan in the 1950s revealed that bacteria had two sorts of chromosomes. We now know that a bacterium contains a main chromosome comprised of about 3000 genes, and that it may also harbour smaller autonomous genetic units called plasmids (Levy et al. 1981; Hollaender 1985). Plasmids contribute in a variety of ways to the bacterial phenotype; it is of particular interest that in a number of bacterial phytopathogens virulence genes are known to be plasmid-borne (Chatterjee 1978). As autonomous elements—separate from the bulk of the bacterial genome—plasmids are important to genetic studies. In addition, plasmid DNA can be readily isolated in its unaltered, circular double-stranded form, and manipulated in vitro.

The second discovery was that of restriction endonucleases. These are enzymes capable of cutting double-stranded DNA at specific sites. Each restriction endonuclease can recognise a specific sequence of from 4–8 bases, cutting the DNA at that sequence (Arber 1974; Roberts 1980).

A combination of these techniques has led to procedures by which selected fragments of any DNA can be spliced into the DNA sequence of a plasmid, and in which state it can be transferred to a different bacterial host, allowed to express, or mutated.

We shall describe how these techniques are being used to investigate the organism causing bacterial wilt, *Pseudomonas solanacearum*, and ways in which the information so obtained can be used for the control of the phytopathogenic aspects of the

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organism. It should be stressed that compared to other species of *Pseudomonas,* the current genetic knowledge of *P. solanacearum* is somewhat meagre, but with the application of recent techniques, there is every reason to believe that genetics can make a substantial contribution to the understanding of the pathology of this organism. Our approach to studying *P. solanacearum* is based on our knowledge of the genetics of other *Pseudomonas* species.

Genetics of Pseudomonads

Most genetic studies on this genus have been carried out with *P. aeruginosa,* although *P. putida* has also been well studied (Holloway 1984; Dean and Morgan 1983). More recently, various investigations have been aimed at developing methods which will be applicable to *Pseudomonas* species in general.

Table 1 is a summary of the basic genetic tools available for the various species of *Pseudomonas.*

A major advance in the genetic investigation of pseudomonads was the discovery of plasmids that had a host range extending over many gram-negative bacteria, in contrast to most plasmids where the host range is limited to one or two genera (see Timmis and Pühler 1979). The host range of these plasmids, the IncP–1 group, is shown in Table 2.

Variants of these plasmids were isolated which had acquired the ability to mobilise the chromosome of a variety of gram-negative bacteria, extending the advantages of this type of genetic analysis to organisms where it was not previously available. These variants, known as Enhanced Chromosome Mobilising (ECM) plasmids have been extensively studied by a range of laboratories (Holloway 1979; Reiss et al. 1983) and the chromosome-mobilising ability of one such variant, R68.45, for different bacteria is shown in Table 3.

The molecular basis of this chromosome-mobilising ability is of particular interest. Now that many different restriction enzymes are known, it is now possible to characterise, to map in fact, DNA molecules in terms of their restriction endonuclease sites. Figure 1 shows the restriction endonuclease map of the wide host range IncP-1 plasmid, R68, which is the parent plasmid of the ECM plasmid R68.45.

When such a map is constructed for R68.45 an important difference is seen. Whereas R68 contains only one copy of the 2.1 kb sequence called IS*21* situated just anticlockwise from the determinant of kanamycin resistance, R68.45 has two copies of this

Table 1. Genetic exchange mechanisms known in pseudomonads.

	Conjugation	Transduction	Transformation
P. aeruginosa	+	+	+
P. putida	+	+	–
P. syringae	+	+	–
P. solanacearum	–	–	+
P. phaseolicola	+	–	–
P. cepacia	–	–	–
P. fluorescens	–	+	–
P. acidovorans	–	+	–
P. maltophilia	–	+	–

Table 2. Host range of IncP-1 plasmids.

Acinetobacter calcoaceticus
Agrobacterium tumefaciens
Alcaligenes faecalis
Azospirillum brasiliense
Azotobacter vinlandii
Chromobacterium violaceum
Erwinia chrysanthemi
Escherichia coli
Klebsiella aerogenes
Klebsiella pneumoniae
Methylobacterium AMI
Methylophilus methylotrophus
Methylosinus trichosporium OB36
Neisseria perflava
Proteus mirabilis
Pseudomonas aeruginosa
Pseudomonas syringae
Pseudomonas putida
Pseudomonas solanacearum
Pseudomonas fluorescens
Pseudomonas extorquens
Rhizobium spp.
Rhodopseudomonas spp.
Salmonella typhimurium
Shigella flexneri
Vibrio cholerae
Zymomonas mobilis

Table 3. Bacteria in which chromosome is mobilised by R68–45.

Pseudomonas aeruginosa	*Klebsiella pneumoniae*
Pseudomonas putida	*Rhizobium leguminosarum*
Pseudomonas syringae	*Rhizobium meliloti*
Agrobacterium tumefaciens	*Rhodopseudomonas sphaeroides*
Azospirillum brasiliense	*Zymomonas mobilis*
Erwinia chrysanthemi	*Methylophilus methylotrophus*
Escherichia coli	*Methylobacterium* AMI

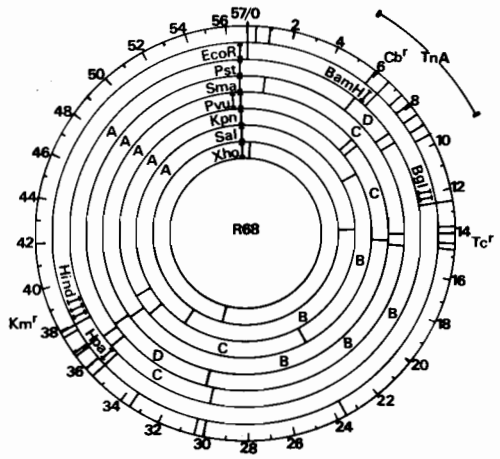

Fig. 1. Restriction endonuclease map of the IncP-1 plasmid R68. (With permission from the Journal of General Microbiology).

sequence as shown in Fig. 2 (Willetts et al. 1981).

When IS*21* adopts this tandem configuration it acquires mobility, being able to transpose from the R68.45 molecule to other DNA molecules, including the bacterial chromosome and this is the reason why this plasmid can mobilise the chromosome in such a wide range of species (Willetts et al. 1981; Reiss et al. 1983).

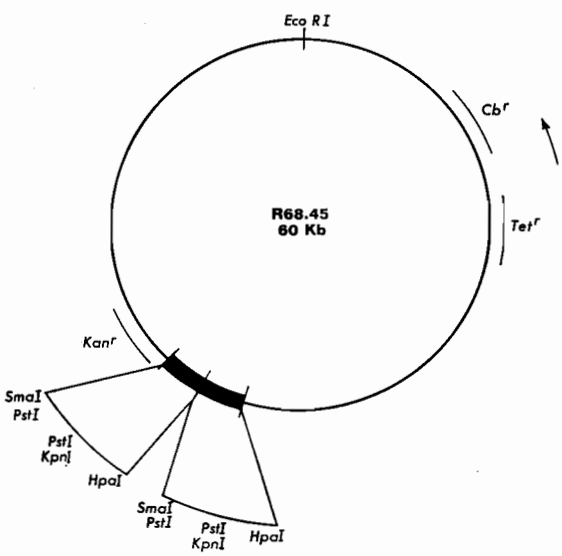

Fig. 2. Restriction endonuclease map of the ECM plasmid R68. 45 showing the tandem IS*21* structure.

R68.45 has another interesting property. It can pick up sections of the bacterial chromosome and insert them into the genome of the plasmid, thus forming what is known as a Prime Plasmid, a hybrid consisting of the entire R68.45 genome and a section of bacterial chromosome which may be as large as 100 kb (Zhang and Holloway, unpublished data). The prime plasmid still retains the wide host range of R68.45 so that it can be transferred to the same wide host range of bacterial genera. Thus these prime plasmids are in essence the product of an *in vivo* cloning mechanism and as a result fragments of bacterial chromosome can be transferred between different genera at will, resulting in novel hybrids by which the expression of bacterial genes in a heterologous background can be studied.

Using these, and other procedures for gene exchange including transduction, it has been possible to construct maps of two species of *Pseudomonas, aeruginosa* (Holloway 1985, Morgan and Dean 1985) and *putida*.

There are several features of interest in these maps. Firstly, unlike *E. coli,* biosynthetic genes are not arranged into contiguous arrangements, but separated at various sites around the chromosome. Secondly, and perhaps of significance for the genetic analysis of virulence, genes having related function are often arranged into clusters, although the genes in the cluster are not contiguous. This has been called supraoperonic clustering (Wheelis 1975).

Genetics of Pathogenicity

A striking feature of *P. solanacearum* strains is their ability to produce copious quantities of an extracellular polysaccharide (EPS), which gives a sloppy fluidal consistency to colonies on solid media. Wilting of susceptible plants is principally due to the blockage of xylem vessels by this viscous polysaccharide (Husain and Kelman 1958). Interestingly, natural isolates of *P. solanacearum* frequently undergo a transition from a non-motile virulent fluidal type, to a less virulent motile form which has both an altered cell-surface lipopolysaccharide and reduced EPS production (Kelman 1954; Kelman and Hruschka 1973; Hendrick and Sequeira 1984). The resemblance of this phenomenon to other examples of 'phase variation' (Silverman and Simon 1983) tempts the speculation that an invertible genetic switch regulates aspects of the *P. solanacearum* pathogenesis process. An array of genes, in addition to those for EPS production, is likely to contribute to virulence. These may facilitate the bacterium to invade plant tissues, proliferate, and evade the defensive responses of the host.

To date, genetic knowledge of *P. solanacearum* is scant, with regard to both its gross genomic organisation and its virulence genes. In spite of this, preliminary reports have shown *P. solanacearum* to be amenable to genetic analyses. Strains of this organism have been transformed with chromosomal material (Boucher and Sequeira 1978, Le et al. 1978); served as recipients for the transfer of broad host-range plasmids (Boucher et al. 1977); and transposons have been used to generate auxotrophic and avirulent mutants (Boucher et al. 1981; Staskawicz et al. 1983). Furthermore, unusually large 'megaplasmids' up to one-third the size of the main chromosome, have been detected in many *P. solanacearum* strains (Rosenberg et al. 1982).

Our objective is to fully characterise the genetic basis of pathogenicity in *P. solanacearum*, using a combination of microbial and molecular techniques. It will be necessary to determine the nature of virulence gene products, where these genes are located on the genome, and how the expression of these genes is controlled.

Transposon Mutagenesis

An important part of the genetic analysis of any complex biological phenomenon is to isolate mutants. In this way the component reactions in the phenomenon can be dissected and studied in isolation. The selection of such mutants is often a difficult problem. With most chemical mutagens, even where selection can be imposed for particular types of mutants, such as auxotrophs, 1% of the mutagenised culture can be identified as mutants. Where no selection can be imposed, the frequency of such mutants is usually 0.01%. Thus to detect mutants of *P. solanacearum* altered in virulence properties a large number of experimental plants are needed.

However, there is a new procedure by which genes such as those for pathogenicity can be readily mutated and marked in such a way that they can be identified without the regular need for plant inoculation. This is by Transposon Mutagenesis. One of the most exciting developments of modern genetics is that there are specific sequences of DNA which can move from one chromosome to another chromosome without the two whole chromosomes undergoing recombination (see Shapiro 1983). Such 'jumping genes' were first found by Barbara McClintock in maize, but are now known to occur in bacteria and other organisms. Transposons are one example of such mobile genetic units, and are distinguished by the fact that they carry genes which determine some feature of the bacterial phenotype, most commonly antibiotic resistance. The process of transposition, from say a plasmid to a bacterial chromosome, yields a transposon copy inserted at a new site, often this interrupts the continuity of one of the genes of the chromosome, thus causing a mutation in that gene. Once the function of that gene has been identified, the movement of the mutated gene following transfer or segregation,can be monitored solely by the antibiotic resistance determined by the transposon.

While some transposons are specific with respect to the sites of insertion into the bacterial chromosome, others are quite random in their insertion properties. Thus, if insertion is random, and as there are about 3000 genes in a bacterial chromosome, then to detect any desired mutant we need to isolate 3000 non-sibling clones selected for insertion of the transposon. In reality, it is less than that. All the insertions into genes affecting biosynthetic functions can be readily screened out, and insertions into 'lethal' genes will not survive. In effect we would expect that about one in every thousand transposed candidates might be a mutant in a gene affecting pathogenicity.

Two important experimental features of transposon mutagenesis are firstly, the choice of transposon and secondly, the vector carrying the transposon. Tn*5* which codes for kanamycin and streptomycin resistance is the most commonly used transposon with a low specificity of insertion and readily scorable antibiotic resistances (Berg and Berg 1983).

For transposon mutagenesis in *Pseudomonas* species, we have used a plasmid called R91-5 as a delivery vector because while this plasmid replicates only in *P. aeruginosa,* it transfers readily to many other organisms, including other species of *Pseudomonas,* but does not replicate in them (Moore and Krishnapillai 1982). Thus R91-5 loaded with Tn*5* can be transferred in to the new host organism, in which the plasmid vector fails to replicate and hence is lost. Selection can be made for retention of the transposon alone which can survive by virtue of its insertion into the chromosome. Transposition of Tn*5* occurs at a frequency of about 10^{-4} per donor parent. Using this system Tn*5* insertion mutants have been isolated in *P. putida, P. syringae* and, more recently, *P. solanacearum.*

When R91-5: :Tn*5* is employed as a delivery plasmid for tranposon mutagensis in *P. solanacearum,* approximately 2% of the Tn*5* inserts yield auxotrophic mutants; these are of various types, indicating that the insertion sites are randomly distributed. These Tn*5*-induced auxotrophs are useful for genetic mapping purposes as they provide reference points, around the genome, which are labelled

by the antibiotic resistances of the transposon. In addition, suitable manipulations may utilise these Tn*5* inserts to create origins for chromosomal transfer.

Close to 0.1% of the Tn*5* inserts examined caused defects in extracellular polysaccharide production and these mutants are unable to induce wilting of tomato seedlings. This does not mean that virulence is solely associated with the production of extracellular polysaccharide. It is likely that other bacterial products are involved, which act either to enhance the effects of extracellular polysaccharide or with entirely different mechanisms.

We propose to study further the arrangement of genes affecting virulence by cloning procedures. The actual cloning vector to be used is a cosmid, which is a plasmid which contains that region of the lambda phage DNA which forms cohesive ends—λcos. The presence of the λcos site enables the plasmid to be packaged into lambda phage heads. A main advantage of the cosmid system is that it preferentially allows cloning of large passenger DNA fragments, sized between 25–45 kb. Moreover, the cosmid has genes from the IncP-1 group which enable it to be replicated and maintained in a wide range of gram-negative bacteria including *Pseudomonas* sp.

By transferring a cosmid bank of cloned genomic fragments from the wild type *P. solanacearum* into the avirulent mutants which have been isolated, DNA sequences encoding functional virulence genes can be identified. Large chromosomal fragments isolated in this manner will be subjected to regional transposon mutagenesis and sub-cloning procedures, to determine whether additional virulence genes are clustered around the gene initially detected. Previous knowledge of gene arrangement in pseudomonads makes such an arrangement likely.

Prospects for Biological Control

Knowledge of the identity of the crucial virulence gene-products, their mode of action, and the mechanisms by which their expression is regulated, will provide the basis on which to devise a biological control strategy.

The concept of biological control is that a genetically engineered strain of bacteria will be applied to plant crops to protect against physical or biological damage. This has been proposed for the control of frost damage. *Pseudomonas syringae* causes nucleation of ice crystals in supercooled water at a temperature slightly below 0 °C. The genes responsible for ice nucleation activity in *P. syringae* are confined to a small genomic region and have been cloned.

By spraying plants with a mutant bacterial culture of *P. syringae* lacking the genes for ice nucleation protection against frost could be achieved (Orser et al. 1983).

Once the mechanisms of virulence of *P. solanacearum* have been understood it is possible that plants could be inoculated with bacterial mutants which would prevent the entry or activity of the normal virulent organisms. This may involve the laboratory construction of a *P. solanacearum* in which virulence genes have either been inactivated or completely deleted. To be an effective control agent, this avirulent derivative would have to 'out compete' field strains. However, in any such procedure, the implications of releasing modified organisms into the environment will need to be thoroughly understood (Stotzky and Babich 1984).

In summary, the techniques of molecular genetics are being applied to establish an understanding of how *P. solanacearum* causes the disease process. This information, combined with the extensive and ongoing investigations on the microbiology and pathogenicity of bacterial wilt, will provide the basis of new strategies for the control of this disease.

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Interaction Between Strains of *Pseudomonas solanacearum*, its Hosts and the Environment

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TAXONOMIC terminology in phytobacteriology has been changing over the past decade and this has caused some confusion in the literature on *P. solanacearum*. Recently, pathovars were defined as pathogens differentiated on the basis of pathogenicity to different host species, by the Subcommittee on Taxonomy of Phytopathogenic Bacteria of the International Society for Plant Pathology. This definition was proposed by the Subcommittee during the VI International Conference on Plant Pathogenic Bacteria in June 1985, and updates the earlier definitions proposed by Dye et al. (1980) and Bradbury (1983).

Unfortunately, in the case of *Pseudomonas solanacearum* many published reports of host range are not useful in identifying pathovars, because they are based on artificial inoculation which is not necessarily the natural host range. The races described by Buddenhagen and Kelman (1964) are generally defined and based on different host species. Races are correctly utilised at an infrasubspecific level and are commonly used by plant pathologists for the fungi. However, this is not recognised for bacteria by the most recent International Code of Nomenclature of Bacteria (Lapage et al. 1975). Vanderplank (1982) considers the physiologic or pathogenic race as a taxon of the lowest order, defined by resistance genes in a given host a microorganism parasitises. *Pseudomonas solanacearum* has also been classified according to a small number of selected biochemical or physiological properties into five biovars (Bv) (Hayward 1964; He et al. 1983; Hua et al. 1984). However, since a better classification of *P. solanacearum* is not yet available, I will here utilise the defined races and biovars and one additional term, strain, to represent groups of isolates that appear to have some property in common (geographical range, colony morphology, transmissibility by insects, apparent adaptation to given temperatures, etc.). Terms such as pathovar and pathogenic group utilised by some authors will be considered here as strains, describing these when appropriate as pathogenic strains, temperature strains, etc.

Imperfect though the race classification scheme may be and as limited in scope the biovar determinations, these two systems are useful, complement each other and have gained acceptance. They are unlikely to be soon replaced by other criteria because the kind of research needed to establish even the elementary 'Schematic Classification for Isolates' proposed by Buddenhagen and Kelman in 1964 is not under way to any extent.

Strains of Race 1

There are many pathogenic strains of race 1, each of which is pathogenic to several hosts including usually at least one solanaceous plant, the sum total of all hosts being estimated at close to 200. Race 1 occurs in the lowland tropics and warm temperate lands, with a few exceptions. Race 1 includes Bvs I, III and IV (Buddenhagen and Kelman 1964; French 1979; Kelman 1953).

Strains of Race 2

Race 2 affects musaceous hosts: plantains, bananas and *Heliconia* spp. (Buddenhagen and Kelman 1964). A summary of the principal characteristics of strains reported for race 2 appears in Table 1. In the pathogenicity column are listed the hosts and symptoms caused by the seven recognised strains. These differ in specificity to hosts such as *Heliconia,* banana or plantain. They cause symptoms ranging from stunting and distortion to rapid wilt. Dissemination by insects causes a different symptom to that from soil or machete spread with other strains, the flower and fruit becoming diseased before the leaves wilt. However, spread may be by

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insects between plantations of one variety of plantain or banana and then by mechanical means to other varieties not as susceptible to contagion by insects.

Race 2 is indigenous to Central and South America and its native host is *Heliconia* (Table 1). The bananas and plantains introduced from Asia and Africa to the Americas provided the selection pressure for new strains to emerge from the indigenous population. Only in the 1960s did bacterial wilt of banana or 'Moko' disease first appear in Asia (Mindanao, Philippines) where commercial interests previously in Central America have established plantations (Quimio 1976).

Insect dissemination has resulted in rapid spread in the Amazon Basin (Brazil, Colombia, Ecuador and Peru) where strains with distinctive colony characteristics in culture have been described (French and Sequeira 1968). Abaca has been reported as a musaceous host in the Philippines, but little is known about the strains infecting this crop (Quimio 1976).

Strains of Race 3

Race 3 (the potato race) has a narrow host range, being restricted to the potato, tomato (especially when planted after infected potato, which seldom occurs) and a few weeds in nature, and having a lower optimum growth temperature (Buddenhagen and Kelman 1964; Graham et al. 1979). When the biovar (Bv) determination tests have been conducted isolates of race 3 have always been Bv II. Thus when an isolate from potato or tomato is Bv II it is considered to be race 3 (Hayward 1964, 1979) even though its natural host range may be unknown.

There is considerable evidence for cool temperature-adapted strains of race 3. It is found principally in the higher latitudes of the world and the higher elevations of the tropics (French 1984). In the past 10 years it has spread into cooler, more temperate lands such as Mexico, Uruguay, Argentina, Chile, Sweden and temperate Australia (Fernandez 1984; French 1984; Fucikovsky 1984; Graham et al. 1979; Olsson 1976). This spread may have been the result of the movement by man of

Table 1. Characteristics of strains of race 2 of *Pseudomonas solanacearum* (based on French and Sequeira 1970; Stover 1972).

Strain	pathogenicity	Origin	Cultural characteristics[a]
R	Stunting and distortion of *Heliconia* only	*Heliconia* in Costa Rica	Elliptical; lacelike slime; slight formazan pigmentation towards centre 2[b]
D	Leaf distortion and slow wilt of banana[c]. Stunting and distortion of *Heliconia*	*Heliconia* and banana in Costa Rica	,, ,,
B	Rapid wilt of banana in Guyana, Venezuela, Costa Rica, Panama, Honduras, Trinidad	Probable mutation of strain D in SW Costa Rica Also South America	,, ,,
H	Moderate wilt on plantain[d] Not pathogenic to banana	Costa Rica	,, ,,
SFR	Rapid wilt of banana and plantain. Insect disseminated in Venezuela, Honduras, Guatemala, El Salvador, Nicaragua, Costa Rica, Colombia	South America (probably Venezuela)	Near round; little slime, lace-like initially; slight formazan pigmentation
SFR-C	As SFR, but a variant in Colombia	Probable mutation of SFR in Amazonas State on plantain	Near round; moderate slime deposition, lace-like initially; slight formazan pigmentation
A	Rapid wilt of plantain and banana. Insect disseminated from Colombia, similar to SFR-C	Amazon Basin in Peru and neighbouring Brazil, on plantain and banana	Near round; plentiful slime, faintly lace-like; formazan in faint helical pattern

[a] Colonies on Kelman's agar medium observed with oblique light after 24 h at 32 °C.

[b] Strain R as per French and Sequeira (1970); Sequeira and Averre (1961) reported rapid formazan pigmentation.

[c] Banana is Musa Group AAA

[d] Plantain is Musa Group AAB

diseased tubers, with low temperature adaptation of the pathogen progressively taking place as selective pressure resulted from progressive movement to colder climates (French 1983).

The low temperatures at the higher latitudes are suggested by the fact that in Argentina bacterial wilt occurs south of the 18 °C average annual isotherm as far south as 28 °S at Balcarce, Buenos Aires Province (Mitidieri 1984). An unpublished report places it now in Trelew, Chubut Province, 43 °S. In Uruguay the average temperature for the month of September (10-year average) in Rocha where wilt is severe that month is 12.4 °C (Garcia et al. 1979). The most northerly latitude for which wilt has been reported is the overwintering of latent infection in *Solanum dulcamara* inoculated at Solna, Sweden, more than 59 °N where soil freezes for 2 months each year (Olsson 1976).

The progressive spread into the cooler highlands in the tropics is also well documented. In Colombia race 3 was endemic up to elevations of about 2200 m, but infected seed was taken to an elevation of 2600 m where the disease was serious at an average temperature of about 13 °C. There was little soil survival the following year (Thurston 1963). In Peru, where wilt occurred at elevations above 2800 m, symptoms were slight (French et al. 1972). At the International Potato Centre (CIP) a Bv II (race 3) strain was isolated from Renacimiento potatoes with mild wilt symptoms that were severely infected with the cyst nematode at Chocon near Jauja (about 3500 m). A Bv (race 1) strain was isolated from the CIP La Victoria, Huancayo farm (3350 m) in wilting Tomasa Condemayta potatoes infected with *Phytophthora erythroseptica*. Seed from apparently healthy plants proved to be latently infected since tubers planted at a lower elevation led to severe wilt in the crop. The seed grown in La Victoria had been provided by the Ministry of Agriculture from a farmer in Huasahuasi whose land was subsequently determined to be infested with *P. solanacearum*. Both Jauja and Huancayo are in the Mantaro River Valley, Junin Department.

In Kenya where the potato was recently introduced, and race 3 is considered to have been introduced with it, it causes wilt in tomato and potato in the elevation range 1200–2400 m, the higher elevation coinciding with a soil temperature average of about 15 °C. However, when infected potato tuber seed was planted at an elevation of 2743 m, wilt resulted (Harris 1972, 1976). In Costa Rica race 3 commonly caused wilt up to an elevation of about 2000 m (16.5 °C average), but symptomless infection occurred at higher elevations (Gonzalez 1976). In Sri Lanka Bv II (race 3) occurs, usually above 1900 m but it has also been found at lower elevations and even near sea level when latently infected seed was planted. Bvs III and IV were detected below 1900 m (Seneviratne 1969; Velupillai and Seneviratne 1983). In India only race 3 was found in the hill country, race 1 elsewhere (Shekhawat et al. 1978). Strains of race 3 from high altitudes in the tropics were found to have lower optimum temperatures than those of race 1, Bv IV (Katayama and Kumura 1984). Moraes (1947) reported from Portugal the first detection of a cold temperature strain with an optimum of 27 °C, several degrees lower than previously reported (Kelman 1953). Thurston (1963) found that two strains of race 3 had an optimum of about 28 °C though their minimum temperatures for growth differed: 12–16 and 16–20 °C. Olsson (1976) in Sweden determined that 10 isolates from a restricted area where race 3 was probably introduced only a few years earlier, had an optimum of 30, two others of about 25 °C. In Australia race 3 isolates grew better at 27 °C whereas race 1 isolates (Bvs III and IV) preferred 37 °C (Hayward 1979). Velupillai (1980) determined that three race 3 isolates from Sri Lanka and Colombia grew best at 30 °C whereas race 1 and 2 isolates from various sources grew best at 35 °C. In China potato isolates and one tomato isolate of race 3, grew better at 27 °C but so did one Bv III and one Bv V, whereas others of Bvs III and V as well as Bv IV grew better at 37 °C (Hua et al. 1984).

In Kenya the optimum for both races 1 and 3 was 30 °C, the minimum 12–15 °C (Harris 1976). French and Gutarra (1974) found that race 3 isolates from Peru behaved like a race 1 from the USA growing better at 30 than 25 or 35 °C. However when a race 3 isolate was 'adapted' for 20 days by growth at 16 °C it later grew faster at 16 and 20 °C and also grew at 12 and survived at 8 °C, whereas the original culture did not.

Nydegger and French (1984) utilising liquid cultures in a temperature gradient incubator with approximately 1 °C intervals, compared lowland tropical isolates of Bvs I and II from potatoes planted in freshly cleared jungle in the Amazon Basin with isolates of the same Bvs from cool climates. Results are shown in Table 2. The average optima were similar for all isolate groups, but slightly lower within both Bv groups when from cooler climates. The lowest optima for both Bv I and II from the lowlands were the same, but the highest considerably greater for Bv I. The lowest optimum for a Bv II from a cool climate was 1.1 °C lower than the lowest for a Bv II from the lowlands.

Table 2. Range and average optimum temperatures (°C) for growth of Biovar I and II isolates of *Pseudomonas solanacearum* from potato, comparing those from virgin Amazon jungle soil with those from cooler climates (highland tropics or non-tropical).

Origin	Biovar I	Biovar II
Amazon jungle	Range 34.3–37.7	34.3–35.7
4 isolates	Average 35.8	35.0
Cooler lands	Range 33.6–36.7	33.2–36.1
7 isolates	Average 35.0	34.6

In the Philippines where race 3 has not been detected until recently (Kloos, these Proceedings), 61 isolates of race 1 from five crops had varying otpima ranging from 31.0 to 36.5 °C, 60% of them being within 33.0 to 34.5 °C (Quimio and Tabei 1979). These are lower than the optima for the Amazon Basin race 1. Results of different *in vitro* growth studies may not be comparable because the optimum temperatures for growth of bacteria may vary according to the media used, and they are reported to be higher in liquid than on solid media (Hayward 1979). Furthermore, results of growth studies in liquid culture did not correlate with in-plant experiments in the work of Ciampi and Sequeira (1980). Therefore, controlled temperature experiments with plants are essential.

Thurston (1963) showed that race 3 isolates caused wilt of potatoes at 18 °C, whereas Zehr (1970) with race 1 isolates did not get wilt at 18–24 °C. In a growth chamber study race 3 caused wilt at 8–20 (average 14 °C; French 1972). Ciampi and Sequeira (1980) inoculated potatoes at 16, 20, 24 and 28 °C with five race 1 and four race 3 isolates. Two race 3 isolates killed all plants at 16, 20 and 24 °C whereas others caused little or no wilt. Though some isolates grew equally well in liquid culture at 16 °C, they were not equally pathogenic at that temperature. Tafur-Santillan and French (unpublished) inoculated potted potato plants with six race 3 isolates when temperatures were 15–26 °C; only two caused wilt, whereas at 25–32 °C they all did to a similar extent.

A Strain of Race 4

Race 4 was described in 1983 by He et al. after conducting a study of 29 isolates from China. One strain (three isolates) from mulberry did not belong to the known Bvs. They produced acid from lactose, maltose, cellobiose and mannitol, but not from dulcitol and sorbitol. They were only slightly pathogenic to potato and eggplant by stem stab inoculation, whereas most other strains of Bv III and IV were very pathogenic (two Bv II and one Bv III were isolated from potato).

Conclusion

The vast literature about race 1 has not been reviewed here in detail since previous papers have done so (Buddenhagen and Kelman 1964). The outstanding feature of this race is that it is variable and widely distributed throughout the lowlands of the tropics and subtropics, being highly damaging to crops. Its numerous pathogenic strains make crop rotation ineffective as a general control measure. Low temperatures reduce its aggressiveness, as noted by the fact that one strain became latent in potato at high elevations, but this race has not spread much into cooler climates.

Race 2 was limited to the American tropics until recently. It is indigenous on native *Heliconias*, but has adapted to the exotic bananas and plantains introduced from other continents (French 1979). The evolution to being insect-spread appears to be relatively recent, a factor which enhanced its rate of spread, advancing into the Amazon Basin. It has spread with the large-scale plantation industry from country to country. During the 1960s it was taken to the Philippines, where it is well established on banana plantations in Mindanao.

Race 3 has apparently few pathogenic strains. The tendency to call this race a low-temperature strain seems valid for most isolates, but some Bv II from the lowlands do not have a lower temperature optimum than some Bv I from the same location. An appropriate question might be: Are all Bv II from the host range standpoint truly race 3?

Race 3 is present throughout most of the potato-growing tropics and much of the subtropics. It is spreading rapidly from country to country, and it may be that most of its distribution is due to spread from an original infestation site. The recent detection of race 1 in as high (cold) a site in the Mantaro Valley of Peru as race 3 suggests it could also spread like race 3 has. This would have serious consequences because of its usually broad host range. Why hasn't race 1 spread like race 3? One possible explanation may be that it does not adapt (mutate) as rapidly to become more cold tolerant, or in nature does not include as much variation for this factor. The vegetative nature of the potato and the cool habitats it does best in make it a suitable carrier of latent infection to cooler climates where the stored tuber gives it an overwintering capacity (though in Sweden *P. solanacearum* has become endemic in *Solanum dulcamara,* even though most strains there do not have as low an optimum tem-

perature for growth as many others elsewhere). Another possibility is that race 3 is more specialised and more aggressive by nature to potato, or that race 1 is more destructive thus the potato carrier is less likely to be taken to other sites. There is little evidence to support either of these contentions. French and Gutarra (1974) showed that a race 3 strain could adapt to colder temperatures in vitro. Race 1 may have the same adaptability.

Races 2 and 3 have been studied systematically because of their importance to two vegetatively grown crops, banana and potato, both of which are of importance to developed countries or their commercial interests. Race 1 has been studied intensively as a pathogen of tobacco and tomato, but otherwise in a more fragmentary way. A concerted effort worldwide with emphasis in the tropics is needed if headway is to be made in better understanding and controlling this race.

The taxonomy of *P. solanacearum* is inadequate. Resistance breeding for crops cannot be well executed without an understanding of the pathogen and its range of variability. Other studies that are needed, such as the ecology of this bacterium, are also handicapped by an incomplete classification system. Thus the highest priority must be given to this task.

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Breeding Potatoes for Resistance to Bacterial Wilt Caused by *Pseudomonas solanacearum*

Peter Schmiediche*

THE first systematic potato breeding program for resistance to bacterial wilt was started in 1967 by Rowe and Sequeira (1972) at the University of Wisconsin. These investigators began their program by intercrossing several resistant diploid clones of *Solanum phureja* from the Central Colombian Collection (CCC), with clones of *S. tuberosum* ssp. *tuberosum*. Field tests of the *S. phureja* x ssp. *tuberosum* hybrids revealed susceptibility of this genetic material to *Phytophthora infestans*. In a further set of crosses, the bacterial wilt-resistant hybrids were therefore crossed with Mexican late blight-resistant clones, combining the two needed resistances. The Mexican germplasm used in this crossing program consisted of ssp. *tuberosum* clones containing late blight resistance genes derived from the wild hexaploid species *S. demissum* (Fig. 1).

In 1969, the University of Wisconsin sent 369 clones, representing 10 families, to Peru where they were to be tested under natural conditions in fields heavily infested with *P. solanacearum*. French and Herrera started a screening program at Huambos in the Department of Cajamarca, Peru (Herrera 1972). By 1974 the Wisconsin material had changed hands three times and only seven of the original 369 clones had survived. Most of the material had been lost due to causes other than bacterial wilt. On the basis of the assembled data clone BR-63.74 was named a variety (Caxamarca) in 1976 (Herrera et al. 1977), and in the following year clone BR-63.65, a sib of Caxamarca, was named Molinera (de la Puente et al. 1977).

A survey conducted by French in Cajamarca in 1981 revealed that local farmers had themselves selected two more Wisconsin clones on their own and had unofficially named them Molinera-II

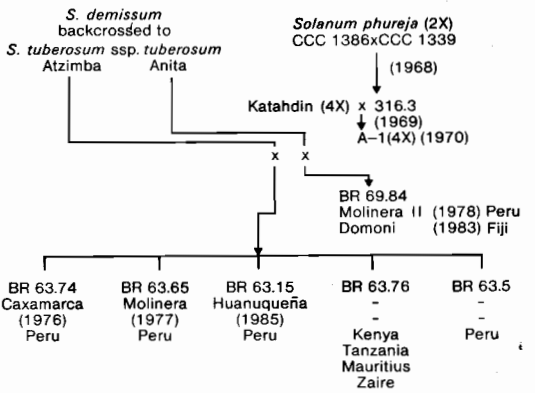

Fig. 1. Pedigree of BR clones with *S. phureja*-based resistance to bacterial wilt.

(thought to be BR-69.84 which is now the variety Domoni in Fiji) and Amapola (CIP 800950) whose pedigree has been lost. Molinera-II had been selected for its culinary quality and Amapola for its high yield and strong resistance to late blight. It is interesting to note that Amapola had been rejected by the National Potato Program of Peru because of its poor tuber shape.

Apart from sending 369 clones of bacterial wilt-resistant genetic material to Peru, the University of Wisconsin sent similar sets of material to 20 countries around the world. Virtually no information about the performance of this material was received from any of the participating countries. After the foundation of the International Potato Centre (CIP), the University of Wisconsin entered into a research contract with CIP and began to supply more bacterial wilt-resistant material. Much of this material is still in CIP's breeding program and is identified by codes like BR, MS, PSP or PSW.

All of the bacterial wilt-resistant material that national potato programs received from CIP during CIP's first 7 or 8 years came exclusively from the

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University of Wisconsin breeding program. CIP's breeding program for bacterial wilt resistance relied exclusively on Wisconsin germplasm as its only source of resistance (i.e. CCC clones of *S. phureja*).

The outstanding success of some of the Wisconsin clones is shown in Fig. 1. BR clones have either become varieties or are extensively grown in farmers' fields in countries as widely apart as Kenya, Tanzania, Zaire, Mauritius, Peru and Fiji. In Colombia the variety Ica Sirena was released, and this clone has the same genetic background as the BR clones shown in Fig. 1. However, its pedigree is not exactly the same as that of the BR clones.

The success of the Wisconsin material is partly due to its high degree of heterozygosity, which results from use of unreduced gametes in the tetraploid x diploid crosses which were necessary components of this breeding program. The bacterial wilt resistance of this material comes from the diploid species *S. phureja*. Expression of this resistance at the tetraploid level becomes even more remarkable when the genomic composition of the tetraploid BR clones is considered. Only one genome, disregarding crossing over during meiosis, is of *S. phureja* origin. Crossing BR clones with other tetraploid clones that are not resistant to bacterial wilt will obviously dilute the *S. phureja*-based resistance even more.

CIP Breeding Program for Bacterial Wilt From 1977 to 1980

By 1977, data received from National Potato Programs in CIP's regions had suggested that resistance based on *S. phureja* was effective against strains of *Pseudomonas solanacearum* that affect potatoes, as long as the resistant material was grown under temperate climatic conditions. Such conditions can be found in tropical highland regions or in the plains of northern India during the winter.

Through a combination of measures, including use of the important resistant variety Molinera, bacterial wilt was eradicated in the highlands of the Department of Huanuco in Peru. Molinera is still grown in the Huanuco area, not because of its bacterial wilt resistance but because of its high degree of resistance to late blight, a continuing serious problem during the wet season.

The bacterial wilt resistance, which was so effective under temperate climatic conditions, was ineffective when the cultivation of potatoes was extended into the hot, humid lowland tropics. Such an extension of potato production into non-traditional growing areas has been commonly observed during the last 10 years. A further disadvantage of the BR material, which became apparent in the first 8 years of its use, was its relative lateness under tropical lowland conditions (French 1979). CIP's breeding program tried to overcome this limitation by crossing two BR clones with relatively early tetraploid material. The first set of crosses to combine earliness with bacterial wilt resistance was carried out in 1977 (Table 1).

Table 1. Pedigrees of bacterial wilt-resistant Group I, 1977.

CIP No.	Pedigree
377830	BR-63.65 x N-574.1
377831	BR-63.65 x Katahdin
377835	BR-63.65 x Atlantic
377838	BR-63.65 x N-522.22
377847	BR-63.74 x Anita
377849	BR-63.74 x N-503.214
377850	BR-63.74 x DTO-28
377852	BR-63.74 x WRF-1923.1
377863	WRF-1919.2 x BR-63.65
377864	Anita x BR-63.65

The progenies of this set of crosses became known as bacterial wilt-resistant Group I.

By the time Group I was generated, data received from CIP's regions confirmed the view that expression of resistance is a function of adaptation, and that bacterial wilt-resistant genotypes would express their resistance if adapted to heat. This consideration led to the generation of Group II which combines the characteristics of Group I (earliness and bacterial wilt resistance) with adaptation to heat (Table 2).

Table 2. Pedigrees of bacterial wilt resistant Group II, 1979.

CIP No.	Pedigree
379648	377835.3 x DTO-33
379666	377831 x Maria Tropical
379669	377835.12 x Maria Tropical
379673	377847.4 x Maria Tropical
379674	377847.5 x Maria Tropical
379676	377850.1 x Maria Tropical
379677	377851.3 x Maria Tropical
379679	377852.1 x Maria Tropical
379681	Serrana x CGN-69.1
379693	377835.12 x CGN-69.1
379695	377847.1 x CGN-69.1

Just before Group II was generated, every breeding program at CIP was facing severe restrictions which had been caused by the occurrence of the potato spindle tuber viroid (PSTV). From then on every progeny that was produced for export either as true potato seed (TPS) or in the form of tuber families had to be obtained from parents that were known to be PSTV-free, and the crosses had to be carried out in special quarantine greenhouses. This drastically limited the choice of parental material for at least 2 years.

The pedigrees of the progenies produced in Group III (Table 3) show the limited amount of parental material available at that time. Group III had been generated in order to restore some of the resistance to bacterial wilt and late blight that had been lost when Group I (which combines earliness and bacterial wilt resistance) and Group II (which combines the characteristics of Group I with adaptation to heat) were produced.

Table 3. Pedigrees of bacterial wilt-resistant Group III, 1979–80.

CIP No.	Pedigree
379690	377835.9 x CGN-69.1
380572	Atlantic x PI/PS bulk
380576	377852.2 x PI/PS bulk
380577	377849.2 x PI/PS bulk
380579	377835.2 x CGN-69.1

Group II was already five generations removed from the original bacterial wilt-resistant *S. phureja* source and three generations away from the Mexican source of resistance to late blight and both resistances had been progressively diluted. However, genotypes with combined resistances were found in each cycle of crossing and subsequent selection in an artificially inoculated medium.

From 1980 to 1985

Between 1981 and 1983 results obtained in Sri Lanka confirmed the hypothesis that the expression of resistance to bacterial wilt is a function of adaptation to heat. This consideration would become an important factor in a modified breeding strategy developed in 1980–81 (Schmiediche 1985).

Until 1980 the only source of resistance to bacterial wilt had been *S. phureja*, which had demonstrated its potential as well as its limitations. The narrow genetic base of this resistance had become an object of concern for breeders, pathologists and geneticists.

At the end of 1980 bacterial wilt-resistant Group IV was generated (Table 4). The most significant difference from the earlier three groups was the use of clone AVRDC 1287.19 from Taiwan. This clone had been developed at the Asian Vegetable Research Development Centre (AVRDC) for resistance to bacterial wilt and adaptation to heat. The source of resistance was, however, not *S. phureja* but the two wild species *S. raphanifolium* and *S. chacoense*. The latter was used with hesitation since its resistance, so effective under Taiwanese conditions, had broken down under field conditions in Peru, where a strain of *P. solanacearum* different from the Taiwan strain was present. However, the chance to introduce a new source of resistance to bacterial wilt into the existing gene pool, and of combining two sources of resistance into one progeny, overrode doubts about the usefulness of this clone in CIP's breeding program.

Table 4. Pedigrees of bacterial wilt-resistant Group IV, 1980.

CIP No.	Pedigree
381046	377849.2 x AVRDC-1287.19
381047	Serrana x BR-63.5
381048	Serrana x 377831, 377852 bulk
381050	377835.9 x AVRDC-1287.19
381064	377852.1 x AVRDC-1287.19
381065	377835.2 x AVRDC-1287.19
381075	Serrana x AVRDC-1287.19
381077	Serrana x BR-69.84 + BR-63.5 bulk

In the meantime, progeny 3B1064 (see Table 4), which combines two specific sources of resistance, has shown its superiority to the traditional material derived from *S. phureja* under heavy infection pressure in Mindanao, Philippines.

Host-Pathogen-Environment Interactions

Pseudomonas solanacearum exists in a range of specific strains with varying degrees of pathogenicity. The system of classification itself is an expression of the complex nature of this pathogen. Whether a given strain of the pathogen will be more or less virulent depends largely on environmental conditions, such as temperature, soil moisture or the degree of salinity of a given soil. In saline soils on the Peruvian coast for instance, the bacterial wilt pathogen has never established itself. Interaction of *P. solanacearum* with other pathogens, such as root-knot nematodes (*Meloidogyne* spp.) is well documented (Jatala et al. 1975; Jatala and Martin 1977a, b; Suatmadji, these Proceedings). Non-solanaceous hosts may also play a role in the survival of the bacteria in the soil which can be altered by cultural and agronomic practices.

The specificity of the pathogen-host-environment interaction has prevented the breeding of genotypes that will retain their resistance under conditions different from those under which they have been selected. A potato clone that was selected in Peru as resistant to a specific strain of a given race of the bacterial wilt pathogen might not exhibit the same resistance to the same strain in another part of the world. This is due to the fact that in CIP's regions soil and environmental conditions are often quite different from those of the test and selection sites in Peru. Even if inoculum samples were taken from infected plants in a specific country or region and brought to Peru in order to screen material for those places at CIP, the problems caused by specificity of the pathogen-host-environment interactions would not be solved. This became clear when a large number of clones were screened against an Indonesian isolate in Peru. When the material selected for resistance to an Indonesian strain in Peru was tested in the field in Indonesia, it was not resistant to bacterial wilt.

Preliminary evidence obtained in CIP's breeding program for resistance to bacterial wilt appears to indicate that the genetic base for resistance is different in each resistant species. The resistance alleles in one species are probably different from those in another species although both determine resistance to the same pathogen. Although the exact mode of inheritance and the number of genes involved is not clear, it is known that bacterial wilt resistance is dominant in potatoes and that families segregating for resistance are relatively easily obtained. Recent evidence obtained in work with wild species suggests that contrary to the major gene inheritance observed in *S. phureja* (although the exact number of genes involved is not known), there might be polygenic inheritance in some of the wilt-resistant wild species.

Considering the complex variability and interactions of the bacterial wilt pathogen it was decided to initiate a breeding program that would develop maximum genetic variability for the trait 'resistance to bacterial wilt' into one genetic population. This would equip the population with a type of broad spectrum resistance that would meet any serious challenge of infection in the field. This goal was to be achieved by combining various sources of specific resistance into one genetic population (Schmiediche and Martin 1982). The idea of breeding a population with this type of broad spectrum resistance is conceptually akin to the principle of breeding genetic populations with field or horizontal resistance to *Phytophthora infestans*. Broad-spectrum resistance was to be combined with earliness and adaptation to heat as potato cultivation expanded into hot and humid regions where the bacterial wilt problem is most serious.

NEW SOURCES OF RESISTANCE

Accessions of *S. sparsipilum, chacoense,* and *microdontum* with resistance to bacterial wilt were identified at CIP about 6 years ago. A population combining resistance from these three species as well as from *S. phureja* was assembled and tested against four strains of races 1 and 3 in a 2-year screening program. The original population consisted of 11 500 seedlings of which only 40 clones, representing eight families, survived the rigours of screening against four different strains of the pathogen.

In the 1980–81 growing season these 40 clones were grown in the Andes at an altitude of about 3200 m and they were intercrossed in 203 different combinations. More than 100 000 seeds were obtained and almost 10 000 seeds from 43 families were sown and the seedlings tested against the particularly severe strain 013 of race 3. Of the 7468 seedlings tested, 2410 survived. The control DTO-33 was destroyed demonstrating the effectiveness of the inoculum. When the original wild population had been tested against the same pathotype, it showed a similar percentage of survival (about 30%), and the increase on a population basis after one cycle of recurrent selection appeared rather small. However, the increase in individual families was quite dramatic as survival rates of up to 67% were recorded. In two parallel screening programs resistance to the root-knot nematode *Meloidogyne* spp. and against the potato tuber moth (*Phthorimaea operculella)* was also identified in this population which is now identified with the code MBN, (the Spanish acronym for marchitez bacteriana nematodos = bacterial wilt and nematode *resistance*).

Before using the broad-spectrum resistance to bacterial wilt present in the MBN population, a strategy had to be developed that would permit quick and efficient transfer of the resistance to the tetraploid level and that would also allow quick and efficient combination with commercially acceptable agronomic and quality traits.

Mendiburu et al. (1974), Peloquin (1983), Leue (1983) and Peloquin et al. (1985) have clearly demonstrated that the most efficient way to use diploid wild germplasm is to cross the selected diploid wild clones with *tuberosum* haploids. This appears to eliminate any wild characteristics in the diploid hybrid population obtained from the wild species x haploid cross.

Consequently the breeding program would have to be carried out at the diploid level in an attempt to 'nobilise' the wild germplasm (Hermsen 1977) before going to the tetraploid level in a tetraploid x diploid (TD) cross. Such 'nobilisation' would considerably reduce the necessity for backcrosses to the tetraploid parent, and the probability of obtaining resistant genotypes with good agronomic characteristics in the F_1 following a TD-cross would theoretically be quite realistic.

The notoriously low male-, and often also female-, fertility of *tuberosum* haploids make the use of this material as crossing partner in the 'nobilisation' of wild germplasm less attractive in practice than in theory. Cultivated diploid potato species were considered in order to achieve 'nobilisation'. Cultivated diploid potatoes are abundantly fertile and easily cross with most wild species from the taxonomic series *Tuberosa* and the series closely related to *Tuberosa*. A population of the diploid cultivated species *S. stenotomum, phureja* and *goniocalyx* had been selected for high yield, high dry matter content and good agronomic characteristics and performance in a breeding program at North Carolina State University (NCSU). This most advanced cultivated diploid germplasm was therefore chosen to be hybridised with the bacterial wilt-resistant wild material. There is little difference genetically between a *tuberosum* haploid and highly developed cultivated diploid clones except that the haploid carries some of the sophisticated quality and agronomic traits customarily present in *tuberosum* varieties.

Before the NCSU cultivated diploid population was crossed with the bacterial wilt-resistant wild population it was screened for resistance to bacterial wilt. Bacterial wilt resistance had been observed in that population under field conditions but it had never been systematically tested. Since *S. phureja* was part of that population, the presence of resistance to bacterial wilt was expected. When this population was tested against strain 013 of race 3 some families had resistance levels of 25–30%.

From the resistant wild MBN population, 167 clones representing 42 families were planted and crossed with bulk pollen from the resistant members of the cultivated NCSU population. In early 1983, 10 000 seedlings of the MBN x NCSU hybrid progeny were planted and 500 clones were selected exclusively for agronomic characteristics and tuber shape. The hybrids, which combined one genome from the wild population and one from the cultivated diploid population, had agronomic traits at least as good as those found in a common Andean cultivated diploid potato. This represented a considerable improvement over the almost pure wild population. This diploid hybrid population was screened against bacterial wilt strain 052 (Biovar III, Race 1) from Taiwan and against strain 204 (Biovar II, Race 3) from Peru. The surviving clones were then intercrossed for a further cycle of recurrent selection. They were also crossed in tetraploid x diploid crosses with the most advanced tetraploid germplasm resistant to bacterial wilt. These crosses were highly successful due to the functioning of an abundant number of unreduced ($2n$) gametes. The first TD progenies were planted in clean fields on the coast of Peru to assess their agronomic and quality potential. A second set of the same progenies was planted in fields heavily infested with bacterial wilt in the Department of Cajamarca, Peru, where the first BR clones had been tested 15 years earlier.

Genetically this tetraploid population contained four specific sources of resistance, and of the four genomes three came each from a different species either wild or cultivated. This combination assured a high degree of heterozygosity and a theoretical broad-spectrum resistance.

The high degree of heterozygosity resulted in a high-yielding, vigorous and most heterotic crop. Family averages of more than 2 kg/plant were the rule and the highest-yielding seedling in the population produced 7.6 kg of tubers. These results were obtained in clean fields on the Peruvian coast. The limitation of this material, its relative lateness, also became apparent in the first growing season. The crop was barely ready for harvest after 126 days which is too late for many tropical sites. The tuber form of more than 90% of the individual clones was *tuberosum*-like with shallow eyes and commercially acceptable shapes. In a field of 1200 seedlings only one had distinctly wild characteristics. As far as yield and tuber shape were concerned, commercial varieties could have been found in this population, and backcrosses to improve agronomic performance and quality characteristics were not needed.

However, the relative lateness of the material made at least one backcross to early and heat-adapted material necessary. Such early and heat-adapted populations were already available at CIP. The excellent agronomic characteristics of the early heat-adapted populations resulted in further agronomic improvement of the first backcross generation (bacterial wilt-resistant tetraploid population x early heat-adapted population). The progenies of

this first backcross generation were tested in a large experiment under heat stress conditions in Peru; the results were being analysed in July 1985. About 200 highly promising selections have been made, and they will be tested further. The first set of seeds has in the meantime gone to CIP's regional program in Southeast Asia for field testing under high infection pressure.

One part of the first set of TD progenies was planted under conditions of heavy infection in Peru. In order to conduct a proper randomised complete block experiment with three replications in two sites, the families to be tested were multiplied by cuttings and the rooted cuttings were transplanted to the field. The two checks for this experiment were Molinera and BR-69-84, which are bacterial wilt-resistant clones with resistance derived from *S. phureja*. Preliminary results are summarised in Table 5. The average yield per plant appears to be rather low, but top-yielding clones with 1.8 kg/plant were also found in this population. The low average yield reflects the fact that all individual plants had been raised from stem cuttings. More important, however, was the heavy attack of the bacterial wilt pathogen on the resistant clones Molinera and BR-69-84. In Molinera, more than half of the plants were lost and clone BR-69-84 was almost destroyed. The highest loss in the new material (on a family basis) was 24.5% and the lowest was 6.5%. These preliminary data suggest that development of broad-spectrum resistance to bacterial wilt appears to be a potentially important defensive strategy for disease control.

Conclusion

The concept of broad-spectrum resistance for bacterial wilt has been developed during the last 4 years. Preliminary data suggest that development of broad-spectrum resistance will be an important strategy in the control of bacterial wilt. Despite the initial success reported with *S. sparsipilum, chacoense,* and *microdontum*, additional sources of resistance should be investigated. By July 1985 at least three more sources, possibly more effective than the ones already in use, had been identified.

Combining all these sources into one resistant super population is not envisaged, since experience with the bacterial wilt pathogen has shown that it is wise to develop populations differing in sets and combinations of sources of resistance. Such populations can be used according to the specific needs of the various regions where the bacterial wilt problem exists. At the moment it appears that breeding programs may be able to meet the challenge of this pathogen wherever potato growers are faced with it.

Table 5. Average yield and losses through wilting of material with broad spectrum resistance to *Pseudomonas solanacearum*.

Families		Yield/plant	Wilted plants %
1.	44A (S-51.1 x bulk 052)	468	14.5
2.	15A (S-20.3 x bulk 052)	393	17.1
3.	21A (S-23.1 x bulk 052)	456	24.5
4.	4A (S-9.2 x bulk 052)	413	10.0
5.	29A (S-26.5 x bulk 052)	378	10.0
6.	14B (S-21 x bulk 204)	416	11.4
7.	22B (S-26 x bulk 204)	458	9.2
8.	35B (S-50 x bulk 204)	543	12.2
9.	20B (S-25 x bulk 204)	457	10.6
10.	10B (S-20 x bulk 204)	431	6.5
11.	Molinera (check 1)	475	56.9
12.	Clone BR-69-84 (check 2)	345	77.2

Test Sites: Heavily infested fields in the Department of Cajamarca, Peru, during the 1984-85 growing season.

Experimental design: Randomised complete block, two sites, three replications.

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Bacterial Wilt of Groundnut: Control with Emphasis on Host Plant Resistance

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BACTERIAL wilt caused by *Pseudomonas solanacearum* is the only important bacterial disease of groundnut. It is a serious problem in major groundnut-producing areas of Indonesia (Schwarz and Hartley 1927; Machmud, these Proceedings), in southern China (Darong et al. 1981), and in restricted areas of Africa (Simbwa-Bunnya 1972). The disease is a potential threat to groundnut production in several other parts of the world, especially in warm humid areas. Effective control measures are to use suitable crop rotations and to grow wilt-resistant groundnut cultivars (Schwarz and Hartley 1950; Porter et al. 1982). Breeders have produced bacterial wilt-resistant groundnut cultivars in several parts of the world (Schwarz and Hartley 1926; Darong et al. 1981). Several screening/inoculation techniques have been used to identify sources of resistance (Darong et al. 1981; Winstead and Kelman 1952), but the wide range of variability in the pathogen populations complicates wilt resistance breeding.

This paper reviews the disease situation in groundnut and recent advances in identifying sources of resistance. Strategies for incorporating genetic resistance to bacterial wilt and to some other important diseases of groundnut into high yielding cultivars are also discussed.

Distribution and Economic Importance

Bacterial wilt caused by *Pseudomonas solanacearum* is common on many crops throughout the tropics and subtropics, but the disease occurs in a relatively isolated fashion on groundnuts. For instance, bacterial wilt of tobacco, tomato, and egg plant is common in India, the Philippines, and in West Africa, but there are no reports of the disease on groundnut in these areas. Bacterial wilt, also called slime disease, of groundnut was first observed in 1905 in Indonesia (Van Breda de Haan 1906) where it was later reported to cause an estimated loss of at least 25% of the crop (Palm 1922). In the United States the disease was first reported in 1912 from Granville County, North Carolina (Fulton and Winston 1914), and was later reported to occur in all groundnut-growing counties of Georgia (Miller 1931; Miller and Harvey 1932). However, bacterial wilt is not at present regarded as an important disease of groundnut in the United States. The disease on groundnut has also been reported from Mauritius (Shepherd 1924; Edwards 1928), South Africa (McClean 1930), Libya (Petri 1931), Somalia (Curzi 1934), Ethiopia (Castellani 1939), Madagascar (Bouriquet 1934), and Japan (Fujioka 1952), but little is known about its present status in these countries.

Bacterial wilt of groundnut is currently known to cause serious damage to the crop in Indonesia (Machmud, these Proceedings), in the south of the People's Republic of China (Darong et al. 1981), and in restricted areas of Uganda (Simbwa-Bunnya 1972). The disease is particularly severe on crops grown in wet soils where incidence commonly reaches 10%. Losses of up to 30% of the crop are experienced in seasons favouring severe disease development (Darong et al. 1981; Simbwa-Bunnya 1972).

Races and Strains of *P. solanacearum*

The existence of strains of *P. solanacearum* varying in virulence and host specificity is well documented. Van der Goot (1924) suggested that the strain of *P. solanacearum* attacking potatoes in Java was distinct from the strain affecting groundnut, and this viewpoint was supported by

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Schwarz (1926). She carried out extensive rotation experiments with groundnut, tobacco, potato, tomato, and eggplant and interpreted differing wilt disease incidences as evidence for strain differences. She considered that the strain of *P. solanacearum* which attacked groundnut, tomato and tobacco at Buitenzorg, Indonesia, was different from the strain attacking potato and eggplant.

In South Africa tobacco is rarely attacked by *P. solanacearum*, although bacterial wilt is often serious on other solanaceous crops and on groundnut (Wager 1944). Experimental evidence that the strain in South Africa is avirulent or weakly pathogenic to tobacco was given by McClean (1930). Two strains of the pathogen have also been reported from southern Rhodesia (Hopkins 1947). One strain cannot attack tobacco but affects potato, groundnut, sunflower and tomato (Dowson 1949). In extensive cross-inoculation tests in the United States, Kelman and Person (1961) identified strains differing widely in pathogenicity to tobacco and groundnut. Certain isolates that were avirulent on tobacco were highly virulent on groundnut, while the reverse was true for other isolates. The reported existence of pathogenic strains of *P. solanacearum* was based mainly on: (i) the apparent immunity or high resistance of plants in a given area that were hosts elsewhere, and (ii) the failure to produce wilt in known host plants by inoculations with pure cultures of the bacterium isolated from the same host plants elsewhere.

Three races of *P. solanacearum* were described by Buddenhagen and Kelman (1964). Four biovars, based on differences in physiological characters, have been described by Hayward (1984). The three races are:

Race 1: affecting solanaceous and other plants including plants in the Leguminosae;

Race 2: affecting triploid bananas and heliconias; and

Race 3: affecting potato.

Recently, He et al. (1983) designated the strains from mulberry in China as race 4 biotype V. The strains from mulberry that they tested were unusual because they were weakly virulent on eggplant and potato and not virulent on tomato, pepper, groundnut, or tobacco. Physiologically, the strains were also unusual in their ability to oxidise lactose, maltose, cellobiose, and mannitol, a combination of traits not found in biovars described previously by Hayward (1964).

Within each of these races there are numerous pathotypes that may be associated with particular geographical locations (Buddenhagen and Kelman 1964; Harris 1972). Several pathotypes, some pathogenic and some non-pathogenic to groundnut, have been described from Uganda (Simbwa-Bunnya 1972). Little effort has been made to describe pathotypes of the bacterium pathogenic on groundnut in areas where bacterial wilt of this crop is a serious problem.

There is a critical need to understand the distribution of distinct pathotypes in nature in relation to ecology and etiology of bacterial wilt of groundnut. We need to have answers to the following questions: (1) whether groundnut in a given area is infected by a uniform and stable population of *P. solanacearum*; (2) whether this population can, under natural conditions, cause disease of crops rotated with groundnut; (3) whether there are other strains present which cannot be detected because the crop cultivar grown is not susceptible to these strains; (4) whether the strain affecting a given host is the one attacking it in other regions. Answers to these questions would enable more realistic inferences to be drawn on questions of origin, relationship, and biology of populations pathogenic to groundnuts.

Disease Cycle and Epidemiology

Bacterial wilt of groundnut is most prevalent and severe in heavy clay soils (Van Breda de Haan 1906; Palm 1922; McClean 1930; Darong et al. 1981) although the disease has also been recorded in red lateritic and light sandy loam soils (Van Hall 1924; Palm 1926; Miller and Harvey 1932). The disease is most severe on groundnut grown in wet soils, and where the crop is grown continuously (Palm 1922; Kelman 1953; Darong et al. 1981). High rainfall, high water tables, and inadequate drainage predispose groundnuts to infection by *P. solanacearum*. Young succulent plants develop critical wilt symptoms much more rapidly than older plants (Palm 1922; McClean 1930; Miller and Harvey 1932). High soil temperatures prevailing early in the growing season favour the development of bacterial wilt in young groundnut plants (Miller and Harvey 1932; Darong et al. 1981). Wilt symptoms appear rapidly if the diseased plants are subjected to a spell of dry weather. The infected plants collapse and die quickly. If the weather remains continuously wet the disease develops and spreads, but symptoms of severe wilt may not appear for some time. Later infected plants may not develop severe wilt symptoms except when subjected to hot, dry weather late in the season.

No thorough studies have been made of penetration of groundnut roots by *P. solanacearum*, but

it is believed that roots may become infected through insect and nematode wounds, lenticels, or rifts in the root cortex made by secondary roots (Kelman 1953). Under field conditions infection of susceptible host plants usually occurs through the root system, and a wound is generally considered essential for entrance of the pathogen (Palm 1922; Kelman 1953).

Many investigators have recognised the importance of rootknot (*Meloidogyne* spp.) and other nematodes in providing wounds for entry of the pathogen into roots. On the basis of histopathological studies of groundnut roots it was concluded that infection occurred in part through insect wounds (Miller and Harvey 1932). However, Miller and Harvey (1932) reported that wounding of roots was not always required for bacterial infection. Two Japanese workers have made similar observations (Nakata 1927; Vong 1937). In their studies this phenomenon of bacterial infection through undamaged roots occurred only when highly virulent cultures were used.

The relationship between host and pathogen from time of entry of the bacterium into the susceptible host plant and the appearance of wilt symptoms has been described in detail by Buddenhagen and Kelman (1964). The xylem trachae of infected plants become filled with bacteria that eventually return to the soil following death and breakdown of plant tissues.

There is no definite evidence of *P. solanacearum* being transmitted through groundnut seed. Palm (1922) isolated the bacterium from groundnut shells and found that it could penetrate the funiculus and sometimes the integuments of the seed. But no bacteria were isolated from embryos. The possibility of the bacterium remaining viable on the outside of dry seed appears to be remote. Further research is needed to determine whether or not *P. solanacearum* can be seed transmitted in groundnut.

Control

Cultural Measures

Rotation of groundnut with crops that are immune or highly resistant to *P. solanacearum* such as corn, soybean, sugarcane and rice has been reported to be an effective means of control of bacterial wilt of groundnut (Schwarz 1926; Kelman 1953; Darong et al. 1981). In Guangdong Province of the People's Republic of China, rotation of groundnut with sugarcane for 2–3 years has been found to reduce bacterial wilt incidence from 60% to below 10%, while rotation with rice could reduce incidence to below 1% (Liang-Gao Zhou, personal communication with D. McDonald 1980). Although crop rotations for shorter periods with immune crops have proved effective in containing the disease, a gap of at least 4–5 years between groundnut crops would probably be most effective for control of the disease on soils that are heavily infested with the pathogen. Little is known as to how these cropping systems affect soil microorganisms in general and the perpetuation/survival of the bacterial wilt pathogen in particular.

In areas where groundnut is grown with irrigation in the dry season, it should be possible to control or greatly reduce levels of the disease by dry season fallowing since the bacterium is highly susceptible to desiccation. The effects of the dry season fallow can be enhanced by cultivation to improve drying out of soil and to reduce weed growth.

A few attempts have been made with limited success to minimise crop losses from bacterial wilt in groundnut by altering dates of planting (Palm 1922) to avoid periods of high temperatures or heavy rainfall that favour bacterial infection and disease development.

Crop sanitation (e.g. burning of crop residues and removal of solanaceous weeds, and cleaning of tools and machinery after operations in infested fields) should help reduce disease levels.

Chemical Control

Soil treatment with sulfur, lime and several other chemicals has not proved useful in controlling bacterial wilt of groundnut (Poole 1936; Kelman 1953).

Plant Quarantine

Since the bacterium is potentially seed-borne, strict control of seed movement should be enforced to avoid the spread of the pathogen on pods or seeds to disease-free areas.

Use of Resistant Cultivars

An effective and practical way of controlling bacterial wilt is to grow groundnut cultivars resistant to *P. solanacearum*.

The first successful attempt to breed groundnut cultivars resistant to bacterial wilt was made in Indonesia. Extensive field trials were conducted in 1921 to evaluate possible sources of resistance in groundnut genotypes from Africa, South America, North America and Indonesia (Hartley 1925). All of these gentotypes, especially the Jumbo and Valencia types, were more susceptible than the best Javanese cultivars, Tjina, Brol and Holle. Among the latter, Tjina was most resistant to bacterial wilt. Van Hall (1924, 1925) reported that Hybrid No. 3, Katjan Toeban, and Pure Line 21 showed relatively

high levels of resistance. However, these cultivars were not immune and showed high mortality of plants under severe disease pressure (Palm 1926). Later, from an extensive breeding program in Java (Indonesia), a highly resistant cultivar, Schwarz 21, was developed by selection from a wilt-resistant groundnut line of doubtful origin (Schwarz and Hartley 1950). However, on the basis of relative resistance and character of the gynophore, it was thought that Schwarz 21 probably originated from the Plumbon seed collection No. 16. This seed population and lot No. 15 from Madjalenkga were the two collections from which the lines with highest resistance were obtained in the original selection work by Schwarz at the Institute voor Plantenziekten. It is of interest that both of these collections were obtained from the Cheribon region of Java where the disease was especially severe. The history of the development of the wilt resistant cultivar Schwarz 21 has been discussed in detail by Schwarz and Hartley (1950). This cultivar has also shown a high level of resistance when inoculated with several isolates of *P. solanacearum* in greenhouse tests (Winstead and Kelman 1952; Jenkins et al. 1966).

Several groundnut cultivars resistant to local strains of the pathogen have been reported from the United States, South Africa, Uganda and the People's Republic of China. In South Africa, the small, two-seeded Natal Common types were found to be more resistant than the Virginia Bunch types (Sellschop 1947). In Mauritius, a local cultivar known as Cabri was observed to be highly resistant to bacterial wilt (Orian 1949). Simbwa-Bunnya (1972) reported three germplasm accessions, PI 341884, PI 341885, and PI 341886 immune to biotypes III and IV of *P. solanacearum* in Uganda.

In extensive inoculation tests of 17 groundnut cultivars in Georgia, USA, using three isolates of *P. solanacearum*, Jenkins et al. (1966) found a high level of resistance to bacterial wilt in Ga.119-20. However, when tested in Hubei Province of China, this cultivar did not show any resistance to bacterial wilt (Darong et al. 1981). This difference in reaction could be due to variation in pathogen and/or host-pathogen-environment interactions.

Many sources of resistance have been reported from the People's Republic of China where an active program of breeding and selection for wilt resistance has been operating since 1972. In the early 1970s two wilt-resistant cultivars, Suei tian and Yui io 589, were bred and released for cultivation in South China (Darong et al. 1981). Over the past 10 years, considerable research effort has been made in China to identify further sources of stable resistance to bacterial wilt of groundnut. From an extensive screening of germplasm accessions and breeding lines under high disease pressure in the field, Darong et al. (1981) reported five cultivars that showed relatively high levels of resistance to the disease. These cultivars were Xie kong chung, Suei tian, Yui io 589, Teishan sanliyue, and Huongzhuan zhili. Of these cultivars, Xie kong chung had the highest level of resistance. The cultivars that gave less than 10% mortality of plants were regarded as highly resistant. Recently, several additional wilt-resistant genotypes have been identified in China (Yeh Wei-Lin 1982 and Guang Rou zheng 1984, both personal communications with D. McDonald).

Genotypes from all sources reported resistant to bacterial wilt are listed in Table 1. Some of these genotypes have also been reported to have resistance to late leafspot and rust in India (Subrahmanyam et al. 1980) and in China (Yeh Wei-Lin 1982—personal communication with D. McDonald). Wilt-resistant genotypes that have been found resistant to other diseases of groundnut are listed in Table 2.

Chinese workers have made some interesting speculations on the origins of wilt-resistant genotypes. They noted a definite relationship between environmental conditions and resistance to bacterial wilt, with most of the resistant genotypes being developed in lower latitudes (Darong et al. 1981). Wilt-resistant cultivars seem to have been bred in areas where the disease occurs in severe form, especially in hot and humid areas, and such environments are most common in low latitudes. However, care should be taken to discover the primary origin when trying to relate wilt resistance in genotypes to where they have been grown. These findings indicate a need for extensive evaluation of germplasm from low latitude areas for sources of resistance to bacterial wilt.

Although it has been suggested that the stability and durability of genetic resistance to bacterial wilt may be suspect due to the possible genetic variability of the pathogen, this is not borne out by the continued resistance of cultivars such as Schwarz 21 that were bred about 55 years ago. However, this consideration is complicated by the absence of critical information on the distribution of the wilt pathogen in farmers' fields and on the genetic composition of local cultivars. There is critical need to determine the distribution of possible different strains of the pathogen in areas where the disease is a serious problem. The inheritance of wilt resistance is not yet well understood. Resistance is nor-

Table 1. Genotypes reported resistant to bacterial wilt.

Genotype/ identity	Country of origin	Criteria* code for resistance	Test locations	References
Schwarz 21	Indonesia	1	Cheribon Buitenzorg (Indonesia)	Schwarz and Hartley (1950)
		2	North Carolina (USA)	Winstead and Kelman (1952)
		2	Georgia (USA)	Jenkins et al. (1966)
PI 267771 (Matjan)	Indonesia	2	Georgia (USA)	
PI 341884	?	2	Uganda	Simbwa-Bunnya (1972)
PI 341885	?	2	Uganda	
PI 341886	?	2	Uganda	
Xie Kong Chung	China	1, 2	Huong An County (China)	Darong et al. (1981)
Suei tian	China	1, 2	Huong An County (China)	
Huongzhuan zhili	China	1, 2	Huong An County (China)	
Yui io 589	China	1, 2	Huong An County (China)	
Teishan sanliyue	China	1, 2	Huong An County (China)	
PI 393531 (ICG 7893)	Peru	1, 2	Guangdong (China)	Yeh Wei-Lin (1982)[a]
NC Ac 17130 (ICG 1705)	Peru	1, 2	Guangdong (China)	
NC Ac 17129 (ICG 1704)	Peru	1, 2	Guangdong (China)	
NC Ac 17127 (ICG 1703)	Peru	1, 2	Guangdong (China)	
PI 393641 (ICG 7894)	Peru	1, 2	Guangdong (China)	
PI 414332 (ICG 7900)	Honduras	1, 2	Guangdong (China)	
Hai-hua 1(HPS) PI 476825	China	?	?	Hammons and Porter (1982)[b]
Yie-You 22 (HP-23) PI 476842	China	-	-	
Yue-you 589 (HP-15) PI 476834	China	-	-	
320-14 (HP-4) PI 476824	China	-	-	
Dingzixili	China	-	-	Guang Rou Zheng (1984)[a]
Jinake	China	-	-	
Yuebeizhong	China	-	-	
Shuikouyazai	China	-	-	
Bairizai	China	-	-	
Bayuehao	China	-	-	
Dunduzai	China	-	-	
Liamzhou	China	-	-	
Yangjiang-pudizhan	China	-	-	
Qujiangdazhiaou	China	-	-	

* 1 = field evaluation at disease hot spots and by artificial inoculation
2 = greenhouse evaluation under artificial inoculation conditions
[a] Personal Communication to D. McDonald
[b] Collected on their visit to People's Republic of China as being resistant to bacterial wilt (cited by Mixon et al. 1983).

mally expressed in terms of high percentage of surviving plants, but this can be influenced by such factors as soil type, soil moisture and temperature, condition of the root system of the host, inoculum thresholds, and virulence of the pathogen.

Late leafspot and rust diseases are recognised as serious problems in countries where bacterial wilt occurs. As pointed out earlier, some bacterial wilt-resistant genotypes have been found to possess high levels of resistance to both late leafspot and rust. However, these genotypes are low yielding and have undesirable pod and seed characters. Emphasis should be placed on incorporating resistances to rust, leafspots and bacterial wilt into high yielding cultivars with agronomic and quality characters adapted to specific environments. At ICRISAT we are collecting a wide range of germplasm resistant to bacterial wilt. Such germplasm could be crossed with sources of resistance to major groundnut diseases and pests, and with high yielding cultivars suited to different environments. Segregating populations from such crosses could be made available for selection in areas where bacterial wilt is a problem.

Research Needs

Bacterial wilt of groundnut has received little attention except in those few regions of the world where it causes obvious economic damage. However, there is no guarantee that the disease will not become important in other regions, and priority should be given to determining the full extent of the distribution of the strains of *P. solanacearum* that attack groundnut. This work could well be done in cooperation with scientists working on bacterial wilt diseases of other tropical and subtropical crops. Little is known of the occurrence of bacterial wilt on groundnut and on wild *Arachis* species in the regions of South America where the genus *Arachis* originated, although such information could be valuable in many ways (e.g. it may be possible to study factors limiting the spread and severity of bacterial wilt in natural populations of wild *Arachis* species).

Surprisingly little is known about the infection process although this is of obvious importance in relation to inoculation methods and study of components of resistance. Research on this process could include investigation of the possible roles of nematodes and pathogenic soil fungi in rendering groundnut plants more susceptible to infection. For such studies it would be useful to have highly specific antisera (preferably monoclonal) to permit the identification of individual strains of the bacterium in soil, in the rhizosphere, and in root and pod tissues. Initially, standard methods of antiserum production such as those used successfully to type strains of *Rhizobium* (Nambiar and Anjaiah 1985) may be used, but if these prove ineffective it should be possible to produce monoclonal antibodies. Antisera could also be used in field surveys as modifications to the ELISA technique to permit antibody plates and buffer solutions to be carried to different locations.

More information is needed on the effects of different cropping systems and rotations on survival of *P. solanacearum* and on the mechanisms involved in decreasing or increasing populations of the pathogen.

Resistance screening of groundnut germplasm and breeding lines should be organised on an international basis. ICRISAT is responsible for collection and maintenance of a world collection of

Table 2. Bacterial wilt-resistant genotypes reported resistant to other groundnut diseases.

Genotype	Reaction to:				
	Bacterial wilt	Late leafspot	Rust	Pythium pod rot	Verticillium wilt
NC Ac 17127	R[a]	R[a b]	R[a b]	?	?
NC Ac 17129	R[a]	R[a b]	R[a b]	?	?
NC Ac 17130	R[a]	R[a b]	R[a b]	?	?
PI 393531	R[a]	R[a b]	R[a b]	?	?
PI 393641	R[a]	R[a b]	R[a b]	?	?
PI 414332	R[a]	S[a b]	R[a b]	?	?
Schwarz 21	R[d]	S[a b]	S[a b]	R[c]	R[c]

[a] Yeh Wei-Lin, personal communication with D. McDonald 1982.
[b] Subrahmanyam et al. 1980.
[c] Frank and Krikun 1968, 1969.
[d] Schwarz and Hartley 1950; Jenkins et al. 1966.
R = Resistant; S = Susceptible; ? = reaction not known.

groundnut germplasm and this currently consists of 11 500 groundnut accessions and 57 accessions of wild *Arachis* species. These could be made available to research workers in countries where bacterial wilt 'hot spots' occur that can be used for field resistance screening. Lines found resistant or tolerant in one region should be retested in other regions because of the possible differences in geographic distribution of pathogenic strains of the bacterium. It may also be possible to organise a project in a country where groundnut is not grown and where *P. solanacearum* is not a problem, to test selected resistant lines for their reaction to inoculation with strains of the pathogen from different regions of the world. Such tests, preferably in conjunction with international disease nurseries would be useful in determining stability of resistance of cultivars to bacterial wilt.

The overall aim should be to combine stable resistance to bacterial wilt with resistance to other economically important diseases and pests that occur in the same regions.

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Complex Diseases Involving Nematodes and *Pseudomonas solanacearum* in Potatoes in the Tropics and Subtropics

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SOILS sustaining plant growth normally harbour a complex of different genera and species of nematode parasites of higher plants. Accordingly, potato crops can always be expected to be associated with a number of plant nematode species. Among nematodes of potato in the tropics and subtropics, there are genera and species that are widespread, possess a wide host range, and are well known for their pathogenicity to other crop species. *Pseudomonas solanacearum* also has a wide host range and is widespread in similar climatic regions. The disease it causes (bacterial wilt, brown rot, bacterial ring disease, watery-eye or sore eye) is a major limiting factor in potato production. There are considerable overlaps in the distributional areas of nematode pests of potato and of the bacterium.

Since Atkinson's (1892) report of a synergism between root knot nematodes (*Meloidogyne* sp.) and a *Fusarium* sp. causing a wilt disease of cotton, there is considerable literature describing associations of nematodes and fungi and their plant pathological interactions in a wide range of crops, including potatoes. The role of nematodes in predisposing plants to fungal disease is well recognised. In contrast, since the discovery of increased disease due to *P. solanacearum* in tomato grown in soil infested with root-rot nematodes in Indonesia (Hunger 1901) there are few articles on interactions of nematodes with bacteria in various plant species; many of these concern pathogens of aerial parts of hosts. Most reports on nematode-bacterial complexes affecting roots are limited to *Meloidogyne* spp. and bacterial wilt in tomato and tobacco. More recently, workers at CIP (International Potato Centre) have published work on interactions of these organisms in potato, mainly under conditions in Peru. The role of other recognised pathogenic nematode genera and species in the culture of potato in the tropics and subtropics has been barely touched.

Pseudomonas solanacearum has been classified on the basis of its host range into 3 races (Buddenhagen and Kelman 1964). Race 1 is pathogenic to many solanaceous plants, including potato. Race 2 is pathogenic on triploid bananas and *Heliconia* spp. and does not attack solanaceous plants. Race 3 is highly virulent on potato and tobacco but not in other solanaceous crops. The bacterium has also been classified on the basis of biochemical and physiological properties into four biovars (Hayward 1976). French (1978) considered four factors as important in the control of bacterial wilt of potato: temperature, bacterial survival in the field, host range and resistance of the host. French also considered other factors that may influence disease incidence: moisture content and flow, soil type and salinity, strain aggressivity, inoculum potential, mechanical damage to hosts, transmission in seed tubers, and nematode interaction.

This paper highlights the importance of nematodes in the control of bacterial wilt in the tropics and subtropics.

Major Nematode Pests of Potatoes

At least 20 genera and 40 species of plant nematodes have been reported to attack potatoes in the tropics and subtropics. They include potato-attacking nematodes from temperate regions such as the potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*), the root lesion nematode *Pratylenchus penetrans* and genera of more restricted distributional areas, such as false root-knot nematodes (*Nacobbus* spp.) from the Andean regions of Peru and the potato-growing areas in Bolivia. Genera and species of actual and potential importance, commonly found in the tropical belt, and some salient features of their life cycle and relationships with their hosts are described below.

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***Meloidogyne* (Root-Knot Nematodes)**

Eight species have been reported to attack potato. The most widely distributed and destructive in potato are *M. arenaria, M. hapla, M. incognita* and *M. javanica.* The species most often cited for Africa and South America are *M. javanica* and *M. incognita.* All four major species have a very wide host range. The mature female is a sedentary endoparasite in the roots and tubers. It secretes a gelatinous matrix (egg sac) and deposits eggs in it. The egg mass may or may not protrude from the root. The first moult occurs in the egg. The second stage female hatches and enters the root tip, usually in the region of elongation. Within a few days, three consecutive moults occur, while the juvenile increases in size to become a vermiform male or a rotund female. Egg sac formation and subsequent egg laying commence 20–30 days from the beginning of the life cycle. Soon after entering the root, the nematode settles to feed on pericyclic cells surrounding its head. These cells enlarge, undergo a series of nuclear divisions and develop cell wall ingrowths to form a cluster of multinucleate 'giant cells'. Hyperplasia in parenchymatic tissues in the vicinity of the giant cells gives rise to the formation of conspicuous galls.

***Pratylenchus* (Root-Lesion Nematodes)**

Worldwide, 12 species have been found to be associated with potatoes. *Pratylenchus penetrans,* a polyphagous nematode, is the most widespread and damaging in potatoes in temperate regions, causing crop losses of 20–50%. It has been identified from potato in the Cameron Highlands in Malaysia (Winoto S., 1976; Winoto S. and Sauer 1982), but its significance in this crop has not been determined. In Peninsular Malaysia, *P. brachyurus* is ubiquitous, occurring in a wide range of wild and cultivated plants; it is probably native to the country because it has been found in virgin dipterocarp forests, the dominant floristic type of the lowlands (Winoto S. 1976). *Pratylenchus coffeae* is also common in Malaysia and Indonesia. In Malaysia both species attack many crops ranging from woody perennials to vegetable crops and have been implicated in poor growth and decline of many crop species.

In the Philippines, root lesion nematodes are one of the most commonly found nematodes in potatoes, but their effects on the crop are not known (Valdez 1978). In South Africa, *P. brachyurus* has been found to cause considerable losses in table potatoes, and to render infested seed tubers worthless (Koen 1967).

Pratylenchus spp. are migratory endoparasites of the root cortex. Its life cycle is simple. Eggs are laid singly in roots or in soil. The first moult occurs in the egg; the second stage juvenile hatches and undergoes three more moults to become an adult. All free-living stages can be infective and can move in and out of the roots freely. Invaded host tissues are broken, discoloured and mostly develop into necrotic lesions.

Rotylenchulus

R. reniformis, the reniform nematode, is extremely widespread in the tropics and subtropics. Its host range includes a large number of crop species. In Malaysia infected root systems of susceptible crops often do not show symptoms of injury except for a general, slightly yellowish discolouration, but growth and yield may be severely reduced (Winoto S., unpublished data). A survey in lowland potatoes in Los Baños, the Philippines, has shown that *R. reniformis* is the predominant nematode (Valdez 1978); however, no records of the nematode's effect on potato are available.

The fully grown adult female of *R. reniformis* is a sedentary, semi-endoparasite; it is embedded with its slender anterior part in the root, while the kidney shaped posterior part protrudes from the root surface. Eggs are deposited in a gelatinous matrix in the soil. After the first moult in the egg, juveniles can without feeding develop through a series of at least three superimposed moults into vermiform, preadult nematodes. Hence, large numbers of vermiform preadults are frequently found in the soil. After infecting and lodging iteself semi-endoparasitically in the roots, the posterior part grows to a kidney-like shape. The nematode lays eggs within 7–9 days after infection. The life cycle takes 20–30 days. *Rotylenchulus reniformis* initially feeds on an endodermal cell, which expands, and by cell wall degradation and lysis fuses with neighbouring pericycle cells to form a giant cell-like, multinucleate syncytium. As many as 100–200 syncytia per female have been recorded in soybean root (Rebois et al. 1970).

***Helicotylenchus* (Spiral Nematodes)**

Spiral nematodes are ectoparasitic on roots. Eight species have been recorded in Peninsular Malaysia. Of these, *H. dihystera* has been found to have the widest host range and distribution; it has also been found associated with potato (Winoto S., 1976; Winoto S. and Sauer 1982). In the Philippines, spiral and root-lesions nematodes are commonly associated with potato (Valdez 1978). The life cycle is as described for root-lesion nematodes, but takes place entirely outside the root.

Nematode/*P. solanacearum* Interactions in Potato

Since Hunger (1901), various other authors have reported cases of increased incidence or severity of wilt due to *P. solanacearum* in the presence of *Meloidogyne* in tomato. Recent papers are those of Napiere (1980) and Sellam et al. (1980). In potato, interactions of *Meloidogyne* spp. with *P. solanacearum* caused wilt-resistant potato cultivars to become susceptible (Jatala et al. 1975, Jatala and Martin 1977), a problem of particular importance in hot climates (French 1978). Such effect has also been shown in interactions between *M. hapla* and *P. solanacearum* in the wilt-resistant Clone 22, but not in Sebago; in both Sebago and Clone 22, there were little or no differences between plants that were inoculated with the bacterium alone and those with both *P. penetrans* and *P. solanacearum* (Winoto S. and Wimalajeewa, unpublished data). The ability of *Helicotylenchus nannus* and another ectoparasite *Rotylenchus* sp. to increase the incidence and severity of bacterial wilt has been demonstrated in work with tomato by Libman et al. (1964).

Possible Mechanisms of Interactions

No data exist to permit a clear insight into the mechanisms of the interrelationships between nematode, bacterium and potato. Johnson and Powell (1969) observed that the presence of *M. incognita* greatly increased the severity of wilt due to *P. solanacearum*; giant cells consistently showed the presence of 'bacteria-like masses' and these cells subsequently degenerated to become virtually empty. Here the possible mechanism resembles one that has been well documented in studies on interactions between *Meloidogyne* spp. and different fungal pathogens on different crop species, namely the predisposing role of host substrates which has been biochemically and/or morphologically modified by the nematode. Considerable information on the possible roles of root-attacking nematodes in interactions with other pathogens has been gained from the study of nematode-fungal complexes. It is reasonable to speculate that they may also operate in nematode-bacterial complexes.

All plant nematodes possess a stylet, well adapted to penetrate the host's epidermis. Surface punctures produce leakage that render the roots more attractive to other organisms. These punctures also facilitate entry of these organisms. Endoparasitic nematodes enter roots with their entire body, causing more extensive wounds and thereby possibly facilitating access to inner tissues for secondary invaders. These roles may be of particular importance to bacteria, because they are generally poorly equipped to actively invade intact roots. While pathologically not the major ones in nematode-fungal complexes, these mechanisms occur more frequently in complexes with bacteria (Lucas et al. 1955; Stewart and Schindler 1956; Libman et al. 1964).

Control of Nematode Pests of Potato

There is a paucity of research and knowledge on the control of nematodes of potatoes in the tropics and subtropics. This is understandable, because the role of nematodes in potato growing has not been fully appreciated and perhaps because potato is a new crop in much of the tropics and cultivated in limited areas.

Rotation with Non-Host Crops or Antagonistic Plants

Any consideration for the application of rotation crops in the cultivation of potato should take into account some general limitations in crop rotation systems: (a) the degree of resistance of the rotation crop and the length of time between susceptible potato varieties determine the degree of control; (b) other nematode species damaging to potato may breed on the rotation crop; and (c) the rotation crop has a low or no market value and does not contribute to farm income.

Crop Residue Amendment and Organic Manuring

Addition of organic matter, for example by ploughing under green manure crops or organic manure, has been known to suppress populations of plant nematodes due to the increase in numbers and activity of natural enemies such as nematophagous fungi or to the release of lower fatty acids from decomposing crop residues, which have a specific nematicidal action (Sayre et al. 1965). A survey in Benquet Province, the Philippines, by Valdez (1978) found that the most common nematodes in potato were species of *Pratylenchus* and *Helicotylenchus* and that their population densities were low. He attributed these low densities to heavy applications of chicken manure, usually about 8–15 tons/ha per planting season.

Biological Control

In Peru, Jatala et al. (1979) reported that the fungus *Paecilomyces lilacinus* is parasitic on eggs of *Meloidogyne* and *Globodera* on potato roots. In studies under field conditions successful use of this fungus has been achieved in the control of *G. ros-*

trochiensis and *M. incognita* in Peru, the Philippines and Panama (Anonymous 1983). In the Philippines the use of *P. lilacinus* has been made a recommended method to control *G. rostochiensis* on potatoes. At CIP, work is continuing with other fungal parasites of nematodes isolated from potato fields.

Physical Methods

In South Africa, immersion of mature potato tubers at 50 °C for 45–60 min killed all stages of *P. brachyurus* without loss of tuber viability; however, immature and mechanically damaged tubers have suffered losses of up to 25% (Koen 1971). In Peru, solar heating of soil infested with *M. incognita* using transparent polyethylene soil mulches raised the temperature by 12 °C and significantly reduced damage due to nematodes on potato (Anonymous 1983). In Victoria, Australia, solarisation of soil has been found to satisfactorily control *P. penetrans* in other crops but control of *M. javanica* has been found to be inadequate (Porter, personal communication). These Victorian observations suggest that solarisation is effective against soil-borne, free-living stages of nematodes such as hatched second stage larvae of *Meloidogyne* and all soil-borne stages of *Pratylenchus*, but not against *Meloidogyne* eggs within the protective gelatinous matrix. If this is true, soil solarisation may also be unsatisfactory when applied to control other nematodes having their eggs in similar matrices in the soil, namely against *Rotylenchulus, Nacobbus* and other saccate, sedentary nematode species. *Heterodera* eggs within cysts are known to be resistant to desiccation and high temperatures in the soil.

In the case of *Meloidogyne,* soil solarisation should, therefore, probably be combined with or supplemented by other methods of control to achieve more satisfactory results. Such methods may include the use of pesticides at a reduced rate of application. Another possibility involves the manipulation of soil moisture level: solarisation may be applied after a period of desiccation, followed by a period of watering of the soil. After desiccation of the soil eggs of *Meloidogyne* are stimulated to hatch in masses on application of water to the soil. The series soil desiccation–moisturisation–solarisation may have to be repeated more than once before egg reserves in the soil are exhausted. Desiccation may be achieved by fallow but it may be applicable in areas of low or seasonal rainfall only.

Chemical methods

Reports from the 1970s mention the effective use of the halogenated hydrocarbon fumigants DD in Alabama, USA (Rodriguez-Kabana et al. 1974), and DD, EDB and DBCP in India (Raj and Nirula 1970, 1972). However, DD has been known to cause a taint, and DBCP to be phytotoxic to potato. Moreover, several such chemicals, including DBCP, have since been banned for health reasons in many countries.

Other types of nematicides reported to be effective against *Meloidogyne* in potato fields are the methyl-isothiocyanate-based carbamate fumigants Dazomet and Vapam (Raj and Nirula 1970), the oxyme N-methyl carbamate, systemic nematicide Aldicarb (Raj and Nirula 1970), which is also effective against *R. reniformis* (Abdul Rahman 1974), and the organophosphate systemics Nellite (Raj and Nirula 1970), fensulfothion and fenamiphos (Abdul Rahman 1974). Much smaller dosage rates of non-volatile systemic chemicals than of soil fumigants are required for nematode control; also, they are not usually phytotoxic, so that they can be applied at or just before planting. Chemical control methods are unlikely to be important in the control of nematodes in developing countries, where host plant resistance is a more economical form of control, when available.

Plant Resistance

In regard to nematodes most commonly found in the tropics, efforts have mainly been concentrated in searching for sources of resistance to one or more of the four earlier mentioned major *Meloidogyne* spp. Jatala and Rowe (1976) screened accessions representing 62 tuber-bearing *Solanum* spp. and found the highest frequency of plants resistant to *M. incognita acrita* in *S. sparsipilum* and four other *Solanum* species. In regard to *P. solanacearum* resistance has been reported in wild tuber-producing *Solanum* spp. and in some hybrid crosses (Thurston 1963; Sequeira and Rowe 1969). Thurston and Lozano (1968) have found resistance to the *P. solanacearum* in *S. phureja* that gives good protection in the field. A breeding program based on *S. phureja* resistance was initiated as a joint effort of the University of Wisconsin, the Potato Programme in Colombia, and CIP, Peru. Cultivars resistant to bacterial wilt have been selected in several countries. The *S. phureja* resistance, however, has failed in the presence of *Meloidogyne* (Jatala et al. 1975), or at high temperatures (French 1978).

Because *Meloidogyne* and *P. solanacearum* are likely to be the major problems in potato-growing in the hot areas of the tropics, efforts were aimed at combining resistance to these organisms and their races, and to other major diseases, namely *Phy-*

tophthora infestans, potato virus Y (PVY) and potato leaf roll virus (PLRV) (Anonymous 1983). Gomez et al. (1983) used *S. sparsipilum* as the source of resistance to *M. incognita, M. javanica, M. arenaria* and *P. solanacearum,* and also neotuberism clones into which bacterial wilt resistance from *S. phureja* had been transferred. Resistance to the three *Meloidogyne* spp. was successfully transferred to the neo-*tuberosum* population. Two cycles of selection were made, the percentage of clones resistant to the bacterium in the last cycle being 16% over the previous cycle. Two groups of selected clones have been formed, both being resistant to the above *Meloidogyne* spp. and *P. solanacearum*. In one of the groups the sources of resistance to bacterial wilt have been combined (Schmiediche, these Proceedings).

It appears that, regionally, breeding for resistance against bacterial wilt commenced in the 1960s has had disappointing results. In contrast, breeding against root-knot nematodes in the early 1980s has been relatively successful, but the resistances found have not been tested in other geographical areas.

Suggested Areas of Research

An assessment should be made of the effects on potato of the following common nematode parasites in the tropics, singly and in combination with bacterial wilt: *Pratylenchus brachyurus, P. coffeae, Rotylenchulus reniformis* and *Helicotylenchus dihystera.*

Investigations could be carried out into the nature of the interrelations between nematode, *P. solanacearum* and potato, with special reference to interactions involving *Meloidogyne.*

Studies could be conducted on the control of nematode pests of potato by growing unfavourable hosts and antagonistic plants, and observing effects on bacterial wilt-nematode complexes in potato; the effects of crop residue amendments in soil and organic manuring on nematode–*P. solanacearum* complexes in potato; biological control of nematode-bacterial wilt complexes using *Paecilomyces lilacinus* and other nematophagous organisms; the effect of soil solarisation in combination or supplemented with other methods on the incidence and severity of diseases due to nematode-bacterial wilt complexes; and testing of potato cultivars bred for resistance against *Meloidogyne* spp. and *P. solanacearum* under local conditions in different regions in the tropics.

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Bacterial Wilt Revisited

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IT is an interesting experience to attempt an overview of a subject one was once vitally involved in as a young man—some 20 years later. I entered the world of bacterial wilt full of youthful enthusiasm and faith that most of the important questions could be answered by hard work and good research. That was in 1957 in the banana plantations and jungles of lowland Costa Rica. Luis Sequeira was already there, already an accomplished biological scientist out of Harvard and a Costa Rican upbringing. We struggled with a difficult company bureaucracy, the lack of laboratories, funds and transport, but also with an urgency for understanding a disease—Moko disease— that was threatening our very existence. We lived with an intimacy of that disease that was almost palpable. We lived immersed in the banana plantations day and night, watched and recorded the progress of death, and attempted to understand. The disease was not an abstraction and we did not have the luxury then of abstracting it in laboratories in reductionist fashion. To us the questions of importance were 1) Where was the inoculum coming from? 2) How was it spread? and 3) How could one stop its spread and the losses? In other words, **we felt we must understand both the evolution and biology of the pathosystem.**

We were lucky. We were at the right place and time in terms of observing the conversion of a natural ecosystem into an agricultural system and thus we were able to unravel the evolution and biology of a major plant disease—not bacterial wilt in general—but Moko disease of bananas.

The focus of this essay then is the framework for understanding of any disease—at whatever reductionist level—down even to the molecular. The understanding has to be based on the **evolution** and **biology** of the system. As I scan the literature of the last 20 years I see little for bacterial wilt in general that gets us closer to this understanding. In my view, the really important questions rarely have been asked, and if they have, they rarely have been carefully researched. This is not to say that good research has not been done, nor that nothing has been learned. But, other than the continuing productivity of Luis Sequeira and his colleagues and students over the years, and some work in Australia, and in a few other locations, I see little that connects the extensive laboratory research with the framework of the reality of disease biology as it exists in nature. And as for understanding pathogen ecology, we are still almost, but not quite, as ignorant as we were 20 years ago.

The Conceptual Problem

'Bacterial wilt' is two words but one conceptual entity. *Pseudomonas solanacearum*, likewise, is a single epithet. Although the literature is replete with 'biotypes' and 'strains', it generally treats 'wilt' as if there is **one** disease and **one** pathogen, created, I presume, by God, all at once, everywhere. We still live in a creationist, Linnean, descriptive, recording of 'static' phenomena which occur around us in our approach to our disease research—no matter whether we are generalists, biochemists, or molecular geneticists.

The reality, of course, is very different. There are many bacterial wilts and there are many '*Pseudomonas solanacearums*'. They have originated and evolved in widely different places and they have different capabilities with both native flora and introduced hosts, and presumably with different soils and environmental conditions.

Reductionist research needs to be based on natural groupings that reflect the plant host-bacterial evolutionary canalising of population structure. The attempt to generalise bacterial wilt as a single

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entity tends to obscure emphasising the local reality and researching the local situation in depth.

For instance, when I first tried to emphasise that Moko disease was not caused by a generalised worldwide *'Pseudomonas solanacearum'* and thus that the literature on bacterial wilt might not apply, this was considered heretical. But indeed, that was the case, and the many references to bacterial wilt of bananas in Asia and Africa were shown to be false (Buddenhagen 1961). Moreover, since the pathogen was really different, the generalities on long-term soil survival and poor effect of rotations on reducing bacterial wilt did not apply, and disease control was found to be much easier than expected.

Evolution and distribution

The vast majority of references to bacterial wilt deal with what I call new-encounter diseases (Buddenhagen 1977). This is where the crop host is introduced into a region in which the pathogen has evolved with different hosts of the natural indigenous flora (or with such plants in a non-host or semi-commensal relationship).

These references deal mostly with tobacco in Japan, tomato in Asia and elsewhere where tomato is not native; on potato in Asia, Africa and North America in lowland locations where it is not native; and less commonly on eggplant and pepper and on bananas and ginger, usually where these also are not native. Even where reports exist of wilt on crops where they are native, such as for tobacco in southeast USA, or eggplant in India, we do not know the situation on the wild progenitors that are growing in the native flora.

The evidence is inescapable that *P. solanacearum* has evolved with non-crop hosts, in many diverse locations, on different kinds of plants, which differ from place to place. We know essentially nothing of this original ecosystem evolution, and we do not even know the role of *P. solanacearum* in nature. Is it a pathogen, a commensal, a rhizosphere inhabitant, a soil inhabitant, an opportunist existing as bursts of growth inside roots of plants not wilting and thus not considered to be hosts? And, by 'it' I mean 'the population' in each area, not the species, nor the strain we recover from the introduced host.

We know only that Moko disease of bananas evolved out of a small part of a pathogen population that was connected in evolution with a banana-related, but American, genus, *Heliconia* (Buddenhagen 1960, 1964; Sequeira and Averre 1961). Extensive isolations from rhizomes of *Heliconia* from the wild in several countries revealed that, indeed, *P. solanacearum* was commonly associated, **whether or not there were disease symptoms,** and that the strains varied from place to place and that most could not cause a disease of banana.

Moreover, *Heliconia* was observed to be healthy in most natural situations and thus we know essentially nothing of the only real natural host-parasite system for *P. solanacearum*. By having a new-encounter host (banana) widely planted in *Heliconia* regions from Peru-Brazil to Mexico, however, we know that Moko disease was independently 'created' in widely divergent locations, by 'different' strains out of much more diverse populations associated with *Heliconia*, and that the epidemiological potential of these different strains differs on banana. And contrarily, that heliconias in many areas harbor *P. solanacearum*, which cannot attack bananas. So, genetic plasticity of the pathogen populations is, in nature, highly circumscribed.

We also know that in most of these same areas, different populations exist also, in the same soils, which have the capability of attacking introduced solanaceous crops, but not bananas nor heliconias. These have been lumped into race 1 (Buddenhagen et al. 1962), and are commonly called the tomato or tobacco race. What their role is with the original flora is entirely unknown. What they evolved with is also unknown. The presumption from their wide host range in Solanaceae and Compositae (and other families) is that they have evolved with one or more of several genera and species of these families, present in every tropical area of the world.

Reports continue to appear of 'new hosts' from endemic flora from many parts of the world (Kishun et al. 1980; Amat et al. 1978; Graham and Lloyd 1978; Chaudhuri and Khatua 1982; Abdullah 1980a; Sulladmath et al. 1975; Pegg and Moffett 1972; Lio et al. 1982). Usually these reports are based on weeds dying in fields where wilt was severe on an introduced crop. It is these weeds (if indeed they are indigenous) which should be examined first in original undisturbed tropical flora, as originators and perpetuators of *P. solanacearum* populations. But again, the key lesson from the many observations is that race 1 and race 2 are different; that they live side by side (sympatrically) in the same areas, but race 2 on bananas is highly restricted in geographical range compared with race 1, indicating that race 1 does not generate (nor exchange 'host range' genes with) race 2 in nature.

Restriction of Range and Population Dynamics

Bacterial wilt is largely a tropical and subtropical disease. The pathogen has been taken to more northerly, or southerly locations, in potato tubers

or in transplants or by other unknown means and it may survive and overwinter in such locations (Olsson 1976). But it does not appear to have evolved, nor to really establish itself outside of the summer-wet tropics and subtropics (I include more northerly tobacco-growing areas, including Japan, whose summers are warm-humid, in 'subtropics'). Why is it restricted to the areas it is? Why is it present in the southeast USA in areas of quite cold winters, apparently indigenously and not present in areas with Mediterranean climates where winters are no colder? (except for locations of frequent introduction with potato tubers, such as in Egypt). Would it do well, say, in California where tomatoes are grown under irrigation, if it were introduced?

Although there are many reports on its survival ability in different soils, sometimes under different temperatures and moistures (Graham et al. 1979; Shamsuddin et al. 1978; Tanaka 1979; Tanaka and Noda 1973; Shekhawat et al. 1979; Quimio and Chan 1979; McCarter 1976; Govindarajan and Gnanamanickam 1980; Graham and Lloyd 1978; Hsu 1977; Nayar and Phyza 1982; Devi et al. 1981; Lloyd 1978; Rat 1978; Ramos 1978; Hara and Ono 1983; Moffett 1981), really thorough studies which are realistic in terms of natural conditions, and specific in terms of physical factors, are largely lacking.

Population dynamics in soil per se must be carefully separated from dynamics in soil-plus-weed-hosts. As soil goes from normal cycles of wet to dry and dry to wet what happens to the pathogen population? As soil temperature (and moisture) changes with the season, what happens? As the food base for saprophytes, derived from organic matter is depleted with time or differs by place, what happens to the population? What is the effect of different soil pH levels on survival?

Then, if local hosts or non-wilting hosts are added to the system, what are the dynamics of the population changes? Good experimentation is required to answer such questions. Rational disease management requires such answers.

The assumption of course is that *in situ* population dynamics determine initial disease levels on recropping. This means we discount reintroduction or reinvasion. Since we know little about dissemination we must be open to the possibility that reintroduction by unknown means occurs and confounds some of the literature on survival. At least we could do population dynamics studies in isolated or protected sites and establish, without question, realistic soil environmental physical factor effects, with and without weed-host effects. Then, if actual field results differed markedly we would know that unknown reinvasion mechanisms were occurring.

This is especially important in relation to disease management in the extensive paddy rice soils of the tropics. Conflicting reports continue to appear of the efficacy or lack of efficacy of a flooded-rice crop in eliminating *P. solanacearum* from the soil (Seneviratne 1976). If flooded-rice really works, there should be more than ample pathogen-free land for all the susceptible solanaceous crops needed in the Asian tropics. Why does it appear not to work at times? Poor research? Or unexpected and unknown re-invasion, or unsuspected life in cryptic hosts?

Granada and Sequeira (1983) have proposed that apparent non-hosts maintain the bacterium, not as rhizosphere populations, but through limited invasion and population expansion within roots of such apparent non-hosts. I consider this hypothesis fits both the requirements of evolution and the many reports of different 'survival' rates under different rotational 'non-host' crops. How general might this phenomenon be? Others have been unable to prove its involvement (Akiew 1985), or have data possibly confirming its existence (Quimio and Chan 1979), but little testing of this hypothesis has been done. A single unique report has appeared of the wilt pathogen causing a leafspot disease (Hayward and Moffett 1978).

Since wilt due to race 1 strains occurs on Pacific tropical islands such as Samoa, Hawaii, and others, which are of fairly recent volcanic origin, and since their indigenous flora does not contain crop-host species or their progenitors, one may ask how and when did the pathogen get there? When I worked in Hawaii I was interested in this question because it is fundamental to understanding evolution, pathogen maintenance through time, and to understanding dissemination (Buddenhagen 1967). Was the pathogen there before the Polynesians? Or did they or the Europeans/Americans or Asians bring it? If so, how? In or with true seed of tomatoes or tobacco? Or did it come naturally with insects or birds? Or with seeds of the progenitors of the indigenous flora, which then became largely endemic? The fact that it is present on 'new' islands means that it has to have been introduced from somewhere. From where? Moreover, from its introduction, either a hundred, a thousand, or millions of years ago, it has had to be maintained. In the system of population growth and attrition, a balance exists wherein death and mutation to non-pathogenic butyrous forms are balanced by reproduction in or with some niche (plants) and/or by back mutation. How does this occur?

To me these are interesting questions, that should be explored, and the more remote islands and island situations should be examined for the answers.

Islands are interesting for evolution, and, indeed, island life diversity was a major inspiration for Darwin. Tropical islands throughout the world have bacterial wilt—from Reunion and Mauritius in the Indian Ocean to many Pacific islands and to the Caribbean (Zahner and Allen 1974; Buddenhagen and Kelman 1964). How similar or different are the pathogen populations on these islands? Have they, too, unique evolutionary stories to tell us? We do not know. But certainly they offer intriguing possibilities. We need comparative studies, but not just of isolates from tomatoes, potatoes and tobacco, but of strains which may be present in the indigenous flora.

The fact of such remote distribution emphasises the continuing validity of a statement in the review of 21 years ago: 'In the case of bacterial wilt it is now evident that the diversity of means of dispersal, spread, and survival has been underestimated in the past' (Buddenhagen and Kelman 1964).

Species, Races and Other Subdivisions of *P. solanacearum*

Twenty-three years ago three major divisions of *P. solanacearum* were proposed (Buddenhagen et al. 1962). The major impetus for this came from the then new research by Sequeira and myself which revealed that Moko disease of bananas was not related to the general wide host *P. solanacearum* diseases. In order to rationalise this we looked further afield, with Kelman, at both the existing literature and at as many isolates as we could obtain. I presented this work at the newly formed Caribbean Division Phytopathology meetings, but it was published, unfortunately, only as an abstract. I attempted for several years to develop a full paper on this work, but with more research and more analysis it became clear that the situation was much more complex, that there were infinite variations among isolates and that much more research was needed in this area. This is just as true today. But, the original separation into three races has proved to be very useful, and the three races still stand today, as a rational base for further work on variation and pathogen differences. Two new races have been proposed, one from ginger in the Philippines as race 4 (Kam and Quimio 1977), and one from mulberry in China (He et al. 1983). Unfortunately, this latter race was suggested to be called race 4 as well (obviously due to an oversight of the previous reference). Thus, two quite different strains are suggested to be race 4.

Hayward (1964) and Harris (1972) took a classical bacteriological approach to subdividing *P. solanacearum* and came up with biotypes (now biovars) or 'biochemical' types based on ability to oxidise various carbon sources and on other bacteriological reactions. Hayward used numbers 1, 2, 3 and 4 and 'biotype' for these subdivisions which appear to have little relation to the evolutionary or existing biology of the strains as pathogens. The exception to the lack of relation is that biotype 2 appeared at first to be essentially equivalent to our race 3. Over the years, many reports have appeared where the meaning of 'biotype' and 'race' appear confused and where the writers appear satisfied to group new isolates into a 'biotype' on a simple nutritional lab test and to consider this to be sufficient for a subspecies grouping. Some have even erroneously equated the nutritional groups with pathovars (Fucikovsky 1978).

To the uninitiated, 'biotype' infers a biological meaning. But, the biological meaning of non-saprophytic pathogens is their pathogenic potential. Their very existence is so dependent, and their population structure is so defined and circumscribed, genetically as well as biologically. Their taxonomy in relation to lab bacteriology is of bacteriological interest. But to be meaningful biologically, taxonomy of plant pathogenic bacteria must be correlated with pathogenic potential or geographic origin, and such correlations are rare.

Apparently, the strains of race 1 in Australia are confined to biotype 1 and thus it would appear that race 1 there differs in its origin. This is of interest and should be examined further. It is difficult to imagine that simple mutations for carbohydrate utilisation could not occur in any genetic stock. To make initial correlations into absolute truths is the easy error, and this is what has happened wherein any biotype 2 is equated automatically with race 3, the potato race.

Other bacteriologists have extended Hayward's phenotypic characterisation to include many more nutritional and biochemical traits useful in classifying other *Pseudomonas* species (Palleroni and Doudoroff 1971). In this and other studies (de Lay 1978), phylogenetic relationships were also explored by DNA hybridisations. On the basis of 169 characters for 26 strains the former authors found a 'moderately homogeneous species, which is at most only remotely related to all other species of the genus studied to date'. Their discussion reflects the bacteriologist's bias: they attempted to rationalise their findings with Hayward's biotypes (unsuccessfully)

but did not try to evaluate race differences—the differences which should be responsible, evolutionarily, for genetic divergence!

Thus, modern classical bacteriology still leaves *P. solanacearum* in limbo as a species. It seems to fit nowhere. Is it really a *Pseudomonas*? Does it have more affinity to *Rhizobium* than we have thought? In my view, relationships should be sought with organisms having similar biological niches—i.e. adaptation to long association with root infection—such as *Rhizobium*. I have long been intrigued by the common production by these two organisms, of polybetahydroxybutyrate as a storage compound.

It would be both nice and useful to have simple tests that would give groupings related to the reason-for-being of our bacterial pathogens. In general, carbohydrate catabolism differences (Hayward 1964; Buddenhagen et al. 1966) and other characters (Keshwal and Joshi 1976; Alagad 1980) do not. Serological groupings so far found generally do not but sometimes may (Colleno et al. 1976; Kojema and Buddenhagen 1969; Digat and Cambra 1976; Morton et al. 1966; Schaad et al. 1978); nor have phage typing or other simple tests proven consistently correlated with pathogen groups.

Thus, possibly we need to step back and analyse what we want, and, in light of modern techniques, explore the most likely approaches in depth to getting what we really want.

Most important questions are related to origin, distribution, dissemination, natural host range, epidemiological potential, and to off-season survival potential in soil and in cryptic hosts. These points require analysis in terms of bacterial population structure for the population (or populations) existing in any given delineated area. These points are those which, taken together with the ecosystem or agroecosystem, determine, through time, the existence or non-existence of *P. solanacearum* and of the importance or non-importance of bacterial wilt.

For population structure analysis one needs to circumscribe an area for sampling, preferably an area that is delineated naturally or by a specific agroecosystem. One needs to sample not only the crop host of concern but also the weeds, the natural flora nearby, and other known hosts which should be grown as bait. For, presumably, the primary crop host is picking up only a sub-sample of a more diverse population locally evolved with other cryptic and/or non-cryptic hosts. With the development of good selective media (Karganilla and Buddenhagen 1972; Granada and Sequeira 1981; Nesmith and Jenkins 1979; Hara and Ono 1983) the limitation is only the time and effort for field sampling itself, which should be extensive.

The most promising new technique for potentially typing meaningfully different pathovars is that of utilising monoclonal antibodies (Alvarez et al. 1985). This technique should be fully explored with *P. solanacearum* races and pathovar strains already known. It should also be applied to examine similarities and differences among groups believed to have been indigenous in different geographical locations.

I believe there is a good chance that monoclonals will work, and that if these are combined with realistic host inoculations, and with whatever can be learned from colony characters, that much can be accomplished toward understanding population structure, population biology and phylogenetic relationships.

Race 2

Our original hypothesis for its origin in Latin America remains unchallenged, but several developments have occurred in the last 15 years which pose interesting new questions.

The insect-transmitted SFR strain did sweep through Central America in the 1960s and the same or similar types were known in Venezuela and Colombia then. But more recently, in the eastern Andes of Peru, a chain of infection linking banana inflorescences on into the Brazilian Amazon has occurred (French and Sequeira 1970). Additionally, Moko disease, strains SFR and A (Schaad et al. 1978), appear to have come south from Guyana into Brazil (Robbs et al. 1981). I suspect these represent several separate origins from jungle *Heliconias* in those areas. Thus we have Peru, Brazil, Grenada (Cronshaw and Edmunds 1980) and Surinam (Power 1976) as new regions or countries with Moko disease, but within Latin America where the occurrences are commensurate with our original hypothesis for origin and our experience on insect dissemination (Buddenhagen and Elsasser 1962). I have recently seen Moko disease on Chato plantains, obviously insect-transmitted, in the jungle areas south of Lake Maracaibo, in Venezuela, as well as separately in the Uruba region of Colombia on French plaintains. The intriguing question to explore is to determine if these insect-disseminated strains in Central and South America are different or the same and which ones are of epidemiological importance, and the regions of importance and threat. Both Sequeira and I have evidence that the insect-transmitted strains differ in different regions.

The early reports of Moko disease in southern Brazil which I discounted as untrue in 1961 in a

review of world distribution appear to stand today still as untrue since they were on bananas in more southerly areas and no subsequent reports have appeared.

Two new events have occurred in Asia regarding Moko disease which require follow-up.

True Moko now occurs in the Philippines, with little doubt that it was introduced on banana rhizomes from Honduras, in about 1968. Previously, a disease of abaca (*Musa textilis*) was attributed to *P. solanacearum*, but it was realised gradually that this was not Moko disease, but was caused by a strain of race 1 (Zehr 1969, 1970; Rillo 1982). The new introduced Moko pathogen has remained confined to commercial banana plantations and nearby local bananas on Mindanao by application of rigid control practices. It is believed to be the B strain of race 2 rather than the SFR strain which has explosive epidemiological potential through insect dissemination. The identity of the strain should be determined with clarity. My view is that it could not be SFR, otherwise it would spread widely throughout the southeast Asian region on the many local bananas. However, without knowing the tightness of the control practices, one cannot be sure. It would be imperative to keep the SFR strain out of Asia.

The second occurrence is a report of Moko disease in southern India (Gnanamanickam et al. 1979). Several brief notes have subsequently appeared, including one on survival of the 'moko wilt pathogen in the soil of Madras' (Govindarajan and Gnanamanickam 1980). Of course it should be obvious that these reports go counter to all we have believed and published regarding the absence of Moko from Asia. If they are correct, it is a major correction or addition to our hypothesis on origin and it would require careful comparison of strains and a search for either an introduction or a local source. However, we do not know, and independent confirmation is needed. If they should prove to be correct, the enigma should be followed up vigorously.

There are several other points relative to bacterial wilt of bananas which are enigmatic and require clarification through further research.

First was the finding many years ago that race 1 in Honduras can vigorously attack certain diploid *Musa* species under natural conditions (Buddenhagen 1962). These species were introduced for breeding purposes and were present only in a breeding nursery. Nevertheless, it was clear that race 1 was very capable on these diploid *Musa*. This means that in Asia, in the homeland of banana evolution, these diploids (and possibly others) should be vulnerable to race 1 in those soils. Yet, no report has appeared of such an occurrence. This may be because no one has looked at dying diploid seeded bananas in their homeland, or it may mean that a special strain of race 1 is required and it, like race 2, is not present. The enigma should be resolved. I suggest a thorough look should be taken in areas of wild *Musa,* and new collections of *Musa* species should be made, along with their pathogens, including isolations from roots and corms of apparently healthy or only mildly affected plants. I suspect *P. solanacearum* race 1 will be found in banana species in tropical Asia. I further suspect that bacterial wilt (but not Moko disease) will be found, if searched for, in certain wild diploids.

A second enigma is the single report from Honduras that the SFR strain of race 2 has many herbaceous dicotyledonous weed hosts (Berg 1971). Although Berg undoubtedly obtained *P. solanacearum* from these weeds it is highly unlikely that they were of the insect-transmitted strain (SFR) of the banana race. That this was probably not so is commensurate with the absence of a source for this inoculum in the weeds and the subsequent rarity of diseased bananas in this area. However, our earlier extensive work on insect transmission in bananas opened up possibilities for thinking about insect spread to flowers in general and this has not been followed up. Could this be a major undetected manner of dissemination? Good realistic research is needed on this point.

Race 3

When we designated the races in 1962 we probably knew least about race 3. Yet over the years the concept that there is a narrow-host-range, highland tropical race that also has been taken to more polar locations in potato tubers, and that is distinct, has been maintained. Moreover, it is the only race that fits (but not always) with a nutritional biotype (type 2) of Hayward (race 3 so far is always of biotype 2 but the reverse is not the case). There is abundant evidence that this race has been widely spread, into Europe, Egypt and elsewhere in potato tubers.

But where is it really indigenous, and where are new records just a reflection of introduction on potato tuber seed? Potato tubers are ideal vehicles for moving this race about, since they may be latent carriers (Ciampi and Sequeira 1980; Ciampi et al. 1980, 1981).

Solanum phureja probably evolved with race 3 in the Colombian and more southern highlands and it is this *Solanum* species which was cultivated by

primitive man to grow in the presence of challenge by race 3. Thus, it is in this species where resistance has been found which is useful, and which has been incorporated into *S. tuberosum*. Where *phureja* genes have been used, potatoes have been developed with good resistance to race 3, as well as to virus Y, leaf roll, and *Phytophthora infestans* (Sequeira, personal communication). *Andigena* and *tuberosum* cannot survive with race 3 in areas where temperatures are conducive to disease. They presumably evolved in areas without race 3 or without temperatures conducive to disease, or both. But, so far, no *Solanum* potato species is known that evolved with race 1 (although some non-potato *Solanums* did so) and not even *S. phureja* can prosper in soils containing race 1.

The key questions to answer with race 3 are: 1) whether it evolved on potatoes in the Andes or on other local flora at lower altitudes in Latin America; 2) whether it evolved independently in different highland locations outside of Latin America; 3) whether race 3 occurrences on potatoes, such as in Australia, the Himalayas, the Philippines, Africa, Central America, Sri Lanka, etc. are due to an indigenous or an introduced pathogen; or 4) whether it evolved also at low altitudes but has been missed because, of our crops, only potatoes will detect it easily and these are either not planted in tropical lowlands or they are attacked more readily by race 1.

And we need to answer the question, based on realistic simulation studies, of what are the population changes of race 3 in soils under conditions of lowland, mid-altitude and highland tropics in terms of both moisture, pH, and temperature. An explanation is required for why this race is apparently confined to the highlands in the tropics. Is this really so, and if so is it due to a more limited host range or to cardinal temperature differences or exactly what? Although on first glance the connection with potato seed movement into many areas is strong (Lloyd 1978), and these areas are either temperate, or if they are in the tropics, highland locations, its apparent restriction from the lowland tropics is not explained. Recently, race 3 was reported as being present in the lowland tropics in Peru (Martin et al. 1981), picked up by planting 'pathogen free' potatoes in a jungle area, from which race 1 was also obtained. The published data do not provide sufficient information to verify that it was indeed race 3, nor that there was no possibility of introduction with the potato seed pieces. Apparently, the researchers equated the carbohydrate nutritional pattern of 'biotype 2' with race 3, a connection that should not be expected to be absolute. More concern is required for findings, such as this, which 'do not fit.' They are either interesting new findings which upset past 'truths' and therefore should be pursued in depth, or they are in error.

Interestingly, in a study of 100 strains from the Philippines in 1976–78 (Tabei and Quimio 1978), from solanaceous hosts including potatoes, only race 1 was found (no race 3) even though on nutritional typing, a few were classed as biotype 2. (The authors did not automatically equate biotype 2 with race 3.)

Race 3 is considered by some to be present in the Philippines. Has it been imported in potato seed pieces recently? Or was it always there? Or is the identity in questions due to assuming the 'truth' of the biotype 2/race 3 connection?

The only fairly good case for race 3 endemism outside of Latin America is presented in a paper (Seneviratne 1969) from Sri Lanka where a good case is made for appearance of race 3 in potatoes in recently cleared highland jungle, with seed pieces imported from western Europe, where bacterial wilt was not known (or admitted). The case is good, but not water-tight and thus we do not know if race 3 is really a ubiquitous *P. solanacearum* highland tropical organism, evolved separately in such ecosystems, or whether it is an Andean organism, moved worldwide since the Conquest or even much more recently. Some believe it may be that this 'potato race' is not originally from potatoes (French, personal communication). It is time we found out, and I am sure it can be done.

Crop Host Range

Interesting reports continue to appear from various parts of the world of new or unexpected crop hosts. One wonders about the validity of some, especially from Russia where *P. solanacearum* is often reported from various legumes, including clovers, and their true seeds (Podkina et al. 1980; Nikitina 1974; Budanova et al. 1976). Anomalous reports also occur where a crop known to be commonly grown free of bacterial wilt disease in tropical or subtropical soils containing *P. solanacearum,* is reported to be attacked in a specific location. Some of these reports one must believe, others one must question. However, even where one must believe the identity of the isolations, one wonders if the disease is real and thus a problem that will become generally important, or if it is a fluke based on a local stress. For instance, what about cowpea and common beans in Brazil (Robbs et al. 1981; Akiba et al. 1981), and cowpea in the Philip-

pines (Quimio 1974). These should be correct but they are anomalous and it should be obvious that if special stress is not involved (one cannot know) then the pathogen is of immense interest as being a unique strain. Yet there is no evidence that this was realised nor that it was saved for further research. What about a report on papaya from India (Seshadri et al. 1977) (probably wrong)? On *Dolichos lablab* from India (Keshwal 1976)? On jute from West Bengal (Sharma and Mukherji 1970)? On *Stylosanthes* in pastures in Australia? What about on cassava in Indonesia (Nishiyama et al. 1980)?

New hosts from China include olive, sweet potato, mulberry and *Casuarina* (this latter was reported earlier as a host from Mauritius), in an article of undoubted validity (He et al. 1983). I note that three are introduced species and are thus new-encounter hosts for residual indigenous Chinese bacteria. Mulberry, the indigenous host, was affected by a strain of sufficient difference that it was designated race 4 by the authors. Unfortunately they also gave it a biotype rating (5) as if the inability of oxidising dulcitol and sorbitol would be pathovar (race) specific.

Ginger has been reported as a host from several countries (Mathew et al. 1979; Zehr 1969; Indrasenan et al. 1982; Pegg et al. 1974; Pegg and Moffett 1971; Hayward et al. 1967; Lum 1973), and although it may be infected by typical race 1 it also may be picking up its own strain in some locations, out of a different indigenous microflora. This has been studied in greatest depth in tropical Australia (Pegg et al. 1974; Pegg and Moffett 1971; Hayward et al. 1967) and in the Philippines (Kam 1977) and it appears that the situation parallels closely that of Moko disease of bananas—with ginger having its own strain, less capable on typical solanaceous hosts. But, like potatoes, which has its own strain (race 3), ginger can also be attacked readily by omnivorous race 1. (This strain was proposed as a new race (race 4) earlier than the strain from Mulberry and thus it has priority and the mulberry race should become race 5.)

An overview of host range reports, of information from China, and of papers dealing with control through rotations, and on host-parasite interaction all lead me to the same basic conclusions and hypotheses: *P. solanacearum* populations evolved in different regions of the world on various members of locally indigenous flora. This association was (and is) one with rhizosphere and/or limited invasion and multiplication in native plants, only sometimes with overt disease symptoms. Survival through time has been dependent on development of bacterial and plant characters for only partial non-recognition by the roots of many plant species, so that the plant/bacterial association would not be too destructive to its natural host populations.

Where crop progenitors were present locally, they, like other flora, developed and maintained sufficient genes for general 'foreigner recognition' to include the locally-evolving *P. solanacearum* populations, as well as other potential invaders. Some crops, now hosts, were derived from native flora in locations where no *P. solanacearum* was present, in dry or high tropical and subtropical regions, or in Mediterranean-like temperate climate regions. In any case, crops moved to new locations encountered, for them, either new *P. solanacearum* populations, or *P. solanacearum* as a species, for the first time. Their diseases are new-encounter diseases, showing the destructive imbalance of such diseases (Buddenhagen 1977). They pick up, out of the potpourri of native strains, only those few which escape their recognition systems, and we attempt to understand pathogen diversity (and capability) on the basis of our crop-host sieves.

Development of Resistant Cultivars and Genetics of Resistance

Plant breeders include breeding for resistance with their many objectives when they are forced to, but it is not always an important concern. If the disease force is major and continuously protracted, sufficient resistance is developed in a given location. If the location is not the best ecosystem for disease development and pathogen survival, that resistance will be insufficient in such a 'better' ecosystem. If that location contains a genetically limited and less-than-maximally aggressive pathogen population, the resistance will be inadequate as the variety is moved to locations providing greater pathogen diversity and aggressiveness.

The record of the last 25 years of breeding of crops that are hosts of *P. solanacearum* is commensurate with these statements. Progress has been made in tobacco (Nakamura et al. 1974; Gillham and Harrigan 1977), tomato (Rao et al. 1975; Sonoda et al. 1980; Coyne and Schuster 1983), potato (Sequeira and Rowe 1969; Rowe and Sequeira 1970; Rowe et al. 1972), and a little with eggplant and chilli (Srinivasan et al. 1969; Rahim and Samraj 1974; Mochizuki and Yamakura 1979), and possibly peanut (Sun et al. 1981) and that is about it. But, the fact that the disease remains important on all these crops, and that people avoid

these crops, or use grafting on resistant rootstocks, in 'good' wilt areas (Tikoo et al. 1979), means that resistance breeding has not been sufficiently successful and that much remains to be done.

How much could be accomplished to remove wilt as an important problem on solanaceous crops by greater concerted efforts at breeding? I believe that much could be accomplished, and that resistance breeding should be intensified and the strategy and methods of approach should be re-examined (Bowman and Sequeira 1982; Keshwal 1978; Ercolani 1984; Buddenhagen and de Ponti 1983; Buddenhagen 1983a, b; Gonzalez et al. 1973). The success of any disease management approach (except for complete eradication) will depend on the level of resistance/susceptibility of the cultivar entering the system. Thus, disease management and resistance are inextricably intertwined, not separate approaches to control.

If resistance is insufficient in a crop rapidly breedable (such as tomatoes, chillies, etc.) one should ask why? The reasons are complex, but they should be examined. First, there are few good local breeding programs where much genetic material is assessed for performance under conditions of realistic challenge to the local strains and environmental conditions. Second, most solanaceous crops require a high component of product 'quality' in terms of shape, taste, colour and texture (even tobacco leaves). Third, yield potential is important, especially to the commercial breeder and the most aggressive breeding programs are, in general, commercial. Fourth, other diseases are of concern, especially viral and fungal diseases. Fifth, too few crosses are made and recurrent selection is limited. And, lastly no one is going back to locations of potential co-evolution to collect germplasm of pathogen strains and of crop progenitors or related species in locations where conditions would favour disease. Taken together, these are formidable brakes to progress. But each can be lessened by appropriate thought and concerted effort.

New collections can be made and screened; more crosses can be made; utilisation of male steriles with recurrent selection can be developed; challenge can be reduced to allow expression of minor genes; segregating populations can be sent out more widely for local selection and recrossing, more local breeding/selection programs can (and should) be developed. Quality targets can be less tight, especially for the smallfarmer. More effort can be made to assemble and study all the resistant germplasm of a given crop, both cultivars and wild species, in several parts of the world.

Resistance, crop by crop, should be reviewed in depth by a competent investigator actually involved with resistance where such research exists in depth. I have not found any such analysis. One gets the impression that for each crop there is much 'in-house' information held by breeders and agronomists, especially by company breeders, which never reaches the general reader. With tomatoes, for instance, various companies are developing varieties with wilt resistance which are privately held. I was recently informed that Peto Seed Co. (Woodland, California) has a superior wilt-resistant tomato for the tropics. Many Japanese companies have breeding programs with several wilt hosts and they sell seed to one and all. Possibly there is a lot more disease resistance available than we commonly realise, and the problem may be largely one of getting seeds of resistant cultivars into the hands of smallholders in the tropics.

I would like to see both a review of resistance breeding and seeds of a set of 'resistant', 'differential' and 'susceptible check' lines assembled for tomato, eggplant, chilli, tobacco, potato, and peanut. I would like to see a few collaborative trials of these sets in different regions where the crop and the disease are important and where different strains are suspected.

From the literature one receives the impression that there is no real concerted effort at recurrent selection to develop high wilt resistance for any given crop and crop area. Most articles give results of testing cultivars for resistance (Rahim and Samraj 1974; Sun et al. 1981; Mochizuki and Yamakura 1979), of indicating sources of resistance, or of inheritance of resistance from a cross of resistant x susceptible.

However, Robinson (1968) discussed breeding for horizontal resistance to bacterial wilt of potatoes in Kenya, an effort that he considered very promising and which apparently was dropped and has never been pursued since, anywhere. This was within *tuberosum* itself. The early work with resistance from *S. phureja*, published in 1969, made it clear that the resistance was very strain-specific (Sequeira and Rowe 1969). Moreover, these authors stressed the need for selection for resistance to a wide variation of strains and that breeding should be carried out for specific areas. This work coming out of Wisconsin, carried out later with support and collaboration from CIP, has been the most successful for potato wilt, with the development of some eight cultivars, released in various parts of the tropics (Sequeira, personal communication). The basic findings in genetics have been that few *S. phureja*

genes are involved, probably only three, independent and dominant, but somewhat strain-specific and with modifiers (Rowe and Sequeira 1970; Rowe et al. 1972). This genetic work and much screening was done with race 1 isolates even though the first need (in my view) was for resistance to race 3. Whether or not recurrent selection breeding has been practised for resistance to race 3 (as opposed to evaluating introduced lines) anywhere is not clear from the literature. In fact, it is not evident that local breeding programs have been developed for potatoes for wilt (and other resistances) utilising concepts and methodologies of horizontal resistance breeding and that even for race 1 resistance, little recurrent selection has been practiced. One may question why race 3 resistance should not be sought directly and studied genetically, and why recurrent selection is not practiced locally.

It is obvious from studies with both tomatoes (Sonoda 1978; Mew and Ho 1977) and potatoes (French and Lindo 1982; Ciampi and Sequeira 1980) that temperature and light influence greatly the expression of resistance genes and that these environmental variables greatly affect both interpretation of genetic inheritance, as well as the expression of field resistance even without strain variation. The odd report continues to appear on resistance where the reader has no idea of the meaning or follow-through on the finding. One such case from 1980 is the 'transfer of brown rot resistance from *S. microdontum* to *S. tuberosum*', from Simla, India (Tyagi et al. 1980). Apparently the International Potato Centre now has a vigorous program on interspecies crossing in potatoes for resistance targets (see Schmeidiche, these Proceedings).

Resistance in other tuber-bearing *Solanum* species was reported earlier (Sequeira and Rowe 1969), but apparently either it has not been utilised, or at least followed up.

Resistance from wild species (or species different from the major crop species) has also been used in tomatoes (Coyne and Schuster 1983). A wild species of eggplant has been suggested as a resistance source in India (Srinivasan et al. 1969) and related wild species have been assessed for resistance in Japan (Mochizuki and Yamakura 1979). An extensive search for resistance in wild crop-related species has not been carried out for most crop-hosts, but presumably, if it were, resistance would be found.

For crops such as chilli, eggplant, tobacco and peanut, occasional reports of resistance appear, usually without information on genetics (except for tobacco). For peanuts, a recent report from China reveals considerable resistance in some cultivars 'originating' in south China (Sun et al. 1981). The general impression I get is that resistance could be developed for most horticultural crops if breeding were carried out thoroughly and properly but that in most cases very little concerted effort and strategic thinking are applied.

Disease Management

How much and how severe the disease is in a given field will depend upon the level of resistance of the cultivars planted, the quantity and distribution of the inoculum in the soil and its particular genetic strain-structure, the amount of inoculum introduced with the planting stock, by insects or water or other means from outside, and on the degree to which temperature/light/moisture favour disease development and spread. Hypothetically, but not well proven, is the possibility that disease levels are also influenced (or are potentially influenceable) by the particular rhizosphere microflora.

Disease over time (from year to year) will also be influenced by all of the above plus the fate of crop-derived pathogen population increase during the time of crop-host absence. The fate of this population will depend on many factors of soil type, soil moisture, soil organic matter, soil temperature—probably mostly as they influence saprophytic microorganism activity and host tissue decay. Dominating the pure soil aspect will be the presence of other plants with their roots, in the off-season, acting to influence the pathogen population either as alternative hosts, cryptic hosts, or through negative or positive rhizosphere effects, and as they influence, especially, soil moisture levels.

Any disease management effort has to be set within the above framework. Any proposed manipulation of the system will have to be considered in practical and economic terms, as well as in giving full consideration to the biology of the system. Moreover, for success, one will have to focus on the crop-pathogen strain (or strains) relationship since, indeed, we are dealing with many different individual diseases in respect to the strain population potential for survival, for host-range, for virulence and for epidemiological potential.

Generally, one sees efforts applied (1) to reduce *in situ* inoculum (rotations, fallow); (2) to avoid reintroduction on seed stock (applied to bananas, potatoes and ginger only); (3) to escape the disease through grafting onto resistant root stocks (resistance without breeding); (4) to reduce *in situ* inoculum with chemicals (decreasing efforts recently);

(5) to utilise cultivars (resistance development is discussed elsewhere); (6) to escape the disease by growing the host crop in new fields or in new locations entirely; (7) to reduce in-field spread (for bananas) by disinfecting tools removing infectable sites and eradicating the inoculum source of already infected plants; and (8) to develop antagonistic biological control mechanisms.

It should be clear from the earlier discussion that increasing the success of the manipulative approaches requires more knowledge of how the environment in its entirety influences the off-season decline or maintenance of the population of the particular strain(s) of importance existing at the site.

Increasing the effectiveness of rotations and fallow requires more precise and realistic local research on soil factors influencing survival and on effects of alternative crops and weeds on population maintenance.

Improving clean seed programs requires better techniques for detection of the pathogen in seed lots, especially of potatoes latently infected. It also requires good organisation, regulation and authority in relation to movement of planting stocks. Ideally, where one wishes to move much material, wilt-free locations could be used for propagation of material originally introduced and indexed through tissue culture methods.

Whether grafting onto resistant rootstocks is really useful will depend purely on local economic considerations and on the continued inadequacy of 'bred resistance'. New reports continue from the tropics of this age-old control method for tomatoes and eggplant.

Of considerable interest is the more recent work on 'biological control' wherein bacteriocinogenicity is sought as a practical means of control (Cuppels et al. 1978; Luo and Wang 1983; Akiew 1985; Kempe and Sequeira 1983; Chen et al. 1981). The basic idea is that a rhizosphere coloniser that would antagonise *P. solanacearum* at the root infection site could be applied to the system and result in reduced infection. Such a system was developed for *Agrobacterium* control (Kerr and Htay 1974) which has been successful when woody perennials were dipped in bacterial solutions before transplanting. This whole research area impinges on genetic engineering approaches wherein it is envisioned to genetically engineer superior antagonists.

Although attractive in terms of innovation and new research, I think a word of caution is in order in terms of our approach to ecosystem management. First, the disease exists when and where it exists because the ecosystem itself is thoughtlessly unconcerned and antagonistic bacteria are not operating sufficiently to eliminate the pathogen. Second, any soil/rhizosphere system is well buffered and influencing it by introduction of a new organism, to cover all infectable sites in a fast-developing root system of a short season annual, is to me, a very long shot. Moreover, other points of difference from *Agrobacterium* are that most hosts are short-season annuals, and one successful infection means host death, i.e. it is a systemic disease. Where true seed is used, sufficient root surface colonisation from a seed-applied bacterium seems remote. It seems to me that the one hope would be where a high cash crop is grown from transplants, a root dip might result in sufficient attachment of an antagonist to result in further colonisation of the developing root system. In the developing world would this be only tobacco? Presumably it could also include potato seed treatment, already tried unsuccessfully (Akiew 1985) or with partial control (Kempe and Sequeira 1983).

A different approach to biological control has been tried: that of inducing resistance by treatment with incompatible bacteria that act by raising the resistance level of the host (Kempe and Sequeira 1983; Sequeira 1984). Potato tubers treated with either incompatible *P. solanacearum*, or by fluorescent pseudomonads produced plants that had a reduction in disease severity under light challenge. The practicality or utility of such induced resistance is, in my view, highly questionable.

However, one should not be too negative, since new discoveries require upsetting old prejudices or old limited knowledge. I would suggest that antagonists be sought from soils where one would expect wilt to be severe on general grounds, but where in fact it is not. There are such places, such as in Georgia and North Carolina, where wilt is severe on newly cleared sites, but then it gradually disappears. Also, one might survey host rhizospheres to see if any bacterium is sufficiently consistent to be considered a candidate for genetic engineering as an antagonist. Although I think this a long shot, it may merit inquiry. In some isolated islands (like Samoa) bacterial wilt is especially severe. Could this be partly due to a lack of an antagonistic rhizosphere organism, that is partially effective elsewhere?

In general, in the tropics I see scope for utilising rotations, and we need more practical and careful research to explore realistic rotations in relation to local strains, especially where rice culture is practiced and where dicot-host weed-smothering crops

such as sugarcane and pastures are possible. I also see scope for improving clean seed operations for potatoes combined with rotations to eradicate race 3 from specific highland or other 'introduced' locations. And, of most importance for all host crops is the revitalisation of breeding for local ecosystem adaptation (Buddenhagen 1983a, b) to include resistance to the local bacterial wilt infraspecies group.

Genetics and Host/Pathogen Interaction

Advances in modern molecular genetics offer new opportunities for understanding compatibility/incompatibility, host resistance, strain specificity, and strain differences. Several laboratories are working vigorously in this area with *P. solanacearum* and we may expect, hopefully, an unravelling of the present confused picture of what really **stops** strain A on host B and **enables** strain A to be virulent on host A, etc.

Fortunately, the question of what makes plants wilt has received less attention in recent years and the more critical question of recognition/non-recognition between pathogen and plant is being examined. In a long series of elegant studies in Sequeira's laboratory this question has been pursued step by step over many years (Graham et al. 1977; Sequeira 1980; Whatley et al. 1980; Duvick and Sequeira 1984a, b; Hendrick and Sequeira 1984; Morales and Sequeira 1984; Sequeira 1984; Stemmer et al. 1984). There has been sufficient continuity so that once shining hypotheses have been scrapped and new ones constructed and tested. The simple original hypothesis that virulence was lost with the loss of production of extracellular polysaccharide (EPS) explained only that. It did not explain specificity, i.e. why EPS-producing members of the tomato race did not attack bananas, etc. An explanation for the hypersensitive response (HR) in tobacco has been pursued diligently and for a while it appeared that lipopolysaccharide (LPS) was critical to this interaction (Whatley et al. 1980). By 1984 this hypothesis was considered not supported by the evidence and 'the initial interaction between rough LPS and tobacco cell walls is (not) the determining factor in HR initiation' (Hendrick and Sequeira 1984).

The basic hypothesis remains that the incompatible reaction (no disease) results from bacterial attachment to plant cell walls and that the compatible reaction (disease) results from lack of attachment and this, in effect, is **lack** of recognition, i.e. a negative function (or a function suppressed).

The various roles of wild and mutated LPS, EPS, pili and plant cell wall agglutinins, lectins, etc. are not resolved. In a recent discussion with Sequeira, he assured me that the problem is much more complex than he thought originally and that he is little closer to understanding specificity than at the beginning. He considers it proven, however, that the HR reaction is due to a positive function. At this time in his laboratory Tn*5* transposons and plasmids are being used for establishing a gene library of mutants for studying the genes involved in recognition/non-recognition.

The present evidence is that genes for pathogenicity in *P. solanacearum* are not resident on simple plasmids. But *P. solanacearum*, like *Rhizobium*, harbors a megaplasmid with molecular weight larger than 4.5×10^8 (Rosenberg et al. 1982). There is some evidence that most of the genes involved in the control of pathogenicity are clustered together on the megaplasmid in an 80 kb area whose deletion also gives resistance to acridine orange (Boucher et al. 1984). The megaplasmid is really not a typical plasmid in terms of handling it; rather it is more on the order of a small chromosome.

Although molecular genetics of plant pathogenic bacteria is in a flurry of activity at this time (Panopoulos and Peet 1985), and it is exciting to a small group of 'in persons', one may ask how this research might be useful.

As a generalist I think the target should be an understanding of host specificity. How many genes and of what function are involved with recognition or non-recognition in the plant/bacterial interactions? A better understanding of this interaction at the molecular level could lead to plant transformation with incompatible-host or with bacterial pathogen genes that would result in host recognition of the now-compatible invader, thereby making it an incompatible invader and thus preventing disease.

Obviously there is much to do, but much unravelling of genes in *P. solanacearum* will, I think, prove to be of interest to a few and of little value in answering fundamental questions that are really important in disease biology and disease control. Hopefully, however, enough connection can be developed with plant biologists and plant breeders so that useful innovation from molecular genetics will reach the **field**, where the biology of the system actually occurs.

The mystery of the fate of the avirulent butyrous mutants in nature was raised in our 1964 review 'whether (these) might exist in soil and have the potential to 'revert' to the virulent fluidal form is not known.' Now, 21 years later, we are just as ignorant on this point but we know that the apparent one-way shift to the butyrous form is not a

fluke. It occurs with all strains and it is a change of several characters at once. Possibly it is a major rearrangement. It would seem that it would lead to the extinction of the pathogenic form in nature unless reversion could occur. Could it be that the butyrous form really is a soil saprophyte and a mechanism of long term population maintenance, awaiting reversion with the appropriate stimulus? I think we should test this hypothesis and apply molecular genetics to understanding the shift from virulence/fluidal to avirulence/butyrous colonial forms.

And now, in 1985, I may add that the development of host resistance remains as the real viable target for research. It is not only the solution to the disease problem, but the pursuit of host resistance has the capacity to be the integrator of basic knowledge at many levels. For it appears that this pursuit will require a better understanding of strain differences and strain ecology as well as host-parasite interaction, all of which appear possible only through modern molecular genetics and related fields.

Before waiting on useful molecular genetics, however, we should reexamine our simple breeding and selection methodologies and strategies and carry on vigorously with more thorough and targeted breeding programs. The breeding team, if they do or could exist, may come up with resistance first that is both useful in the field, and useful ammunition for the molecular geneticist to attempt to understand.

Retrospect on Retrospect

Most of the questions we asked 'In Retrospect' in our review of 1964 can still be asked, because the answers have not been obtained. Apparently asking the questions did little to stimulate a search for the answers, or the questions were too naive or difficult.

Hundreds of articles have appeared on *P. solanacearum* or on wilt since then and thus much time, effort and money have been spent in many places. Many groups found support and did work. I sense, however, that much work has been done that really did not help answer the important questions. Of course the key is to identify what is important to find out, and this is a subjective activity.

If work led or leads to local reduction of disease or to a deeper understanding of disease biology at any level, it should be considered worthwhile. We do know more than we did, but I think not enough more. What was true in 1964 is still true today, and on that note I conclude from 'In Retrospect' of 1964—

'Good progress has been made in understanding the effects of the pathogen on the advanced and intermediate stages of pathogenesis; but the most critical phases of the first steps are still elusive. The subtle interactions of plant and pathogen that enable the tobacco race to be pathogenic on tobacco and not on banana (and vice versa) are still unknown. The subtle differences between resistant and susceptible varieties that give them these attributes are not understood.

The butyrous mutants, which have lost virulence and polysaccharide, are not just simple single-character mutants; they differ in many ways from the virulent form. Why do they appear as one-way mutants from the fluidal virulent form? What is their fate in nature?

Why is it (the pathogen as a species) almost world-wide in distribution? Why is this bacterium so distinctive with respect to its ability to attack so many hosts in such diverse families? How do thousands of plant species remain unaffected among wilting hosts on soils abundantly infested? How have all the diverse strains and pathotypes evolved, and how malleable are these pathotypes under the selection pressure of different cropping systems or natural host succession? What is the relation of this bacterium as a pathogen of crop plants to the natural flora and fauna and to the soil beyond the fraction of it that we know in what is actually a very narrow ecological framework? We have considered and evaluated (many things)—but answers to most of the broad and the deep questions escape us today and remain for the future. This disease is an excellent model system for investigations at all levels and we hope this review will stimulate further research with *Pseudomonas solanacearum* into the nature of virulence, strains, and host-parasite ininteraction.'

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