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9. ABSTRACT

The two objectives of this conference were: 1) to define the research or programs needed to develop more effective controls vis-a- vis P. solanacearum. Emphasis was put on strain identification and variability of the organism, soil ecology, the genetics of disease resistance in various hosts, and improved methods for development of resistant varieties. 2) To assess current resources, personnel, and facilities available in the world. Plans for continuation and expansion of current programs were considered, and specific needs for funding ongoing research programs and proposed task forces were evaluated. Included in these proceedings are all the papers presented at the conference, as well as a list of the conference participants, the conference schedule, and outline reports of the three task force committees (biotype differentiation and variability; breeding for disease resistance; and ecology and cultural control methods). No general conclusions or recommendations are given.

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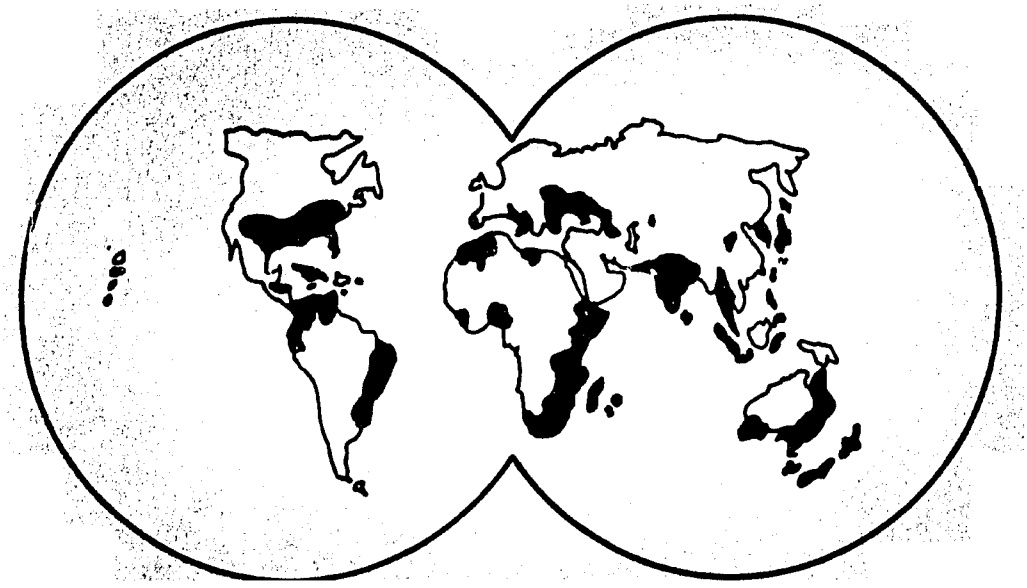

NORTH CAROLINA STATE UNIVERSITY

RALEIGH, NORTH CAROLINA

JULY 18-JULY 23, 1976

PROCEEDINGS

OF THE

FIRST INTERNATIONAL

PLANNING CONFERENCE AND WORKSHOP

ON THE

ECOLOGY AND CONTROL

OF

BACTERIAL WILT

CAUSED BY

PSEUDOMONAS SOLANACEARUM

LUIS SEQUEIRA AND ARTHUR KELMAN
EDITORS

RALEIGH, NORTH CAROLINA

JULY 18-24, 1976

Sponsored by the University of Wisconsin-Madison, North Carolina State University, Raleigh, and the International Society for Plant Pathology. Supported by grants from the Agency for International Development, The University of California/AID Pest Management and related Environmental Protection Project, and the International Potato Center.

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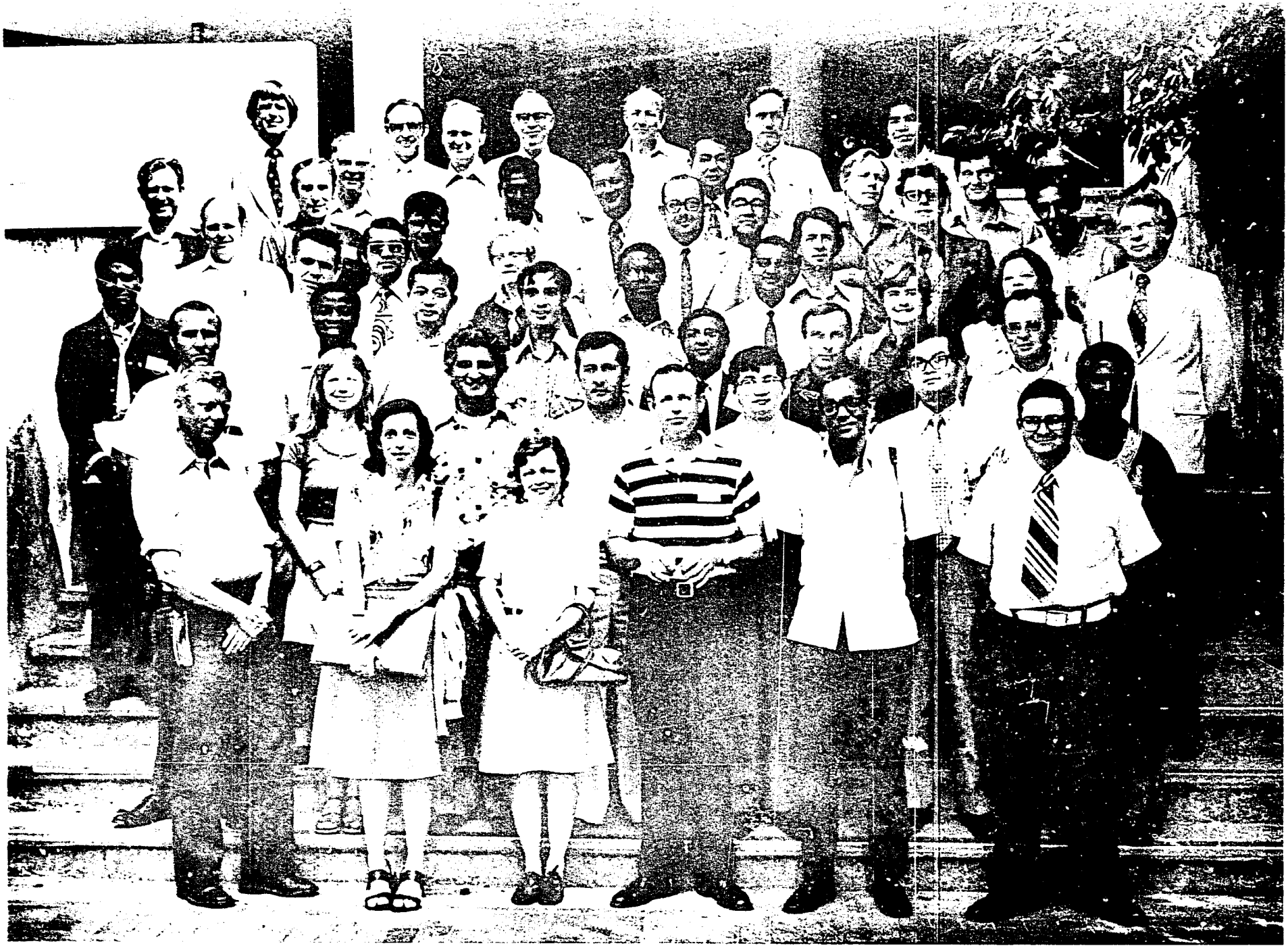

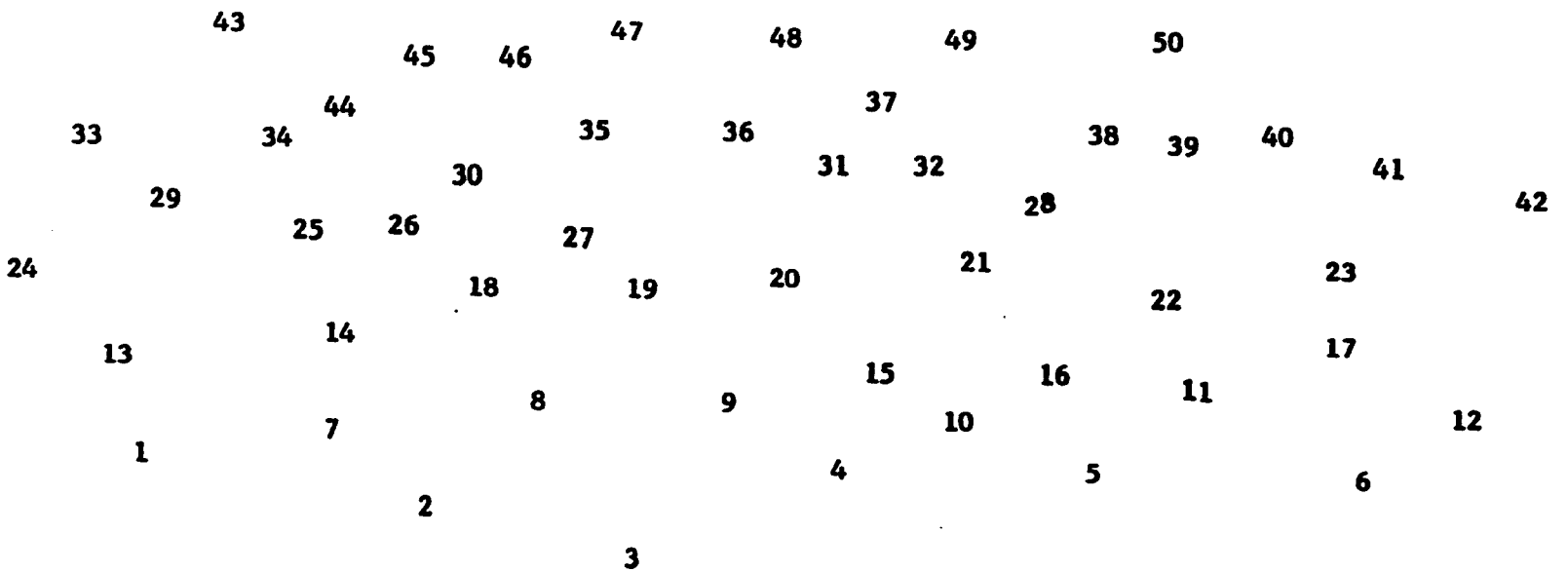

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40. Harris
41. Ribeiro
42. Aycock
43. Jenkins
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45. Apple
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MISSION OF THE CONFERENCE

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At the First International Congress of Plant Pathology held in London in 1968, an informal meeting was held by a number of research workers interested in bacterial wilt. At this meeting, the suggestion was made that planning should be initiated for an international conference to assess the status of research and to consider areas for emphasis in future studies on this important disease. A second informal meeting of interested workers was held during the Third International Conference on Plant Pathogenic Bacteria at Wageningen in 1971. With these meetings providing the impetus and the encouragement and support of colleagues, plans were initiated for a workshop and planning conference to be held in 1976. Dr. Luis Sequeira kindly agreed to serve as the Program Director, and we are deeply indebted to him for his leadership and commitment in the organization of the program and in completing the various grant applications, as well as serving as the contact person for travel of the participants and discussion leaders.

Initial financial support for the conference was provided by a grant from the University of California/Agency for International Development Pest Mangement and Related Environmental Protection Project. We wish to acknowledge with appreciation the advice and assistance provided by Drs. David Schlegel and Ray F. Smith of the UC/AID Project in the course of development of plans and their implementation.

We also acknowledge with appreciation the additional support for the conference that was provided by a travel grant from the Agency for International Development. The assistance provided by Dr. Edward Rice in connection with the administrative procedures of this grant is also acknowledged with appreciation.

The major burden in completing the local arrangements for this conference rested with the staff and faculty of the Department of Plant Pathology of North Carolina State University. Thus, we are particularly grateful to Dr. Samuel F. Jenkins, Jr., who held the primary responsibility for making the plans for housing, meals, tours, and the laboratory and greenhouse exercises, to Dr. Robert Aycock, Chairman of the Department of Plant Pathology, for his support and encouragement, and to Dr. J. L. Apple, Associate Dean of the College of Agriculture, who provided invaluable advice and assistance in completing essential administrative matters in connection with the conference and in obtaining financial aid.

This is the first workshop and conference of this type developed under the sponsorship of the International Society for Plant Pathology (ISPP). As an officer of this Society, I am pleased to extend to you greetings from the Executive Committee, the members of the Council and the Committee on Phytobacteriology. It is our hope that this workshop will serve as a model for similar meetings which will focus the attention of leading international scientists on the key diseases that currently decrease production of major food crops. The success of this conference format and the type of proposals that are developed from this workshop, as well as their implementation, will also provide guidelines for future workshops that may be developed under the sponsorship of ISPP.

Most international societies have directed their primary emphasis to the organization of international congresses which are sponsored at five- and six-year intervals. Althoughwe all recognize the value of such meetings in enhancing the exchange of scientific information, there is an unrealized opportunity within the grasp of scientists, and plant pathologists in particular, to take a more active role in the resolution of major international problems. We are hopeful that the organization of the task force committees and the specific research proposals that will be developed as a result of this meeting will greatly speed progress in development of improved effective controls for bacterial wilt, as well as in delineating procedures that may be helpful to other groups.

Eighty years have passed since Erwin F. Smith first described the causal agent of bacterial wilt. With the development of improved procedures for laboratory studies on Pseudomonas solanacearum, we should no longer experience the difficulties that motivated Smith to state that he had seen more variations in virulence and had more failures in the way of inoculations with P. solanacearum than with almost any other organism. Notwithstanding eight decades of research and observations summarized in approximately 1,400 publications, we continue to experience heavy losses all over the world because of the lack of truly effective controls and full knowledge of the biology and ecology of this destructive pathogen.

Thus, this is a historic occasion to those of us who have been involved in studies of bacterial wilt. It is the first and largest gathering of scientists with one common research objective: the study of Pseudomonas solanacearum and its control. Probably no other bacterial pathogen causes more economic loss than the wilt pathogen. Certainly no other bacterial disease has resulted in more deaths of individual plants than bacterial wilt. The epidemics of rice bacterial blight caused by Xanthomonas oryzae have been very destructive in recent years; however, this pathogen does not have the long history of continuous inroads in the production of a range of diverse food crops that characterizes the wilt pathogen. Also, P. solanacearum is a far more insidious pathogen, for once certain strains become established in the soil, profitable production of susceptible crops may be ended.

In the developing countries of the world, bacterial wilt has limited the production of food crops at all levels, from tomatoes grown by the peasant farmer in his home garden to the production and export of major agricultural products such as potatoes and bananas. This disease exemplified the impact that plant diseases can have on the socio-economic development of tropical countries. Potato cultivation, for instance, is limited in many tropical countries to extremely high elevations (usually above 8,000 ft in the Andean Region) because of the devastating effects of bacterial wilt at lower elevations. Even at high elevations, the crop can be threatened by the so-called "low-temperature strain" which may be endemic in Peru and Colombia. This strain represents a potential threat to the potato industry in Europe and North America. The recent evidence that P. solanacearum can overwinter in Sweden provides us with an increased awareness of the danger to the potato-growing regions of Western Europe.

Factors limiting research:

Investigations on P. solanacearum have been hampered by the lack of information on the many different strains of the pathogen that apparently exist throughout the tropics and subtropics. The ability to distinguish between closely related strains of the pathogen can be important in terms of the proper application of control measures. Identification of specific strains of the pathogen is particularly essential in programs designed to test host germ plasm. In potato, for instance, some hybrids are resistant against some, but not all, strains of the pathogen. The numerous hosts affected by this pathogen, its wide geographic distribution, the intrinsic complexities of strain differentiation in this variable species, and the lack of communication among researchers have resulted in the development of several systems of classification at the subspecific level, including races, strains and pathotypes, on the basis of widely different criteria.

This confusing situation can be corrected. To the research worker attempting to devise some rational control measures at an isolated location in the tropics, the bewildering systems of classification present a forbidding picture and he is not likely to attempt identification of his strains. Furthermore, if he sends his cultures to different laboratories, he is likely to obtain different, and sometimes conflicting, determinations.

Soil-borne pathogens such as P. solanacearum are also extremely difficult to control by conventional means. We still know relatively little about the characteristics of P. solanacearum that enable certain strains to survive almost indefinitely in some soils even under bare fallow conditions, whereas others can be reduced effectively by relatively short-term rotation and fallow periods in certain areas. The use of chemicals and antibiotics has been impractical in most instances. The inherent complexity of the soil environment and the wide host range of the pathogen make it difficult to determine the soil management or cultural practices that will be effective. It is essential also that the relationship between P. solanacearum and root-invading nematodes be more clearly defined if improved wilt-resistant varieties can be used in nematode-infested soils.

Breeding for disease resistance:

Control through the development of disease resistance is the only method that offers a practical solution to most of the diseases caused by P. solanacearum. The remarkable success of wilt-resistant tobacco varieties in North Carolina and peanut varieties in Indonesia has provided the hope that improved breeding techniques can be applied to develop bacterial wilt resistance in other important crop plants. Wilt-resistant varieties of tomato and resistant clones of potato are now available and are being tested in various parts of the world. However, it is clear that varieties developed with high levels of resistance at a given location may not survive in different geographic areas because of the presence of specific strains of the pathogen or because resistance is not expressed under certain environmental conditions. On the basis of the high resistance demonstrated in a number of different tobacco-growing regions for the tobacco varieties developed in North Carolina to bacterial wilt, there was hope that high levels of resistance in one area would also be effective for other host plants in other areas as well. Unfortunately, it now appears that races differ markedly in their virulence to different breeding lines of tomato and potato.

Inheritance of resistance:

The exact genetic pattern of inheritance of resistance to the various strains of the wilt pathogen has not been defined for any major host, although excellent progress has been made in the studies on inheritance of wilt resistance in potato. Although work on resistance to potato is continuing at Wisconsin and elsewhere (with the support of the International Potato Center) and efforts are continuing to improve wilt resistance in tomato (at North Carolina, Florida, Taiwan and Hawaii), it is clear that unless the strain situation can be clarified and the genetics of resistance can be defined, progress will continue to be slow.

Conference mission:

With this brief overview of selected problem areas, we should now turn our attention to the mission of this conference. The objectives of the task force planning conference and workshop on P. solanacearum will be two-fold: 1) We shall attempt to define the research or programs that are needed to facilitate the development of more effective controls. Our emphasis should be on what needs to be done in the following areas: strain identification and variability of the organism, soil ecology, and genetics of disease resistance in various hosts, and improved methods for development of resistant varieties. 2) An assessment will be made of the current resources, personnel and facilities that are available in the world. Plans for continuation and expansion of current programs should be considered. Specific needs for funding ongoing research programs and new projects should be evaluated. At the conclusion of the workshop, the proposed task forces, working through the Secretariat of the International Society for Plant Pathology, will seek support from international agencies or local governments in an attempt to expand research aimed at solving specific problems.

We need to evaluate the feasibility of establishing a center for research on P. solanacearum. At this center, cultures from as many geographical locations as possible could be stored. These can be used by research workers to develop perspectives on the origin, evolution, and pathogenic potential of the various strains of the bacterium.

The task force committees should consider prospects for the establishment of a clearinghouse for requests on information regarding P. solanacearum. Personnel of this center could guide researchers in developing countries to possible sources of materials or funds required to develop control measures at their particular location. Specifically, this center would expedite and encourage research on P. solanacearum by workers at key locations in the tropics, and would attempt to place research workers in contact with plant breeders, bacteriologists, epidemiologists, etc. in this country or abroad. The center could provide opportunities for study, on a short-term basis, by qualified research workers wishing to obtain further training.

The program that has been outlined for this conference as well as the long-term objectives are rather ambitious in scope. We are confident that the challenge of the problem and the need to make more rapid progress in our research on P. solanacearum will provide the impetus so that the goals of this conference will be reached.

SYSTEMATICS AND RELATIONSHIPS OF PSEUDOMONAS SOLANACEARUM

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INTRODUCTION: This account of the systematics of Pseudomonas solanacearum E.F. Smith will review present understanding of the position of this important plant pathogen in relation to other species of the large and ubiquitous genus Pseudomonas. Those physiological and biochemical properties will be discussed which have shown constancy or variation in studies on isolates of P. solanacearum from different hosts and territories, and which have been used either in broad taxonomic studies by microbiologists engaged in general taxonomic studies, or which have been used specifically for classification of P. solanacearum at the sub-specific level. No attempt will be made to review the research which has been carried out on host range, which together with differences in colony shape, texture and size, as well as the nature of growth and formazan deposition on tetrazolium medium (24) have been widely and successfully used as differential criteria for the designation of races and strains (5, 10). Similarly, studies on phage-typing, bacteriocin production, and serology which hold out promise of alternative systems of identification at the infrasubspecific level have been excluded because of their coverage elsewhere in these proceedings.

Several new approaches to the systematics of microorganisms have led to greater understanding of relationships at the specific, generic and higher levels. In the past 20 years the concepts of numerical taxonomy have made a contribution and, more recently, studies on nucleic acid homology using bulk DNA:DNA or ribosomal RNA:DNA reassociation have aided this area of research. Studies on the genetics of bacteria, particularly on gene transfer accompanied by recombination, have also made an impact but mainly in the Enterobacteriaceae rather than among pseudomonads and related bacteria.

NUMERICAL TAXONOMY: Several broad taxonomic studies of Pseudomonas and related genera have included reference strains of P. solanacearum. Colwell and Liston (8) included 5 isolates in an extensive examination of the phenotype of species of Pseudomonas and Xanthomonas. Fluorescent pseudomonads, Xanthomonas and P. solanacearum, formed three separate clusters at comparable levels of percentage similarity. The extent of cross relationship with the fluorescent pseudomonads was greater than with Xanthomonas.

In a study of plant pathogenic and saprophytic pseudomonads Sands et al. (37) found that four isolates of P. solanacearum formed a cluster well separated from the fluorescent pseudomonads. Some other plant pathogens and certain saprophytes were joined in the cluster including P. solanacearum; all except P. stutzeri were capable of producing poly-β-hydroxybutyrate inclusions. This study was the first to apply to P. solanacearum the technique of nutritional screening, using a wide range of carbon sources in defined medium, introduced by Stanier et al. (43) for the differentiation of the green, fluorescent pseudomonads.

NUCLEIC ACID HOMOLOGY STUDIES: Examination of the reassociation of single stranded bulk DNA from different pseudomonads or of RNA with DNA have confirmed and extended conclusions drawn from studies involving numerical taxonomy.

There is a wide divergence in the base sequence of DNA from different pseudomonads. P. solanacearum has been shown to be remote from not only the fluorescent pseudomonads but also many other plant pathogens and saprophytes of the genus Pseudomonas (28). Two isolates of P. solanacearum included in a study of reassociation of bulk DNA showed little or no homology with a range of fluorescent and other pseudomonads (29). The use of r-RNA:DNA reassociation showed more distant relationships where none were evident with bulk DNA (32). Five homology groups were described of which P. solanacearum formed one together with P. cepacia, P. marginata, P. caryophylli, P. pseudomallei, P. mallei and P. pickettii. The latter species, from human clinical sources, showed the closest relationship with P. solanacearum on the basis of nucleic acid homology studies (35).

This method of approach has added greatly to understanding of interrelationships, but the information available is incomplete. Other non-fluorescent pseudomonads which contain poly-β-hydroxybutyrate inclusions may be related to P. solanacearum. The species which have so far not been examined in nucleic acid homology studies include: P. alboprecipitans, P. stizolobii, P. andropogenis, P. rubrilineans and P. rubrisubalbicans.

GENERAL CHARACTERIZATION OF P. SOLANACEARUM AND DIFFERENTIATION AT THE SUB-SPECIFIC LEVEL: Contributions from many sources have led to an improvement in knowledge of the phenotype of P. solanacearum, of which the principal features are given in Table 1. There are a number of variable properties which invite discussion.

Salt Tolerance: Isolates of P. solanacearum show an unusually low, perhaps for Pseudomonas, uniquely low, level of tolerance of nutrient media containing salt. Pseudomonas spp. usually grow readily in nutrient media containing 3% or higher concentrations of sodium chloride, whereas there is general agreement that isolates of P. solanacearum from all sources do not grow at 2% concentration of NaCl and are often inhibited by concentrations of between 0.5% and 1.7% sodium chloride. Differences in the salt sensitivity of isolates of P. solanacearum have been observed (12, 46, Table 8).

Pigment Production: P. solanacearum isolates lack fluorescent, phenazine or carotenoid pigments. A brown to black, diffusible pigment is often produced in media by many isolates from solanaceous hosts. Variable results have often been obtained; this is partly a reflection of the extreme variation in the techniques used. Some workers have used the medium of Kelman (24) without tetrazolium salt, sometimes supplemented with 0.1% L-tyrosine, others have used liquid media such as nutrient broth or tryptophan broth. The production of a tar black or brown pigment on sterilized potato plugs has been considered to be an important diagnostic feature of the species. In general agar media incorporating tyrosine as a precursor of melanin pigment production have given the most consistent results. P. solanacearum isolates have been shown to undergo variation in pigment production in culture (10). El-Helaly et al. (9) found that an avirulent strain of P. solanacearum was more active in producing a brown pigment in culture than a virulent strain. Both strains required an aromatic amino acid for the production of pigment at different pH levels; increase in alkalinity did not result in a significant increase in the density of the pigment. Mono- or disaccharides were not required for pigment production but high concentrations (1-2%) of glucose and fructose completely inhibited pigment production in both strains due to decrease in pH. Thurston (44) using Colombian isolates from potato obtained only slight browning at a temperature of 20-
duced definite browning.

Some of the earlier work suggested that banana isolates [i.e., race 2 of Buddenhagen et al. (6)] were characterized by the absence of a brown pigment when cultures were grown on tyrosine agar (4, 40). However, later work has shown that there are exceptional musaceous strains which produce pigment (10). No musaceous isolates showed tyrosinase activity on first isolation, but two isolates developed high and low activity, respectively, on storage in vitro for long periods. Lack of ability to produce tyrosinase is not limited to isolates from musaceous hosts but has been encountered in isolates from other hosts (13, 34).

Temperature Relations: Wide variations in temperature optima, minima and maxima have been found by different workers for P. solanacearum (23, 44, 46). Smith (41) reported that isolates of P. solanacearum from the USA had an optimum temperature of 35-37°C; most other workers have reported temperature optima somewhat lower than this (cf. 46). Quinon et al. (34) noted differences in temperature optima among Hawaiian isolates. Tomato and ginger isolates grew best at 34°C, whereas an isolate from bird-of-paradise (Strelitzia reginae) grew best at 37°C. There have been many reports that isolates from potato conforming to race 3 in the system of Buddenhagen et al. (6) possess a markedly lower temperature optimum of 27-28 C (23, 14, 44, 16, 46).

Observations of temperature optima are particularly sensitive to variations in technique, such as the use of liquid or solid media, complex or defined, with large or small inocula. There are reports of bacteria showing higher temperature maxima in complex than defined medium, perhaps because of the protective effect of organic matter, and other reports that growth in liquid medium may occur at temperatures as much as 2°C higher than obtained in solid medium. Probably all tests should be carried out in a liquid medium in waterbaths rather than on solid media in incubators.

Biotypes: There are differences in the cultural and physiological properties among isolates of P. solanacearum which various authors have considered sufficient to establish sub-specific categories(5). E. F. Smith (42) designated a variety asiaticum on the basis of ability to produce acid in a litmus milk medium containing cream, but none of Smith's isolates has survived to the present day, and the relationship of var. asiaticum remains obscure.

Hayward (16) established four biotypes on the basis of ability to utilize three hexose alcohols, mannitol, sorbitol and dulcitol and to produce acid from three disaccharides, cellobiose, maltose and lactose (Table 2). It should be noted that the term "biotype" is contrary to the recommendations of the revised International Code of Nomenclature of Bacteria (26a) regarding the terminology used for infrasubspecific terms. It is recommended that the suffix "-var" or "-form" be used to replace "-type" to avoid confusion with the strict use of the term "-type" to mean nomenclatural type. According to this recommendation the term biovar should be used instead of biotype.

A reassessment of the biotype classification can be made in the light of more recent work. The type of variation forming the basis of the classification, in which isolates either utilize all three hexose alcohols or none of them, or all three disaccharides or none of them, is unusual and does not, in the author's experience, occur in any other species of Pseudomonas. If the kind of variation observed were without significance all biotypes should occur in all areas; however, several studies indicate that this is not the case. There are many localities where biotype II alone occurs and others

where biotypes III and IV occur but none of the other biotypes (17, 33, 39, 46). Nevertheless other evidence indicates that the biotype classification contains insufficient information to be of general predictive value. For example, the fact that biotype I includes pathovars of P. solanacearum which are as divergent, from the point of view of the plant pathologist, as race 2 affecting banana and many strains of race 1, according to the system of Buddenhagen et al. (6) suggests that the biotype classification may be an over-simplification of a complex situation.

The sub-division into biotypes was not wholly supported by an extensive phenotypic characterization of 26 representative isolates, using nutritional screening of sources of carbon for growth and nucleic acid homologies (30), or by studies on comparative zone electrophoresis of selected enzymes (2). Both studies provide some support for a division into two groups corresponding to combined biotypes I and II and combined biotypes III and IV.

Harris (13) showed that when the information content of the classification was increased, i.e., by increasing the number of tests applied, particularly carbon utilization tests, the differences between the biotypes become less clearcut (Table 3). He divided 100 isolates into three main groups on the basis of several correlated features which corresponded to biotype I, biotype II and a combined biotype III and IV. Biotypes II and combined III/IV were quite uniform and differed in 10 characters. But biotype I was heterogeneous and could be divided into a spectrum of nine sub-types, in which one end resembled biotype II and the other resembled biotype III/IV (13, cf. Table 3). These results and those of Palleroni and Doudoroff (30) confirm that there is substantial biochemical variation within P. solanacearum.

Zehr (46) made a study of 35 isolates of P. solanacearum from the Philippines, most of which conformed in character to biotypes III and IV, together with a single isolate of biotype I (the K-60 strain from North Carolina) and an isolate of biotype II (S-208 from Colombia). On application of numerical taxonomy to the results of 56 tests, isolates K-60 and S-208 were shown to be distinct from all Philippine isolates and also to be of relatively low similarity (76.8%) to one another. These two isolates were also readily differentiated from Philippine isolates by colony form and pigmentation.

Status of Biotype II: Biotype II is the low temperature biotype corresponding to race 3 of Buddenhagen et al. (6), and is primarily a pathogen of potato although known to occur on alternate immune, carrier or resistant weed hosts (17, 36). Examination of the growth of 12 isolates of each of the biotypes II, III and IV from Australian sources in static culture in a defined salts medium (43) at 27°C and 37°C has shown that biotype II produces more rapid growth at 27°C than at 37°C, whereas biotypes III and IV produce more rapid growth at 37°C than at 27°C (Figs. 1 and 2). Biotype II isolates also show differences in salt tolerance, rate of pitting in pectate gels at high, low and intermediate pH (20), and in phosphatase activity, compared with biotypes III and IV (Tables 7 and 8), and there are differences in the pattern of carbon source utilization (13, 30).

Biotype II is the sole biotype, usually on potato or tomato only, at the most northerly and southerly limits of the geographical distribution of P. solanacearum (16). The studies by Seneviratne (39) on the distribution of biotypes II, III and IV in the hill country of Sri Lanka (Ceylon) indicate a distribution with altitude which corresponds to the distribution according

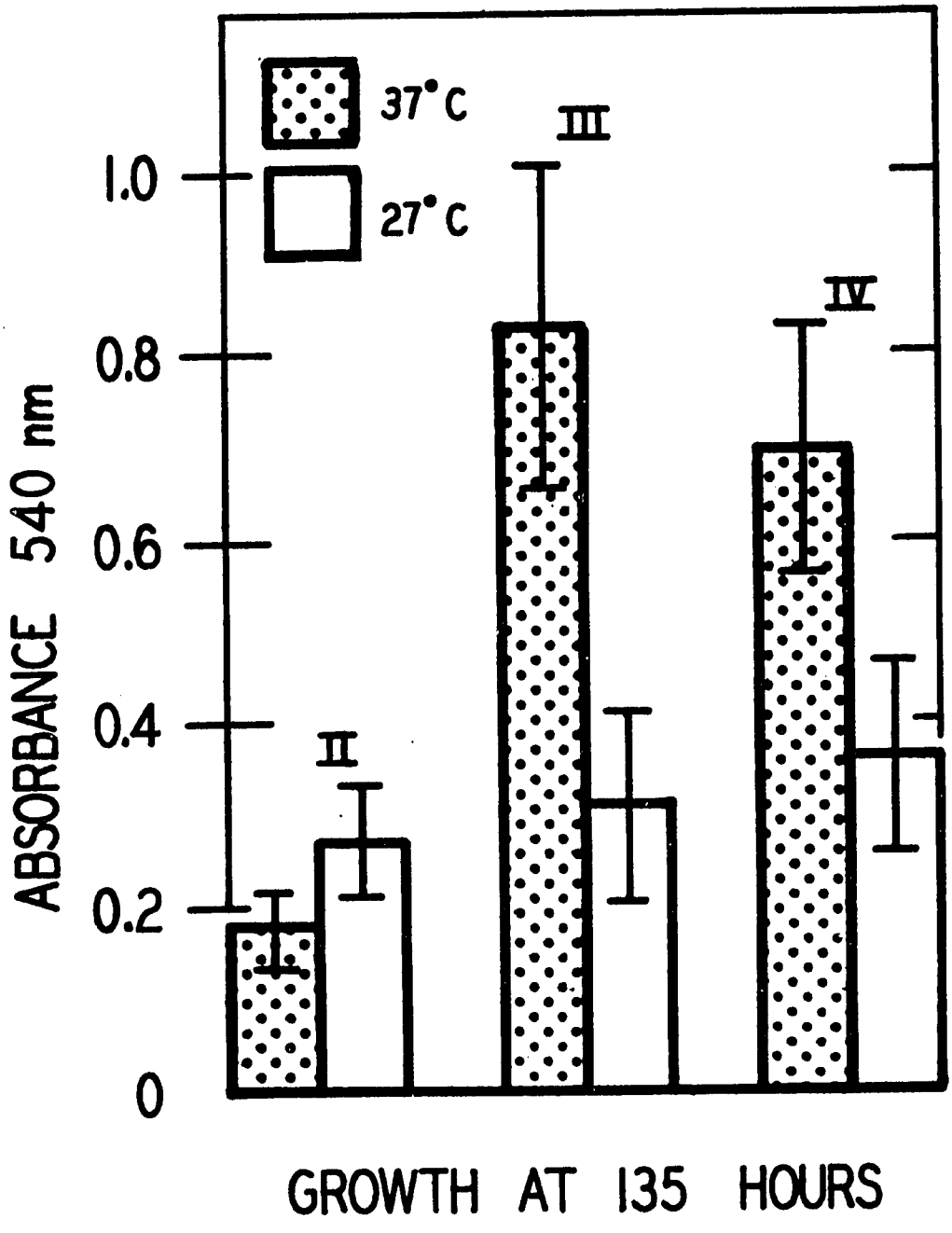

Figure 1. Growth in static culture of Pseudomonas solanacearum biotypes II, III and IV (12 cultures each) at 37°C and 27°C for 135 hr in a defined medium containing 0.25% glycerol as carbon source.

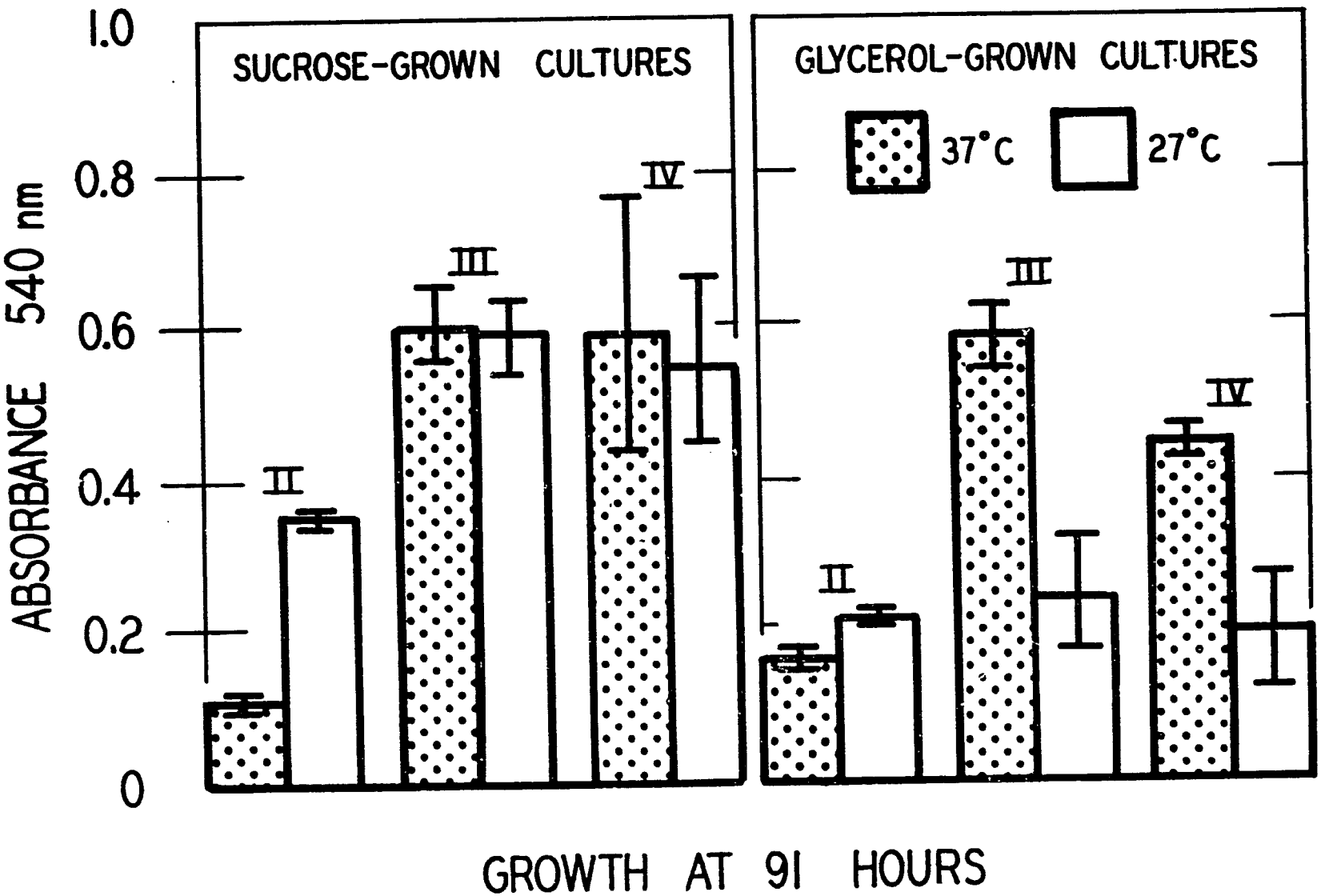

Figure 2. Growth in static culture of Pseudomonas solanacearum biotypes II, III and IV (three cultures each) at 37°C and 27°C for 91 hr in a defined medium containing either 0.25% sucrose or 0.25% glycerol.

to latitude. Seneviratne found that biotype II was obtained from only one host, potato, from cultivations established at elevations of 6,200 ft. and over in virgin lands in the hill country wet zone with a cool temperate climate. He concluded that biological and environmental factors play a part in determining the distribution of biotypes.

Other Variable Features: Variations have been reported in the antibiotic sensitivity of P. solanacearum (12, 44, 45). A comparison of 36 Australian isolates has shown a measure of uniformity in results (Table 6). Biotype IV isolates were sensitive to oleandomycin (5 µg), biotype III isolates resistant or weakly sensitive and biotype II isolates uniformly resistant. Biotype II isolates were slightly less sensitive to streptomycin than biotypes III and IV.

There are inconsistencies (Table 4) in the measurements of the guanine plus cytosine content of the DNA in P. solanacearum; the results of Murata and Starr (27) and of Khan and Sen (26) are 5-6% lower in G + C content than those obtained by Palleroni and Doudoroff (30) and Jackson (21).

CONCLUSIONS:

Several new approaches to the systematics of Pseudomonas have been applied to P. solanacearum. Studies in defined mineral salts medium of the sources of carbon for energy and growth have been useful and productive in that a much wider range of differentiae has become available (28). Several workers have shown that there are differences in the pattern of carbon utilization among isolates of P. solanacearum (12, 13, 30, 46). Most studies of this kind have made use of isolates of diverse origin in time and space; more needs to be known about variations in carbon utilization pattern among strains occurring in a particular area. Differences which occur may provide a useful tool in epidemiological studies. It may be possible to devise selective or enrichment media on the basis of particular selective carbon sources which, together with antibiotics or other inhibitors, may serve to isolate particular clones or strains of epidemiological importance.

Buddenhagen and Kelman (5) have admirably summarized the objectives and problems associated with biotype, race or strain separation in P. solanacearum. The goal should be a working classification at the sub-specific level which will have sufficient information content and therefore predictive value to be of assistance to plant pathologists in their studies on the ecology and etiology of infection, and the movement and detection of P. solanacearum between territories and in the field. Characterization should be as broadly based as possible and not limited to studies on host range, colony form and other cultural features, valuable though these approaches have been in observing the evolution of races and strains in Central America and the West Indies (5). In this regard there is an urgent need for the standardization of media and test conditions for systematic studies. In some respects the problems associated with the systematics of P. solanacearum parallel those obtaining with other major wilt pathogens of wide host range such as Fusarium, or in the field of soil microbiology with the recognition of species of Rhizobium where reliance on host range alone has not led to a stable or satisfactory system of classification. Jeffrey (22) has stated the case for systematics in the following terms: "The task of systematics is to produce systems of classification which best express the various degrees of overall similarity between living organisms. Such systems are used in biology for

the storage, retrieval and communication of information and for the making of reliable predictions and generalizations. They are based on as broad as possible study of the variation of living organisms and aim to establish groups, the members of which possess the largest number of common features and exhibit therefore the greatest overall similarity.

The possibility of constructing such systems, of course, depends upon the occurrence of different features associated in definite combinations in different living organisms. If features all varied independently of one another, then each feature considered would produce a different way of grouping organisms and no grouping based on greatest overall similarity would be possible. However, this is not so, and it is possible to construct systematic groupings that are based on multiple correlations of common features and which reflect greatest overall similarity. This is in general a result of the fact that all living organisms are related to one another to a greater or lesser degree by way of evolutionary descent, and it is this evolutionary relationship that makes possible the establishment of meaningful systematic groupings."

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LITERATURE CITED

1. BALLARD, R. W., N. J. PALLERONI, M. DOUDOROFF, R. Y. STANIER and M. MANDEL. 1970. Taxonomy of the aerobic pseudomonads: Pseudomonas cepacia, P. marginata, P. alliicola and P. caryophylli. J. Gen. Microbiol. 60: 199-214.

2. BAPTIST, J. N., C. R. SHAW and M. MANDEL. 1971. Comparative zone electrophoresis of enzymes of Pseudomonas solanacearum and Pseudomonas cepacia. J. Bacteriol. 108:799-803.

3. BRYAN, M. K. 1915. A nasturtium wilt caused by Pseudomonas solanacearum. J. Agr. Res. 4:451-457.

4. BUDDENHAGEN, I. W. 1960. Strains of Pseudomonas solanacearum in indigenous hosts in banana plantations of Costa Rica, and their relationship to bacterial wilt of bananas. Phytopathology 50:660-664.

5. BUDDENHAGEN, I. W. and A. KELMAN. 1964. Biological and physiological aspects of bacterial wilt caused by Pseudomonas solanacearum. Ann. Rev. Phytopathol. 2:203-230.

6. BUDDENHAGEN, I. W., L. SEQUEIRA and A. KELMAN. 1962. Designation of races in Pseudomonas solanacearum. Phytopathology 52:726 (Abstr.).

7. BURKHOLDER, W. H. and M. P. STARR. 1948. The generic and specific characters of phytopathogenic species of Pseudomonas and Xanthomonas. Phytopathology 38:494-502.

8. COLWELL, R. R. and J. LISTON. 1961. Taxonomic analysis with the electronic computer of some Xanthomonas and Pseudomonas species. J. Bact. 82:913-919.

9. EL-HELALY, A. F., M. K. ABO-EL-DAHAB and M. A. EL-GOORANI. 1969. Chromogenesis in cultures of Pseudomonas solanacearum. J. Phytopath. UAR. 1:1-11.

10. FRENCH, E. R. and L. SEQUEIRA. 1970. Strains of Pseudomonas solanacearum from Central and South America: a comparative study. Phytopathology 60:506-512.

11. FUERST, J. A. and A. C. HAYWARD. 1969. Surface appendages similar to fimbriae (pili) on Pseudomonas species. J. Gen. Microbiol. 58:227-238.

12. GRANADA, G. A. and L. SEQUEIRA. 1975. Characteristics of Colombian isolates of Pseudomonas solanacearum from tobacco. Phytopathology 65: 1004-1009.

13. HARRIS, D. C. 1972. Intra-specific variation in Pseudomonas solanacearum. pp. 289-292 in H. P. Maas Geesteranus (ed.) Proc. 3rd Int. Conf. Plant Pathogenic Bact., Wageningen, The Netherlands. 365 p.

14. HARRISON, D. E. 1961. Bacterial wilt of potatoes, I. Field symptoms of the disease and studies on the causal organism, Pseudomonas solanacearum variety asiaticum. Australian J. Agr. Res. 12:854-871.

15. HAYWARD, A. C. 1960. A method for characterizing Pseudomonas solanacearum. Nature 186:405-406.

16. HAYWARD, A. C. 1964. Characteristics of Pseudomonas solanacearum. J. Appl. Bacteriol. 27:265-277.

17. HAYWARD, A. C. 1975. Biotypes of Pseudomonas solanacearum in Australia. Australian Plant Path. Soc. Newsletter 4:9-11.

18. HENRICHSEN, J. 1975. The occurrence of twitching motility among Gram-negative bacteria. Acta.Path. Microbiol. Scand. Sect. B 83:171-178.

19. HENRICHSEN, J. and J. BLOM. 1975. Examination of fimbriation of some Gram-negative rods with and without twitching and gliding motility. Acta. Path. Microbiol. Scand. Sect. B 83:161-170.

20. HILDEBRAND, D. C. 1971. Pectate and pectin gels for differentiation of Pseudomonas sp. and other bacterial plant pathogens. Phytopathology 61:1430-1436.

21. JACKSON, J. F. Unpublished observations.

22. JEFFREY, C. 1973. Biological nomenclature. Edward Arnold, London 69 p.

23. KELMAN, A. 1953. The bacterial wilt caused by Pseudomonas solanacearum. A literature review and bibliography. N.C. Agric. Exp. Stn. Tech. Bull. 99. 194 p.

24. KELMAN, A. 1954. The relationship of pathogenicity in Pseudomonas solanacearum to colony appearance on a tetrazolium medium. Phytopathology 44:693-695.

25. KELMAN, A. and J. HRUSCHKA. 1973. The role of motility and aerotaxis in the selective increase of avirulent bacteria in still broth cultures of Pseudomonas solanacearum. J. Gen. Microbiol. 76:177-188.

26. KHAN, N. C. and S. P. SEN. 1974. Further observations on genetic transformation in Pseudomonas. J. Gen. Microbiol. 83:251-259.

26a. LAPAGE, S. P., P. H. A. SNEATH, E. F. LESSEL, V. B. D. SKERMAN, H. P. R. SEELIGER and W. A. CLARK (Editors). 1975. International Code of Nomenclature of Bacteria. Bacteriological Code. 1976 revision. American Society for Microbiology, Washington, D.C. 180 p.

27. MURATA, N. and M. P. STARR. 1973. A concept of the genus Xanthomonas and its species in the light of segmental homology of deoxyribonucleic acids. Phytopath. Z. 77:285-323.

28. PALLERONI, N. J. 1975. General properties and taxonomy of the genus Pseudomonas. Pages 1-36 in P. H. Clarke and M. H. Richmond (eds.) Genetics and Biochemistry of Pseudomonas. John Wiley, London and New York 366 p.

29. PALLERONI, N. J., R. W. BALLARD, E. RALSTON and M. DOUDOROFF. 1972. Deoxyribonucleic acid homologies among some Pseudomonas species. J. Bacteriol. 110:1-11.

30. PALLERONI, N. J. and M. DOUDOROFF. 1971. Phenotypic characterization and DNA homologies of Pseudomonas solanacearum. J. Gen Microbiol. 107:690-696.

31. PALLERONI, N. J., M. DOUDOROFF, R. Y. STANIER, R. E. SOLANES and M. MANDEL. 1970. Taxonomy of the aerobic pseudomonads: the properties of the Pseudomonas stutzeri group. J. Gen. Microbiol. 60:215-231.

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

33. PRUNIER, J. P. 1967. Differenciation en types biochimiques de souches de Pseudomonas solanacearum (Smith) Smith. Phytiat-Phytopharm. 16: 199-204.

34. QUINON, V. L., M. ARAGAKI and M. ISHII. 1964. Pathogenicity and serological relationships of 3 strains of Pseudomonas solanacearum in Hawaii. Phytopathology 54:1096-1099.

35. RALSTON, E., N. J. PALLERONI and M. DOUDOROFF. 1973. Pseudomonas pickettii, a new species of clinical origin related to Pseudomonas solanacearum. Int. J. Syst. Bacteriol. 23:15-19.

36. REVIEW OF APPLIED MYCOLOGY. 1968. 47: No. 2358.

37. SANDS, D. C., M. N. SCHROTH and D. C. HILDEBRAND. 1970. Taxonomy of phytopathogenic pseudomonads. J. Bacteriol. 101:9-23.

38. SCHAAD, N. W., C. I. KADO and D. R. SUMNER. 1975. Synonymy of Pseudomonas avenae Manns 1905 and Pseudomonas alboprecipitans Rosen 1924. Int. J. Syst. Bacteriol. 25:133-137.

39. SENEVIRATNE, S. N. DE S. 1969. On the occurrence of Pseudomonas solanacearum in the hill country of Ceylon. J. Hort. Sci. 44:393-402.

40. SEQUEIRA, L. and C. W. AVERRE. 1961. Distribution and pathogenicity of strains of Pseudomonas solanacearum from virgin soils in Costa Rica. Plant Dis. Reptr. 45:435-440.

41. SMITH, E. F. 1896. A bacterial disease of the tomato, eggplant and Irish potato (Bacillus solanacearum nov. sp.). U.S. Dept. Agr., Div. Veg. Phys. and Path., Bul. 12:1-28.

42. SMITH, E. F. 1914. Bacteria in relation to plant diseases. Vol. 2. Carnegie Inst., Washington. 309 p.

43. STANIER, R. Y., N. J. PALLERONI and M. DOUDOROFF. 1966. The aerobic pseudomonads: a taxonomic study. J. Gen. Microbiol. 43:159-271.

44. THURSTON, D. H. 1963. Bacterial wilt of potatoes in Colombia. Am. Potato J. 40:381-390.

45. WARDLAW, C. W. 1961. Banana diseases, including plantains and abaca. Longmans, Green & Co., Ltd., London. 648 p.

46. ZEHR, E. I. 1970. Cultural, physiological and biochemical properties of isolates of Philippine Pseudomonas solanacearum. Philippine Phytopathology 6:29-43.

Table 1. Profile of Pseudomonas solanacearum (Smith, 1896) Smith 1914

I. Morphology

Gram-negative rods, ca. 0.5 - 0.7 x 1.5 - 2.5 μm

Motile with 1 - 4 polar flagella; virulent isolates are mainly non-flagellated and non-motile, avirulent isolates usually flagellated and highly motile (25).

Polar fimbriae present (11,25,19), with which twitching motility and spreading growth on solid media are associated (18).

Cells contain inclusions of poly-β-hydroxybutyrate which are sudanophilic and refractile under phase microscopy (15).

Cells commonly show bipolar staining (41).

II. Cultural characteristics

Colony variation common; from fluid, opaque to non-fluid butyrous; intermediate colony types occur.

Differences between colony types and levels of virulence related to presence, absence or amount of an extracellular polysaccharide slime (24).

Brown, diffusible "pigments" often produced on media containing tyrosine; some strains produce no pigment (10).

Fluorescent, phenazine or carotenoid pigments not produced.

III. Physiological and biochemical properties

Aerobic, respiratory metabolism; catalase positive and Kovacs' oxidase positive.

Chemoorganotrophs.

No organic growth factor requirements.

None grows autotrophically in atmospheres of H_2, CO_2 and oxygen.

Reports of optimum temperature for growth variable; 27°- 37°C depending on strain (14, 23, 34, 44, 46).

Maximum temperature ca. 39°C.

Minimum temperature 10-15°C (23).

Nitrate reduced to nitrite; some strains produce gas from nitrate, i.e., they denitrify (13, 16, 30).

Low tolerance of saline media; no growth in nutrient liquid medium containing 2% NaCl (3, 7, 16, 46, 12).

Table 2. Differentiation of Pseudomonas solanacearum into biotypes on the basis of oxidation and utilization of carbohydrates (16)

Characteristic	Biotype I	Biotype II	Biotype III	Biotype IV
Oxidation of (acid production from):				
Glucose[2] and sucrose[2]	+[c]	+	+	+
Lactose[a]	-	+	+	-
Maltose[a]	-	+	+	-
Cellobiose[a]	-	+	+	-
Mannitol[b]	-	-	+	+
Sorbitol[b]	-	-	+	+
Dulcitol[b]	-	-	+	+
Denitrification (Gas from nitrate)	V	-	+	+

[a] Not utilized as sole carbon source in defined medium (30,13)

[b] Utilized as sole carbon source in a defined medium

[c] Negative reaction = -; positive reaction = +; some strains give a positive reaction, some give a negative reaction = V

Table 3. Biochemical subdivisions of biotype I (12)

Character	Biotype II	Subdivisions of Biotype I									Biotypes III/IV
		A	B	C	D	E	F	G	H	J	
Gas from nitrate	-	-	-	-	-	-	-	-	+	+	+
Tyrosinase	+	-	-	+	-	+	-	-	-	-	+/-
Tween 80 hydrolysis	+	+	+	+	+	-	+	+	-	+	+
Utilization of substrates as sole source of carbon and of energy:											
Tryptophan	-	-	-	+	-	+	+	+	+	+	+
Hippurate	+	+	+	+	+	+	+	+	-	-	-
Malonate	-	-	-	-	-	-	-	+	-	-	-
Fructose	+	+	+	+	-	+	+	-	-	+	+
Inositol	-	-	+	+	-	+	+	+	+	+	+
Trehalose	-	+	-	-	+	-	+	+	-	+	+
Lactate	-	-	-	-	-	-	-	-	-	+	+

Table 4. Guanine plus cytosine content of the deoxyribonucleic acid (DNA) of *Pseudomonas solanacearum* and some related pseudomonads

Species	Number of strains	%G + C in DNA	Method	Reference
P. andropogonis	1	57.8	t	(38)
P. stizolobii	1	59.2	d	(27)
P. solanacearum	1	61.8	d	(27)
P. solanacearum	1	63.3	t	(26)
P. pickettii	3	64.0	d	(35)
P. caryophylli	3	65.3±0.6	d	(1)
P. caryophylli	3	66.0	d	(31)
P. cepacia	6	66.9±0.9	d	(1)
P. solanacearum	26	66.0 - 69.0	d	(30)
P. solanacearum	2	67.0 - 69.0	d	(21)
P. marginata	3	68.5±0.8	d	(1)
P. alboprecipitans	3	71.6 - 72.8	t	(38)

The results are expressed as average molar (guanine plus cytosine) per cent, %G + C, or as the mean value and standard deviation calculated for each group of isolates.
Method d, density gradient centrifugation in CsCl
Method t, thermal denaturation (result calculated from T_m values)

Table 5. Systematic study of some Australian isolates of *Pseudomonas solanacearum*: Hosts of origin of cultures

Cultures	Hosts
Biotype II (12 isolates)	Potato (12 isolates)
Biotype III (12 isolates)	Tomato (2 isolates)
	Xanthium pungens (3 isolates)
	(Compositae)
	Rapistrum rugosum (1 isolate)
	(Cruciferae)
	Potato (2 isolates)
	Chilli (1 isolate)
	(*Capsicum annuum*)
	Eggplant (1 isolate)
	(*Solanum melongena*)
	Tobacco (1 isolate)
	Pultenaea villosa (1 isolate)
	(Leguminosae)
Biotype IV (12 isolates)	Ginger (11 isolates)
	(*Zingiber officinale*)
	Tomato (1 isolate)

Table 6. Antibiotic sensitivity of some Australian isolates of *Pseudomonas solanacearum*

Antibiotic	Biotype II (12 isolates)	Biotype III (12 isolates)	Biotype IV (12 isolates)
Penicillin (1.5 µg)	R	R	R
Streptomycin (10 µg)	S	S	S
Tetracycline (10 µg)	S	S	S
Chloramphenicol (10 µg)	R-WS	R-WS	R-WS
Erythromycin (10 µg)	S	S	S
Nalidixic acid (30 µg)	S	S	S
Kanamycin (30 µg)	S	S	S
Oleandomycin (5 µg)	R	R-WS	S
Neomycin (10 and 30 µg)	R	R	R
Lincomycin (10 µg)	R	R	R

R - Zone of inhibition absent or insignificant; S - Wide zone of inhibition (< 15 mm); R-WS - Resistant or weakly susceptible (zone of inhibition >15mm).

Table 7. Physiological and biochemical properties of some Australian isolates of *Pseudomonas solanacearum*

Property	Biotype II (12 isolates)	Biotype III (12 isolates)	Biotype IV (12 isolates)
Acid production from:			
Glucose, fructose, galactose, sucrose, glycerol, and inositol	+	+	+
Mannitol, sorbitol and dulcitol	-	+	+
Lactose, maltose, and cellobiose	+	+	-
Trehalose	-	+	+ (11/12)
Raffinose and salicin	-	-	-
Hydrolysis of aesculin, starch, chitin and alginate	-	-	-
Dihydroxyacetone from glycerol	-	-	-
3-ketolactoside from lactose	-	-	-
Pitting in carboxy methyl cellulose gel	+	+	+
Pitting in polypectate gel (pH 4.9 - 5.1)	Slow	Rapid	Rapid
Pitting in polypectate gel (pH 6.9 - 7.1)	Slow	Rapid	Rapid
Pitting in polypectate gel (pH 8.3 - 8.5)	Slow	Rapid	Rapid

Table 8. Physiological and biochemical properties of some Australian isolates of *Pseudomonas solanacearum*

Property	Biotype II (12 isolates)	Biotype III (12 isolates)	Biotype IV (12 isolates)
Denitrification (gas from nitrate)	-	+	+
Destruction of 0.01% sodium nitrite	+	+	+
Ammonia from arginine	-	-	-
Arylsulphatase	-	-	-
Phosphatase	+	-	-
Gelatin hydrolysis (beneath growth)	+ (11/12)	+	+ (11/12)
Urease	+	+	+
Deamidation of acetamide	-	-	-
Hydrolysis of Tween 80 &	+	+	+
Tributyrin	-	-	-
Utilization of citrate	+	+	+
Utilization of malonate	-	-	-
Utilization of lactate	-	+	+
Oxidation of gluconate	-	-	-
Growth at pH 4.5	-	-	-
Growth at pH 5.5	+	+	+
Growth at pH 9.0	-	-	-
Growth in 1% NaCl medium	+	+	+
Growth in 1.4% NaCl medium	+ (5/12) ± (4/12) - (3/12)	+ (12/12)	+ (12/12)
Growth in 1.7% NaCl medium	- (12/12)	+ (8/12) ± (4/12)	+ (3/12) ± (7/12) - (2/12)
Growth in presence of 0.025% phenol	+	+	+
Growth in presence of 0.1% phenol	-	-	-
Growth at 10°C	-	-	-
Growth at 15°C	+	+	+
Growth at 37°C	+	+	+
Growth at 42°C	-	-	-

APPROACHES TO A GENETICAL STUDY OF INTERACTIONS BETWEEN PSEUDOMONAS SOLANACEARUM AND ITS HOSTS

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INTRODUCTION

A plant disease such as Bacterial Wilt is the phenotypic expression of interactions between a host plant and a pathogen. In order to analyze such a complex process, one would like to introduce variations into one member of the pair and to study the resulting effects on the expression of the disease. Bacterial genetic allows a study of inheritance and expression of hereditary characters of the pathogen and therefore should be a very useful tool for the study of the disease.

The first step in a genetical study of Pseudomonas pathogenicity is the isolation of mutants. Two different methods can be used which lead to different approaches of the relationships between the bacteria and its host.

a) Isolation of bacterial mutants which differ from the wilt type by properties easily recognizable "in vitro," for example, mutants which are no longer able to synthesize a particular metabolite. The pathogenic properties of such mutants can then be examined and conclusions concerning the relationships between bacterial functions or structures and virulence can be derived from the comparison of the "in vitro" and the "in vivo" properties of the mutants.

b) Direct isolation of nonpathogenic mutants.
After a mutagenic treatment, individual clones can be used to inoculate plants. In such a way, one can detect mutants which are no longer able to produce the wilt symptoms. Cytological or biochemical methods can then be used in order to define the step which is blocked in the infective process.

Once mutants which can give information on the relationships between the bacterium and its hosts have been obtained, one would like to number and map the genes which are involved. Construction of hybrid strains would allow a study of the interactions between the products of different genes which contribute to a given phenotype. Both of those goals can be attained only if we have the means for transfer of genetic material between bacteria.

I - ISOLATION OF P. SOLANACEARUM MUTANTS

1 - Isolation and properties of mutants recognizable "in vitro"

a - Mutation from the fluidal to the butyrous colony type

This spontaneous "mutation" was described (Kelman, 1954) a long time ago and was shown to be responsible for the loss of virulence of cultures of Pseudomonas maintained in vitro. Several characters are changed as a result of that mutation (Kelman and Hruschka, 1973) so that it is difficult to say whether it is a pleiotropic point mutation or a stable modification affecting a whole collection of genes. In the latter case, one could think of the loss of a plasmid. The inheritance pattern of colony type following a transfer of genetic material could help to decide between those two hypotheses.

On the other hand, recent work on the hypersensitive reaction elicited by the butyrous colony type cells points to the probable modification which leads to loss of virulence (Sequeira, these proceedings).

b - Isolation and properties of auxotrophic mutants

The penicillin method for selection of auxotrophic mutants after mutagenesis (Davis, 1948) has been an invaluable tool in bacterial genetics. This method has been used with slight modifications in order to obtain mutants of P. solanacearum strain K60 (Coplin, 1972) and strain Kourou (Pitrat, 1972; Message, Boucher, Boistard, unpublished results). The pathogenic properties of the Kourou mutants have been examined and are recorded in Fig. 1.

The mutants which require one particular metabolite can be grouped as follows:

i) All the mutants behave in the same way: for example all the aspartic acid-requiring mutants are virulent. All the tryptophan-requiring mutants isolated by Coplin are avirulent. That could mean that those mutants which are avirulent simply cannot grow inside the plant. Coplin demonstrated that the quantity of tryptophan present in the plant sap was insufficient to allow the growth of tryptophan-requiring mutants. If tryptophan is fed to plants infected with tryptophan-requiring mutants, virulence is restored (Coplin et al, 1974).

ii) Some mutants are virulent, others are avirulent. For example, one of the mutants which requires arginine for its growth is as virulent as the wild type; another is totally avirulent. The possibility that a second mutation not concerned with arginine biosynthesis is responsible for that behavior can be ruled out by the fact that the prototrophic revertants regain their virulence. The simplest explanation is that in the plant, bacteria do not utilize arginine for their growth, but instead a precursor which is provided by the plant is transformed into arginine. A metabolic block in the avirulent mutants may prevent the utilization of that precursor and thus would be a block in the last steps of the biosynthetic pathway. On the contrary, the mutation in the virulent mutants does not prevent the utilization of the precursor and thus affects one of the early steps. That such is the case is indicated by the fact that the virulent arginine requiring mutants can grow in the presence of proline. This means that the block is prior to the synthesis of a common precursor for proline and arginine. We have still to rule out the possibility that the virulence of this class of mutants is due to the occurrence of reverse mutations in the plant.

iii) All the adenine-requiring mutants are avirulent but the revertants isolated from one of them, GMI 1107, differ from each other in their virulence (Fig. 2). That could be explained by the fact that one of the enzymes of the adenine pathway is bi-functional. The mutation leading to adenine requirement would have altered both metabolic and pathogenic functions of that enzyme. The reverse mutation to prototrophy would restore only one of the functions (adenine synthesis) with various suppressive effects on the altered pathogenic function.

c) "Mutants" induced by acridine orange

In an attempt to decide whether the transition from the white fluidal to the butyrous colony type was due to the loss of a plasmid, we treated white fluidal Kourou cultures with acridine orange, a drug which is known to eliminate certain classes of plasmids (Hirota, 1960).

Instead of enhancing the frequency of occurrence of the butyrous colony type cells, we observed the appearance with a high frequency of cells which require methionine for their growth and release a brownish pigment in the culture medium. Such mutants still have the fluidal phenotype. As for the transition from the white fluidal to the butyrous red colony type, the genetical basis of the hereditary change is not known presently: is it due to a mutation to acridine orange resistance with pleiotrophic effects as would be likely the case for a mutation affecting bacterial cell wall, or is it due to the loss of a plasmid? By the methods we used, we could not detect any supercoiled DNA in the wild type Kourou cells which was absent from the methionine-requiring mutants.

Whatever the genetical basis for that change, the pathogenic properties of the methionine-requiring mutants are profoundly affected. They are completely avirulent but whereas the wild type Kourou cells induce the hypersensitive response when infiltrated into tobacco leaves, the methionine-requiring mutants neither induce that reaction nor protect from the hypersensitive response when the leaf is challenged later on with wild type cells. According to Sequeira's hypothesis (these proceedings) methionine-requiring mutants could be modified in their lipid A structure in such a way that they are no longer recognized by the tobacco lectins.

Although several attempts were made under various conditions, no such mutants could be obtained from strain K60. Nor could they be isolated from the Kourou strain using other curing agents such as ethidium bromide or sodium dodecyl sulfate.

2 - Direct isolation of nonpathogenic mutants

In a preliminary experiment, a Kourou culture was treated with nitrosoguanidine, a very potent mutagen. After allowing expression of the mutations and segregation of the mutants, one hundred individual clones were isolated. Each clone was used to inoculate separately two tomato plants grown aseptically on vermiculite and mineral nutrient solution. One of those clones did not produce any wilt symptoms in either plant and was studied further. Except for its lack of pathogenicity, that mutant retained the characteristics of the wild type cells and particularly its white fluidal colony type.

It can be predicted that, in the case of this class of mutants, it will be very difficult to correlate a bacterial modification with the loss of virulence. On the other hand, their behavior in the plant could provide valuable information on the infective process. Our working hypothesis is that the infective process is conditioned by a succession of steps, each of which is controlled by one or several bacterial genes. Isolation of various nonpathogenic Pseudomonas mutants would allow identification of barriers to the infection which the nonpathogenic mutants cannot overcome. Knowledge of these barriers in turn would help in the identification of the plant genes which contribute to horizontal resistance and in their use in a breeding program.

Furthermore, by isolating avirulent mutants blocked in a late step of the infective process, and therefore likely to compete efficiently with the wild type strain in the plant, one could have the means for biological control of the disease.

II - ATTEMPTS TO ESTABLISH A SYSTEM OF GENETIC TRANSFER

The purpose of a system of genetic transfer for the analysis of pathogenic properties of *Pseudomonas* is twofold:

i) To number, locate and identify the genes which contribute to the pathogenic properties of the bacterium. It would be of interest, for example, to know whether one particular step in the infective process is under the control of several bacterial genes. After the isolation of several mutants blocked at the same step, mapping of the mutation sites could answer this question.

ii) To construct hybrid strains in order to study the interaction of genes.

Three systems of genetic transfer are known in bacteria: transduction, transformation and conjugation (Hayes, 1968). Attempts to utilize each of these systems have been made in *P. solanacearum*.

1. Transduction

As early as 1955, there was a report on hereditary changes brought about by the infection of *P. solanacearum* with a temperate phage (Okabe and Goto, 1955). The results described as a proof for transduction could best be interpreted in terms of conversion since all the bacteria which were lysogenized by the temperate phage acquired the new characters, indicating that the foreign genetic material did not replace resident genes through a recombination process but instead were added to the host genome.

Temperate phages able to infect Kourou cells were isolated and their transducing properties for various auxotrophic markers were examined (Boucher, 1973). One of the phages had transducing ability but these results have to be confirmed because the transduction frequency was low, around 10^{-7}. Furthermore, definitive proof of genetic transfer, that is, the acquisition of unselected markers, was lacking.

2. Transformation

Transformation of the K60 strain was demonstrated by the use of auxotrophic and drug resistance markers (Coplin, 1972). However, as in the case of transduction, the frequency was low, around 10^{-7}, with a maximum of 10^{-5} for some markers. No transfer of unselected markers could be shown.

3. Conjugation

Two different approaches have been used in order to get a conjugation system in *P. solanacearum*:

a) Search for a naturally-occurring conjugation system

By appropriate crosses between strains with multiple markers, it was possible to demonstrate a genetic transfer system in strain K60 (Boucher and Sequeira, in preparation). Furthermore, by an analysis of the genetic constitution of the recombinants, transfer of unselected markers could be shown, which rules out the possibility of reverse mutations as an explanation for the occurrence of bacteria possessing the selected markers. Although definitive proof that the transfer is mediated by conjugation is still lacking, there is no doubt about the existence of such genetic transfer. Furthermore, the occurrence of cotransfer renders that system usable for a genetic analysis of K60.

b) Introduction of a sex factor into Pseudomonas solanacearum

The autotransferable drug resistance factor, RP4, a sex factor originating from Pseudomonas aeruginosa (Saunders and Grinsted, 1972) and endowed with a broad host range (Datta and Hedges, 1972) was introduced into Kourou cells (Message et al., 1975) and more recently into K60 cells (Boucher and Sequeira, unpublished results). But contrary to what was found in Rhizobium (Meade, personal communication) and in Escherichia coli (Denarie and Michel, personal communication) it was not possible to demonstrate any transfer of chromosomal genetic markers during conjugation mediated by RP4 between Kourou cells. Genetic transfer, if any, occurred with a frequency smaller than that of spontaneous reversions. It has been shown (Denarie et al., in press) that by introducing a zone of homology between the RP4 sex factor and the chromosome of Escherichia coli, one could get a very efficient polarized transfer. We are seeking to introduce such a region of homology between the RP4 sex factor and Pseudomonas solanacearum by "in vitro" recombination between the sex factor and chromosomal fragments. One advantage of conjugation mediated by RP4 would be the possibility of interstrain genetic transfer, whereas a natural conjugation system is restricted by the host range of the resident sexual factor.

CONCLUSION

The existence of auxotrophic mutants of P. solanacearum makes it possible to study the metabolic relationship between the bacteria and its hosts. As exemplified by the preliminary study of arginine-requiring mutants, this system may provide an answer to the question of what is utilized by the bacteria for their growth in the plant: is it the terminal product of a metabolic pathway or, alternatively, intermediate precursors?

Particular mutants with distinctive in vitro properties such as the butyrous colony type mutants or the methionine-requiring mutants produced in the presence of acridine orange could help in elucidating the process of recognition between the plant and the parasite which occurs during the hypersensitive reaction.

Finally the systematic isolation of nonpathogenic mutants could provide a means to define successive steps in the infective process and eventually help in the identification of plant genes which contribute to horizontal resistance.

But, as was pointed out for the butyrous colony mutants or the methionine-requiring mutants, a precise understanding of the cellular changes which are responsible for the occurrence of those new phenotypes would be greatly facilitated by genetic analysis. The same need applies to the characterization of the various nonpathogenic mutants which we are in the process of isolating.

Among the three systems of genetic analysis which are known, conjugation seems the most promising. We hope that it will be confirmed that it occurs spontaneously in strain K60 or that it can be produced artificially by in vitro recombination between a sex factor and fragments of chromosomal DNA.

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REFERENCES

Boucher, C. 1973. Recherche d'un systeme de transduction generalisee chez Pseudomonas solanacearum. D.E.A. Faculte des Sciences Paris-Sud.

Coplin, D. L. 1972. Pseudomonas solanacearum: genetic transformation and virulence of biochemical mutants. Thesis. The University of Wisconsin.

Coplin, D. L., L. Sequeira and R. S. Hanson. 1974. Pseudomonas solanacearum: virulence of biochemical mutants. Can. J. Microbiol. 20:519-529.

Datta, N., R. W. Hedges. 1972. Host ranges of R factors. J. Gen. Microbiol. 70:452-460.

Davis, B. D. 1948. Isolation of biochemically deficient mutants of bacteria by penicillin. J. Am. Chem. Soc. 70:4267.

Denarie, J., C. Rosenberg, Beatrice Bergeron, C. Boucher, M. Michel and Mirta Barate de Bertalmio. (In press). RP4::Mu plasmids: obtention, conjugative transfer into Klebsiella pneumoniae, Pseudomonas solanacearum and Rhizobium meliloti and evaluation of potentials as a tool for "in vivo" genetic engineering of Gram-negative bacteria. In DNA insertions. Edited by A. I. Burkhari, J. Shapiro and S. Adhya. New York: Cold Spring Harbor Laboratory.

Hayes, W. 1968. The Genetics of Bacteria and their Viruses. 2nd edition. Blackwell Scientific Publications, Oxford and Edinburgh.

Hirota, Y. 1960. The effect of acridine dyes on mating type factors in Escherichia coli. Proc. Natl. Acad. Sci., U.S.A. 46:57-64.

Kelman, A. 1954. The relationship of pathogenicity of Pseudomonas solanacearum to colony appearance on a tetrazolium medium. Phytopathology 44:693-695.

Kelman, A. and Judith Hruschka. 1973. The role of motility and aerotaxis in the selective increase of avirulent bacteria in still broth cultures of Pseudomonas solanacearum. J. Gen. Microbiol. 76:177-188.

Message, Brigitte, C. Boucher and P. Boistard. 1975. Transfert d'un facteur R, RP4 dans une souche de Pseudomonas solanacearum. Ann. Phytopathol. 7:95-103.

Okabe, N. and M. Goto. 1955. Studies of Pseudomonas solanacearum X: genetic change of the bacterial strains induced by the temperate phage T-C200. Rep. Fac. Agric. Shizuoka Univ. 5:57-62.

Pitrat, M. 1972. Relation entre virulence et besoin nutritionnel de quelques mutants auxotrophes de Pseudomonas solanacearum E. F. Smith. D.E.A. Faculte des Sciences de Paris VI.

Saunders, J. R. and J. Grinsted. 1972. Properties of RP4, an R factor which originated in Pseudomonas aeruginosa S 8. J. Bacteriol. 112:690-696.

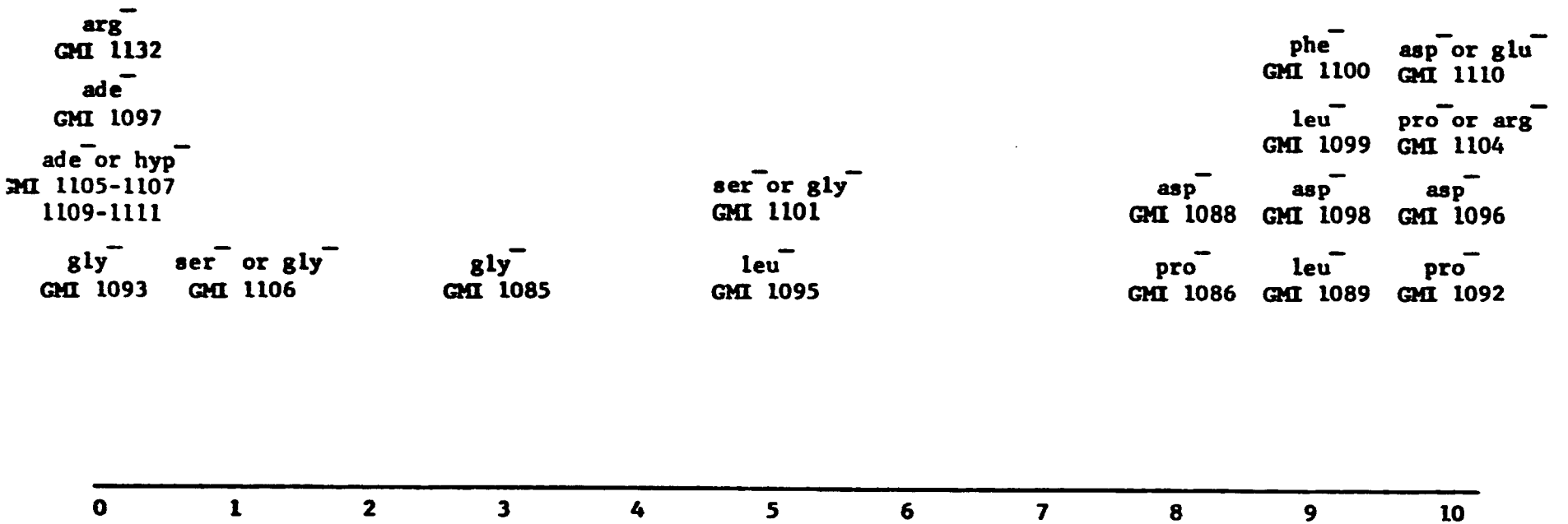

Fig. 1. Virulence of auxotrophic mutants.

Sterile, pregerminated seeds of tomato are grown in test tubes in sterile soil. A culture of each auxotrophic mutant is used to inoculate ten plants at the time the seeds are planted. Each tube receives 10^8 bacteria resuspended in distilled water. The figures indicate the number of wilted plants after one month.

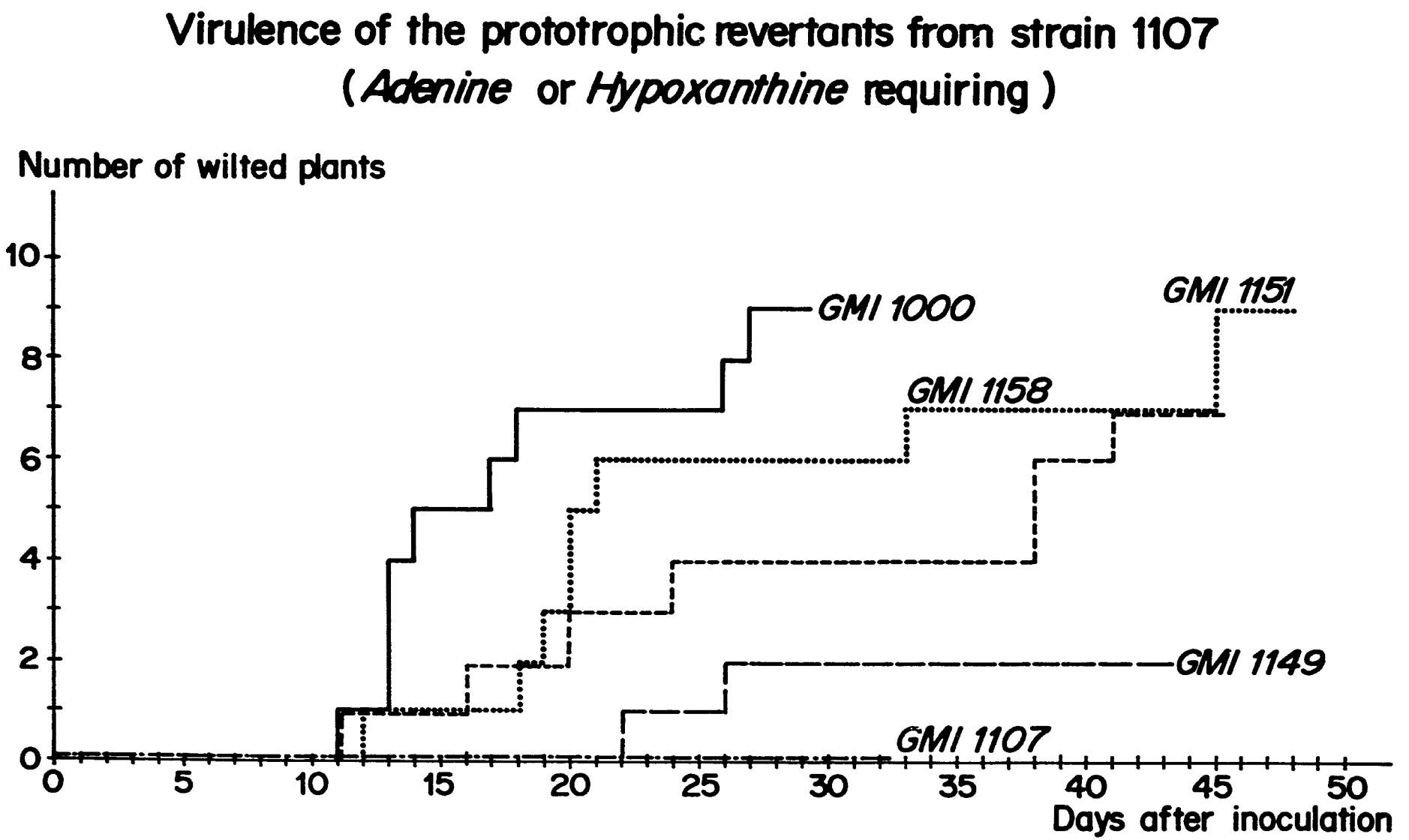

Fig. 2. The conditions are the same as in Fig. 1. The cultures used for inoculation were the wild type strain (GMI 1000), the adenine-requiring mutant GMI 1107, and three independent reverse mutants able to grow in the absence of adenine. Total number of wilted plants versus time after inoculation are plotted.

ASSESSMENT OF PLANT RESISTANCE BY INFECTIVITY TITRATION

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Despite its potential for multiple applications to the study of different facets of plant-pathogen interactions (2-9, 11, 14-18, 24, 28), infectivity titration has found surprisingly little use as a tool for assessing plant resistance to bacterial infection.

Infectivity titration is a relatively simple procedure, the applicability of which is by no means restricted to plants infected by bacteria, but extends to all infective systems -- i.e., systems in which a response follows multiplication of the inoculated agent within the subject. According to a well established protocol, it is usually carried out in four steps: (i) a test preparation (e.g., a bacterial suspension) is serially diluted; (ii) each dilution is inoculated into a separate group of subjects (e.g., healthy plants); (iii) the response of each subject within each dose group is recorded; and (iv) the data are condensed into some estimate of the magnitude of the effect observed.

Whereas steps (i) and (ii) can be standardized with reasonable objectivity with respect to factors such as spacing of dilutions, number of subjects inoculated with each dilution, etc., steps (iii) and (iv) always introduce some degree of subjectivity into the exercise. The objective effect observed is the derangement of the normal condition of the subject, but the nature and magnitude of the effect can only be expressed subjectively either in terms of pathogenicity of the microorganism or of resistance of the subject, according to the specific interest of the investigator. Estimates of pathogenicity, i.e., measurements of virulence, are formally independent of host resistance, but this condition tends to be obscured by the fact that, in any given type of infection, the pathogenic manifestation of the microorganism varies with the state of the host: the lower the resistance, the higher the virulence, and vice-versa (23). Obvious as it may be, this is a key consideration to be borne in mind in assessing plant resistance to bacterial infection by infectivity titration. When estimates of pathogenicity are made, resistance must be specified, and, conversely, virulence must be specified if estimates of resistance are to be made. In this context, it may be noted, reliable estimates of the resistance of the host rather than the pathogenicity of the microorganism are usually easier to obtain, owing to the fact that microbial populations can, by drying, deep-freezing, etc., be preserved (temporarily at least) from changes in factors that determine virulence, whereas host populations are more prone to variations in non-specific resistance associated with environmental conditions, for example.

Given a stable bacterial population, infectivity titration is apt to yield estimates of the relative resistance of two or more populations of subjects either in terms of differences in the dose of the bacterium that induces the same indicating effect, or of differences in the indicating effect induced by equal doses of the bacterium. Any indicating effect can be used, as long as it is related to disease severity. It may be a host effect (e.g., death, wilting, local lesion formation), or a pathogen effect (e.g., survival or multiplication in the host tissues). Both host and pathogen effects can be distinguished into quantal and quantitative effects. Quantal effects occur

as all-or-none responses, which, by definition, can only take either of two mutually incompatible forms (e.g., alive or dead, healthy or diseased, turgid or wilted); quantitative effects occur as responses that can take any of a series of values (e.g., severity of disease, time to response). It may be appropriate at this point to mention that infectivity titration using quantitative responses can achieve greater precision than using quantal responses (19, 21), but has found comparatively more limited application.

Infectivity titration using quantal responses

The usual procedure to determine quantal responses is to measure the proportion of inoculated subjects that respond to any given dose. A dose-response curve is thus obtained, which is characterized by the value of the ED50, i.e., the dose that causes 50% of the subjects to respond, and by its slope at the ED50 point.

Two situations can be encountered in practice. In a few infective systems, the subjects are completely susceptible and invariably respond to inoculation with one bacterium. In this case, the shape of the dose-response curve is determined solely by the random distribution of viable bacteria among individual doses. As bacterial densities used in these and similar experiments are compatible with a Poisson distribution of the bacteria in the suspension, the proportion, Q, of subjects that do not respond to inoculation with a mean dose of d viable bacteria, i.e., the proportion of subjects that receive $n = 0$ viable bacteria, is given by $Q = e^{-d}$, the first term of the Poisson series. The proportion of subjects that do respond to d is, of course, given by $R = 1 - Q = 1 - e^{-d}$. These systems, in which the subjects are completely susceptible, are recognized very simply by the fact that the ED50 equals 0.69 viable bacteria ($Q = e^{-0.69} = 0.5$). In other words, an infectivity titration yielding an ED50 = 0.69 viable bacteria indicates that the subjects are completely susceptible -- a conclusion remarkably more difficult to reach using different approaches.

In most infective systems, the subjects are only partially susceptible, as indicated by the fact that they do not invariably respond to inoculation with one viable bacterium. This situation is open to different interpretations that have been formalized in two chief hypotheses, i.e., the "hypothesis of the individual effective dose" and the "hypothesis of independent action" (22). These hypotheses, that have stemmed from the statistical treatment of entirely different situations, have not only theoretical but also considerable practical interest. In particular, in studies of plant resistance, it is of the utmost importance that infective systems to which either hypothesis applies be distinguished since all the evidence available so far indicates that the course of infection of a partially resistant plant by a compatible bacterium conforms to the hypothesis of independent action, whereas interactions of bacteria with plants involving a hypersensitive reaction conform to the hypothesis of the individual effective dose if macroscopically visible disease symptoms are used as the indicating effect (11). In the author's experience, the same holds true when responses are recorded at the microscopic level, but independence *in vivo* among incompatible bacteria inoculated at subclinical doses has been reported also (26, 27).

The recognition of infective systems described by either hypothesis is simplified by the fact that each of the two hypotheses predicts two basic aspects at least of the outcome of infection, i.e., the general form of the dose-response curve and the composition of the bacterial population present in the subjects that respond to inoculation at any given dose. This is a fortunate

state of affairs because, even without being entirely familiar with the statistical theory behind both hypotheses, one can obtain conclusive evidence not only about the nature, but also about the distribution of resistance in an unknown population of plants. This is done by simply making an infectivity titration and determining the form of the dose-response curve and the composition of the bacterial population in the plants that respond to inoculation of a given dose. For the sake of simplicity, the two hypotheses will be considered separately.

The hypothesis of the individual effective dose postulates the existence of an individual effective dose (IED) for each subject, so that if the number of viable bacteria inoculated is less than the IED for any given subject, none of the bacteria will multiply and the subject will not respond, whereas, if the number of viable bacteria in the dose equals or exceeds the IED, all inoculated bacteria will multiply and the subject will respond. Provided that errors in dosage can be ignored, the shape of the dose-response curve predicted by the hypothesis depends solely on the distribution of the IED among the subjects, i.e., on the level of susceptibility of each subject, so that the slope of the curve will take the value ∞ if the subjects do not vary in susceptibility, and any value from 0 to ∞ if they do vary. Furthermore, since all viable inoculated bacteria are assumed to multiply in any subject given a dose greater than its IED, the hypothesis predicts that all the subjects responding to inoculation will contain the progeny of more than one bacterium, i.e., a mixture of different clones, independently of the size of the dose. As inoculated bacteria can be considered to cooperate and "saturate" host defenses, the hypothesis of the individual effective dose is known also as the "hypothesis of cooperative action".

The alternative hypothesis, i.e., the hypothesis of independent action, postulates that the bacteria act independently after inoculation so that the fate of one does not affect the fate of the others. The mean probability ($1>\underline{p}>0$) per inoculated bacterium of multiplying to induce response is therefore independent of $\underline{d}$, the number of viable inoculated bacteria, and depends only upon random events governed by chance *in vivo*. If the subjects do not differ in susceptibility, i.e., if $\underline{p}$ is the same for all of them, the proportion, $\underline{Q}$, of subjects that do not respond to inoculation of a mean dose of $\underline{d}$ viable bacteria is given again by the first term of the Poisson series, $\underline{e}^{-\underline{pd}}$. The resulting dose-response curve is predicted to be a slightly asymmetrical sigmoid curve that reduces to a straight line of unit slope when a response metamer, i.e., $\log_{10} -\log_e(1 - \underline{R})$ is plotted against log dose (1, 25). If the subjects differ in susceptibility, i.e., if $\underline{p}$ is not the same for all of them, the dose-response curve is no longer a straight line of unit slope, but is predicted to take different shapes that are determined by the distribution of $\underline{p}$ among the subjects. Fig. 1 illustrates several types of dose-response curves characteristic of systems to which the hypothesis of independent action applies. Curve A is characteristic of systems in which $\underline{p}$ is not distributed, i.e., systems in which all the subjects have the same susceptibility: it is a straight line of unit slope and crosses the 50% response line at the ED50 point ($\underline{d}_o$). Curve B, which consists of a plateau and two branches with asymptotes of unit slope, is characteristic of systems in which $\underline{p}$ has a two-point distribution, i.e., systems in which there are two groups of subjects mixed together, one more "susceptible", the other more "resistant": the height of the plateau determines the proportion of "susceptible" subjects (20% in the example), the point at which the asymptote to the upper branch crosses the 50% response level gives the ED50 for the "resistant" group ($\underline{d}_2$), and the value corresponding to the point at which the asymptote

to the lower branch crosses the 50% response level (d'), multiplied by the proportion of "susceptible" subjects, gives the ED50 for the "susceptible" group. Curve C is characteristic of systems in which p has a continuous distribution, i.e., systems in which susceptibility differs from subject to subject: it has a slope of less than unity and log ED50 is uniformly distributed over a more or less extended dose interval (2 logs in the example, as indicated at the bottom of the graph). With respect to composition of the bacterial population in the subjects responding to inoculation, the hypothesis predicts that those responding after inoculation of a dose containing many ED50's do so following the multiplication of many inoculated bacteria and therefore contain a mixture of different clones, whereas those responding after inoculation of one or less than one ED50 do so as a result of the multiplication of only one inoculated bacterium and therefore contain a pure clone, if the subjects do not differ in susceptibility. The predicted effect of differences in susceptibility among the subjects is to increase the proportion of subjects containing a mixed population after inoculation of $\leq$1ED50, and to decrease that proportion after inoculation of many ED50's.

The distribution of individual plant resistance to a given pathogen within different varieties of the same species, the relative resistance of these varieties to inoculation with graded doses of the same pathogen, and the interference of resistant manifestations of the plant with the chemical control of a bacterial disease, are among the factors that have been investigated by the use of infectivity titration. Several cases in point are illustrated by the results of infectivity titration of Corynebacterium michiganese (E. F. Sm.) Jens., the agent of bacterial canker of tomato, on three cultivars of tomato, i.e., Florentina, C1402 and Roma (5). Experiments using seedlings indicated that Florentina was composed of plants that did not differ in susceptibility (ED50 = $3.0\text{-}7.0\times10^4$ viable bacteria), whereas C1402 comprised 38-50% of "susceptible" plants (ED50 = $1.4\text{-}2.7\times10^3$ viable bacteria) and 42-50% of "resistant" plants (ED50 = $1.0\text{-}1.6\times10^5$ viable bacteria), and Roma 5-6% of "susceptible" (ED50 = $2.7\text{-}8.0\times10$ viable bacteria) and 94-95% of "resistant" (ED50 = $8.0\text{-}22.0\times10^3$ viable bacteria) plants. These and other experiments (6) indicated that the course of infection conformed to the hypothesis of independent action and that differences in plant age, plant nutrition and the route of inoculation did not affect the form of the dose-response relation, i.e., the distribution of susceptibility among the plants appeared to be under the control of genetic factors -- a fact that was demonstrated by genetic analysis several years later. Furthermore, by use of infectivity titration, not only could the distribution of susceptibility within each of the three varieties be characterized fully, but gross errors of judgment over the relative resistance of the three varieties arising from the use of only one dose of inoculum could be avoided. For instance, by testing at a dose level of 10^3 viable bacteria, Florentina (R = 0.03) would appear more resistant than C1402 (R = 0.4), but, by testing at 10^5 viable bacteria, the situation would be reversed (R = 1.0 for Florentina and 0.5 for C1402).

Another pertinent example of assessment of disease resistance by infectivity titration can be illustrated from a study of the reason why chemical sprays successfully controlled blossom blight of pear (cultivars Kaiser and William) induced by Pseudomonas syringae van Hall, but failed to cause any significant increase in fruit yield (7, 13). The answer was found by analyzing the dose-response curves obtained from infectivity titrations for the totality of the flowers and for the flowers growing from the distal and from the proximal portion of the blossom axis, respectively. This showed that the former curve, the shape of which was indicative of a two-point distribution

of susceptibility, resulted from the superimposition of two straight lines of unit slope, each corresponding to one group of flowers of uniform susceptibility: the apical one with an ED50 of $3.3\text{-}4.0 \times 10^2$ viable bacteria for Kaiser and $3.8\text{-}4.6 \times 10^2$ viable bacteria for William, and the basal one with an ED50 of $1.6\text{-}1.9 \times 10^4$ viable bacteria for Kaiser and $9.3\text{-}22.0 \times 10^4$ viable bacteria for William. On unsprayed trees in nature, up to 50% of flowers were infected, but the impressive reduction of blossom blight caused by spraying had no appreciable effect on fruit yield because, under the prevailing conditions of inoculum potential at the time of the control trials, the only flowers that responded were those belonging to the most susceptible group, i.e., the apical ones, that are known from tree physiology to be unimportant for pear fruit production.

Infectivity titration using quantitative responses

Response time (also called time to response, or latent period, or incubation period), i.e., the interval between inoculation and occurrence of a given response in the subject, is virtually the only quantitative effect that has been used in infectivity titration experiments with plant pathogenic bacteria. It is measured easily, and, in compatible systems, it bears a demonstrable relation to the behavior of the bacteria *in vivo* during the interval between inoculation and response -- which may be related in turn to plant resistance.

Evidence from examination of several compatible systems suggests (i) that doubling times of the bacteria *in vivo* are independent of the mean dose of inoculum, d, within broad limits, and (ii) that when the bacteria reach given population thresholds *in vivo*, then host response follows (10, 12). Employing these two assumptions, several features of the variation of response time with varying inoculum dose can be predicted and subsequently checked by infectivity titration to obtain further information on the aspects of resistance reflected by this type of indicating effect, when the hypothesis of independent action applies. The predictions (20, 21) are: (i) that the mean response time is inversely related to log *d* for values of the dose that exceed the ED50; (ii) that the mean response time tends to constancy for $d < 1ED50$; (iii) that the log dose response time curve asymptotes the abscissa axis at high doses; and (iv) that the slope of the linear portion of the response time curve is determined by the mean rate of increase of the bacteria that multiply *in vivo* to initiate the response.

The four predictions listed above, along with other features of the distribution of individual response times predicted by a development of the hypothesis of independent action known as the birth-death model, have been confirmed in a study of the dynamics of infection of *Pseudomonas phaseolicola* (Burkh.) Dows. in partially resistant populations of bean (10). However, more work is needed to characterize response time more fully as a quantitative effect observed in plants challenged by phytopathogenic bacteria before its generalized use in infectivity titration aimed at assessing plant resistance can be recommended.

LITERATURE CITED

1. ARMITAGE, P. 1959. An examination of some experimental cancer data in the light of the one-hit theory of infectivity titrations. J. Nat. Cancer Inst. 23:1313-1330.

2. BOELEMA, B. H. 1973. Infectivity titrations with some plant pathogenic bacteria. S. Afr. J. Sci. 69:332-336.

3. CIVEROLO, E. L. 1975. Quantitative aspects of pathogenesis of Xanthomonas pruni in peach leaves. Phytopathology 65:258-264.

4. CROSSE, J. E., R. N. GOODMAN and W. H. SHAFFER, JR. 1972. Leaf damage as a predisposing factor in the infection of apple shoots by Erwinia amylovora. Phytopathology 62:176-182.

5. ERCOLANI, G. L. 1967. Bacterial canker of tomato. I. Analysis of some factors affecting the response of tomato to Corynebacterium michiganese (E.F. Sm.) Jens. Phytopathol. Mediterr. 6:19-29.

6. ERCOLANI, G. L. 1967. Bacterial canker of tomato. II. Interpretation of the aetiology of the quantal response of tomato to Corynebacterium michiganese (E.F. Sm.) Jens. by the hypothesis of independent action. Phytopathol. Mediterr. 6:30-40.

7. ERCOLANI, G. L. 1970. Analisi della susceptibilita individuale dei fiori del pero a Pseudomonas syringae var. Hall. Phytopathol. Mediterr. 9:35-38.

8. ERCOLANI, G. L. 1970. Bacterial canker of tomato. III. The effect of auxotrophic mutation on the virulence of Corynebacterium michiganense (E.F. Sm.) Jens. Phytopathol. Mediterr. 9:145-150.

9. ERCOLANI, G. L. 1970. Bacterial canker of tomato. IV. The interaction between virulent and avirulent strains of Corynebacterium michiganense (E.F. Sm.) Jens. in vivo. Phytopathol. Mediterr. 9:151-159.

10. ERCOLANI, G. L. 1972. Dynamics of infection by Pseudomonas phaseolicola in partially resistant populations of bean. Phytopathology 62:756.

11. ERCOLANI, G. L. 1973. Two hypotheses on the aetiology of response of plants to phytopathogenic bacteria. J. Gen. Microbiol. 75:83-95.

12. ERCOLANI, G. L., and J. E. CROSSE. 1966. The growth of Pseudomonas phaseolicola and related plant pathogens in vivo. J. Gen. Microbiol. 45:429-439.

13. ERCOLANI, G. L., H. OBERHOFER and K. RAMOSER. 1967. Orientamenti per la lotta contro la necrosi batterica dei fiori del pero in Alto Adige. Ind. Conserve 42:260-264.

14. KOIZUMI, M. 1971. A quantitative determination method for Xanthomonas citri by inoculation into detached citrus leaves. Bull. Hort. Res. Sta., Japan, Ser. B, No. 11:167-179.

15. LAYNE, R. E. C. 1968. A quantitative local lesion bioassay for Corynebacterium michiganense. Phytopathology 58:534-535.

16. LELLIOTT, R. A. 1973. Dose-response experiments on infection of apple plants. Abstract No. 0937 in Abstracts of Papers, Second Int. Cong. Plant Pathol., 5-12 September 1973, Minneapolis, Minnesota. (unpaged).

17. LIPPINCOTT, J. A., and G. T. HEBERLEIN. 1965. The quantitative determination of the infectivity of Agrobacterium tumefaciens. Am. J. Bot. 52:856-863.

18. LIPPINCOTT, J. A., and B. B. LIPPINCOTT. 1967. The induction of necrotic lesions on primary pinto bean leaves by Pseudomonas savastonoi. Phytopathology 57:1142-1143.

19. MEYNELL, G. G. 1957. Inherently low precision of infectivity titrations using a quantal response. Biometrics 13:149-163.

20. MEYNELL, G. G., and E. W. MEYNELL. 1958. The growth of micro-organisms in vivo with particular reference to the relation between dose and latent period. J. Hyg. (Camb.) 56:323-346.

21. MEYNELL, G. G., and E. W. MEYNELL. 1970. Theory and practice in experimental bacteriology, 2nd ed., Cambridge University Press, Cambridge, England, 347 p.

22. MEYNELL, G. G., and B. A. D. STOCKER. 1957. Some hypotheses on the aetiology of fatal infections in partially resistant hosts and their application to mice challenged with Salmonella paratyphi-B or Salmonella typhimurium by intraperitoneal injection. J. Gen. Microbiol. 16:38-58.

23. MILES, A. A. 1955. The meaning of pathogenicity. Pages 1-16 in J. W. Howie and A. J. O'Hea, ed. Mechanisms of microbial pathogenicity, Cambridge University Press, Cambridge, England, 233 p.

24. PEROMBELON, M. C. M. 1972. A quantitative method for assessing virulence of Erwinia carotovora var. carotovora and E. carotovora var. atroseptica and susceptibility to rotting of potato tuber tissue. Pages 299-303 in H. P. Maas Geesteranus, ed. Third Int. Conf. Plant Pathogenic Bacteria Proc., Centre Agr. Publ. Doc. (PUDOC), Wageningen, The Netherlands, 365 p.

25. SHORTLEY, G., and J. R. WILKINS. 1965. Independent-action and birth-death models in experimental microbiology. Bact. Rev. 29:102-141.

26. TURNER, J. G., and A. NOVACKY. 1974. The quantitative relation between plant and bacterial cells involved in the hypersensitive reaction. Phytopathology 64:885-890.

27. VAN DER PLANK, J. E. 1975. Principles of plant infection. Academic Press, New York, N. Y., 216 p.

28. ZIELKE, R. 1976. Der Schwellenwert von Infektionen mit Pectobacterium carotovorum var. atrosepticum (van Hall) Dowson zur Erzegung eines latenten und akuten Nassfäulebefalls an Kartoffelsprossen und -knollen. Arch. Phytopathol. u. Pflanzenschutz 12:27-41.

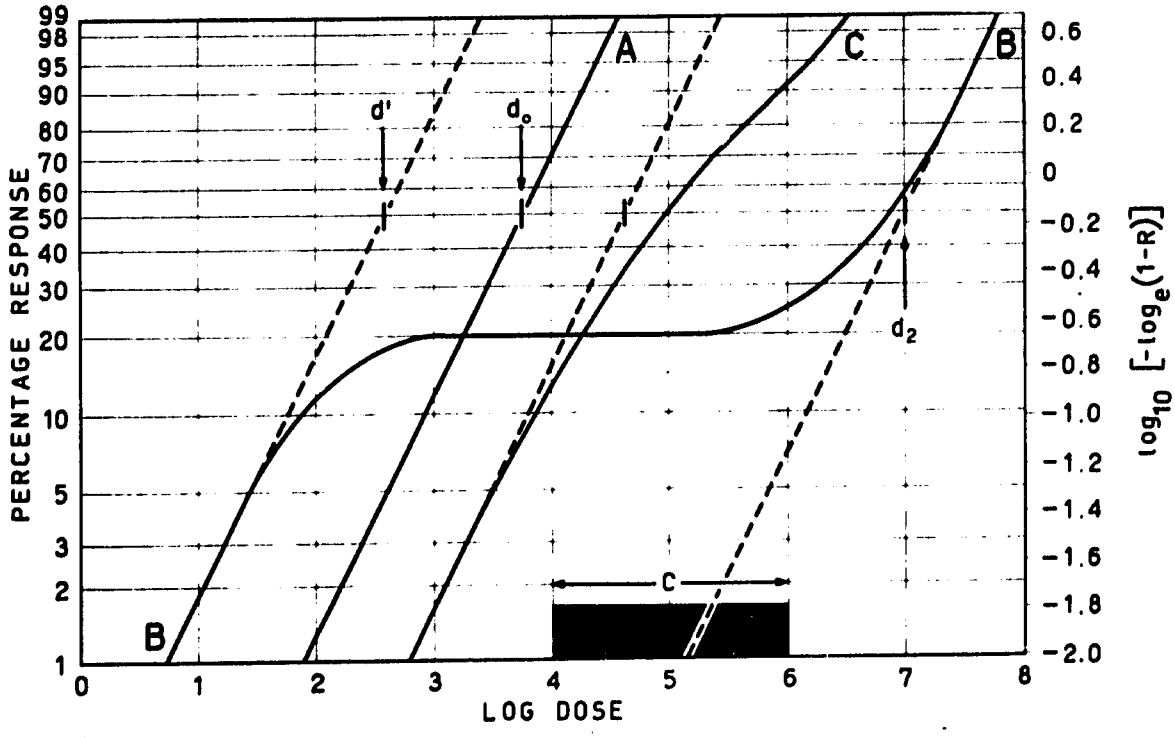

Fig. 1. Hypothetical examples of dose-response curves for a population of subjects of uniform susceptibility (A) and for two populations of subjects the susceptibility of which has a two-point and a continuous distribution (B and C, respectively). (From Ercolani, Ref. 5).

SPECIFICITY OF ANTIGENS IN PSEUDOMONAS SOLANACEARUM E. F. SM. AND APPLICATION OF SEROLOGY FOR STUDYING BACTERIAL WILT

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ABSTRACT

A survey of techniques and corresponding results obtained by several researchers in the field of Pseudomonas solanacearum serology has been undertaken. Most workers have been trying to obtain race or strain-specific antisera, or to demonstrate as a possibility the race or strain antigen specificity in view of differentiating serotypes.

However, antigen structure of the bacterial cell is too complicated to be studied from whole cells. A more successful study needs to extract relatively pure antigen entities and then to obtain the corresponding antisera. The results obtained using classical techniques (agglutination, immunodiffusion, immunoelectrophoresis, immunofluorescence) suggest that: 1. Extracellular polysaccharide and somatic antigens from several P. solanacearum strains are closely related (except for a few strains). These antigens can help in clearly differentiating the "solanacearum" species from the other phytopathogenic Pseudomonas species but are not helpful in differentiating races and strains of P. solanacearum. 2. On the other hand, P. solanacearum glycoprotein antigens seem to be the most specific for differentiating races and strains. In this case, if corresponding antisera are conveniently absorbed, they can give very specific reactions which provide the means for differentiation of races and strains.

This specificity can be used particularly for detecting bacteria in the soil and in the host-plant, but also for studying epidemiological aspects of bacterial wilt.

Among the phytopathogenic Pseudomonas spp., P. solanacearum E. F. Sm. has special grouping within the non-fluorescent pigment producers (25). The species of this bacterium is complex because it consists of several races differing in host range and pathogenicity (13). Each of the three races presently identified represent many strains (23). If the characteristics of the species are well determined (13) the specific characters of races are, on the other hand, less clear and those of the strains remain very difficult to identify. It is well-known, however, how a precise evaluation of the pathogenic potential is needed at first to identify P. solanacearum races and strains. In the same way, for a better understanding of epidemiology, it would be useful to differentiate, using a simple technique, races 1 and 3 affecting solanaceous and race 2 causing wilt of musaceaous hosts.

At present pathotype (10), colony type (10), biochemical type (13), are the major characters used to differentiate races and strains.

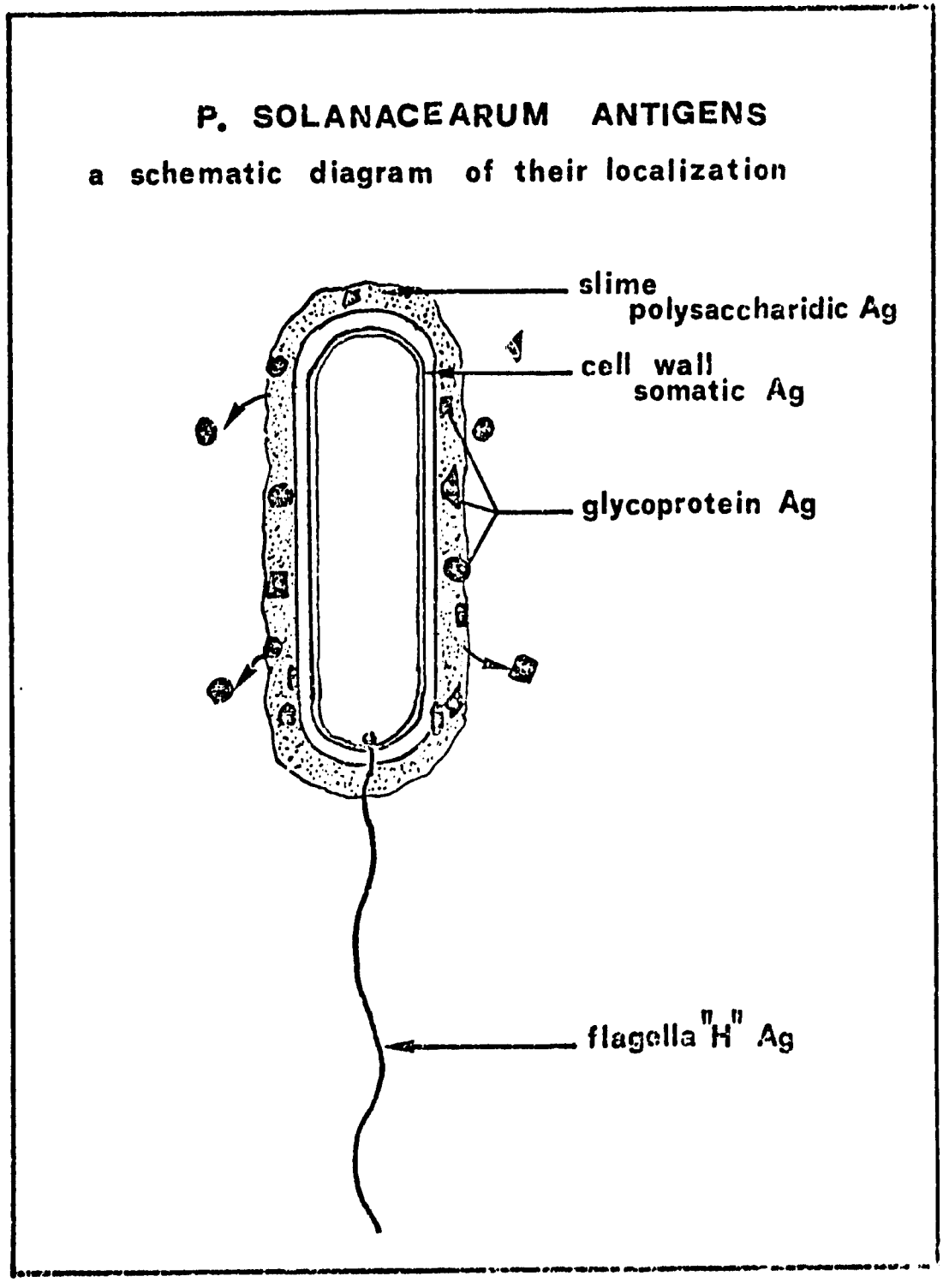


Figure 1. A schematic diagram of the localization of the antigens of P. solanacearum.

If the problem of antigen specificity could be resolved, serology would also help to identify races and strains using serotypes.

The purpose of this paper is to summarize the work conducted in the field of serology of P. solanacearum with emphasis on the antigen specificity of races and strains.

I. THE STUDY OF SEROLOGICAL CHARACTERS OF THE SPECIES P. SOLANACEARUM

For the taxonomist, it is interesting to know whether there is a serological relationship between P. solanacearum and the other phytopathogenic or saprophytic bacteria. For the plant pathologist, it is useful to know whether this species could be serologically related to the other phytopathogenic bacteria affecting the same host-plant.

MATERIAL and METHODS

The techniques used by researchers for serological studies on P. solanacearum are very similar to those used generally in the serology of phytopathogenic pseudomonads.

1. Preparation and injection procedure of antigenic material: (see Table 1). The antigenic material is usually prepared from whole bacterial cells. The whole cells are living (7), heat-killed 100°C for 2 hours (15) or formalin-fixed (12). Sonicated cells were used once (17). These antigens are injected intravenously, but doses and frequency of injections were variable among the various authors. A diagrammatic representation of a cell of P. solanacearum shows localization of antigens (see Fig. 1).

2. Antiserum testing: The obtained antisera were tested by several techniques. The classical agglutination technique was the most frequently used. However some different techniques were tried such as: ring-test (21), hemagglutination (22) and complement-fixing test (4).

On the other hand, antiserum was tested with antigen extracts other than the whole bacteria, such as: polysaccharides (22), sonicated cells (17) and somatic antigens (7).

RESULTS and DISCUSSION

According to the results obtained by various researchers (see Table 1), the following conclusions can be made:

1. The bacterium is a good immunogen: Antiserum titers are indeed generally high (up to 1/8192 for the agglutination technique and 1/40920 for the immunofluorescence technique).

2. The serological characters of the species are homogeneous: Fifty isolates from the races 1, 2 and 3 originating from several countries in the world were tested by the agglutination technique and all gave positive results (7). However, it must be noticed that some variations are observed with the agglutination technique according to whether colony types are "fluidal" or "opalescent", i.e. according to whether cells are with or without extracellular polysaccharide. For a given isolate, cells from fluidal (smooth) colonies can be agglutinated with difficulty and sometimes agglutination is inhibited entirely. Furthermore, with "smooth" colonies, tiny agglutinates are obtained while opalescent (rough) types agglutinate more easily with large agglutinates.

Table 1. Various methods of immunizing rabbits with P. solanacearum E.F.Sm. and corresponding antisera

Antigen		Antiserum		
Nature	Injection procedures	Titer and testing techniques	Specificity	Ref.
W Formalin-fixed (4.10^9/ml) 2 strains	i.v.: 0.5 ml, 0.5, 1.0, 1.0, 2.0, 2.0 at 3 day intervals 5 ml 8 days later. 3.0 ml later	1/1280	NO for 2 strains	12
W Formalin-fixed (10^7 to 10^8/ml) 9 strains	i.v.: 1 or 2 ml twice weekly or 2 successive days during 4 weeks	Titer not indicated; ring-test with polysaccharide extracts as antigens	NO for 59 strains	21
W 3 strains (tomato, ginger, Bird of Paradise)	Not described	Agglutination	Relationship among the 3 strains. Strain specific antigens are suspected	23
S (5.10^8/ml) 3 strains (one from each race)	i.v.: 1 ml, 2, 3, 4, 5, on the 1st, 4th, 8th, 12th, 19th	1/8192 race 1 1/4096 race 2 and 3 Agglutinations	More relationship among race 2 and 3 than either race has with race 1 for 3 strains	17
W Living (2.10^9/ml)	i.v.: 0.5 ml, 1.0, 2.0 at 4 day intervals	1/320 Agglutination	NO for 35 strains	6
W Living (2.10^9/ml)	i.v.: 0.5 ml, 1.0, 2.0 2.0 at 2 day intervals, every week during 30 days then a new series of injections	1/2560 Agglutination; immunoelectrophoresis with O Somatic antigens	3 serotypes identified for 23 strains. Relative specificity for the SFR strain and one Tomato strain	7
W Formalin-fixed and heat killed 7 strains	i.v.	Titer not indicated Immunodiffusion	Relative specificity for the SFR strain and one Ginger strain	15
Glycoprotein extract (5 mg/ml) protein UV dosage 11 strains	i.v.: 0.5 ml on the 1st day 0.8 ml on the 2nd day	1/1280 Agglutination 1/40920 Immunofluorescence Immunodiffusion	Yes: if absorbed, specificity for races and strains	

W Whole cell. S Sonicated cell. i.v. intravenously.

In a comparison of P. solanacearum with Salmonella, Okabe and Goto (19) suggested that, in a given isolate, fluidal colonies would possess two antigenic systems on the surface named A and B, whereas the opalescent ones would only possess the B system. This hypothesis rests on the following results obtained by cross-absorption:

Antigen \ Antiserum against	Fluidal type	Opalescent type
Fluidal type	+	-
Opalescent type	+	+

Suggesting that the fluidal ⟶ opalescent variation would be associated with the loss of the A system (19).

3. The glycoprotein antigens of P. solanacearum can be easily differentiated from the other phytopathogenic bacterial species: With a specific antiserum prepared from bacterial glycoprotein extracts, we have not found any positive cross-reaction using the agglutination (on microscope slides with antiserum 1/100) or the immunofluorescence (indirect method) techniques with the following bacteria: Agrobacterium radiobacter var. tumefaciens; Arthrobacter tumescens; Corynebacterium michiganense, C. sepedonicum; Erwinia carotovora var. atroseptica, E. carotovora var. carotovora, E. carotovora var. chrysanthemi, E. herbicola; Pseudomonas caryophylli, P. fluorescens, P. lachrymans, P. marginalis, P. mors-prunorum, P. mors-prunorum persicae, P. phaseolicola, P. pisi, P. putida, P. rubrilineans, P. rubrisubalbicans, P. savastanoi, P. syringae, P. tabaci, P. tolaasi, P. tomato, P. viridiflava; Xanthomonas albilineans, X. campestris, X. phaseoli, X. vasculorum and X. vesicatoria.

Formerly Harrisson and Freeman (12) found a serological relationship between P. solanacearum and Corynebacterium sepedonicum. That result could be due to the nature of antiserum made from whole bacteria. These observations indicate the potential value of serodiagnosis in identifying P. solanacearum.

II. THE PROBLEM OF SEROLOGICAL SPECIFICITY FOR RACES AND STRAINS

Serum specificity is sometimes questionable. The often-heard criticism of serology is that frequently the serological characters cannot be related to pathogenicity characters.

However, a relationship could be shown between these two characters if the precise nature of pathogenicity characters was known. In other words, if it would be possible to extract and to purify molecules related to or controlling pathogenicity, it would be possible to use them as antigens. Antisera for the pathogenicity molecules could be obtained and a precise relationship between serology and pathogenicity could be finally established.

In order to become concrete, this hypothesis needs a systematic analysis of antigenic entities of the bacterium. Among these, we have now studied the somatic antigen and the extracellular glycoprotein antigen.

MATERIAL and METHODS

The isolates used in this study are listed in Table 2.

1. Preparation and injection procedures of antigens:

Somatic antigen: The somatic antigens from 24 strains were extracted according to the method outlined in Table 3. Every extract was adjusted to a concentration of 5 mg protein per milliliter (Biuret reaction).

Glycoprotein antigen: The glycoprotein antigens from 11 strains were extracted according to the method in Table 4.

Each extract was adjusted to a concentration of 5 mg protein/ml. In some cases, the extract was concentrated by dialysis against Carbowax or by evaporation. For these two antigens, the usual injection procedure was very short: 0.5 ml intravenously on the first day and 0.8 ml on the 2nd day.

These antigens are very toxic for rabbits and mice. For these animals, toxicity causes a hypervascularisation and frequently some serious necroses at the point of injection. The loss of hair for mouse and the paralysis of hind quarters for rabbit are common. If the antigen concentration is too high (i.e. more than 10 mg/ml), the animal frequently dies within two days of the injection. If the antigen concentration is at the limit of toxicity, the animal can survive but its condition becomes quickly cachexic. It could be possible to reduce these problems by decreasing number, concentration and frequency of injections, or even by using intradermic and intravenous injections alternatively.

2. Antiserum testing: The kinetics of appearance of antibodies was followed every day by ear-bleeding (see Fig. 2). Tests were performed with classical serological methods, i.e. agglutination, Ouchterlony immunodiffusion and radial (Mancini) immunodiffusion, immunoelectrophoresis and immunofluorescence against the whole bacteria and bacterial extracts.

3. Antiserum absorption: Antisera were absorbed by whole cells subsequently from each isolate or simultaneously by a "pool" of isolates according to the usual technique (14). Several cycles of absorption and a careful filtration were necessary.

4. Immunodiffusion (ID) and immunoelectrophoresis (IE) techniques: Gel diffusion was made of agar 1.2%, sodium chloride 0.85%, sodium azide 0.02% in distilled water. Ouchterlony ID, radial ID and IE were performed according to the usual methods (11, 16, 20). For radial ID, the melted gel diffusion was mixed with prewarmed antiserum so that the final dilution of antiserum was 1/40. Whole bacteria and glycoprotein extracts were used as antigens in the wells.

5. Immunofluorescence (IF): IF reaction was performed according to the usual method (5). A Leitz Orthoplan microscope with ultra-violet epi-illumination system was used as observation system.

RESULTS

1. Somatic antigens: Agglutination and immunodiffusion techniques were unable to allow us to differentiate clearly the somatic antigens of different races and strains of *P. solanacearum*.

However, we obtained some characteristic patterns by immunoelectrophoresis technique (see Fig. 3). Indeed a few isolates such as the SFR and two tomato isolates (Guy to 2 and PR 80) give a particular line pattern.

Table 2. Isolates of *Pseudomonas solanacearum* used in comparative studies of serological specificity[a]

No. collection	Race	Host	Location	Year isolated
K60	1	Tomato	USA, N.C.	1953
MAR	1	Tomato	Martinique (FWI)	1966
26	1	Tomato	USA Georgia	1954
GUY	1	Tomato	French Guiana	1967
PR 80	1	Tomato	USA, Puerto Rico	
70	2(SFR)	Plantain	Colombia, Ibague	1965
168	2	Banana	Panama, Armuelles	1958
178	2(SFR)	Plantain (Chato)	Colombia	1961
192	2	Plantain (Sapo)	Peru, Amazonas	1968
80	3	Potato (*S. phureja*)	Colombia, Las Palmas	1965
82	3	Potato	Colombia, Tibaitata	1965
119	3	Potato	Costa Rica, Paraiso	1965
145	3	Potato	Australia, Old Toowoomba	1965
153	3	Potato	Australia, Old Kingaroy	1966

[a] These isolates, representative of each of the three races, form part of the collection maintained at the Dept. of Plant Pathology, University of Wisconsin, Madison. The isolates MAR, GUY, PR 80 are from our own collectio

Table 3. "O" somatic antigen extraction with trichloroacetic acid (Modified from Staub, A. M. in Methods in Carbohydrate Chemistry 5:92-93)

1. *P. solanacearum* 48 h culture on LPG Agar in Roux bottles are harvested in sterile water (10 ml per Roux bottle) and washed twice in sterile water in a weighed centrifuge tube. The packed cells are weighed and suspended in 5 times their weight of 0° C water.

2. Equal volume of 0.5 N trichloroacetic acid is added. The mixture is cooled to 4° for 3 h and then centrifuged

3. Supernatant ← → Pellet eliminated
is neutralized to pH 6.5 and precipitated with 2 volumes of ethanol at -4° C
↓

4. Precipitate
is sedimented overnight at -4° and centrifuged
↓ ↘

5. Pellet — Supernatant eliminated
is dissolved in 0.1 the original volume of sterile water and dialyzed for 4 days against distilled water
Then it is centrifuged at 27 000 x G
↓ ↘

6. Supernatant — Pellet eliminated (bacterial debris)
= "O" Somatic antigen.

Table 4. Glycoprotein antigen extraction with ammonium sulfate

1. *P. solanacearum* 48 h culture on LPG Agar in Roux bottles are harvested in sterile water (10 ml per Roux bottle) shaken vigorously for 30 minutes, centrifuged at 3000 g for 15 minutes and then at 6000 g for 15 minutes.

↓ ↓

2. Supernatant is filtered[a], neutralized at pH 7, and precipitated with equal volume of saturated ammonium sulfate solution at +4° C. — Pellet eliminated

↓

3. Precipitate is sedimented overnight at +4° and centrifuged at 20,000 x G for 15 minutes

↓ ↓

4. Pellet is dissolved in original volume of sterile water and dialyzed for 4 days against distilled water. — Supernatant eliminated

= Crude "glycoprotein" fraction named "*glycoprotein antigen*"

[a] The possibility of antigenic contamination (especially by cell wall material or bacterial debris) must be kept in mind.

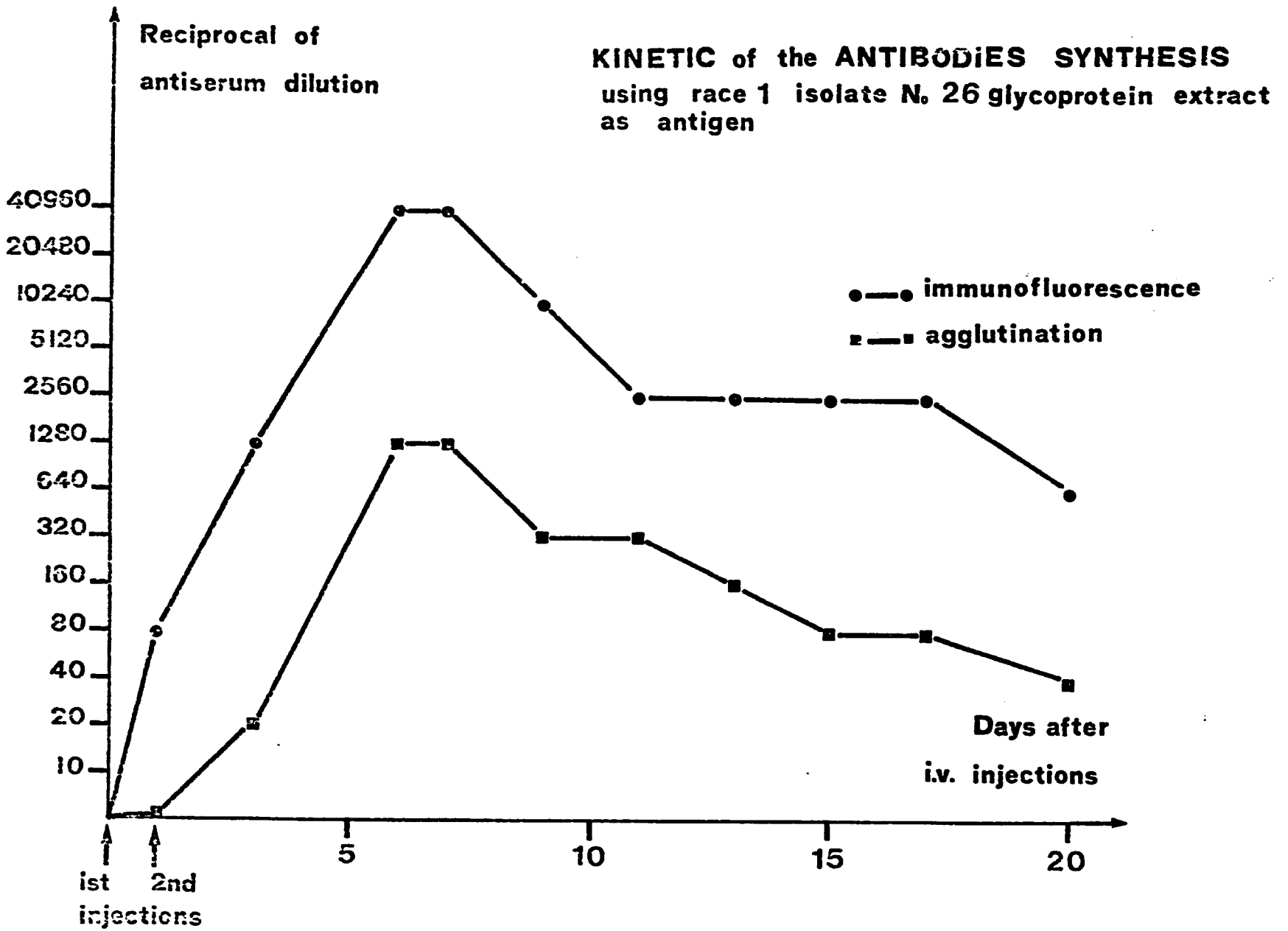


Figure 2. Kinetics of the synthesis of antibodies using race 1 isolate No. 26 glycoprotein extract as antigen.

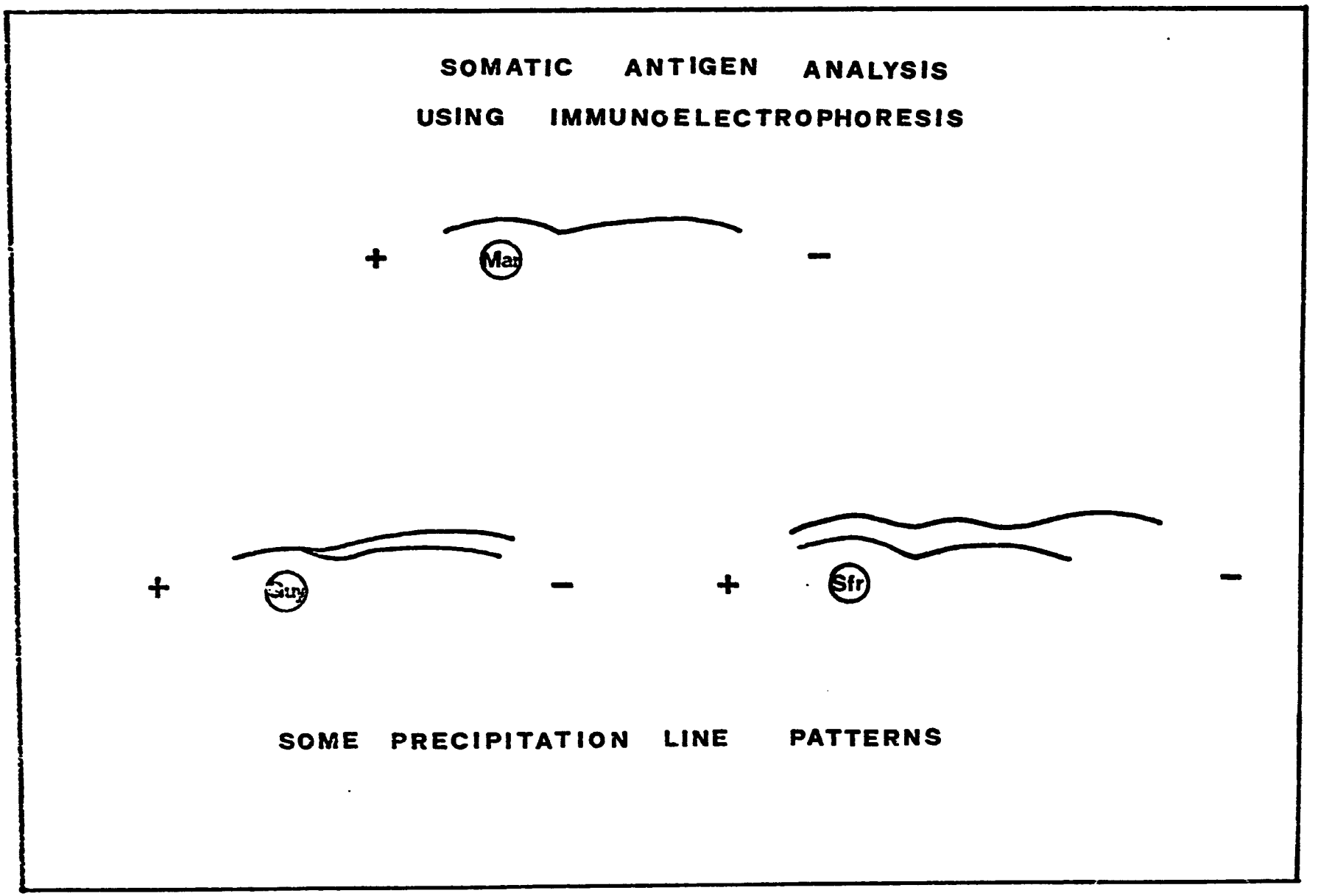


Figure 3. Somatic antigen analyses using immunoelectrophoresis showing patterns of some precipitation lines.

2. Glycoprotein antigens: All the glycoprotein extracts gave a positive Molisch reaction and a few of them (such as isolate 168) had a high catalase activity.

1) Agglutination technique (see Figs. 4, 5): With race 1 isolate 26 unabsorbed antiserum, all the isolates from race 1 and 3 were agglutinated but, by subsequent absorption, strain-specific antiserum became evident from dilution 1/80. With race 3 isolate 80 unabsorbed antiserum, all the isolates from the three races were agglutinated, except for isolate 145. However, after absorption by the isolates 26, 178, 168, 153, strain-specificity of antiserum becomes also evident from dilution 1/10 except for isolate 153 which seems very closely related to isolate 80.

2) Immunodiffusion techniques (see Figs. 6,7): Ouchterlony immunodiffusion techniques enabled us to understand better the subtle serological relationships between the strains.

When using an unabsorbed antiserum in the center well, we obtained the following results: With whole bacteria as antigens in the surrounding wells, one line was close to each strain corresponding to the homologous antiserum, i.e. the strain-specificity of that line was evident and one circular line was close to the center well corresponding to a common antigen for the three strains.

With glycoprotein extracts as antigens in the peripheric wells, only strain specific lines close to antigen wells are visible. On the other hand, when an absorbed antiserum was used in the center well, we obtained only one line close to antigen well corresponding to the homologous strain both with whole bacteria and with glycoprotein antigens.

In this case, absorbed antiserum seems to become relatively strain-specific.
Radial immunodiffusion technique using an absorbed antiserum (made up from race 2 isolate 168) indicated that immuno-precipitates are only obtained from the wells corresponding to race 2.

3) Immunoelectrophoresis technique (see Fig. 8): With whole bacteria as antigens, the complexity of the whole cells is indicated by the number of lines visible on the slides. In contrast, antigen simplicity and strain-specificity of glycoprotein extracts are evident; only two arcs are obtained and there are no reactions with heterologous glycoprotein from the other strains.

4) Immunofluorescence technique (see Fig. 9): From the results obtained by this very sensitive technique, we observed that:

1° Strain flagella are labelled by weakly diluted antiserum (< 1/640). At the highest dilutions (1/20 480 for instance) there is no more labelling whereas bacterial cells are visible. Therefore, the antisera contain relatively less numerous antibodies against flagella than against the glycoprotein of the bacterial cells.

2° The antiserum absorption decreases the antiserum titer and is more or less efficacious among the races and isolates. In some cases (with antiserum 26 for instance), the absorbed antiserum become specific from a low dilution (1/20), but generally strain-specificity becomes only evident about 1/320.

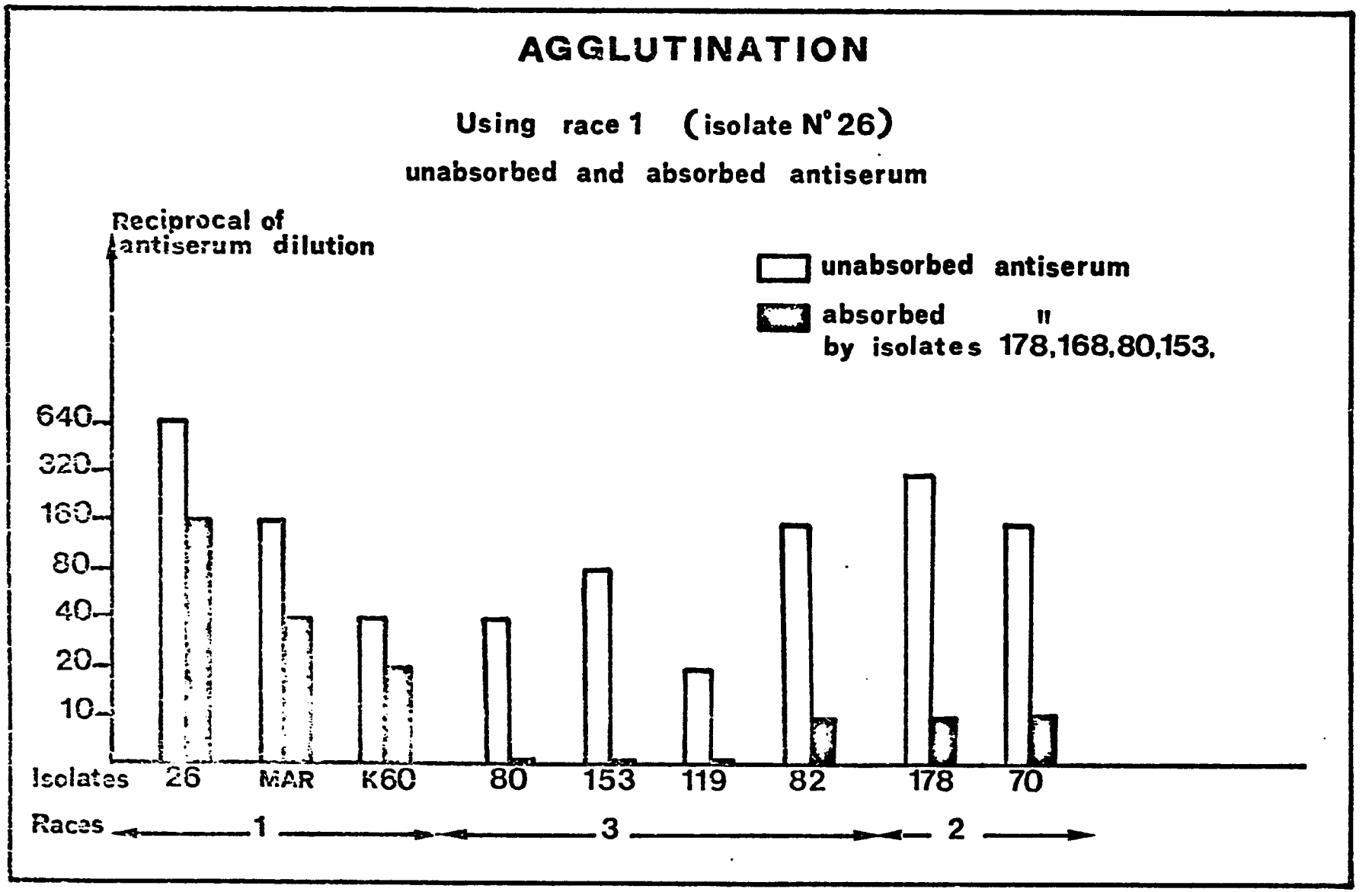


Figure 4. Agglutination patterns using isolate 26 (Race 1) unabsorbed and absorbed antiserum.

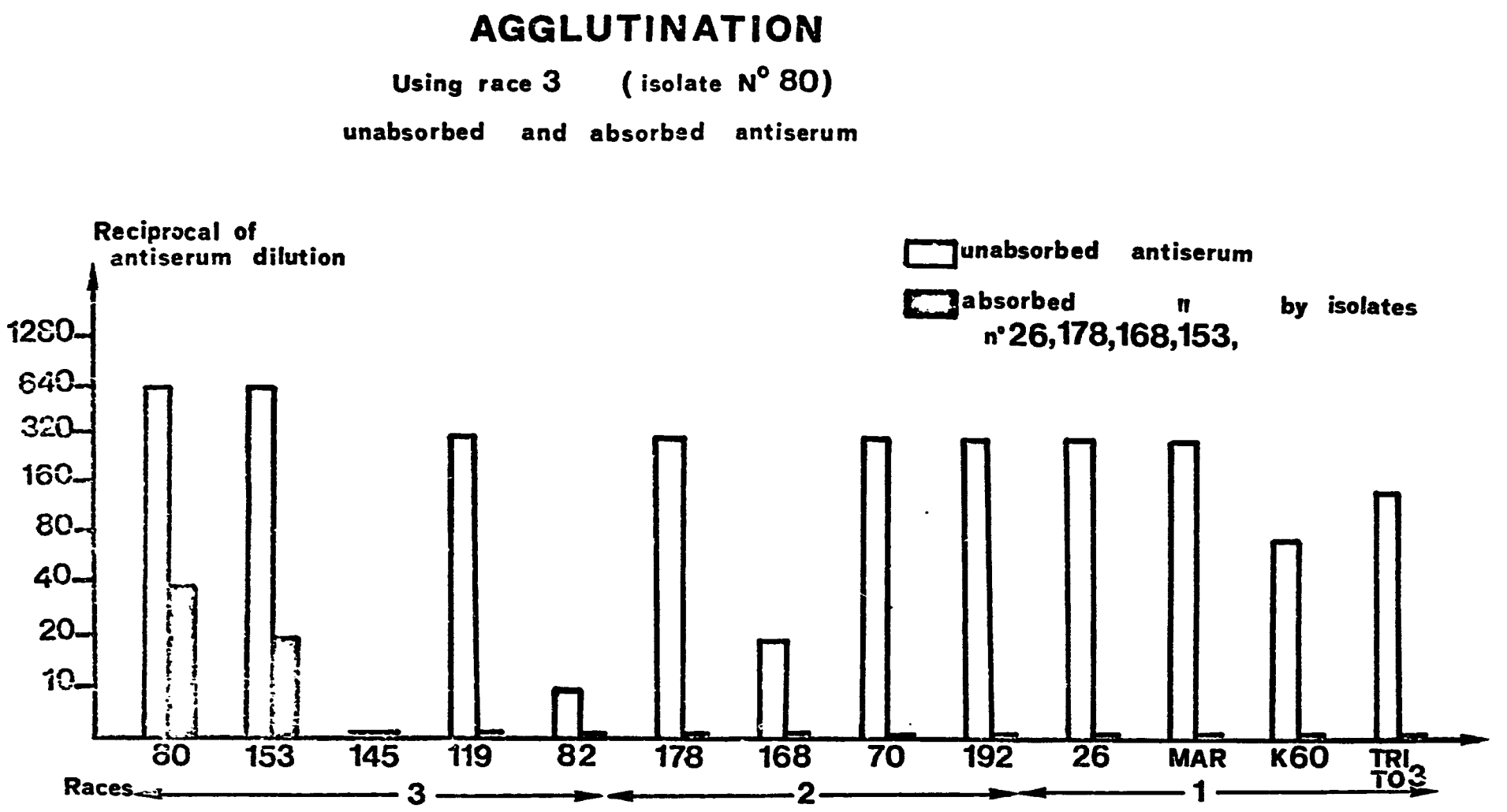


Figure 5. Agglutination patterns using isolate 80 (Race 3) unabsorbed and absorbed antiserum.

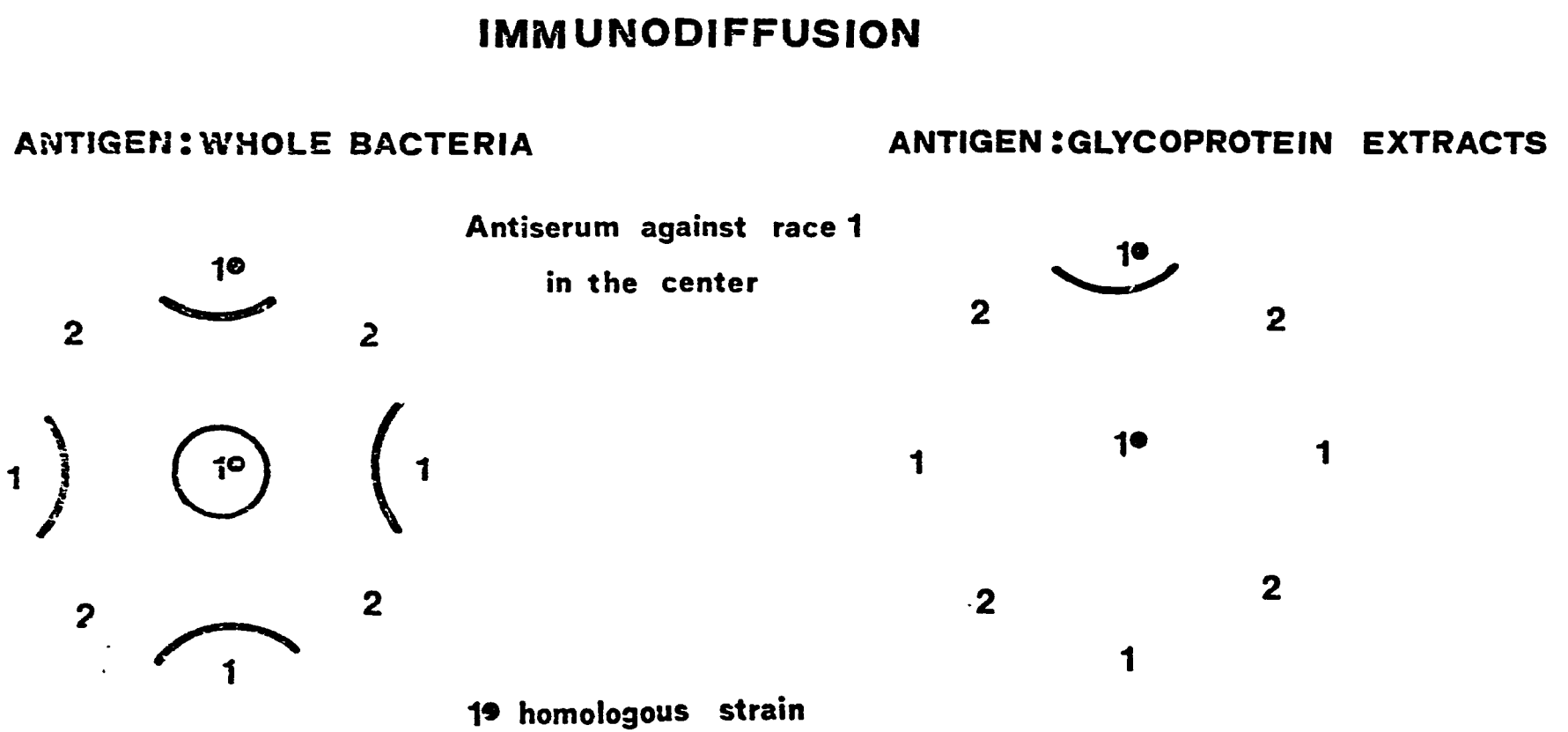


Figure 6. Immunodiffusion patterns of antigens from whole bacteria and glycoprotein extracts of race 1 showing race and strain specificity.

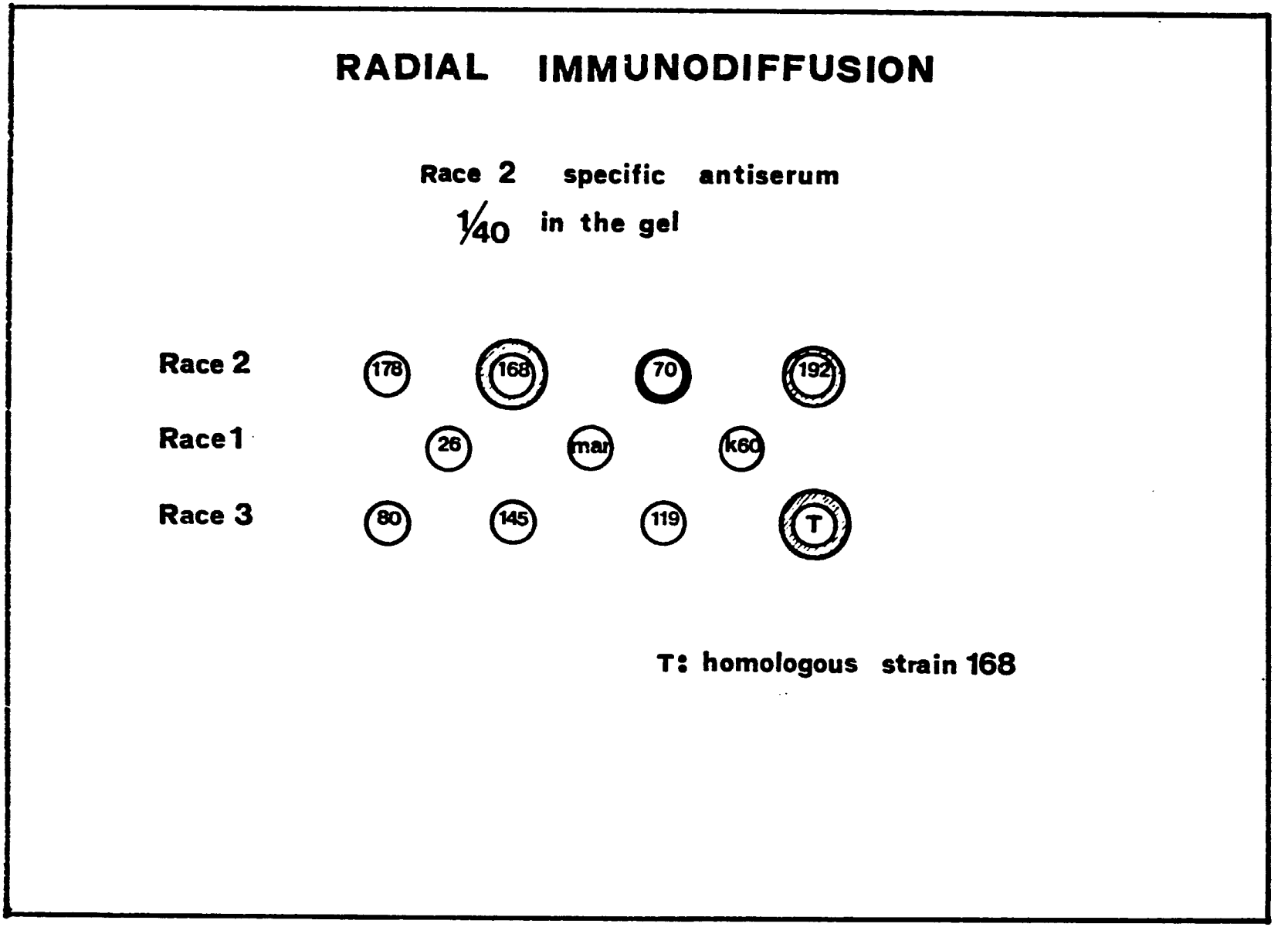
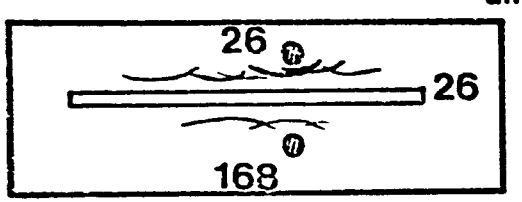


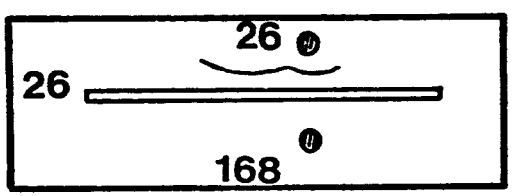
Figure 7. Radial immunodiffusion patterns with race 2 specific antiserum (1/40) in the gel.

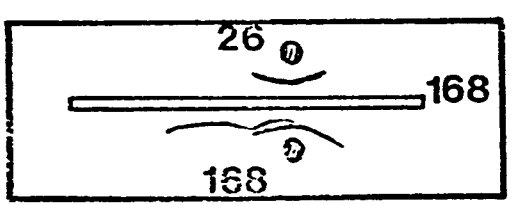
IMMUNOELECTROPHORESIS

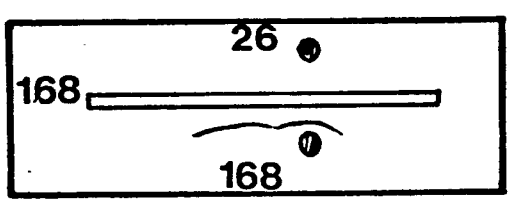
ANTIGEN: whole bacteria — glycoprotein extracts

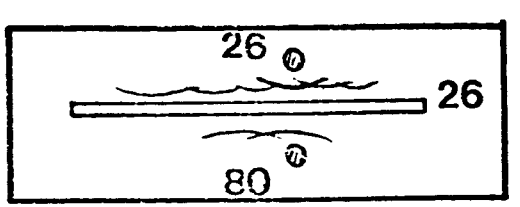
unabsorbed antiserum

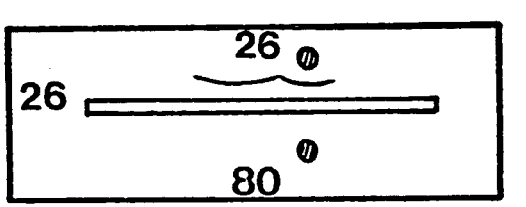












COMPLEXITY of the WHOLE CELLS

SIMPLICITY of GLYCOPROTEIN EXTRACTS

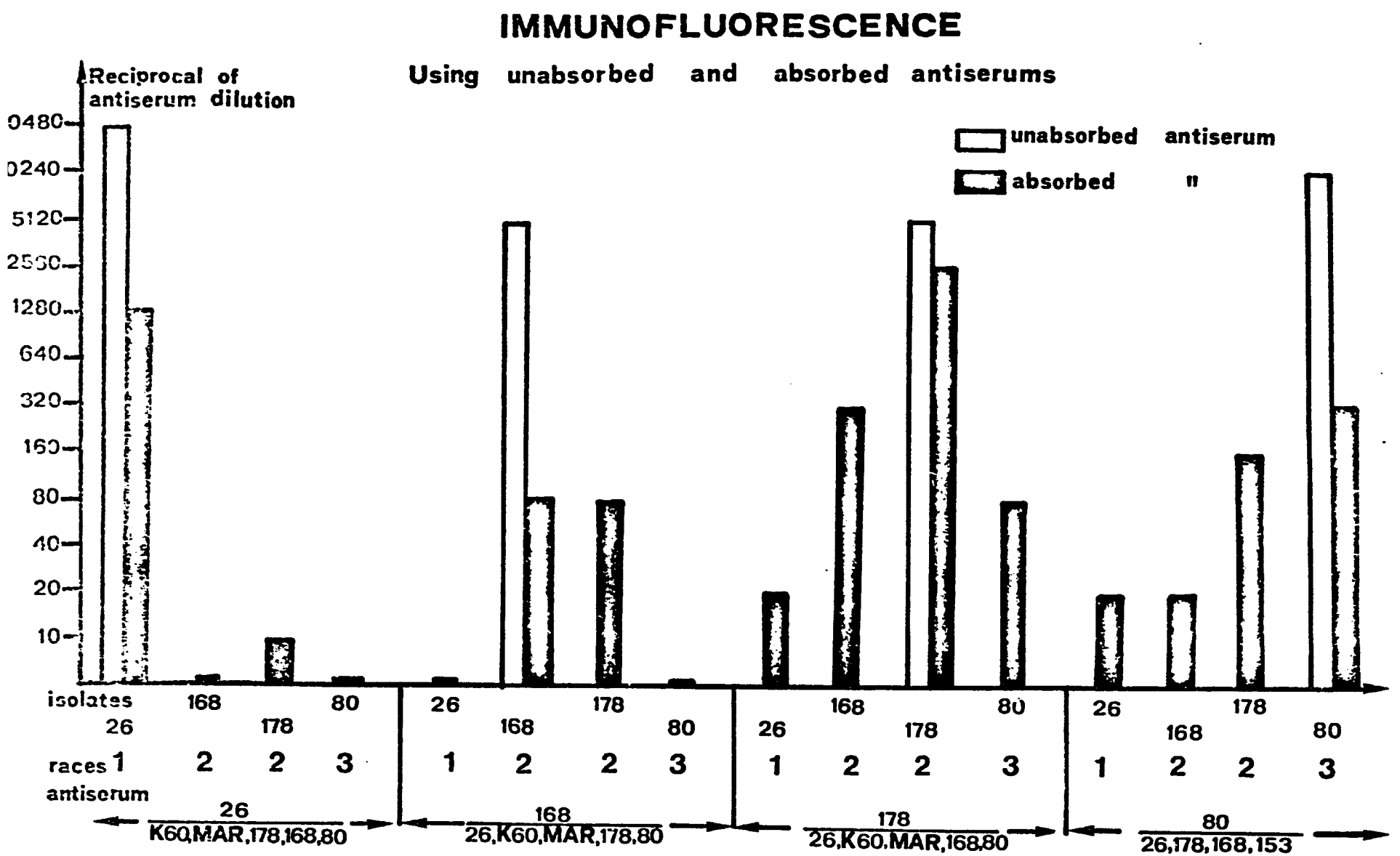


Figure 9. Immunofluorescence patterns using absorbed and unabsorbed antisera.

DISCUSSION

Each technique employed indicated a relative specificity of glycoprotein extracts, bringing simultaneously complementary results. The results of agglutination and immunofluorescence studies indicate that glycoprotein antigens are located on the bacterial cell surface and on the flagella. The results of immunodiffusion and immunoelectrophoresis studies indicate that there are common antigens among strains. In addition there are also strain-specific antigens.

Some of these strain-specific antigens are extracellular diffusible glycoproteins sometimes with catalase activity. Their spread in the host-plant during the infectious process is an intriguing possibility.

In each race, each strain possesses its own antigenic characters, suggesting that the strains have been evolving in wholly different ways, yielding finally that unusual pathogenicity variation.

Although this serological study has been made from relatively few strains, we believe that research on P. solanacearum serology will make it possible to obtain relatively specific antisera in the future.

Work is in progress regarding the extraction and the purification of antigenic molecules possibly correlated to pathogenicity.

III. APPLICATION OF SEROLOGY FOR STUDYING BACTERIAL WILT

Serology could become useful in the following fields of research:

1. Diagnosis and detection: In isolating bacteria, P. solanacearum colonies can be confused with saprophytic colonies. Since it is very easy to examine cells from isolated colonies on the medium, agglutination tests can be made and allow a more efficacious screening of the colonies. Otherwise, agglutination tests can be used directly with exudates from severely infected plants. In this way, the bacterium can be directly detected in potato, tomato (9), eggplant, sweet pepper and banana.

A more subtle detection technique is immunofluorescence which provides a means for detecting the bacterium in the plant, water and soil (1). In this case, practical methods for a bacteriological analysis of soil have to be improved since many serological cross-reactions with saprophytic soil-bacteria could occur. The IF technique can be also used in detecting the bacterium in the host-plant and in studying the rate of spread of the pathogen.

Since P. solanacearum can survive without producing symptoms in potato tubers or in banana rhizomes, a method could be developed for analyzing these plant organs especially when imported or exported.

2. Taxonomy and epidemiology: From our results, race and strain-specific agglutinating antisera can be obtained by absorbing them and using a convenient routine-dilution. Immunodiffusion technique will be more precise in differentiating the subtle serological relationships among the strains. Maps showing the current status and the present localization of races and strains in a country can be plotted and will aid in the evaluation of pathogenic potential.

3. Disease resistance and host-pathogen relationship: The bacterial cell immunolabelling provides a means of following the spread of the bacterium from the roots to the top of the host-plant.

Frequently bacterial wilt resistant hybrids (tomato, potato) may be symptomless carriers of P. solanacearum. The very sensitive immunofluorescence technique provides a means for detection of small numbers of bacteria in the tolerant host-plant.

Relationships between the bacterial cell and the host-plant cell are poorly known, however. For instance, does the bacterial cell have to be fixed on a particular site of the host-plant cell in order to become pathogenic?

The immunolabelling techniques such as IF, immunoenzyme and immunoferritin techniques can contribute in clarifying the unknown cellular relationships, in particular by observing tissue sections.

Fields of investigation on bacterial wilt which need to be explored in the future are numerous and we believe that the application of serology will be one of the areas increasing in importance.

LITERATURE CITED

1. BERNIAC, M. and A. COLENO. 1974. The use of immunofluorescence in detecting phytopathogenic bacteria. Symposium on Crop Protection in the Caribbean. St. Augustine, Trinidad.

2. BUDDENHAGEN, I. W., L. SEQUEIRA and A. KELMAN. 1962. Designation of races of Pseudomonas solanacearum. Phytopathology 52:726. (Abstr.)

3. BUDDENHAGEN, I. W. and A. KELMAN. 1964. Biological and physiological aspects of bacterial wilt caused by Pseudomonas solanacearum. Ann. Rev. Phytopathol. 2:203-220.

4. COLENO, A., L. HINGAND and B. RAT. 1976. Some aspects of the serology of Pseudomonas solanacearum. Conference on bacterial wilt. Raleigh, N.C. July 1976 (in press).

5. COONS, A. H. and M. H. KAPLAN. 1950. Localization of antigen in tissue cells. II. Improvements in a method for the detection of antigens by means of fluorescent antibody. J. Exp. Med. 91:1.

6. DIGAT, B. and A. ESCUDIE. 1967. Reconnaissance du fletrissement bacterien aux Antilles. Phytiatrie-Phytopharmacie 16:187-197.

7. DIGAT, B. 1968. Why and how to distinguish the Pseudomonas solanacearum strains, causal agent of the bacterial wilt of solanaceous and musaceous crops in the Caribbean zone. In Proceedings of the Caribbean Food Crops Society, 6th annual meeting (Trinidad) 6:86-91.

8. DIGAT, B. 1966. Retablissement chez la tomate infectee par le Pseudomonas solanacearum E.F.Sm. C.R. Acad. Agric. France:1005-1009.

9. ESCUDIE, A. and B. DIGAT. 1965. Diagnostic rapide du fletrissement bacterien des Solanees par le test de sero-agglutination. Agronomie tropicale (Paris) n° 6-7:640-644.

10. FRENCH, E. R. and L. SEQUEIRA. 1970. Strains of Pseudomonas solanacearum from Central and South America: a comparative study. Phytopathology 60:506-512.

11. GRABAR, P. and P. BURTIN. 1960. Analyse immunoelectrophoretique, applications aux liquides biologiques humains. Masson et Cie edit. 294 p.

12. HARRISON, D. E. and H. FREEMAN. 1961. Bacterial wilt of potatoes. II. Serological relationship of two strains of Pseudomonas solanacearum and a culture of Corynebacterium sepedonicum. Austr. J. Agr. Res. 12:872-877.

13. HAYWARD, A. C. 1964. Characteristics of Pseudomonas solanacearum. J. Appl. Bact. 27:265-277.

14. KAUFMAN, F. 1950. The diagnosis of Salmonella types. C. Thomas, Springfield.

15. KOJIMA, E. S. and I. W. BUDDENHAGEN. 1969. Antigenic relationships of strains of Pseudomonas solanacearum. Phytopathology 59:1035-1036 (Abstr.)

16. MANCINI, G., A. O. CARBONARA and J. F. HEREMANS. 1965. Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 2:235.

17. MORTON, D. J., S. F. JENKINS, JR. and P. D. DUKES. 1965. Serological relationship among races 1, 2 and 3 of Pseudomonas solanacearum. Phytopathology 55:1069 (Abstr.).

18. MORTON, D. J., P. D. DUKES and S. F. JENKINS, JR. 1966. Serological relationships of races 1, 2 and 3 of Pseudomonas solanacearum. Pl. Dis. Reptr. 50:275-277.

19. OKABE, N. and M. GOTO. 1954. Studies on Pseudomonas solanacearum. VI. A comparative study of the colony type organisms F. and O.p. Shizuoka Univ. Fac. Agr. Rept. 4:41-60.

20. OUCHTERLONY, O. 1958. Diffusion-in-gel methods for immunological analysis. p. 1-78. In Progress in Allergy (D. Kallos ed.) Vol. V. S. Karger, Basel.

21. PEREZ, J. E. 1962. The use of Fuller's formamide method in the serological identification of Pseudomonas solanacearum. J. Agr. Univ. Puerto Rico 46:144-153.

22. PEREZ, J. E. 1967. Hemagglutination by bacterial phytopathogens. J. Agr. Univ. Puerto Rico 51:337.

23. QUINON, V. L., M. ARAGAKI and M. ISHII. 1964. Pathogenicity and serological relationship of three strains of Pseudomonas solanacearum in Hawaii. Phytopathology 54:1096-1099.

24. STAUB, A. M. 1965. Bacterial lipido-proteino-polysaccharides ("O") somatic antigens. Extraction with trichloroacetic acid. p. 92-93. In Methods in Carbohydrate Chemistry. R. C. Whistler (ed.), Vol. V.

25. STOLP, H., M. P. STARR and N. L. BAIGENT. 1965. Problems in speciation

RESISTANCE TO BACTERIAL WILT (PSEUDOMONAS SOLANACEARUM)

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The history of finding sources of and breeding for resistance to bacterial wilt is a fascinating one and is still in progress. Leaders in plant pathology who develop and articulate the principles which we teach to our students could learn much by studying the history of resistance to *Pseudomonas solanacearum*.

I am going to begin with resistance to *P. solanacearum* in peanuts. The first successful attempt to breed or select for resistance to *P. solanacearum* was made with peanuts in Java (now Indonesia). Dutch workers published on differences in resistance among peanut varieties in Indonesia as long ago as 1910 (see review by Kelman, 1953). Marie Schwarz and C. Hartley, among other workers, made extensive tests with material from the Americas, Africa and Asia, especially Indonesia. Eventually a line named Schwarz 21 was selected and released in 1927. The line from which Schwarz 21 derived its resistance was not positively known, but it is likely that it came from West Java near Cheribon where the disease had been especially severe. Although additional work has been done with peanuts, Schwarz 21 is still widely planted in Indonesia and has been used as a source of resistance in breeding. There has been no evidence that more virulent strains of *P. solanacearum* have developed since the introduction of Schwarz 21 that can attack it.

The story of breeding for resistance in tobacco is equally interesting and encouraging (see review by Kelman, 1953). I will not go into the history of Granville wilt or the great economic losses it caused. Stevens initiated a search for resistance in 1904. Efforts to find resistance by numerous U.S. and Dutch workers in Indonesia were unsuccessful. In 1934 another major effort was made to find resistance and Clayton, Foster and Smith checked the resistance in greenhouse trials of numerous wild *Nicotiana* species and over 1000 collections of *N. tabacum* from Mexico, Central and South America. None of the wild *Nicotiana* species had resistance, but one line - T.I. 448A from Colombia, S.A. - was found to be highly resistant, not only to *P. solanacearum* but also to tobacco mosaic. In 3 years of field trials T.I. 488A rarely was more than 10% infected whereas others were usually 100% infected. Crosses were made involving T.I. 448A and tobaccos of desirable agronomic quality and the variety Oxford 26 was introduced in 1945. In Oxford county Oxford 26 yielded over 1000 lb cured leaf per acre compared to 238 lb for a susceptible variety. Smith and Clayton's studies of the mode of inheritance led them to conclude that resistance was governed by multiple recessive factors, i.e. was polygenic. Subsequent breeding work in N. Carolina gave resistance to black shank and Fusarium wilt also. This resistance, 30 plus years later, is still holding up to *P. solanacearum*. Matsuda and coworkers in Japan have reported on what may be a different resistance in tobacco due to major genes.

Eggplant

Resistance to bacterial wilt has been found and bred into commercially acceptable varieties in Puerto Rico, the Philippines, Ceylon, Malaya, India and South Africa. At North Carolina, resistant eggplant varieties such as Kopek and Natale have been introduced. Little information was found on the mode of inheritance of this resistance however.

Pepper

Peppers seem to have more resistance than other crops, and reports of varieties with resistance have been made from Puerto Rico, the Philippines, Ceylon and elsewhere.

Bananas

The devastation of the moko disease of banana and plantain has been most serious in the Americas where the SFR strain of race 2 occurs. Many observations on the relative susceptibility of bananas and plantains have been reported in the literature. The role of insects in transmitting the bacterium by visiting the exudations from the male floral bract scars is a key to understanding resistance in bananas and plantains. Those clones where the male bracts persist have resistance because insect transmission of the bacterium is reduced. All commercial varieties are susceptible to race 2 after mechanical transmission, i.e. by machete, pruning operations or artificial inoculation. A degree of resistance was found in 34 of 345 Musa accessions tested by artificial inoculation in Honduras. The variety Pelipita (ABB) was highly resistant and is recommended as a disease-resistant substitute for the susceptible Bluggoe (ABB) which has been so seriously attacked in Central and South America by the SFR strain (Stover and Richardson, 1968).

Tomato

Tomato is not quite the success story that was presented for peanuts and tobacco. Nevertheless, resistant varieties have been found. Varietal differences to bacterial wilt were noted in early breeding investigations, and breeding efforts have been made in various parts of the world, including Puerto Rico, the Philippines, Guadeloupe, Ceylon, Fijii, Australia, Brazil, Trinidad, South Africa and Florida, North Carolina and Hawaii in the U.S.A.

At the North Carolina Agricultural Experiment Station an intensive breeding program was begun in 1936. In 1943 a cross between the Louisiana Pink and Beltsville No. 3814, a tomato selection from Puerto Rico, yielded several lines with promising levels of resistance. Long and tedious work, which included the adding of the resistance from PI 129080, a *Lycopersicon pimpinellifolium* selection from Colombia, and the contribution of germplasm from tomato varieties Pan America, Marglobe, Rutgers, STEP 174, and Manalucie, resulted in one of the first tomato varieties which combines favorable horticultural quality with significant bacterial wilt resistance. These varieties, Venus and Saturn, were released in 1972 by Henderson and Jenkins. Singh, in a Ph.D. thesis of work done in Hawaii, reported that the resistance of these varieties was polygenic, but most of the genes were recessive. In Hawaii another source of resistance PI 127805A (*L. pimpinellifolium*) was found in 195 and utilized in breeding for resistance through nine generations by Gilbert and Quinon. Although combining this resistance with fruit quality has been a problem, potentially commercial lines have been developed. The nature of inheritance again seems to be complex; partial dominance for resistance gives way to resistance which appears to be conditioned by recessive genes in more mature plants (Acosta *et al.*, 1964). This resistance also is influenced by temperature and at the high temperatures (for instance 88 to 92°F) at low elevations, the resistance can break down. Considerable work has been done in the Philippines in recent years in attempts to find commercially acceptable varieties of tomatoes for resistance to bacterial wilt. Deanon and Rodriguez recently reported a resistant line (1169) with a considerable yield of marketable fruit.

Digat and Deriex report that a cross between a small-fruited variety from Puerto Rico and Floralou showed significantly high levels of tolerance in Guadeloupe, Martinique and French Guiana. Three breeders are now working there on this project. Workers at the University of Puerto Rico also reported two F8 lines from this small fruited variety which have high levels of resistance. Digat and Deriex suggest that bacterial wilt resistance seems to be determined by one recessive gene which seems to be simply inherited, in contrast with the Hawaii and North Carolina sources of resistance. Unfortunately, at high temperatures much of the resistance in tomatoes breaks down.

Bacterial wilt of tomatoes still remains a serious problem in much of the hot humid tropics. A coordinated world-wide effort to screen all available tomato germplasm (wild and cultivated) in the world is needed to determine if better sources of resistance are available.

Potatoes

Since the 1930's workers in the U.S., Indonesia, Ceylon, Puerto Rico, Portugal, and Brazil have looked for resistance to P. solanacearum in cultivated potatoes and, although varietal differences in susceptibility were noted, high levels of resistance were not found.

From 1947 to 1950 Nielsen and Haynes in North Carolina tested nearly 9000 potato clones in a major program to find resistance, but only moderate levels of resistance were found. In Colombia, Lozano and Thurston found that 6 clones out of 190 Solanum phureja accessions had high levels of resistance. They also tested 238 S. tuberosum, 364 S. andigena, 226 interspecific hybrids, and 43 wild species but no resistance was found among them. S. phureja is a cultivated diploid potato (24 chromosomes) in contrast to the cultivated potatoes of N. America and Europe (S. tuberosum - 48 chromosomes) and S. andigena (48) the most important potato of the Andes.

R. A. Robinson and A. H. Ramos carried out a large scale program of screening and mass selection for horizontal resistance in the field in Kenya. Although resistant potato clones were found, apparently they found no material which maintained a sufficiently high level of resistance under Kenyan conditions.

A program was initiated in Wisconsin by Sequeira and Rowe in 1967 using the resistant Colombian S. phureja lines to develop germplasm that would be useful to potato breeding projects in countries where bacterial wilt was a problem, and to study the inheritance of resistance. Under this program a first series of crosses was made in 1968 with the objective of combining wilt resistance and acceptable horticultural type. A second series of crosses in 1970 combined these characteristics with late blight resistance derived from cultivars developed in Mexico. These clones have been sent for field testing to numerous tropical countries and in many instances have performed well, but reliable results have not always been forthcoming. With the formation of CIP (International Potato Center in Lima, Peru) and its assumption of responsibility for the coordination of the potato bacterial wilt project, the program has continued to produce material that should be of value for programs interested in breeding for resistance to both wilt and late blight of potatoes. A resistant variety, Caxamarca, was released in 1975 in Peru.

Sequeira, Rowe and Gonzalez have shown that resistance is due to perhaps as many as 10 major genes. Thus resistance appears to be polygenic and highly complicated.

One method of controlling bacterial wilt, especially in tomato, should be mentioned. That consists of grafting susceptible hosts onto resistant rootstocks. Nolla in Puerto Rico grafted susceptible eggplant on rootstocks of Solanum torvum. In Java S. torvum was found the most promising as a rootstock. In Hawaii, Obrero, Aragaki and Trujillo found a resistant rootstock of L. pimpinellifolium which was useful for tomatoes. I was told that grafting on eggplant is used on a large scale in Thailand and Panama for tomatoes.

There are many pitfalls in breeding for resistance and it is important to keep in mind the various factors that can affect resistance in the growth cabinet, the greenhouse, and especially in the field. In the time available we cannot do much more than mention these factors; the excellent monograph of Dr. Kelman plus various review articles give more detailed information on them.

1. Temperature - The affect of temperature on P. solanacearum is well known, but it should be kept in mind that different races and/or isolates may react differently, i.e. race 3 tolerates cooler temperatures. Pre-inoculation temperatures seem to make little difference.

2. Soil type - Makes a big difference in the field.

3. Soil moisture and rainfall - Disease is more serious under wet conditions and in moist soils. Buddenhagen and Kelman reported that high soil moisture influenced wilting as follows:

a. By increasing survival of bacterium in the soil
b. Increasing infection
c. Increasing inoculum release from host plants and spread through soil
d. Increasing disease development after infection

4. Soil pH - The disease occurs in both acid and alkaline soils, but differences do occur.

5. Relative humidity - Results have been contradictory.

6. Light intensity - There is some conflict in available reports, but, in general, low light results in more severe disease.

7. Photoperiod - Increased susceptibility - short photoperiod. In most tropical and subtropical regions where P. solanacearum is serious, daylengths may not be short enough to seriously alter resistance. However during extensive periods of cloudiness, often characteristic of tropical wet seasons, reduced light intensity might increase susceptibility of some plants.

8. Nematodes and other pathogens

9. Isolate or race used

10. Vigor of test plants

11. Age of plants used

12. Inoculum concentration

13. Root vs. stem inoculation methods

14. Stand density - Generally it has little effect.

All of these factors must be considered in evaluating resistance under greenhouse or field conditions.

References

Acosta, J. C., J.C.Gilbert and V. L. Quinon. 1964. Heritability of bacterial wilt resistance in tomato. Proc. Am. Soc. Hort. Sci. 84:455-462.

Henderson, W. R. and S. F. Jenkins. 1972. Venus and Saturn: tomato varieties resistant to southern bacterial wilt. N.C. Agr. Expt. Sta. Tech. Bull. 444, 13 p.

Kelman, A. 1953. The bacterial wilt caused by Pseudomonas solanacearum. A literature review and bibliography. N.C. Agr. Expt. Sta. Tech. Bull. 99, 194 p.

Matsuda, T. and Y. Ohashi. 1973. Inheritance of resistance to bacterial wilt disease of tobacco. II. Inheritance of resistance originated from T.I. 448A and relation between those and Rps gene. Japan J. Breed. 23:175-180.

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

Robinson, R. A. 1968. The concept of vertical and horizontal resistance as illustrated by bacterial wilt of potatoes. Comm. Mycol. Inst. Phytopathol. Paper 10, 37 p.

Rowe, P. R. and L. Sequeira. 1970. Inheritance of resistance to Pseudomonas solanacearum in Solanum phureja. Phytopathology 60:1499-1501.

Rowe, P. R., L. Sequeira and L. C. Gonzalez. 1972. Additional genes for resistance to Pseudomonas solanacearum in Solanum phureja. Phytopathology 62:1093-1094.

Sequeira, L. and P. R. Rowe. 1969. Selection and utilization of Solanum phureja clones with high resistance to different strains of Pseudomonas solanacearum. Am. Potato J. 46:451-462.

Smith, T. E. and E. E. Clayton. 1948. Inheritance of resistance to bacterial wilt in tobacco. J. Agr. Res. 76:27-32.

Stover, R. H. and D. L. Richardon. 1968. Pelipita, an AB Bluggoe type plantain resistant to bacterial and fusarial wilts. Plant Dis. Reptr. 52:901-903.

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

SPECIFICITY IN THE INFECTION OF PLANT HOSTS BY *PSEUDOMONAS SOLANACEARUM*

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INTRODUCTION

Isolates of *Pseudomonas solanacearum* E.F.Sm. that are pathogenic to musaceous hosts (Race 2), or are avirulent (B-1), cause a rapid hypersensitive reaction (HR) when infiltrated in the intercellular spaces of tobacco leaves (6). In contrast, most compatible (Race 1) isolates cause slow, progressive necrosis of host tissues and the bacteria invade the adjoining healthy tissues, including the vascular system. Differences in symptom expression are associated with marked differences in the kinetics of bacterial growth in host tissues. If introduced at 10^8 cells/ml, populations of incompatible bacteria remain steady for about 6 hr and then decline precipitously, at a time when the HR is not yet visible. With compatible bacteria, populations show an exponential increase within a few hours after infiltration, reach a peak at 36-48 hr, and then decline in the center of the lesion where necrosis first becomes evident (11).

These marked differences in the interaction of *P. solanacearum* races (or variants) and the tobacco (or potato) host tissues have provided a useful model to study the question of host-pathogen specificity. It is apparent that the host "recognizes" differences in constituents of the bacterium and reacts accordingly. The following is an attempt to summarize the information that we have been able to obtain on the details of this early recognition event.

ULTRASTRUCTURE OF THE BACTERIUM-HOST CELL WALL INTERACTION

Initial efforts centered on the ultrastructural details of the relationship between compatible (K-60) and incompatible (B-1 variant of K-60) forms of the bacterium and tobacco mesophyll host cell walls. Samples obtained at 3 hr after infiltration, at the time when the HR becomes irreversible (9), and examined under the electron microscope, showed that there are very marked differences in the relationship of these bacteria to the host cell wall (13). With K-60, numerous bacteria were observed dividing freely in the intercellular spaces. Although bacterial cells were often in contact with host cell walls, no attachment was evident. As pathogenesis progressed, there was obvious disruption of the host cell wall, as would be expected because of the ability of the bacterium to produce substantial amounts of pectolytic and cellulolytic enzymes (4, 5). However, by 12 hr after infiltration, there were no marked changes in fine structure of the host cell.

In contrast with K-60, B-1 cells did not divide in the intercellular spaces; where they came in contact with the host cell wall, they were surrounded by granular and fibrillar material by 3 hr after infiltration. By 7 hr, a thin, fibrillar pellicle had covered the bacteria completely, and granular material accumulated in large amounts in the space within the pellicle and the cell wall surrounding the bacterium. The reaction, involving both attachment to and envelopment by the host cell wall, is very similar to that des-

pellicle appears to be cuticular, but this has not been established with certainty. The granular material apparently originates from vesicles that accumulate in the space between the plasmalemma and the cell wall, at the point of attachment of the bacterium.

By 12 hr after infiltration with B-1 cells, the HR is essentially complete; host cells collapse and organelles are deranged. Attachment and envelopment of incompatible bacterial cells appear to be a prerequisite for initiation of the HR. However, once attached, the bacterium must be metabolically active if the HR is to proceed. This was determined from experiments in which heat-killed B-1 cells were used. These cells are attached and enveloped in exactly the same fashion as are live cells, but there is no HR. In fact, tobacco leaf cells treated with heat-killed bacterial cells become resistant to infection by bacteria and viruses (7, 12). In leaves previously treated with heat-killed cells, B-1 cells introduced subsequently apparently do not attach to the host cell wall and, therefore, are not enveloped (13). This may be one of the reasons why the HR is not induced in such leaves.

The attachment and envelopment of incompatible forms of P. solanacearum offer an attractive explanation for the reduction in populations of these bacteria that occurs before the HR is induced. Conversely, the lack of attachment of compatible forms explains their ability to multiply rapidly in the intercellular spaces. The process of attachment must consist of a recognition phenomenon in which constituents of the outer cell wall of bacteria bind to putative receptors on the host cell wall. Preliminary investigations have given support to this hypothesis.

THE RECOGNITION PHENOMENON

Recognition events in biological systems generally involve lectin-like glycoproteins of one member and a polysaccharide of the other. Plant cell walls are known to contain lectins; a lipopolysaccharide (LPS) constitutes the outermost component of the cell wall of gram-negative bacteria. We have studied the interaction of these two components, using lectin extracted from potato tubers and tobacco leaves, and LPS extracted from both virulent and avirulent forms of P. solanacearum.

Lectin was extracted from potato tubers by a procedure modified from that of Marinkovich (8). The lectin was extracted with ethanol:1.25 N HCl, precipitated with acetone, bound to SE-cellulose, and released by elution with NaCl. The lectin was purified to homogeneity. The assay system consisted of equal volumes of distilled water suspensions of bacteria (10^9 cells/ml) and lectin (100 ug protein/ml) placed as drops on plastic dishes. Agglutination was determined under the microscope and scored on a 1-4 scale, with 4 as maximum agglutination.

Fifty isolates of P. solanacearum from different regions and different hosts, representing all major races and biotypes, were tested for their ability to bind to potato lectin. All virulent forms failed to agglutinate significantly even after 1 hr. On the other hand, all avirulent forms, many generated from the same virulent isolates tested previously, agglutinated rapidly when mixed with lectin (10). The only exceptions consisted of two virulent isolates from Australia that gave variable results; this appeared to be due to the rapid formation of avirulent forms in culture.

Similar results were obtained with lectin extracted from tobacco leaves by infiltration with saline solution, although only a limited number of isolates of P. solanacearum have been tested.

One of the major differences between virulent and avirulent forms of the bacterium is the formation of extracellular polysaccharide (EPS); virulent forms are smooth, avirulent ones are invariably rough (slime-less). This suggested that EPS might interfere with binding of virulent cells on tobacco cell walls and, similarly, with agglutination by potato or tobacco lectin. Inhibition of agglutination of B-1 cells by relatively low amounts of purified EPS (1-10 ug/ml) from K-60 has been demonstrated (Sequeira and Graham, unpublished).

It seemed likely that binding sites for potato lectin exist on the bacterial LPS. Purified LPS for B-1 cells precipitates readily when mixed with potato lectin. Potato lectin binds to carbohydrates that contain internal N-acetyl-glucosamine molecules (14) and these groups are generally found in the constituent portions of the LPS of most gram-negative bacteria: the O-polysaccharide, the R-core, and the lipid A. Since rough forms do not have the O-polysaccharide portion, it is evident that the binding site for potato lectin in the B-1 LPS must reside in either the R-core or the lipid-A portion. In agglutination tests, the most active moiety of the B 1 LPS is the lipid-A, a region generally associated with biological (endotoxin) activity in animal systems.

Purified LPS from K-60 cells does not precipitate rapidly when mixed with potato lectin, but this may be due to the formation of aggregates. Treatment with deoxycholate, which causes dissociation of aggregates, results in rapid precipitation of the LPS when potato lectin is added (Sequeira and Graham, unpublished).

CONCLUSIONS

In spite of the limited data available, the hypothesis that incompatible forms of P. solanacearum are immobilized on host cell walls via a lectin-LPS recognition system has received support. The system that we propose involves binding of the bacterial LPS to a lectin present on the host cell wall; since LPS from all gram-negative bacteria is similar in structure, binding to many types of bacteria, including saprophytes, would occur. That this is the case has been demonstrated (13).

Virulent forms of P. solanacearum apparently are not immobilized by the cell wall because the EPS, which they produce in copious amounts, is an effective inhibitor of lectin-LPS binding. It seems plausible that, through evolution, certain forms of P. solanacearum have developed a system that effectively bypasses the general defense reaction of certain hosts. The mechanism we propose is similar to that described for nodulating and non-nodulating strains of Rhizobium in their interaction with legume roots (1, 2), except that in the latter the virulent forms are the ones that are attached. Symbionts, however, may have become adapted to attachment and are able to invade the host thereafter.

There is an apparent contradiction in that some strains of P. solanacearum are rapidly attached by tobacco cell walls (e.g. strain S-210, Race 2) but are not agglutinated by tobacco lectin in vitro. The EPS from these strains

appears to be slightly different from that of compatible forms and exhibits different inhibition kinetics when tested in a lectin-LPS system (Graham and Sequeira, unpublished). There may be quantitative and/or qualitative differences in the action in vivo of this EPS, as compared with that of compatible forms but the mechanism involved is not clear at present.

In spite of some obvious inconsistencies, the recognition system involving three components, two from the pathogen (LPS + EPS) and one from the host (cell wall lectin), offers an explanation for the high degree of host specificity exhibited by certain forms of P. solanacearum. Slight changes in conformation of any of these constituents may determine the ability of the pathogen to grow in the host. Work now in progress should establish whether such a system is of general importance in specificity of host-parasite interactions.

REFERENCES

1. BOHLOOL, B. B. and E. L. SCHMIDT. 1974. Lectins: a possible basis for specificity in the Rhizobium-legume root nodule symbiosis. Science 185:269-271.

2. DAZZO, F. B. and D. H. HUBBELL. 1975. Cross reactive antigens and lectin as determinants of symbiotic specificity in the Rhizobium-clover association. Appl. Microbiol. 30:1017-1033.

3. GOODMAN, R. N., P. Y. HUANG and J. E. WHITE. 1976. Ultrastructural evidence for immobilization of an incompatible bacterium, Pseudomonas pisi, in tobacco leaf tissue. Phytopathology 66:754-757.

4. HUSAIN, A. and A. KELMAN. 1958. The role of pectic and cellulolytic enzymes in pathogenesis by Pseudomonas solanacearum. Phytopathology 48:377-386.

5. KELMAN, A. and E. B. COWLING. 1965. Cellulase of Pseudomonas solanacearum in relation to pathogenesis. Phytopathology 55:148-155.

6. LOZANO, J. C. and L. SEQUEIRA. 1970. Differentiation of races of Pseudomonas solanacearum by a leaf infiltration technique. Phytopathology 60:833-838.

7. LOZANO, J. C. and L. SEQUEIRA. 1970. Prevention of the hypersensitive reaction in tobacco leaves by heat-killed cells of Pseudomonas solanacearum. Phytopathology 60:875-879.

8. MARINKOVICH, V. A. 1964. Purification and characterization of the hemagglutinin present in potatoes. J. Immunol. 93:732-741.

9. SEQUEIRA, L. 1976. Induction and suppression of the hypersensitive reaction induced by phytopathogenic bacteria: specific and nonspecific components. p. 289-306. In R. K. S. Wood and A. Graniti, eds. Specificity in Plant Diseases, Plenum Press, N. Y. 354 p.

10. SEQUEIRA, L. and T. L. GRAHAM. 1976. Agglutination of avirulent strains of Pseudomonas solanacearum by potato lectin. (Abstr.) Proc. Am. Phytopath. Soc., 3: (in press).

11. SEQUEIRA, L. and L. M. HILL. 1974. Induced resistance in tobacco leaves: the growth of Pseudomonas solanacearum in protected tissues. Physiol. Plant Pathol. 4:447-455.

12. SEQUEIRA, L., S. AIST and V. AINSLIE. 1972. Prevention of the hypersensitive reaction in tobacco by proteinaceous constituents of Pseudomonas solanacearum. Phytopathology 62:536-542.

13. SEQUEIRA, L., G. GAARD and G. A. DE ZOETEN. 1976. Interaction of bacteria and host cell walls: Its relation to mechanisms of induced resistance. Physiol. Plant Pathol. (in press).

14. VAN DRISSCHE, E. and L. KANAREK. 1975. Purification and characterization of the potato (Solanum tuberosum) lectin. Arch. Int. Physiol. & Biochem. 83:414-415.

CURRENT STATUS OF THE POTATO BROWN ROT RESEARCH IN EGYPT

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ABSTRACT

The first record on the occurrence of the brown rot disease of potato in Egypt was in 1925. The bacterium (Pseudomonas solanacearum E. F. Smith) responsible for the disease has been isolated in Egypt only recently (1961). The disease is widespread in Egypt causing noticeable damage to the potato crop. Brown rot represents one of the most serious problems for potato exportation. The plant quarantines in certain importing countries, especially in U.K., are quite alert. Pseudomonas solanacearum does not occur on hosts other than potato in Egypt. Identification of the prevailing race(s) of P. solanacearum in Egypt was carried out in 1975. All the isolates collected from different localities of different Governorates in Egypt were pathogenic to potato plants, but were not pathogenic to tobacco and banana plants. All the isolates caused only a yellowish discoloration of the infiltrated area of tobacco leaves by 48 hr after inoculation and produced low melanin pigments on a tyrosine agar medium. These results indicate that the tested isolates belong to race 3.

A change toward non-pathogenicity took place also in cultures of P. solanacearum isolated in Egypt. Several investigators have attempted to illustrate certain physiological differences between virulent and avirulent strains of P. solanacearum including catalase, dehydrogenases and tyrosinase activities.

Progress in the development of control measures for brown rot in Egypt has been slow. Preliminary investigations indicate that drenching the soil with solutions of Dithane M-22, Dithane M-45, Chlorthion and Ceresan decreased the incidence of potato wilt disease that developed in soil artificially infested with P. solanacearum. However, further studies are needed before recommending these chemicals for field trials. Some reduction in losses can be obtained in Egypt by the use of disease-free seed. Therefore, it is forbidden, by law, to produce potato seed in infested areas. These areas are to be used for the production of potato for human consumption only. Also, the use of disease-free seed for cultivation and adequate crop rotation has been recommended. These methods reduced disease incidence greatly.

Although potato is a relatively new crop in Egyptian agriculture its acreage has been increasing rapidly. It is grown twice a year in summer and in Nili crops, with a total acreage of 65,629 feddan (one feddan is about 4200 m^2) in 1971. The summer crop is usually grown from certified seed imported from certain European countries, particularly Holland. The importing procedure is followed mainly to avoid losses due to tuber-borne virus diseases. The Nili crop is grown from seed produced locally during the summer. The total yield of both potato crops was estimated in 1971 to be 450,596 tons. About 80% of the total yield was consumed locally, while the remaining 20% was exported to different countries.

Brown rot disease is considered one of the serious diseases of potato in Egypt. Briton-Jones in 1925 (4) was the first to record this disease in Egypt. The bacterium (Pseudomonas solanacearum E. F. Smith) responsible for

the disease was isolated in Egypt only recently in 1961 (24, 27). Gehring (13) in Germany isolated P. solanacearum from imported Egyptian potato tubers showing brown discoloration of the vascular ring. Lelliott (15) in England mentioned that the number of brown-rotted samples collected from Egyptian consignments suggested that the disease was widespread in Egypt. Mickail et al. (19) found that the disease was widespread during the Nili season (planted in August) contrary to the summer season (planted in January). Generally, it could be concluded that the mean percentage of infection in the first season was three times higher to that of the second season. Two major factors may be involved in this situation. The first is environmental which is related to the predisposing conditions usually prevailing during the Nili season. The second is the disease-free seed tubers imported for cultivation during the summer season, which may play an important role in the reduction of disease incidence during that season. Mickail et al. (19) also found that a marked variation in the mean percentage of infection had occurred according to the locality. In the Nili season in 1972, the Kalyubia Governorate showed a higher rate of infection (13.5%) than the Behera Governorate (0.04%).

Brown rot represents one of the most serious problems for potato exportation in Egypt. The plant quarantines in certain importing countries, especially in the United Kingdom, are quite alert to this problem.

A shift toward non-pathogenicity took place in cultures of P. solanacearum isolated in Egypt (8). Several investigators, in Egypt, have attempted to illustrate certain physiological differences between virulent and avirulent strains of P. solanacearum. Abo-El-Dahab and El-Goorani (2) concluded that a virulent strain of P. solanacearum had higher catalase activity than did the avirulent mutant. On the other hand, no difference in catalase activity was determined between the virulent strains and the avirulent mutants of the other four isolates tested. The results obtained by the authors contradict the conclusions reached by Digat (7) who concluded that the avirulent mutants of P. solanacearum are lacking in functional catalase, and suggest that the bacterial catalase does not bear any significance as far as virulence of this bacterium is concerned. El-Helaly et al. (10) pointed out that an avirulent strain of P. solanacearum was more active than the virulent one in the production of the water soluble brown pigment(s) in cultures. Rushdi et al. (22) concluded that the avirulent strains were more active in their dehydrogenase enzyme than the virulent strains in presence of all hydrogen donors tested. Mickail (21) reported that avirulent cultures of P. solanacearum induced HR lesions within 24-48 hours of infiltration into tobacco leaves. The leaves injected with virulent bacteria did not induce HR lesions.

Abo-El-Dahab and El-Goorani (1) reported that growth of the virulent strain was inhibited by the growth of virulent or avirulent strains, but growth of avirulent strain was not inhibited. They pointed out that both the virulent and the avirulent cells can produce a growth inhibitory principle(s) when grown on a casein hydrolysate medium. The amount of such principle(s) produced by the virulent strain could inhibit growth of its own type, but was not enough to inhibit the avirulent strain. Recently, Cuppels et al. (6) in U.S.A. found that strains of P. solanacearum synthesize bacteriocin-like compounds that specifically inhibit other strains of the same species.

Identification of the prevailing race(s) of P. solanacearum, which does not occur on hosts other than potato in Egypt, was carried out by Abo-El-Dahab et al. (3). All the isolates tested, collected from different localities in

Egypt, were pathogenic to potato plants but were not pathogenic to tobacco and triploid banana plants. These results, according to Buddenhagen et al. (5), indicate that the isolates tested belong to Race 3. Also according to the leaf infiltration technique (16) all isolates were found to belong to Race 3. All produced low melanin pigments on tyrosine agar medium and presumably had low tyrosinase activity, a characteristic of Race 3 (12, 26). Biochemical studies indicated that all isolates belong to biochemical biotype II (14). Hayward (14) also found that isolates of P. solanacearum obtained from Egyptian potatoes imported to England, to Scotland in 1959, 1960, 1961 and 1962, and to Sweden in 1959, were of biochemical biotype II.

Progress in the development of control measures for brown rot in Egypt has been slow. Preliminary investigations indicate that drenching the soil with solutions of Dithane M-22 (9, 11), Dithane M-45 (9), Chlorthion (9) and Ceresan (23) decreased the incidence of potato wilt disease in soils artificially infested with P. solanacearum. However, further studies are needed before recommending these chemicals for field trials. Mehiar (18) found that the presence of Meloidogyne javanica along with P. solanacearum markedly increased the prevalence of wilt symptoms in potato plants. Treating the soil with the nematicide V.C. 13 significantly decreased the percentage of wilted plants. Certain investigators indicated that potato varieties varied greatly in their susceptibility to infection with the bacterial wilt organism (17, 25). Some reduction in losses can be obtained in Egypt by the use of disease-free seed. Therefore, it is forbidden, by law, to produce potato seed in infested areas. These areas are to be used only for the production of potato for human consumption. Also, the use of disease-free seed for cultivation and adequate crop rotation have been recommended. These methods reduce disease incidence greatly (20).

LITERATURE CITED

1. ABO-EL-DAHAB, M. K. and M. A. EL-GOORANI. 1969. Antagonism among strains of Pseudomonas solanacearum. Phytopathology 59:1005-1007.

2. ABO-EL-DAHAB, M. K. and M. A. EL-GOORANI. 1972. Catalase activity of virulent and avirulent strains of Pseudomonas solanacearum. Phytopathology 62:294-295.

3. ABO-EL-DAHAB, M. K., M. A. EL-GOORANI and E. E. WAGIH. 1976. Race identification of Pseudomonas solanacearum E. F. Smith in Egypt. Zbl. Bakt. Abt. II (Accepted for publication.)

4. BRITON-JONES, H. R. 1925. Mycological work in Egypt during the period 1920-1922, Egypt. Min. Agr. Tech. and Sci. Serv. Bul., 49, 129 p.

5. BUDDENHAGEN, I. W., L. SEQUEIRA, and A. KELMAN. 1962. Designation of races in Pseudomonas solanacearum. Phytopathology 52:726 (Abstract).

6. CUPPELS, D., R. HANSON and A. KELMAN. 1975. Production of bacteriocin-like compounds by Pseudomonas solanacearum. Proceedings of the American Phytopathological Society 2:56-57.

7. DIGAT, B. 1971. The significance of catalasic activity in Pseudomonas solanacearum. E. F. Sm. Third International Conference on Plant Pathogenic Bacteria. Wageningen, The Netherlands.

8. EL-GOORANI, M. A. 1967. Studies on potato tuber rots in U.A.R. Ph.D. Thesis. Faculty of Agriculture, Alexandria University, Alexandria, Egypt. 136 p.

9. EL-GOORANI, M. A., M. K. ABO-EL-DAHAB, and E. E. WAGIH. 1976. Tests in vitro and in pots with certain chemicals for inhibition of Pseudomonas solanacearum. Zbl. Bakt. Abt. II (Accepted for publication).

10. EL-HELALY, A. F., M. K. ABO-EL-DAHAB, and M. A. EL-GOORANI. 1969. Chromogenesis in cultures of Pseudomonas solanacearum. J. Phytopathology, U.A.R. 1:1-11.

11. EL-HELALY, A. F., M. K. AGO-EL-DAHAB and M. K. EL-KAZZAZ. 1969. Effect of some fungicides on certain phytopathogenic bacteria. J. Phytopathology, U.A.R. 1:81-92.

12. FRENCH, E. R. and L. SEQUEIRA. 1970. Strains of Pseudomonas solanacearum from Central and South America: A comparative study. Phytopathology 60:506-512.

13. GEHRING, F. 1962. On an occurrence of Psuedomonas solanacearum E. F. Smith in Egyptian imported potatoes and on a simple serological demonstration method for this bacterium in heavily infected tuber material, Nachr Bl. Dtsch. Pflsch Dienst, Stuttgart, 14:27-29. (Rev. Appl. Mycol, 41:671).

14. HAYWARD, A. C. 1964. Characteristics of Pseudomonas solanacearum. J. Appl. Bact. 27:265-277.

15. LELLIOTT, R. A. 1964. The occurrence of brown rot (Pseudomonas solanacearum) E. F. Smith in potatoes imported into England from the Mediterranean region. Phytopathologia Mediterranea 3:123-124.

16. LOZANO, J. C. and L. SEQUEIRA. 1970. Differentiation of races of Pseudomonas solanacearum by a leaf infiltration technique. Phytopathology 60:833-838.

17. MAHMOUD, S. A. Z., S. M. TAHA, A. M. ABDEL-HAFEZ, K. Y. MICKAIL and N. S. FARAG. 1974. Susceptibility of some commercial potato varieties to bacterial wilt disease in A.R.E. Agricultural Research Review 52:95-99.

18. MEHIAR, F. F. 1972. Studies on the association of certain nematodes with certain soil borne phytopathogenic bacteria. Ph.D. Thesis, Faculty of Agriculture, Alexandria University, Egypt. 95 p.

19. MICKAIL, K. Y., F. BISHAY, N. S. FARAG and A. E. TAWFIK. 1974. Evaluation of bacterial wilt disease of potato in A.R.E. during the years from 1967-68 to 1971-72. Agricultural Research Review 52:89-94.

20. MICKAIL, K. Y., F. BISHAY, N. S. FARAG and A. E. TAWFIK. 1975. Study on the distribution of brown rot disease of potato in Egypt during the year 1974/75. The Third Conference of Microbiology, Cairo 4-9 November, 1975.

21. MIKHAIL, M. S. 1974. Further studies in the bacterial wilt disease of solanaceous crops. M.Sc. Thesis, Faculty of Agriculture, University of Cairo, Egypt. 93 p.

22. RUSHDI, M. K., F. A. DARWEISH and A. AMIN. 1974. Dehydrogenase activit and polysaccharide production in virulent and avirulent strains of Pseudomonas solanacearum. The First Congress of the Egyptian Phytopathological Society, Cairo, April, 1974.

23. RUSHDI, M. K., F. A. DARWEISH and A. AMIN. 1974. Effect of certain fun cides, insecticides and antibiotics on the virulence of Pseudomonas solanacearum with possible application for the control of the brown ro disease. First Congress of the Egyptian Phytopathological Society, Cairo, April, 1974.

24. SABET, K. A. 1961. The occurrence of bacterial wilt of potatoes caused by Pseudomonas solanacearum (E. F. Sm.) E. F. Sm. in Egypt. Min. of Agric. Extension Department. Tech.

25. SABET, K. A. and F. M. BARAKAT. 1971. Studies in the bacterial wilt disease of potato. II. Relation of certain soil conditions and host variety to infection. Agricultural Research Review 49:155-169.

26. THURSTON, H. D. 1963. Bacterial wilt of potatoes in Colombia. Amer. Potato J. 40:381-390.

27. ZIETOUN, F. M. 1961. The effect of organic phosphorus compounds on certain phytopathogenic bacteria. M. Sc. Thesis, Alexandria University, Egypt.

BACTERIAL WILT OF POTATO AND TOMATO IN THE NORTHERN STATES OF NIGERIA

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Bacterial wilt of the Solanaceae, incited by Pseudomonas solanacearum (E.F. Smith) E.F. Smith was first reported in 1958 when wilted potatoes showing typical symptoms of the disease were collected at Riyom on the Jos Plateau and the organism isolated had the morphological and cultural characteristics of P. solanacearum (Keay, personal communication).

Climate of the Area: The Northern states of Nigeria lie between latitudes 6° 27' north and 14° north and longitudes 2° 44' - 14° 42' east of Greenwich (2). The greater part of this area forms an undulating plain lying at a general elevation varying from 457-704m. This plain is surrounded by a small plateau which forms the geographical centre of the Federation of Nigeria and which rises to an altitude of about 1,828m east of Jos.

The dominant features of rainfall in Nigeria are its seasonal character. From April to September, moist south-west trade winds predominate while the dry north-east trade winds predominate from October to March. The rain usually falls with considerable intensity and 40 to 60 mm per hour is common.

The mean minimum air temperatures during the wet season range from 19° to 22°C and the mean maximum temperatures from 27° to 35°C. Between November and February, i.e. during part of the dry season, the mean minimum air temperatures range between 12° and 17°C and the mean maximum temperatures between 30° and 34°C. At the end of the dry season (March-May), night temperatures above 21°C and day temperatures above 32°C are common just before the onset of the rains. The area under discussion is approximately 729,812 square kilometres, most of which is savannah.

Occurrence and Distribution of Bacterial Wilt: Since the first report in 1958, P. solanacearum has been reported on several crops, principally on tomato, potato and sweet peppers. There has been an unconfirmed record on groundnut (peanut). It is only on potato and tomato that bacterial wilt has become a serious problem, however. To date, distribution of the disease is restricted to the following areas: low-lying areas (fadama) along the banks of rivers and streams in Kaduna State, the Jos plateau and some areas on the Mambilla plateau, Gongola State. The disease has been reported only on land on which potatoes had previously been grown. Hence, spread by infected tubers is considered to be the means by which the disease was initially introduced to this region and also the method of dissemination from field to field. Most fields of potatoes do have bacterial wilt, usually 1-5% incidence; occasionally the incidence is as high as 50-75%. On tomatoes, disease incidence is more erratic and depends to a large extent on environmental factors, particularly soil moisture. In soil heavily contaminated with the pathogen and under conditions favorable for disease development, up to 50% or more of the stands have been found to have wilted. Bacterial wilt, therefore, now constitutes a very major threat to both tomato and potato production.

Host Range: Investigations (1) have shown that isolates of P. solanacearum in the northern States of Nigeria are host-restricted. In several artificial

inoculation tests in the greenhouse, only the following were readily infected: tomato, potato and eggplant. Groundnut (peanut) wilted in one instance but not in subsequent trials. Attempts to induce wilt in tobacco, 'hot' peppers and the local eggplant, Solanum incanum, failed, showing the rather restricted host range of local isolates. Some non-solanaceous hosts have also been artificially inoculated with P. solanacearum and found to be immune. These were Coleus dysentericus Baker and a Plectranthus sp. (both of which are local tuber-bearing plants), bean (cowpea), Vigna unguiculata Wallp. and cotton (Gossypium hirsutum Linn.).

The following weeds have been found to harbor P. solanacearum under field conditions: Ageratum conyzoides Linn., a Commelina sp., Vernonia pauciflora (Wild) Less, a Vigna sp. and Solanum nodiflorum. This last host actually wilted in the field due to infection by P. solanacearum.

Research on Control Measures: In the past few years, several cultivars and selections of potato and tomato have been introduced into Nigeria and screened for resistance to bacterial wilt. Screening for resistant potatoes has been carried out on the Jos plateau through the cooperative effort of international programs and local institutions. Several progenies from S. tuberosum x S. phureja crosses have shown promising degrees of tolerance (Suchomel, personal communication). However none has been released to farmers because of some variability in the degree of tolerance of these selections under varying environmental factors and infection pressures. There is also the difficulty of introducing improved potato cultivars to mostly peasant farmers who traditionally grow a mixed lot of potatoes and save their own seed potatoes. Similarly on tomatoes, several cultivars and selections have been and are being screened for bacterial wilt resistance, but to date no bacterial wilt resistant cultivars have become part of commercial practice.

Investigations are currently being conducted to study the survival of P. solanacearum in upland soils which naturally dry out during the dry season. Although survival from season to season has been confirmed in low-lying areas which do not dry out appreciably during the dry season, this has not been verified for upland soils. This knowledge is essential as a pre-requisite for planning soil management strategies for curtailing the incidence of bacterial wilt in upland soils where the disease has not been important previously. The disease could increase in importance in the near future since production of potatoes is being pushed in some areas.

In the absence at the moment of effective methods for controlling bacterial wilt, emphasis is placed on avoidance. The cultivation of potato and tomato on the same sites is discouraged because of the very important role played by diseased potato tubers in the dissemination and contamination of new sites by the pathogen.

Literature Cited

ERINLE, I. D. 1976. A preliminary report on bacterial wilt in Nigeria. Institute for Agricultural Research, Zaria, Nigeria. Misc. Bul. (in press).

PSEUDOMONAS SOLANACEARUM-INDUCED WILT DISEASES IN NIGERIA

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OCCURRENCE

The disease was first reported in Nigeria on tobacco (Harrison Special) in 1963 from Ago Are in Oyo State (Agboola, unpublished data). Later in the same year there was a more serious occurrence of the disease in the tobacco-growing area around Ogbomosho, also in Oyo State. The disease has now become quite common all over the country. The strain present is considered to be restricted to solanaceous hosts. The wilt disease has also been recorded on tomato, eggplant and pepper. The potato is now being grown extensively in the Jos area where the climate is cooler than in other parts of the country. This crop is now one of the greatest reservoirs of Pseudomonas solanacearum in Nigeria today.

Inoculation procedures: Two methods have been used by the author to introduce P. solanacearum into the conducting tissues of healthy solanaceous plants: root and stem injury. In the first method flamed cork borers of suitable diameters were used to drill holes in the soil as near the stem as possible, thereby injuring some roots. A suspension of the organism was then poured into the hole which was later covered with soil. In the controls, similar holes were bored, but sterile water was added. In the second method some inoculum direct from an agar slant was taken on a flamed needle with a slightly broad tip. This was placed on the stem and pricked into it with the needle, the process being repeated twice per plant. Plants inoculated by the root injury method wilted from 10 to 12 days, while those inoculated by the "pin-prick" method wilted between eight to 10 days. The method of placing a drop of a suspension of the organism on the stem and injecting it into the plant by pin pricks did not cause wilting. Tomato, several Solanum spp and several varieties of peppers were successfully infected with the tobacco isolate of P. solanacearum.

Maintaining cultures of Pseudomonas solanacearum: Difficulties that have arisen in the study of Pseudomonas solanacearum concerned its rapid loss in pathogenicity when maintained in culture. Kelman (Phytopathology 44: 693-695, 1954) attributed loss in virulence of cultures to mutation and selection which result in populations dominated by weakly virulent types, differing in colony morphology from the wild form. Using Kelman's medium, it was possible to pick out pathogenic and non-pathogenic strains on the basis of colony morphology in cultures obtained in Nigeria from tobacco. The two types were transferred to separate agar slopes and eventually inoculated into healthy tobacco plants. The isolates forming red colonies did not induce visible disease symptoms in tobacco plants, whereas the cultures forming whitish colonies induced wilt in about two weeks.

CONTROL

The control of bacterial wilt has been approached in several ways. It is a soil-borne disease and eradication of soil-borne inoculum is an obvious possibility for annual crops grown from seed. Dry season bare fallow has

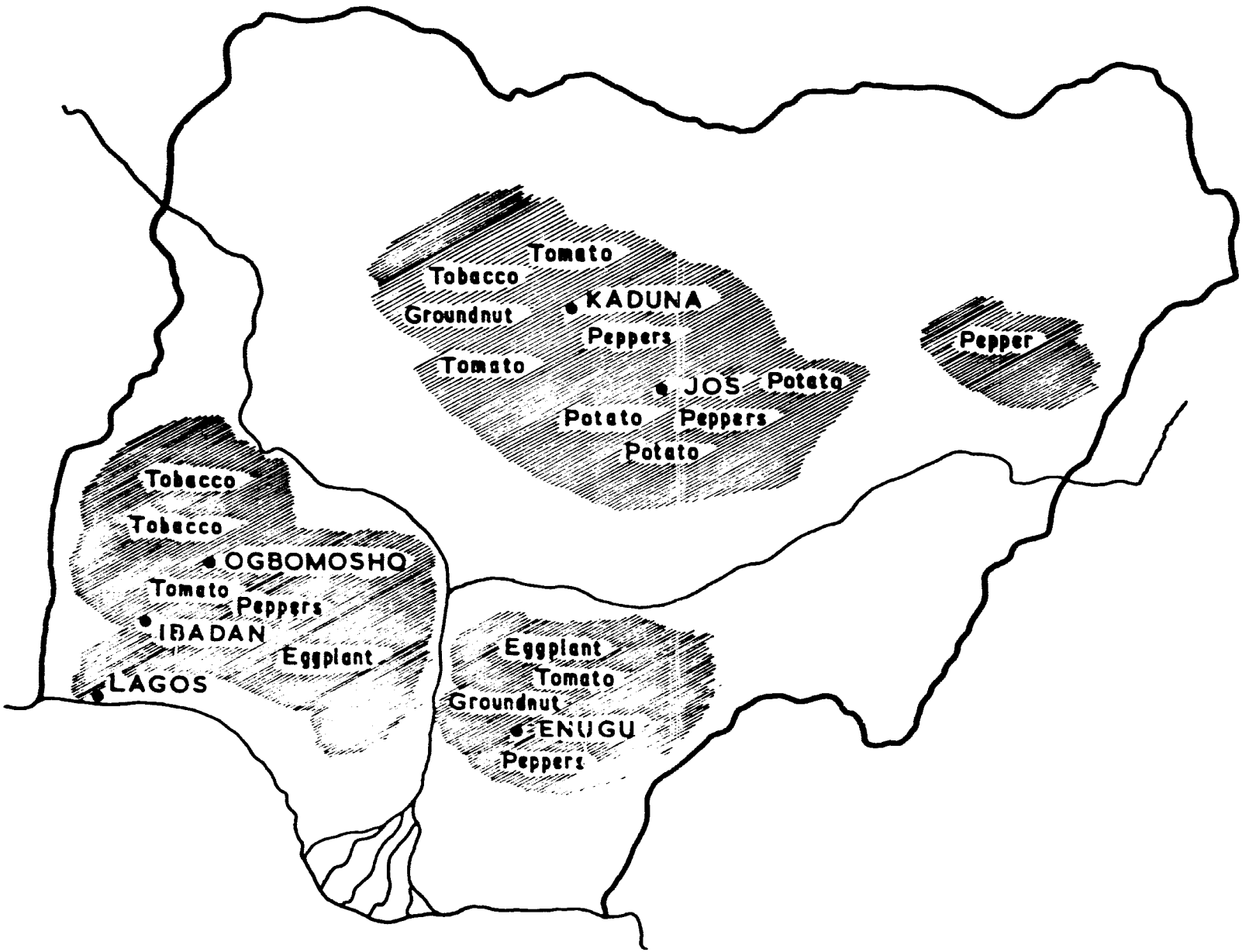
results in less disease in the succeeding crop in Nigeria. It is, however, only possible, in the absence of resistant varieties, to recommend rotation treatments after comprehensive trials in the areas in which they are to be used. In potato a cheap and simple control can be effected by selecting propagating materials from wilt-free plants (Robinson, 1969, personal communication). Where propagating materials have to be cut, a valuable adjunct to this is the frequent disinfection of cutting implements.

During screening trials by the author, tobacco varieties Oxford 26; Dixie Bright 27, 101 and 102; Golden Wilt; T.I. 488A; Kutsagar and N.C. 95 showed resistant reactions. These varieties were not immune to the disease, although they stood up well under ordinary conditions. However, if tobacco is grown continuously on the same land, infection may build up to the point that these varieties are damaged. The remedy is to grow the resistant varieties in short rotations with other crops. Eradication of weeds, good drainage, liberal use of nitrogen fertilizers, keeping tomato, pepper or eggplant seedlings from infested areas have also been known to help. In fields where nematodes were present, the use of nematicides provided by the Nigeria Tobacco Company reduced bacterial wilt appreciably.

ACKNOWLEDGEMENTS

I wish to thank the Director, National Cereals Research Institute, Ibadan, Nigeria, for providing the facilities for this study and allowing its publication. I also thank the Federal Military Government of Nigeria for providing the funds for me to attend this meeting.

MAP OF NIGERIA SHOWING DISTRIBUTION OF WILTS CAUSED BY PSEUDOMONAS SOLANACEARUM



STATUS OF POTATO BROWN ROT IN URUGUAY

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Estacion Experimental Las Brujas
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Uruguay is located on the Atlantic Ocean, between Brazil and Argentina, 30-35° latitude South and 50-58 long West. It has a moderate climate with an average temperature of 7.2°C (min.) during winter and 29.4°C (max.) during summer. The average annual rainfall is 1.100-1.200 mm. with a major amount of rainfall during winter.

The more important potato production areas are located in the east, along the coast on the Atlantic Ocean; south, on the "Rio de la Plata;" NW, along the Uruguay River; and a central area.

The general type of soils in these areas are:

East:	loamy sandy - sandy loamy	pH 4.8 - 5.5
South:	silty loam - silty clay loam	pH 5.5 - 6.2
NW:	sandy	pH 5.0 - 5.5
Central:	sandy	pH 4.5 - 5.5

Practically the only varieties grown in the country are Kennebec and Pontiac.

We have two annual planting seasons -- summer and spring. The first one utilizes imported Canadian seed (certified); and the second one utilizes potatoes obtained from the first crop.

The grower not only plants seed of the categories mentioned above, but he may also use seed of 2nd, 3rd or 4th multiplication cycles; in many cases he has certified and first multiplication seed in the same field so that disease problems, especially virus diseases, are more severe.

In general, the spring harvest is for human consumption and the fall harvest is for seed use for the spring planting season.

Uruguay imports about 2 million dollars (10 tons) of potato seed each year, thus this Experiment Station decided to establish a program to produce national seed of improved quality for the spring planting and to diminish imports in the future. The east area was selected first on the basis of its favorable ecological conditions and its relative isolation from virus sources. Leaf roll in Kennebec and Rugose Mosaic in Red Pontiac were the more important problems at that time.

Since 1973, personnel from the Experiment Station have been working in the area. Growers informed us that for several years they had seen a disease in potatoes that caused a sudden wilt of the plant and a special rot of the tubers. In 1974 and for the first time for Uruguay the personnel working in Plant Pathology together with Dr. Richard Stuckey (M.S.U.) and Dr. W. T. Hooker (M.S.U.) identified the disease as that caused by *Pseudomonas solanacearum*.

Present Status

The disease is spreading rapidly in the East and Central areas. We think that it may also be present in other areas of the country because

there is no control of the movement of seed amongst areas.

During the 1975 spring season the disease spread quickly because of weather conditions. Humidity and temperature were favorable factors for the development of this disease. The crops that were in their maximum growth at that time were the more severely attacked.

We need to know:

a. the present distribution of the disease.
b. history of the infested fields.
c. the behavior of the varieties Kennebec and Red Pontiac with respect to *P. solanacearum*.

We started a survey in the potato growing areas beginning with the most affected. The preliminary results for part of the east area are: of 29 growers 58.6% had *Pseudomonas*, causing 100% loss in some cases, i.e. some growers had a yield of 1,500 Kgs/Ha as compared with an average yield for the area of 20,000 Kgs/Ha.

The increase of *Pseudomonas* infection was due not only to the favorable weather conditions but also to an increase in the number of new growers who planted potato seed from the first generation obtained in an area which was infested. The imported seed arrived free of disease but this was infected soon because the potatoes were planted in infected fields and/or were in contact with infested tools, warehouses, etc.

Reports by the technical personnel working at the Experiment Station in the Central area are alarming. They found the disease in most of the potato areas and in some fields they found up to 80% of the plants infected. Thus, they consider brown rot as the principal problem of the area at this time. From the information obtained through the survey we also want to determine the possible origin of the disease in our country. There is a possibility that the disease came through infected tubers from Southern Brazil where the disease is endemic. Many growers had planted Brazilian seed for several seasons.

Work performed at the Experiment Station Las Brujas

At the Experiment Station we are conducting research to study the characteristics of the pathogen in our country and the behavior of the different inoculum sources from Kennebec and Red Pontiac. We are trying to determine if there is some kind of tolerance or resistance in the Red Pontiac variety because field observations have indicated that it shows no symptoms in infested fields where Kennebec volunteers were dying at the same time. The Red Pontiac tubers didn't show symptoms. Up to this time we have not had the opportunity to see foliage symptoms of wilt in Red Pontiac in the field. However, while examining tuber samples of Red Pontiac from different growers, we found a few tubers with symptoms of brown rot. Some had external symptoms (with exudate through the eyes) but many of them, after cutting, showed symptoms that resemble those produced by *Fusarium oxysporum*. Part of the vascular ring had a brown discoloration and was confined to the invaded vessels. In contrast with the vascular discoloration of Kennebec tubers that were predominantly gray, this discoloration often extends into the surrounding parenchymatous tissue which became soft and watery. It coincides with the description of Nielsen & Haynes (Am. Pot. J. 50:260-267, 1960).

Up to this time studies in the laboratory with several isolates of the bacteria from Red Pontiac and Kennebec tubers have shown no differences in

gram reaction, colony appearance in a tetrazolium medium (TTC), nutrient agar and PDA, milk test, and pigmentation on potato slants. They both showed a conspicuous brown pigment when grown in TTC medium with 2.5 gr of dextrose; almost no pigment with 10 gr dextrose.

In the greenhouse we inoculated the varieties Kennebec and Red Pontiac with inoculum from tubers of the same varieties and the results are summarized in Figs. 1, 2 and 3.

The disease index used was the same as described previously by Winstead and Kelman (Phytopath. 42:628-634).

Note that in the 3 tests isolates from Red Pontiac had a more severe effect on the inoculated plants, especially in the Red Pontiac variety.

We will continue to investigate the characteristics of both isolates and the causes of these differences.

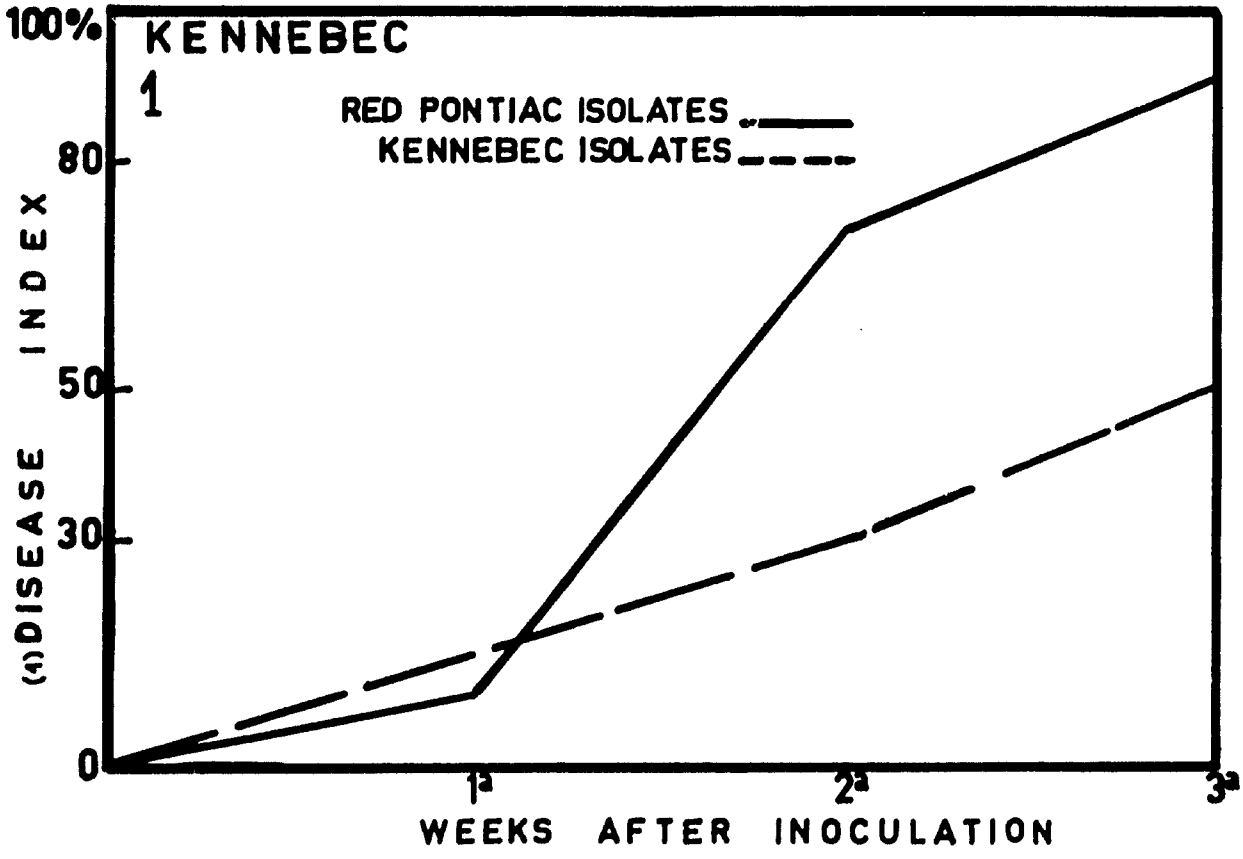

Figure 1. Disease severity in Kennebec potatoes inoculated with isolates of P. solanacearum from infected Red Pontiac and Kennebec potatoes.

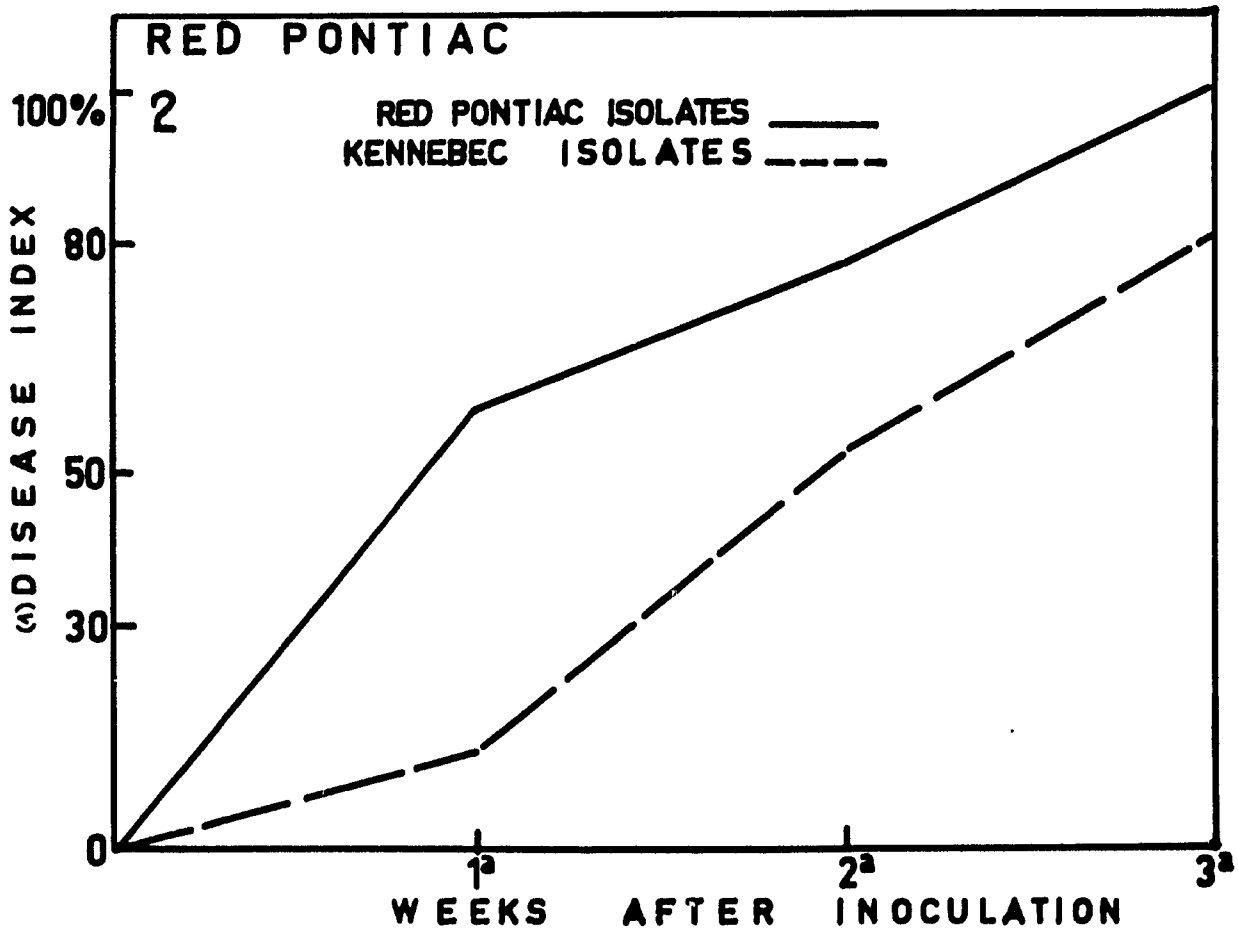

Figure 2. Disease severity in Red Pontiac potatoes inoculated with isolates of P. solanacearum from infected Red Pontiac and Kennebec potatoes.

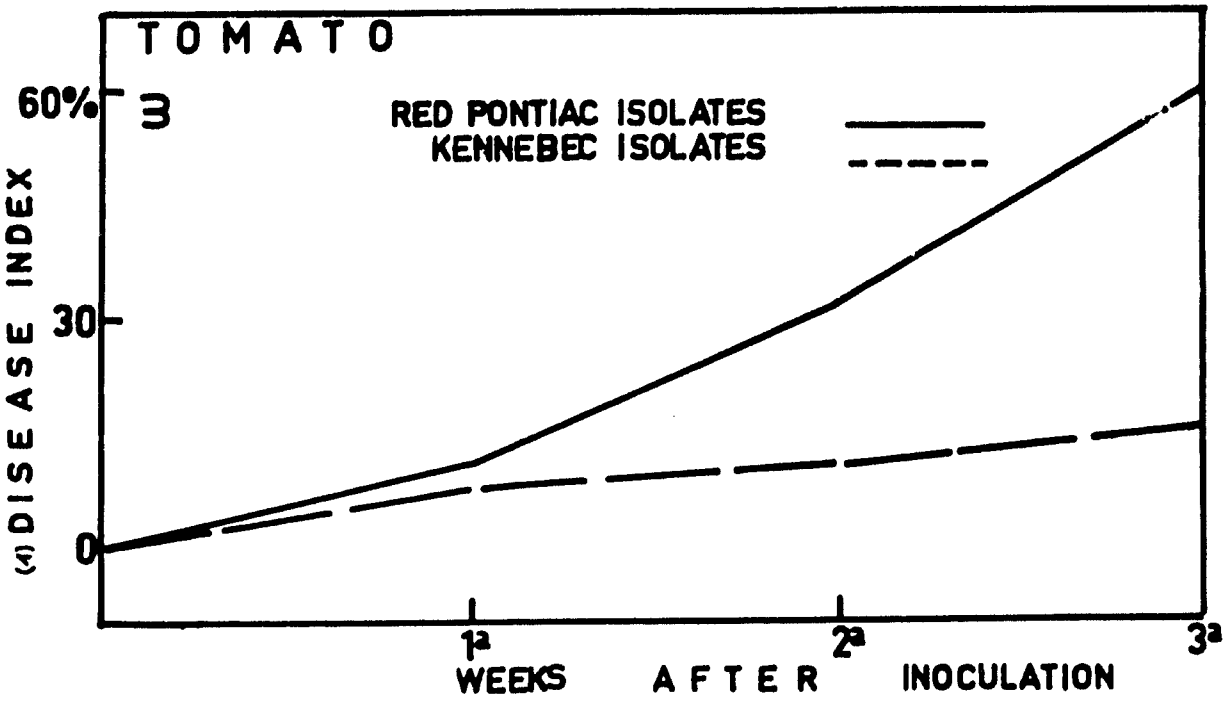

Figure 3. Disease severity in tomato plants inoculated with isolates of P. solanacearum from infected Red Pontiac and Kennebec potatoes.

BACTERIAL WILT IN KENYA WITH PARTICULAR REFERENCE TO POTATOES

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Strains of Pseudomonas solanacearum in Kenya

In Kenya *Pseudomonas solanacearum* causes important diseases of potato, tomato, eggplant and pepper. Robinson and Ramos (1964) first reported the existence of two strains which can be distinguished in host and geographic range as well as in a number of laboratory tests.

One strain is very uniform and corresponds with Hayward's (1964) biotype 2 or the race 3 (the potato race) of Buddenhagen *et al.* (1962). Isolates of the second strain are more variable and conform to biotypes 3 or 4; they would probably be included in race 1 (the tomato race). The former strain occurs between ca. 4,000 feet (1,200 m) and 8,000 feet (2,400 m) and is a field pathogen principally of potato and tomato; the latter occurs from the coastal strip up to ca. 6,000 feet (1,800 m) and causes field diseases of tomato, eggplant and pepper. In experimental plots at the National Agricultural Laboratories in Nairobi these pathogens also attack the weed species *Cleome monophylla*, *Portulaca oleracea*, *Solanum nigrum* (both strains), and *Datura stramonium* (the latter strain only). However, only *S. nigrum* is known to be affected by *P. solanacearum* outside the N.A.L.

In glasshouse inoculation experiments there are no absolute host differentials but quantitative differences of host susceptibility largely reflect the distribution of the two strains in the field (see Table 1).

Neither strain was pathogenic to tobacco or groundnut, although a few biotype 3/4 isolates did grow in the vascular tissues of tobacco when they were stab inoculated.

The two strains differ markedly in biochemical characters and in their patterns of susceptibility to a range of bacteriophages and bacteriocins from exotic isolates of this species. Biotype 3/4 isolates are usually lysogenic, biotype 2 isolates are not. The strains also differ in their effect on infiltrated tobacco leaves. The temperature/growth relations of the strains were similar except that type 3/4 isolates grew slightly faster at intermediate temperatures. The optimum temperature for growth was 30°C and the minimum was between 12 and 15°C.

As will be seen, the strain affecting potatoes and tomatoes is comparable with a strain described by Thurston (1963) in that it can cause disease at lower temperatures than other strains of this nomenspecies. There is good evidence that this strain (or a group of similar, very closely related strains) is widely distributed through the cooler limits of distribution of *P. solanacearum* in both latitude and altitude. The disease which this strain causes in Kenyan potato crops is the subject of the rest of this paper.

Bacterial wilt of potatoes in Kenya

Distribution

The potato is a major starchy food crop in Kenya and its importance is steadily increasing. One of the more important problems of potato production

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is bacterial wilt. This disease was introduced in the early 1940's and, in 20 years, had reached alarming proportions (Robinson and Ramos, loc. cit.).

Some 50 to 60,000 hectares of potatoes are grown each year through a wide agroclimatic belt extending from ca. 4,000 feet (1,200 m) up to 9,200 feet (2,800 m) over a large area of the central and western Highlands. Bacterial wilt is widely distributed through the potato areas but there is a conspicuous effect of altitude on the incidence and severity of outbreaks. The disease is most serious at intermediate elevations, that is, between 5,000 (1,500 m) and 7,000 feet (2,100 m), although even here disease incidence within crops does not often exceed 5%. In the marginal potato areas below 5,000 feet the disease is somewhat less serious but, more significantly, above 7,000 feet wilt becomes decreasingly important with altitude until at 8,000 feet (2,400 m) and above the disease is rarely, if ever, seen. Table 2 shows the relation between altitude and wilt outbreaks.

Epidemiology

In bacterial wilt of potatoes there are two sources of primary inoculum: the bacteria introduced within affected seed; and bacteria surviving in the soil from previous outbreaks. Field observations suggested that the worst wilt outbreaks were invariably associated with contaminated seed. In order to assess the relative importance of soil-borne inoculum and to investigate its eradication by crop rotation, field experiments were set up in four localities at 4,900 (1,500 m), 5,600 (1,700 m), 5,800 (1,800 m) and 8,200 feet (2,500 m). The results of these experiments were assessed by planting potatoes and recording the incidence of wilt in the growing crop (Table 3).

The results show that, in general, site factors and the length of the rotation are more important in determining the wilt potential of contaminated land than is the nature of the rotation. Even where successive potato crops were grown, only at one site were high levels of disease maintained, suggesting the existence of limiting site factors (see Table 4). Short (one year) rotations were not sufficiently effective in reducing wilt potential whilst longer (two year) rotations were generally effective at three sites and effective with some crops at the most wilt-conducive site.

Of many weed plants, including known suscepts of P. solanacearum, from all sites screened during the course of these experiments only one specimen of Solanum nigrum was infected. It would seem that weed hosts are of little significance in the carry over of soil-borne inoculum.

Ecology

Apparently conditions ideal for bacterial wilt development exist only in limited parts of the range of potato cultivation in Kenya. This probably explains why the disease has not become as generally destructive as was feared at one time.

Laboratory studies show that the growth rate of the pathogen is highest at 30°C and declines to zero at between 15 and 12°C. When the data from Table 2 is presented in relation to soil temperature the limiting effect of low soil temperature on the disease is apparent (see Table 5). The ceiling for wilt corresponds with temperatures of around 15°C and the optimum is about 21°C. Why wilt declines in soils of higher soil temperature is not certain, but it is possible that other factors such as reduced rainfall become limiting.

Thus, any area may be considered to have a certain wilt risk or susceptibility which is determined by the combined effects of environmental factors, some known and others unknown.

Control

By applying the 15°C parameter it is possible to demarcate areas in Kenya suitable for potato growing which are too cold for wilt. By producing seed in these areas which, incidentally, are by most criteria superior for seed production, the seed-borne source of the disease can be eliminated.

Experiment suggests that even in the most wilt-prone areas heavily infested land can be restored to potato cultivation after a two-year grass rotation. A longer period may be required for other rotations. Any one of several non-host rotations are, more or less, equally suitable for reducing soil inoculum in sites less favorable to bacterial wilt.

By combining a seed programme with appropriate crop rotations bacterial wilt should be easily controlled by Kenyan potato growers.

References

Buddenhagen, I. W., Sequeira, L. and Kelman, A. 1962. Designation of races in *Pseudomonas solanacearum*. Phytopathology 52, 726 (Abstr.).

Hayward, A. C. 1964. Characteristics of *Pseudomonas solanacearum*. J. Appl. Bact. 27, 265-277.

Robinson, R. A. and Ramos, A. H. 1964. Bacterial wilt of potatoes in Kenya. E. Afr. Agric. For. J. 30, 59-64.

Thurston, H. D. 1963. Bacterial wilt of potatoes in Colombia. Am. Pot. J. 40, 381-390.

Table 1. The susceptibility of various hosts in inoculation tests with two Kenyan strains of *P. solanacearum*

Host	Biotype	
	2	3/4
Tomato	+++	+++
Potato	+++	(+)
Eggplant	++	+++
Pepper	(+)	+
Solanum nigrum	+++	+++
Cleome monophylla	(+)/++*	++
Portulaca oleracea	+	+
Datura stramonium	-/(+)*	+

*Different results in different seasons.

Table 2. Incidence and severity of bacterial wilt in relation to altitude in Kenya

Altitude zone (1000 feet)	No. of plots examined	Total acreage examined	% acreage in severity category*			
			0	1	2	3
8.5+	7	7.5	100	0	0	0
8.0-8.5	21	22.8	100	0	0	0
7.5-8.0	16	26.8	78	22	0	0
7.0-7.5	23	20.8	55	24	16	5
6.5-7.0	20	14.9	28	51	13	8
6.0-6.5	29	27.3	45	46	9	0
5.5-6.0	22	12.0	58	36	4	2
5.0-5.5	20	9.8	15	47	27	11
5.0-	10	7.0	76	13	11	0

* Severity categories: 0 = no wilt seen, 1 = <1% plants wilting, 2 = 1 - 5% plants wilting, 3 = >5% plants wilting

Table 3. The incidence of wilt on contaminated land after various crop rotations

Site and rotation	Mean % plants affected by wilt (4 replicates) of harvest after rotation with different crops							
	Potatoes	Maize	Peas	Bare fallow	Grass	Cabbage	Leeks	Pyrethrum
Njabini (8,200 ft)								
Short	13.3		11.5					
Long		0.8	0.4	0.4	0	2.2	0	0

	Potatoes	Maize	Beans	Bare fallow	Grass	Carrots	Sweet potatoes
N.A.L. (5,800 ft)							
Short	23.1	5.2	11.0				
Long		0.7	2.8	0	1.0	1.1	0.7
Kigari (5,600 ft)							
Short	93.1	69.9	48.9				
Long		7.3	7.7	11.8	0.4	2.8	2.2
Embu (4,900 ft)							
Short	8.9	6.3	2.4				
Long		0	0	0	0	0.4	0

Table 4. Wilt trends in successive potato crops at four sites

Season after contamination	Mean % wilting (4 replicates) Sites			
	Embu	Kigari	N.A.L.	Njabini
First	2	67	71	69
Second	6	97	34	36
Third	9	93	23	13

Table 5. The incidence and severity of bacterial wilt in relation to soil temperature

Soil temperature range °C	No. of plots examined	Total acreage examined	% acreage in severity category			
			0	1	2	3
12.5 - 13.5	4	3.5	100	0	0	0
14.0 - 15.0	12	17.4	100	0	0	0
15.5 - 16.5	24	17.9	90	10	0	0
17.0 - 18.0	36	47.2	63	32	3	2
18.5 - 19.5	43	29.0	40	42	15	3
20.0 - 21.0	27	18.4	20	49	26	5
21.5 - 22.5	18	13.3	65	23	8	4
23.0 - 24.0	4	2.3	100	0	0	0

BACTERIAL WILT OF THE POTATO IN INDIA

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The bacterial wilt incited by Pseudomonas solanacearum is endemic in about 60 percent of the total potato-growing area in India and causes 10-50 percent losses in yield. In the plateau area and West Bengal, race 1 of the pathogen is widespread and, hence, the disease affects potato, tomato, brinjal and chillies. In the hills, only the race 3 has been recorded so far. The race 2 and the pathotypes capable of affecting tobacco and ginger and the low temperature strain on potato have not been recorded so far. In the northern and eastern hills, the pathogen appears to perpetuate in soil. The perpetuation of the pathogen in plateau areas, southern hills, and eastern hills will be discussed in the light of observations made. The possibility of disease control through host resistance and prophylaxis will be discussed.

BACTERIAL WILT OF POTATO IN COSTA RICA

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In Costa Rica, most potatoes are grown on volcanic slopes 1,400-3,400 m above sea level. Average minimum-maximum temperatures are 13-26°C and 6-17°C for the lower and upper regions, respectively. Potatoes are generally rotated with pastures. Bacterial wilt caused by Pseudomonas solanacearum occurs up to about 2,000 m high (10-23°C). There are occasional symptomless infections in plants grown above this level. Severe outbreaks of bacterial wilt are infrequent even at lower regions; some fields may show high incidence of wilt one year and none the next. When the seed source of infected fields has been traced, it almost always turned out to be a low-elevation planting.

It is apparent that constant rotation and increased use of seed produced at the higher, cooler regions has resulted in an overall decrease in bacterial wilt incidence. Thus, healthy seed rather than resistant varieties may be enough to control bacterial wilt in traditional potato regions of Costa Rica. However, this may not be true for elevations lower than 1,400 m where potato cultivation is possible. Therefore, testing for resistance has been continued.

Efficiency of test plots for wilt resistance within commercial fields has been erratic. Farmers seldom plant two consecutive years in infested land, and those that do usually carry on other soil-borne diseases. Also, wilt inoculum decreasing during the dry season (which varies from 1-4 months) and what remains is very irregularly distributed.

A small (65 m^2) test plot is now maintained on the campus of Universidad de Costa Rica. Inoculum level and distribution are controlled by alternating tests of experimental material with plantings of a susceptible variety; irrigation is provided during the dry season. A nonsusceptible crop usually has to be planted toward the end of the rainy season, when inoculum levels become too high to detect present levels of resistance.

Tests are under way to determine the survival of P. solanacearum, races 1 and 3, after potato plantings about 800 m high (temperature 18-28°C) in regions with long and short dry seasons.

CURRENT STATUS OF BACTERIAL WILT RESEARCH IN COLOMBIA

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The three races of Pseudomonas solanacearum have been detected in Colombia.

Race 1 has been reported causing bacterial wilt in several tobacco-growing areas of the country, mainly the Departments of Santander and Bolivar. Research work to incorporate resistance in new commercial varieties of tobacco is in progress using resistant materials such as T.I. 448 A and the cultivar "Costillo Negro".

Race 2, causing the "moko" disease of bananas and plantains, is the most important and widespread bacterial problem at this moment in Colombia. Cultural and chemical control measures to eradicate infestations have been undertaken in some areas of the country during the last four years with partial success. The variety of plantain "Pelipita" (Musa ABB), identified as highly resistant to moko in Central America, has been introduced recently. At present, it is under observation in several regional trials throughout the country to evaluate its resistance under natural conditions of infection and to determine the degree of adaptation to several ecological habitats where native, susceptible plantain is grown. Survival studies under the Espinal ecological conditions (Department of Tolima) have shown that the bacterium does not remain viable more than 4 months in fallow soil.

Race 3 is present in the commercial potato-growing areas, located east of the Department of Antioquia. Bacterial wilt has been observed in this region for many years. The disease is encountered at elevations at 2200 m or below. After the discovery by Thurston and Lozano (1968) that certain Colombian clones of Solanum phureja had high levels of resistance, the Potato and Plant Pathology Programs at ICA have continued testing new clones obtained at Tibaitata and Wisconsin, respectively. The resistance of 13 clones from Wisconsin is being assessed under the ICA-CIP project at two locations: Popayan (Department of Cauca) at 1800 m and Rio Negro at 2200 m. Field experiments to study the survival of the bacterium in this area showed less than 1 percent of bacterial wilt incidence after 6 months of fallow.

BACTERIAL WILT OF TOMATO AND EGGPLANT IN INDIA

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Among the bacterial diseases of vegetable crops in India, bacterial wilt caused by Pseudomonas solanacearum E.F. Smith is one of the most important, causing serious losses in tomato and eggplant (brinjal) (Kelman, 1954; Das and Chattopadhyay, 1955; Rao et al., 1975). It is serious in Karnataka, Kerala, Orissa, Maharashtra, Madhya Pradesh, Bihar and West Bengal states of India. Periodic surveys in farmers' fields in Karnataka state revealed widespread occurrence of bacterial wilt, the incidence ranging from 10-50% in tomato and 15-60% in eggplant. In some areas, the disease is very serious; sometimes it destroys the crop totally. The disease is more severe and appears early on young plants during summer (March - June) than in rainy (July - October) and winter (November - February) seasons.

Assessment of yield losses in eggplant due to bacterial wilt was made by comparing the yield data of a resistant (Pusa Purple Cluster) and susceptible (Pusa Purple Long) variety grown on infested plots, and of a susceptible variety grown on infested and wilt-free plots following paired treatment experimental design. Yield losses between 75 to 81% were recorded.

Periodic observations were made for natural occurrence of the disease on host plants other than those of solanaceous origin. The disease was observed on three weeds: Phyllanthus niruri, Cleome monophylla and Acanthosperma hisipidum. The pathogen from these weeds was isolated and cross-inoculation tests showed that the isolates from these hosts were pathogenic to tomato and eggplant and caused wilting within 7-10 days, whereas it took about 20 days for complete wilting on their respective hosts. Natural infection was more prevalent on P. niruri and C. monophylla; the former was also found to be a symptomless carrier.

Comparison of various isolates of P. solanacearum from tomato, eggplant and potato from Karnataka, Kerala, Tamil Nadu, Orissa, Maharashtra, Punjab and Himachal Pradesh states was made. Based on the reaction of tobacco leaves inoculated by infiltration technique of Lozano and Sequeira (1970) and on the differential hosts tomato, eggplant and potato inoculted by stem injury technique of Winstead and Kelman (1952) the isolates from Karnataka, Kerala, Orissa and Maharashtra were identified as race 1 affecting tomato, eggplant and potato. The isolates of potato from Punjab, Himachal Pradesh and Tamil Nadu states were identified as race 3, i.e. potato race (Buddenhagen et al., 1962; Buddenhagen and Kelman, 1964).

Various antibiotics (aureomycin, streptocycline, achromycin) and chemicals (vapam, formalin, bleaching powder) were evaluated for their efficacy against bacterial wilt. In case of vapam and formalin the infested soil was treated with the chemicals as per the usual procedure followed for soil disinfectants. Other chemicals were used as soil drenches. In case of streptocycline, the seedlings of the check variety were also dipped in the solution for about 10 minutes before planting. The final disease incidence (%) in each treatment was as follows: formalin, 12; bleaching powder, 62; streptocycline, 70; vapam, 66; and control, 83. Sterilization of seed beds with 4% formalin helps in raising disease-free seedlings, but soil disinfection on a large scale is not practical.

Preliminary evaluation of a large number of local varieties of tomato, including the cultivars such as Pusa Ruby, S 12, HS 101, 102 has not provided any source of resistance. Therefore, a number of varieties and lines which were found resistant in the U.S.A. (Henderson and Jenkins, 1972) and the Philippines were introduced and tested for their reaction under our conditions. In the greenhouse, plants were inoculated when they had 8-10 leaves, following the root injury technique of Winstead and Kelman (1952). The inoculum consisted of 48 hour growth of bacterial cells adjusted to O.D. 0.5. Field evaluation of the lines was made on a heavily infested plot maintained at our research station, where the wilt incidence was about 96% in a susceptible variety grown during the previous season. One row of the susceptible variety Pusa Ruby was planted between every two rows of the test lines. Out of the 23 varieties/lines and 4 F_1 hybrids tested, only one line CRA.66 selection A had a very low disease index of 5.0 and 8.0 in both greenhouse and field tests, and was rated as resistant. All other lines had higher disease indices and were rated as susceptible. Some plants of CRA.66 selection A developed wilt symptoms, but they recovered subsequently. The high susceptibility of most of the test lines indicated the existence of pathogenic strains with varying virulence and the need for careful screening of resistant lines against local isolates and under local climatic conditions.

CRA.66 selection A is a bitterless (fruits) selection made by Dr. Gilbert (Gilbert et al., 1974; Acosta et al., 1964) from a Trinidad line CRA.66. It is an indeterminate line bearing green-shouldered and light green fruits with an average fruit weight of 27 grams. It is being utilized currently in our breeding for bacterial wilt resistance program.

In an evaluation of wilt resistance in eggplant, 7 exotic wilt-resistant varieties (Akiba et al., 1972; Daly, 1972; Winstead and Kelman, 1960) and 14 Indian cultivars were screened. In greenhouse tests, out of 7 exotic varieties Dingras Multiple Purple (DMP), Sinampiro and 72-4-L-404 had disease indices of 3, 4 and 8, respectively, while 74-11-L-64-438, Dumaguete Long Purple (DLP) and Kopek showed a disease index of 20, 24 and 30, respectively, and Matale had a disease index of 50. Out of 14 indigenous collections, Pusa Purple Cluster (PPC) showed a disease index of 9 and all others had disease indices above 75%. In field tests, DMP showed 2% infection against 4%, 6% and 6% in Sinampiro 72-4-L-404 and PPC, respectively. 74-11-L-64-438, DLP, Kopek and Matale showed 20, 33 and 48% mortality. PPC is an indigenous variety developed for commercial cultivation by Indian Agricultural Research Institute, New Delhi, India. Fruits are somewhat oval, 10-12 cm long with deep purple color and born in clusters. In view of its resistance combined with its commercial acceptance, this variety is a good alternative for planting in areas in which the bacterial wilt pathogen is endemic. Other varieties are being used in the breeding program at present.

SELECTED REFERENCES

ACOSTA, J. C., J. C. GILBERT and V. L. QUINON. 1964. Heritability of bacterial wilt resistance in tomato. Proc. Am. Soc. Hort. Sci. 84:455-462.

AKIBA, F., R. RIBEIRO, L. A. B. CASTRO, C. F. ROBBS, O. KIMURA and S. SUDO. 1972. Resistance to bacterial wilt in the eggplant variety Nihon Nassu. Archivos da Universidade Federal Rural de Rio de Janeiro 2(2):17-21.

BUDDENHAGEN, I. W. and A. KELMAN. 1964. Biological and physiological aspects of bacterial wilt caused by Pseudomonas solanacearum. Ann. Rev. Phytopath. 2:203-230.

BUDDENHAGEN, I. W., L. SEQUEIRA and A. KELMAN. 1962. Designation of races in Pseudomonas solanacearum. Phytopathology 52:726.

DALY, P. 1972. Research at IRAT/Martinique to produce a variety of eggplant tolerant to Pseudomonas solanacearum. Agronomie Tropicale 27(4):462-472.

DAS, C. R. and S. B. CHATTOPADHYAY. 1955. Bacterial wilt of eggplant. Indian Phytopath. 8:130-135.

GILBERT, J. C., J. S. TANAKA and K. Y. TAKEDA. 1974. 'Kewalo' tomato. Hort. Sci. 9(5):481-482.

HENDERSON, W. R. and S. F. JENKINS. 1972. Venus and Saturn. N.C. Agric Exp. Sta. Tech. Bull. 444, 13 pp.

KELMAN, A. 1954. The bacterial wilt caused by P. solanacearum. N.C. Agric. Exp. Sta. Tech. Bull. 99, 194 pp.

LOZANO, J. C. and L. SEQUEIRA. 1970. Differentiation of races of P. solanacearum by a leaf infiltration technique. Phytopathology 60: 833-838.

RAO, M. V. B., H. S. SOHI and S. K. TIKOO. 1975. Reaction of wilt resistant tomato varieties and lines to P. solanacearum in India. Pl. Dis. Reptr. 59:734-736.

WINSTEAD, N. N. and A. KELMAN. 1952. Inoculation techniques for evaluating resistance to P. solanacearum. Phytopathology 42:628-634.

WINSTEAD, N. N. and A. KELMAN. 1960. Resistance to bacterial wilt in eggplant in North Carolina. Plant Dis. Reptr. 44:432-434.

BACTERIAL WILT IN SOLANACEOUS CROPS GROWN IN RICE FIELDS

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ABSTRACT

In certain parts of Sri Lanka, such as the Welimada and Kandy districts, solanaceous crops, including potatoes and tomatoes, are grown in rice fields during the "dry" season, when the supply of water is inadequate to sustain a crop of rice. Although Pseudomonas solanacearum is regarded as an aerobic organism, flooding of rice fields for several weeks during the rice-growing season does not control the disease. The pathogen is present in these soils and can survive there despite cultural practices which accompany rice cultivation. It was present in fields which had been planted to rice every year and had not been cultivated with solanaceous crops for over 5 years.

Three biotypes of P. solanacearum (biotypes 2, 3 and 4, according to Hayward's classification) were obtained from rice fields.

Diseased plants were not distributed uniformly in the field and generally occurred in patches which frequently were in close proximity to water holes and irrigation drains.

In experimental plantings, there was no conclusive evidence of a rapid increase in the population of P. solanacearum in the fields following harvest of the rice crop. In plantings made at weekly intervals from 2½ to 9 weeks after the rice harvest, the percentage of plants which wilted ranged from 2-18 percent. There was no correlation between the time of planting and the percentage of wilt observed.

Yields of potatoes ranging from 6-10 tons per acre were obtained. Potatoes, therefore, can be grown during the dry season in rice fields although bacterial wilt cannot be overcome altogether even by submergence for a period during the rice growing season.

In Sri Lanka the choice of crops is often influenced by the availability of water. During the Maha season, when rain is received from the north-east monsoon, rice is extensively cultivated in most parts of the country. During the Yala season from April to September when rain from the south-west monsoon is received mainly in the south-west part of the island, the available water is insufficient for rice cultivation in certain areas. During this period, other crops are grown in rice fields in the Welimada and Kandy districts which are important vegetable growing regions.

Pseudomonas solanacearum is regarded as an aerobic organism and flooding is a practice that reportedly is effective in controlling soil borne pathogens. In rice cultivation, fields are submerged for several weeks (usually about 4½ to 5½ months in the districts referred to above). In this study, two aspects were investigated -- the incidence of Pseudomonas solanacearum in rice fields, and the prospects for potato cultivation in rice fields in the Welimada district. This region is climatically suitable for potato cultivation, but

bacterial wilt is a serious disease in the highlands. In the Welimada district and elsewhere in the hill country dry zone, potato is a preferable alternative to some of the crops (mainly beans, cabbage and radish) grown in rice fields during the "dry" Yala season.

The incidence of Pseudomonas solanacearum in rice fields

In areas such as Talatuoya and Marassans in the Kandy district, tomato is the principal solanaceous crop grown in rice fields during the Yala season. Bacterial wilt occurs in these areas and the severity of the disease is variable. The disease was observed in fields which had carried a rice crop every year, but which according to the farmers, had not been cultivated with any solanaceous crops for five years or more. In these fields the incidence of the disease could not be attributed to the use of infected seedlings or contamination through irrigation water. The organism was present in the soil and it was not eliminated by flooding of the fields during part of the rice-growing season. The percentage of plants affected by wilt was variable, generally 5-10 percent, but in some cases more than 25 percent. In the Welimada district where potato and tomato are grown in rice fields during the Yala season, the situation was similar. Also, it was observed that infected plants were not distributed uniformly in a field. They generally occurred in patches in close proximity to water holes and irrigation drains.

Several isolates of P. solanacearum obtained from wilting potato and tomato plants in rice fields in the Welimada and Bandarawela districts of the hill country dry zone were examined to determine which biotypes, according to Hayward's classification, occur in these districts. Biotype 3 appeared to be the type characteristic of the hill country dry zone and was obtained from the majority of diseased potato and tomato plants collected in the region. However, in a transitional area between wet and dry hill country zones, biotypes 2 and 4 were detected in potato, each from a single location (Havarianmalwella and Gorandiyantenna).

Prospects for the cultivation of potato in rice fields

The Uva basin on the eastern side of the centrally-situated mountain range in Sri Lanka constitutes the hill country dry zone, with gently rolling downs at an elevation of about 4000 ft. This region has an annual rainfall of about 65 inches mainly from the north-east monsoon. In this region, rice cultivated during the Maha season is generally harvested in June and these fields can be prepared for planting vegetables by the second half of July. If potato is to be grown instead, the crop must be planted so as to avoid the strong winds in July/August. Again, it must be ready for harvesting before the heavy Maha rains and it is desirable to have the crop lifted by mid October. This fixes the second half of July and early August as the planting period for potato.

Experiments were carried out at two locations, Boralanda and Havarianmalwella, to determine the incidence of bacterial wilt. Also, to ascertain whether increased incidence of wilt is correlated with an increase in the period between harvesting of the rice crop and the planting of the potato crop. This could be interpreted as due to an increase in the population of Pseudomonas solanacearum in rice fields following the rice harvest.

At each location, five plantings were done at weekly intervals, the first of these being completed as early as possible after harvesting the rice crop. The first planting at Boralanda was done on 15 July and at Havarianmalwella on 22 July, 2½ and 4 weeks after harvesting the rice crop, respectively.

At each location, five plots, 12 x 20 ft each, were planted with the variety Gineke at each weekly planting. In each plot, 100 tubers were planted in 10 rows of 10 tubers each, with a spacing of 2 ft between rows and 1 ft within the rows. The total area planted at each location was approximately 1/10 acre. Fertilizer dressings were applied at 3, 4½ and 1 cwt per acre of ammonium sulfate, concentrated superphosphate and muriate of potash, respectively. Protective sprays were applied against late blight and insect pests.

Observations on the incidence of wilt were made at weekly intervals, accurate assessments being possible in all plantings up to 70 days. Plants showing symptoms of bacterial wilt were removed after the weekly counts. Plants infected by Rhizoctonia were also removed. Because of the scattered occurrence of wilt at Boralanda, two separate figures are given for this location (Figs. 1 and 2), one set for wilt incidence in the entire field comprising all five blocks, the other for the incidence in four of the five blocks, excluding the block with localized patches of heavy wilt incidence. The yields at the two locations (Tables 1 and 2) were calculated from the number of plants remaining at the time of lifting. However, many plants affected by bacterial wilt and Rhizoctonia, which were removed in the later stages of growth, had well formed tubers suitable for human consumption.

The results and conclusions from these studies may be summarized as follows

(1) Flooding of fields during part of the rice growing season does not eliminate Pseudomonas solanacearum from these soils.

(2) The incidence of bacterial wilt at Havarianmalwella was lower than at Boralanda. The lowest incidence of wilt in the first planting at both locations was 1.0 percent and 2.0 percent at 70 days, respectively. In the last planting, the corresponding figures were 2.4 percent and 8.2 percent. Differences in the incidence of wilt in the five plantings at each location were not pronounced, except for one block at Boralanda with localized patches of heavy infection. These observations suggest that a rapid increase in the population of Pseudomonas solanacearum in the soil does not occur in the period immediately following the rice crop during the dry season.

(3) With the generally low incidence of bacterial wilt and favorable conditions during the growing season, good crops were obtained at both locations. Yields ranged from 6.07 - 8.70 tons per acre at Boralanda and from 9.23 - 10.76 tons per acre at Havarianmalwella. Yields of this order are considered satisfactory.

(4) Because of conditions imposed by the patterns of cultivations and the climatic characteristics of the hill country dry zone, potatoes must be planted during the second half of July and early August. From these experiments it is clear that from the point of view of bacterial wilt this is a suitable planting season.

Acknowledgements

The writer is grateful to Messrs P. L. E. Pannila and M. Haniffa for their assistance in this work; also to Messrs D. A. M. Punchibanda, D. A. M. Ratnayake and V. M. Rupasinghe, in whose fields the experiments were carried out, for their cooperation.

Reference

Hayward, A. C. 1964. Characteristics of *Pseudomonas solanacearum*. J. Appl. Bact. 27, 265-77.

Table 1. Incidence of diseases and yields at Boralanda

Planting	Plants affected by bacterial wilt, %		Plants affected by *Rhizoctonia*, %		Plants harvested %	Age of crop at harvest, days	Yield, tons per acre
	Up to 70 days	After 70 days	Up to 70 days	After 70 days			
15 July	2.0	4.2	0.0	0.0	93.8	84	7.96
22 July	13.4	7.2	0.0	0.0	79.4	77	6.07
29 July	11.0	0.8	0.0	0.2	88.0	90	8.70
5 August	18.2	0.2	0.0	0.0	81.6	83	8.17
12 August	8.2	0.0	0.0	0.0	97.8	76	8.59

Table 2. Incidence of diseases and yields at Havarianmalwella

Planting	Plants affected by bacterial wilt, %		Plants affected by *Rhizoctonia*, %		Plants harvested %	Age of crop at harvest, days	Yield, tons per acre
	Up to 70 days	After 70 days	Up to 70 days	After 70 days			
22 July	1.0	3.2	1.6	5.6	88.6	84	9.53
29 July	3.6	0.0	2.4	0.0	93.2	77	9.80
5 August	1.4	0.0	1.4	0.0	96.6	84	10.76
12 August	2.0	0.0	0.2	0.0	97.6	77	9.59
19 August	2.4	0.0	0.4	0.0	97.2	70	9.23

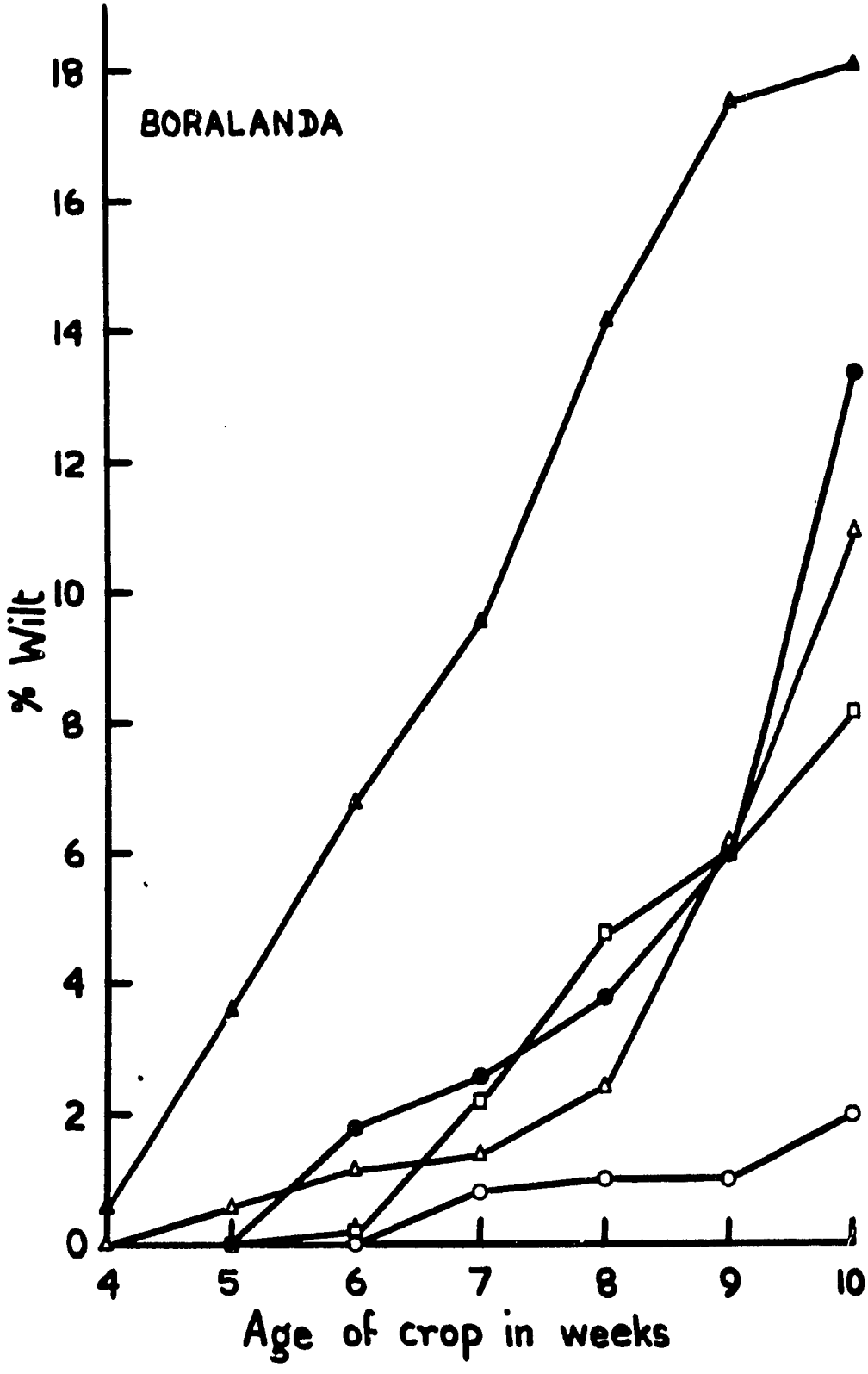

Fig. 1. Incidence of bacterial wilt at Boralanda in entire experimental area of field.—o—, planting on 15 July; —●—, planting on 22 July; —△—, planting on 29 July; —▲—, planting on 5 August; —□—, planting on 12 August.

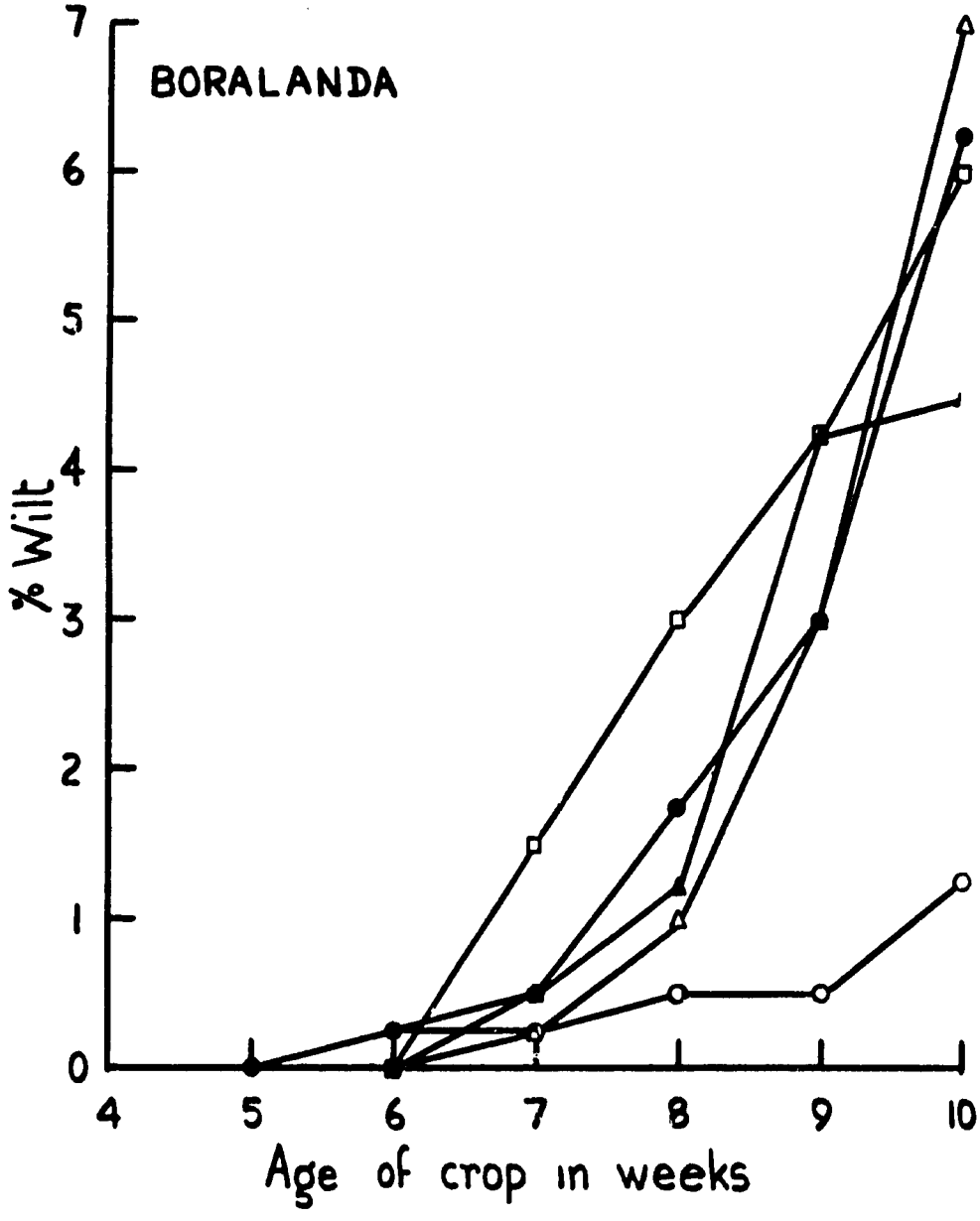

Fig. 2. Incidence of bacterial wilt at Boralanda in field excluding block with localized patches of heavy infection.——o——, planting on 15 July; ——●——, planting on 22 July; ——△——, planting on 29 July; ——▲——, planting on 5 August; ——□——, planting on 12 August.

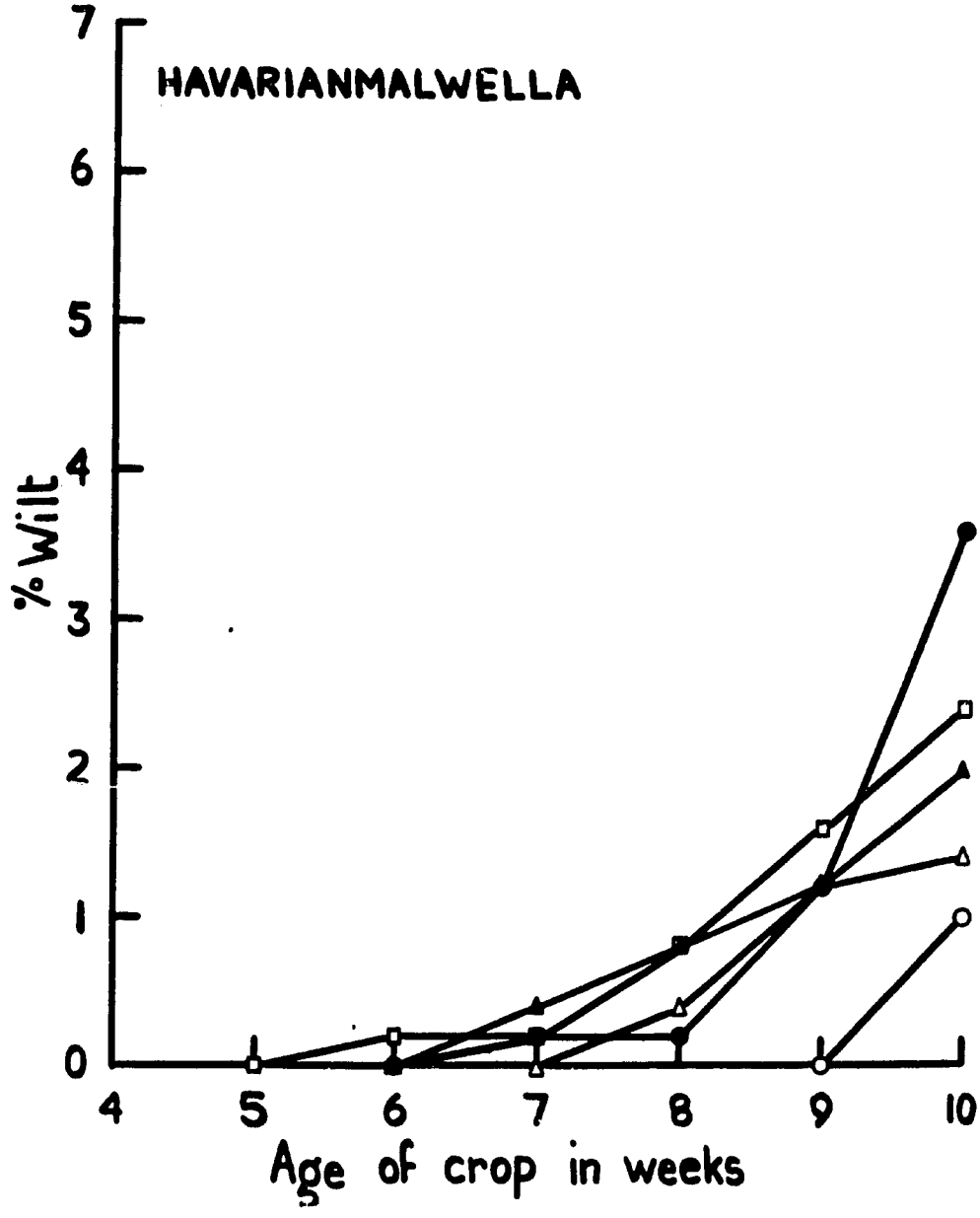

Fig. 3. Incidence of bacterial wilt at Havarianmalwella. ——○——, planting on 22 July; ——●——, planting on 29 July; ——△——, planting on 5 August; ——▲——, planting on 12 August; ——□——, planting on 19 August.

BACTERIAL WILT OF STRAWBERRY IN JAPAN

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Occurrence of bacterial wilt of strawberry was first observed by a farmer at Kambara-cho, Shizuoka Pref. in 1933. The result of its etiological study was reported by Tanaka in 1936. The economic importance of the disease, however, has not been recognized until recently. In 1973, we observed severe damage of strawberry seedlings due to bacterial wilt at Hamaoka-cho, Shizuoka Pref. The disease occurred in seedbeds on 1- to 2-month-old seedlings from June through August. Although it was not common, the disease was also observed on 3- to 4-month old seedlings just after these were transplanted in fields in September. Wilting of leaves was acute in younger seedlings, whereas in mature seedlings, chronic and mild wilting developed from lower leaves. All cultures isolated from the diseased seedlings belonged to pathotype 6a (Okabe and Goto, 1961), which was also highly virulent on tobacco, tomato and eggplant. Inoculation tests further proved that pathotype 4, which showed severe infection on tomato and eggplant, but not on tobacco, could not induce wilting of strawberry seedlings. Pathotype 9, which was weakly pathogenic on *Arachis* and tobacco, also could not cause the disease on strawberry. In some strawberry seedlings which escaped disease development in the inoculation tests with pathotype 6a, *Pseudomonas solanacearum* was detected the next spring at relatively low populations in the vascular tissues of the stems. Studies are currently under way to determine whether or not plants which carry the pathogen could serve as infection sources in the next season and why strawberry seedlings gain resistance to *P. solanacearum* so quickly as they mature.

THE BACTERIAL WILT PROBLEM IN THE PHILIPPINES

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In the Philippines, bacterial wilt caused by Pseudomonas solanacearum E. F. Sm. affects, under natural conditions, the following crops: abaca, banana, eggplant, pepper, potato, tomato, tobacco, peanut, ginger, castor bean, cowpea and bush lima bean.

The disease is very destructive on eggplant, tomato, pepper, castor bean and ginger. Tobacco rotated with upland crops or vegetables is more commonly and severely affected than tobacco rotated with paddy rice. It has not yet been observed in commercial potato plantings at high elevations, but is common and severe on potatoes grown in the lowlands. Abaca wilt is severe on 2- to 3-month-old plants, but, in nature, it seems to be a complex disease in which P. solanacearum is involved. Moko disease, although becoming important in commercial giant Cavendish banana plantations, is not important yet in local banana varieties. Bacterial wilt is not yet a serious problem on peanut, and, on cowpea and bush lima beans, it is regarded as a minor disease.

The most widely distributed strain of P. solanacearum consists of isolates from solanaceous crops (except tobacco), peanut, castor bean, cowpea and bush lima bean. The abaca, banana, ginger and tobacco isolates, though highly virulent (except those from ginger) to solanaceous crops, are less or slightly virulent to other crops except to their original source plants. There are different pathovars of the pathogen in the country.

Recent observations and developments in research on the disease as it occurs on Philippine crops are briefly discussed.

STRAINS OF *PSEUDOMONAS SOLANACEARUM* AND CURRENT RESEARCH ON BACTERIAL WILT IN MAURITIUS

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Bacterial wilt is of major economic importance on tomato, potato and tobacco in Mauritius. Research work has been mainly on strain differentiation and variability, disease resistance in commercial crop varieties and epidemiology in relation to cropping sequence and soil conditions.

Physiological, biochemical and pathotype studies were performed on about 75 isolates collected from different host plants throughout the island. Only biotype 3 was characterized among the isolates. The isolates have been classified into four pathotype groups according to their capacity to infect different crops upon natural and artificial inoculation. Infiltration experiments revealed the presence of races 1 and 3 of the pathogen in the island.

In epidemiological studies, the importance of moisture and inoculum potential was specially evident. Whatever the cropping sequence, there was a similar trendin the susceptibility of crops in rotation studies conducted at different localities. Commercial potato, tomato and tobacco varieties were currently tested for resistance to bacterial wilt under optimum disease conditions.

OVERWINTERING OF PSEUDOMONAS SOLANACEARUM IN SWEDEN

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In 1972, 1973 and 1975 brown rot caused by Pseudomonas solanacearum was found in Bintje potato tubers from a farm in Hokopinge, situated in Skane between 55° N and 56° N. The cases were from different fields in different years; however, all had been irrigated with water from the same ditch. In 1974, when no potatoes with brown rot were found, no irrigation had been performed. The initial origin of this outbreak has not been determined.

In Bintje potatoes from another farm, in Ostra Ljungby at about 56° N, tubers with brown rot were found in 1973 and 1975 but not in 1974. Irrigation had been carried out with water from the river Pinnan in both years when the disease appeared, but not in 1974. The frequency of brown rot in different fields varied from 5 per cent with visible symptoms down to latent infection, established by stem-inoculation into young testplants using suspensions made from several hundred infected heel-ends in a Turmix blender. The testplants used were: eggplant of the variety Black Beauty, and tomato of the variety Dansk Export.

Moreover, in the years when the disease appeared, no diseased potatoes were found in the crop from some non-irrigated fields on the farm described above. The river used for irrigation has no connection with the ditch mentioned previously.

In 1973 a neighbor in Ostra Ljungby had used river water for irrigation in his garden and the disease was detected by inspectors also in his crop. The variety was Greta. In 1974 and 1975, he did not use irrigation and his crop had no brown rot.

All three growers had used seed potatoes of different origin. Sufficient numbers of potatoes from the same origin were examined by experienced inspectors and in one case more than a thousand potatoes were tested for latent infection by stem-inoculation into testplants in the greenhouse. No bacteria were found. Thus the seed potatoes were probably free from the disease.

Hosts and overwintering

In order to find out what species might be of interest as hosts in Sweden, we have stem-inoculated and watered several kinds of plants with dense suspensions of Pseudomonas solanacearum. For results to date see Table 1. The suspensions for use in the field were made from diseased plants from the greenhouse.

Natural hosts might be expected among those attacked after watering with suspensions. Solanum dulcamara, also called bitter nightshade or bittersweet, is perennial and has been demonstrated to be a natural host. Sometimes this host displays external symptoms, and sometimes not. In the map of the river Pinnan, Fig. 1, the black triangles and squares show locations where S. dulcamara was demonstrated to contain the bacteria in 1974 and 1975. Moreover, the bacteria have been isolated in three samples in 1976. The S. dulcamara produces adventitious roots that reach the river.

All known cases of P. solanacearum infecting S. dulcamara, as well as infected potatoes in the region of the river, are located downstream of a place where a potato peeling factory was situated until 1967. In that year the factory moved downstream.

I believe that the bacteria may have overwintered in S. dulcamara for at least ten years. This is based upon the assumption that the bacteria which originally attacked S. dulcamara came from the peeling factory, where imported potatoes probably were used. The waste water did go out directly from the factory into the river without passing through any ponds. Alternatively, a big garbage dump further upstream may have been the focus of infection. It has not been possible to isolate the bacteria from S. dulcamara growing upstream from the place where the factory was situated until 1967. It is easy to infect S. dulcamara artificially by watering with suspensions from Swedish strains and a strain isolated from Mexican potatoes. Young plants in our greenhouse sometimes die quickly as a result of root infections, without any bacteria in the stem.

Overwintering in S. dulcamara has also occurred at 59° N in Solna near Stockholm, in this case in artificially infected plants growing in soil.

Temperature data

The soil temperature in Solna was below 0° C for about two months during the first winter, 1974-1975, when overwintering was demonstrated (see Fig. 2). During the following winter the temperature was somewhat lower and overwintering in artificially infected S. dulcamara was demonstrated.

Unfortunately we have no temperature reading instruments in the river where naturally attacked S. dulcamara are growing. The temperatures are probably somewhat higher than those in Solna.

The optimum temperature for growth of most Swedish strains on a sucrose/peptone medium (Hayward, 1960, Nature 186:406) is about 30° C. Two strains out of 12 seemed to have a lower optimum, closer to 25° C. All strains grew sparsely at 12° and none at 10° C.

Summary

1. P. solanacearum can overwinter in S. dulcamara in Sweden at least as far north as 59° N.

2. Irrigated potatoes have been attacked on a farm between 55° N and 56° N where water from a ditch is used for irrigation. No attacked S. dulcamara have been found in the ditch so far.

3. Potatoes from fields at about 56° N, irrigated with water from a river where naturally infected S. dulcamara are growing, have repeatedly been infected by P. solanacearum. We do not yet know whether the bacteria can multiply in other perennial wild hosts, or whether they can overwinter in the soil or in the mud in the river.

Table 1. List of plants susceptible to Swedish strains on the basis of inoculation and watering experiments

Plants	Greenhouse		Field
	Inoculation	Watering	
Chenopodiacae			
Beta vulgaris L. "Monohill"	+	-	-
Compositae			
Eupatorium cannabinum L.	+	+ 1	
Cruciferae			
Brassica napus L. v. *napus* "Oro"	+	+ 1	-
Leguminosae			
Glycine max (L.) Merr.	+ 1		-
Phaseolus vulgaris L.	+ 1		-
Pisum sativum L.	+ 1		-
Vicia faba L.	+ 1		-
Solanaceae			
Browallia speciosa Major	+ 1		
Capsicum sp.	+		
Datura stramonium L.	+		
Hyoscyamus niger L. v. *niger*	+		
" " v. *pallidus* (Kit.) Rchb.	+		
Lycopersicum chilense Dun.	+		
" *esculentum* Mill. "Dansk Export"	+	+	+
Nicandra physaloides (L.) Gaertn.	+ 1		
Nicotiana alata Lk & Otto v. *grandiflora* Comes	+ 1	-	-
Salpiglossis sinuata R. & P.			+
Solanum capsicastrum Link	+		
" *dulcamara* L.	+	+	+
" *luteum* Mill.	+	+	
" *melongena* L. "Black Beauty"	+	+	
" *nigrum* L.	+		+
" *tuberosum* L. "Bintje", "Saturna", "Up-to-date"	+		+
Tropaeolaceae			
Tropaeolum majus L. v. *nanum*	+	+	+
Urticaceae			
Urtica dioeca L.	+	-	

+ *P. solanacearum* reisolated

- *P. solanacearum* not reisolated

1 Only latent infection

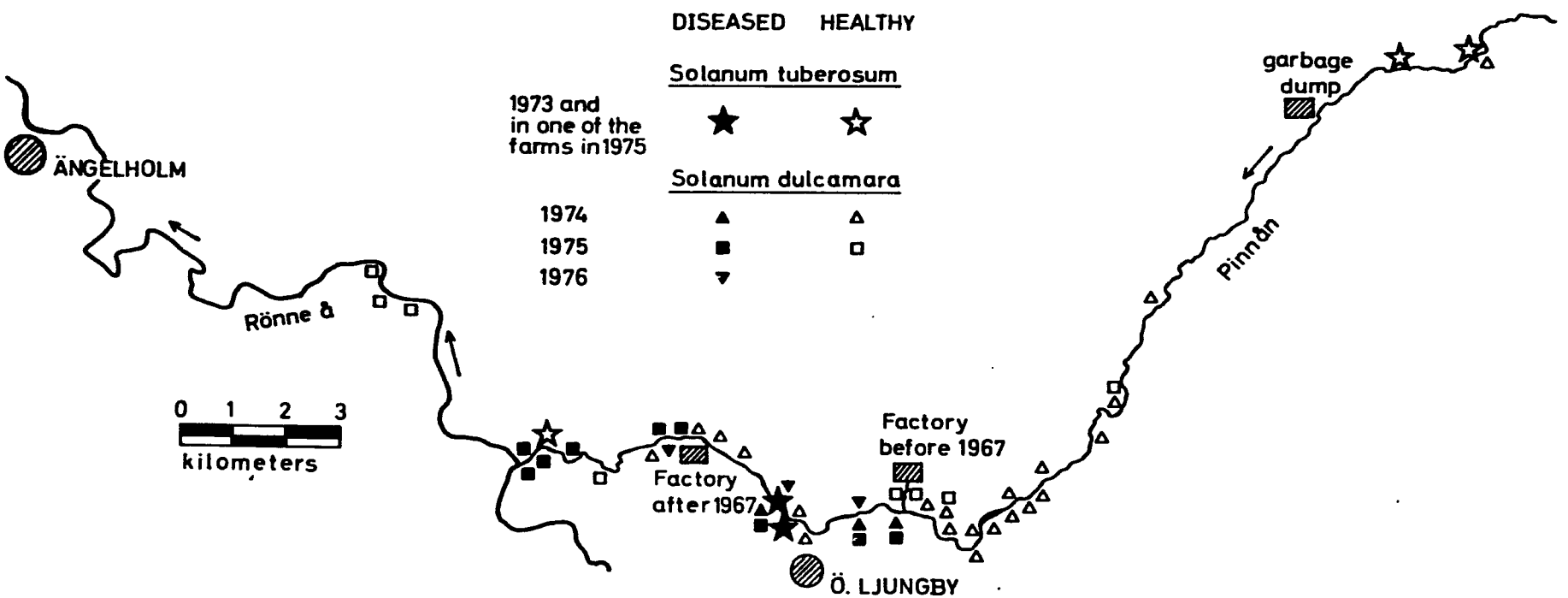

Fig. 1. Map of the river Pinnan showing the position of plants infected, and not infected, by *Pseudomonas solanacearum*.

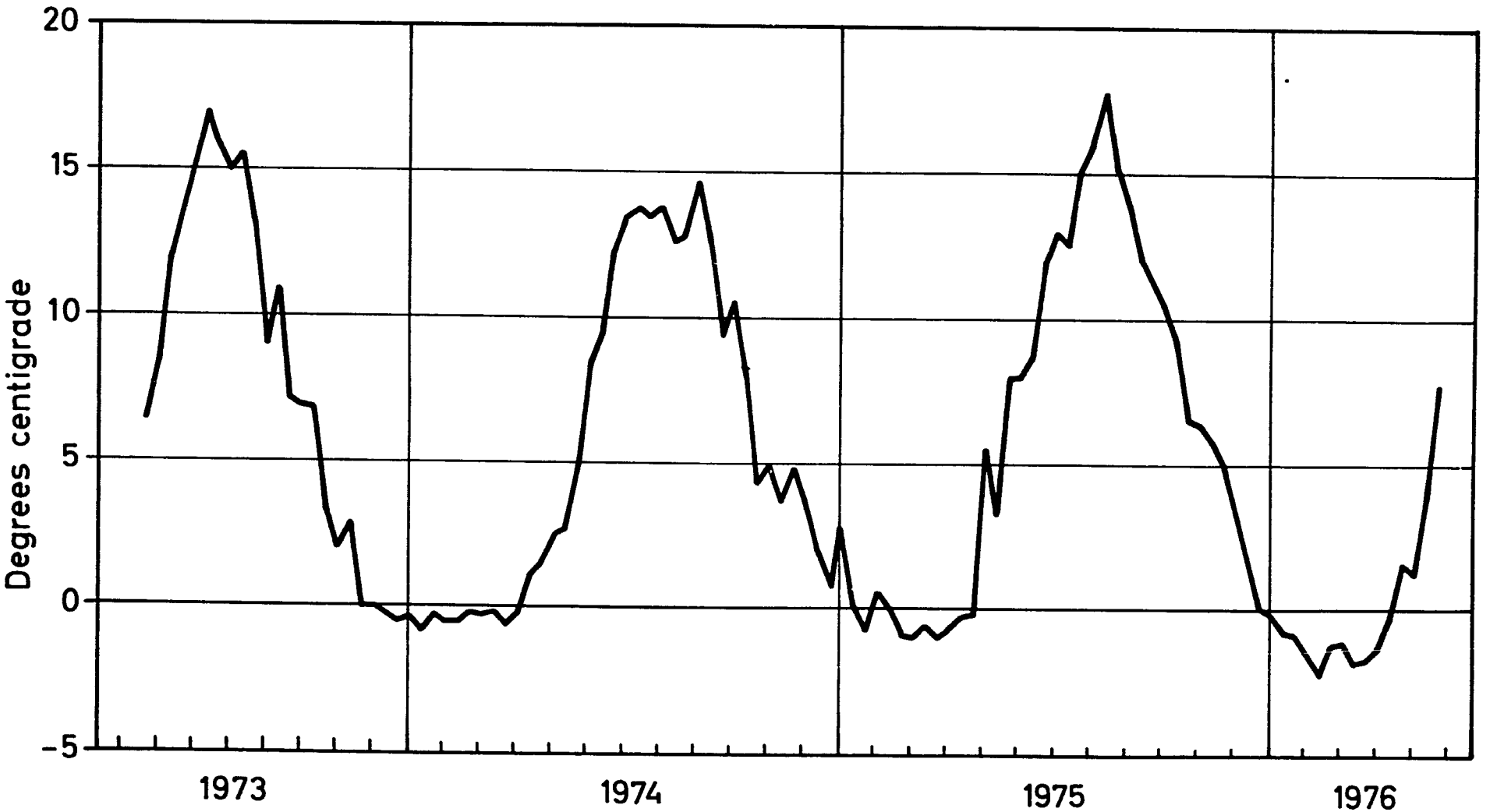

Fig. 2. Soil temperature 5 cm below the surface every tenth day at 7 a.m. at Solna.

SOME ASPECTS OF THE SEROLOGY OF PSEUDOMONAS SOLANACEARUM E.F. SMITH

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Pseudomonas solanacearum E.F. Smith is a bacterium causing a wilting disease of plants that is especially damaging in tropical countries. Three races (Buddenhagen, 1962) are distinguished by host range: race 1 on solanaceous plants, race 2 on musaceous plants, and race 3 on potato. Many aspects of the biology of this bacterium have been examined. The literature on its antigenic structure shows some lacunae and is sometimes inconsistent.

Moraes (1947) first used serology for diagnosis. In 1956 Okabe and Goto compared two colony types F and op and concluded that variation from F to op is followed by the loss of an antigen. Harrison and Freeman (1961) compared two strains of *P. solanacearum* and one strain of *Corynebacterium sepedonicum* Skapt and Burk. They found an antigen common to the two species which showed great homogeneity for the two *P. solanacearum* strains. All of these workers used the slide agglutination technique. Perez (1962) used a tube precipitation technique ("ring test") with polysaccharides extracted by Fuller's formamide. He compared 99 strains and concluded that the polysaccharide antigen were species specific. Morton *et al.* (1965, 1966) used serology for diagnosis and claimed that race specific antisera can be obtained. Digat and Escudie (1967) isolated the somatic antigens of several strains and found them to be heterogeneous. Kojima and Buddenhagen (1969) found the same results on the polyosidic antigen. Coleno *et al.* (1971) showed that the protein part of the somatic antigens fixed complement. Recently Digat and Camara (1976) claimed that strain and race specificity exists.

These studies suggest a good species specificity linked with the somatic antigen. They are, however, in part contradictory and sometimes questions can be raised about the methodology that was used. The work we present is not a quest for race specificity. There is, *a priori*, no reason why serotypes should correspond with races unless the determinants of race virulence are serologically distinguishable; such an assumption would be hazardous. We think, however, that a good knowledge of antigenic structure could be of value in certain cases and at least could lead to a valuable evaluation of interspecies and intraspecies relationships. This paper deals with antigenic determinants.

MATERIALS AND METHODS

Strains. Most of the strains that we have used were obtained from A. Kelman and B. Digat. Table 1 presents the main characteristics of these strains. On LPGA medium (yeast, 5 g; peptone, 5 g; glucose, 10 g; agar, 18 g) they are S form. However, a precise study of colony morphology throughout the year in Guadeloupe showed that during the first months we find more often hyper-mucous colonies on LPGA medium which agglutinate neither with sera prepared following Digat and Escudie (1967) nor with "O" antisera. This colony type can be lost in culture or changes to the S type.

Thus, we have distinguished three forms based on colony morphology: M (hyper-mucous); S (F type); and R (B type).

Antigens. The immunizing strain was cultured on LPGA medium for 24 h at 30° C. The culture was then washed and suspended in saline (0.85%). The bacterial suspension (2 x 10^9 cells/ml) was then treated in three ways:

1. Mixed with formalin (final concentration 0.5%) and after 24 h, washed and resuspended in saline at 2 x 10^9 cells/ml (= formalized suspension).
2. Maintained for 24 h in 96% ethanol, then washed twice and suspended in saline (0.85%) at 2 x 10^9 cells/ml (= alcohol treated suspension).
3. Treated for 2 h at 120° C, then washed and suspended in 0.85% saline at 2 x 10^9 cells/ml (= heat treated suspension).

M antigen. A hyper-mucous colony was cultured on LPGA medium for 24 h. The culture was collected, suspended in saline 0.85%, slightly sonified and washed well three times. The supernatants of the second and third washing were combined. The slime was precipitated with ethanol (final concentration 60%) overnight at 4° C. The pellet was taken up in 20 ml saline (0.85%) and dialyzed against saline (0.85%) and the titre was determined.

Antisera. These were made using rabbits (2 kgs weight). For agglutinating sera nine intravenous injections with live bacteria (2 x 10^9 cells/ml) three times a week were followed by a ten day rest and then by six 2 ml intravenous injections at the same interval preceded by a 0.5 ml subcutaneous injection. For anti-M sera the extract (5 µgN/ml) was injected in the neck. Five ml of an equal mixture of extract and Freund's adjuvant was injected once a week for 3 weeks; then nine 3 ml injections of extract three times a week. In all cases the rabbit was bled by cardiac puncture eight days after the last injection.

Agglutination. The chosen technique was slow agglutination in test tubes. We mixed 0.5 ml dilute antisera with 0.5 ml bacterial suspension (heat treated or formalized). The tubes were read after incubation overnight at room temperature. Absorptions were performed following the Kauffman technique (1954) with living bacteria. The check of specific agglutination was made by the Castellani scheme (1902). Preparation of monovalent antisera was done by absorption at 1/200 with the heterologous strain.

Precipitation. Double diffusion technique (1954) was used as described by Ouchterlony and Nilsson in D. M. Weir (1968).

RESULTS

Antigens. Among other pseudomonads the following antigenic types are known: envelope, capsule, and mucous (P. mallei and P. pseudomallei). These antigens are characterized (Chambon and Fournier, 1956) by their stability. We treated the hypermucous strain with ethanol (50%), formalin (0.5%), hydrochloric acid (N), and by heating (2 h at 120° C). All treatments except formalin produced suspensions that gave S agglutination in an S antisera. On the other hand, microscopic observation of bacteria in M antisera did not reveal any capsule swelling. This suggests the existence, sometimes, of an M type antigen (analogous to the M antigen of P. pseudomallei and located in or underneath the slime). This antigen seemed to be characterized by the following properties: filamentous agglutination partially dissociable and proceeding to an O inagglutinability; susceptible to heat (2 h at 120° C),

ethanol (50%), and hydrochloric acid (N); and resistant to formalin (0.5%).

Serotyping

Serotyping with formalized suspensions: In three cases we have detected specific antigens (Table 2), which we have designated arbitrarily by number. In spite of the choice of immunizing strain among the three races, we can see that the antigens are heterogeneously distributed among the different strains used in the study. There is no correlation with race or origin (Table 3). The frequency with which these antigens occurs in the three races is characteristic of each race among the isolates we examined (Table 4).

Serotyping with heat treated suspensions: With these antigens the results are almost identical with those obtained with formalized type antigens. We detected specific determinants (Table 5) which we designate by letters. They are irregularly distributed among the strains independently of race or origin (Table 6). Their frequency in races did not show the differences between the races observed with formalized suspensions (Table 7).

The precipitation in gel allowed the detection of specificity corresponding to the whole polyosidic structure. There were three different antigens shared among the three reference strains 712, 145, Au reu. It is easy to define serological groups which do not correspond with races (Table 8). However, it seems that most of the race 3 strains are characterized by the 145 antigen. This antigen is also borne by a P. mallei reference strain.

DISCUSSION

The antigenic structure of P. solanacearum seems to be analogous to that of P. pseudomallei and P. mallei in at least two aspects. The M type antigens correspond to those described by Chambon and Fournier (1956). Among P. pseudomallei cultures this antigen is characteristic of aging cultures and is found also in the "suicidal cultures" (Nichols, 1930). We can also isolate colonies of P. solanacearum with this antigen, when cultural conditions are unfavorable.

The polyosidic antigens correspond to those detected in other phytopathogenic pseudomonads (Kojima and Buddenhagen, 1969; Lucas and Grogan, 1969). We find again a relationship with the P. mallei - P. pseudomallei group since the specific polyoside of P. solanacearum 145 is apparently shared by strain 3709 of P. mallei. This polyoside is present in most of the P. solanacearum strains whatever the race. Comparison with the serological study of P. mallei and P. pseudomallei by Fournier (1967) shows that the situation is similar for these two species. They exist as two serological groups: 1) homogeneous with all P. pseudomallei strains and some P. mallei strains (Gravitz and Miller, 1950) (strain 3709 belongs to this group), and 2) heterogeneous with other P. mallei strains. It could be of interest to compare P. solanacearum strains with members of this second group.

The results show that serotype schemes can be readily erected but that, with the antigens we have used, these are not correlated with classification based on other criteria such as host range or chemovar.

ACKNOWLEDGMENTS

We thank A. Kelman, B. Digat and J-P. Prunier for courtesy in providing the strains studied of P. solanacearum.

We thank also R. A. Lelliott for the comprehensive and kindly review of the article.

Table 1. Strain used in the study

Strain	Origin	Geographical origin	Race	Host-plant
Tri mysore	Digat	Trinidad	1	banana
Guy to 1	Digat	Guyane	1	tomato
J_2	Digat	Japan	1	tomato
Duclos 1	Coleno	Guadeloupe	1	tomato
Au	Prunier	Reunion	1	eggplant
65 715	CNBP*	Upper Volta	1	tomato
Mar to 1	Digat	Martinique	1	tomato
Tahiti	Prunier	Tahiti	1	tomato
Gua to 1	Digat	Guadeloupe	1	tomato
Tri	Digat	Trinidad	1	tomato
Gua to 4	Digat	Guadeloupe	1	tomato
Au reu	Digat	Reunion	1	eggplant
707	Digat	Tahiti	1	tomato
Tri to 3	Digat	Trinidad	1	tomato
69 914	CNBP	Guadeloupe	1	potato
192	Digat	Peru	2	banana
178	Digat	Colombia	2	banana
368	Digat	Panama	2	banana
135	Kelman	Colombia	2	banana
138	Kelman	Costa Rica	2	banana
70	Kelman	Costa Rica	2	banana
Heliconia	Digat	Costa Rica	2	Heliconia
80	Kelman	Colombia	3	potato
81	Kelman	Colombia	3	potato
82	Kelman	Colombia	3	potato
119	Digat	Colombia	3	potato
145	Kelman	Australia	3	potato
P. mallei 3709	Dodin			

* CNBP = Collection Nationale de Bacteries phytopathogenes: INRA Domaine de Bois-l'Abbe Angers - Beaucouze France.

Table 2. Agglutination between formalized antigen of representatives of the three strains of P. solanacearum and their absorbed antisera. Antigenic determinants designated by number.

Antiserum	Antigen		
	Ps 80 (race 3)	134 (race 2)	Duclos 1 (race 1)
134	+(3)	-	-
Ps 80 absorbed with Duclos 1	+(2)	-	-
Ps 80	-	+(4)	+
134 absorbed with Duclos 1	-	+(5)	-
Ps 80	-	+	+(6)
Duclos 1 absorbed with 134	-	-	-

Table 3. Antigenic determinants of isolates of P. solanacearum as determined by mirror cross absorption with formalized antigens

Strain		Race	Determinants			
			2	4	5	6
80	(Kelman) Colombia	3	+	-	-	-
81	(Kelman) Colombia	3	+	-	-	-
82	(Kelman) Colombia	3	+	-	-	-
tri mysore	(Digat) Trinidad	1	+	+	-	+
Guy to 1	(Digat) Guyana	1	+	+	-	-
J	(Digat) Japan	1	-	-	-	+
119	(Digat)	3	-	-	-	-
712	(Digat)					
Duclos	(Coleno) Guadeloupe	1	-	+	-	+
69 914	(CNBP) Guadeloupe	1	-	+	-	-
Aubergine	(Prunier) Reunion	1	-	+	+	-
192	(Digat) Peru	2	-	+	+	+
65 715	(CNBP) Upper Volta	1	-	+	+	+
Mar to	(Digat) Martinique	1	-	+	+	-
Tahiti	(Prunier) Tahiti	1	-	+	+	+

Table 4. Frequency of 2-4-5-6 antigenic determinants (percentage)

Antigen	Race 1	Race 2	Race 3
2	15	0	60
4	85	100	20
5	60	100	0
6	75	90	10

Table 5. Agglutination between heat treated antigens of representatives of the three strains of P. solanacearum and their absorbed antisera. Antigenic determinants are designated by letter.

Antisera	Ps 80 (race 3)	134 (race 2)	Duclos S (race 1)
134	+ (b)	-	+
Ps 80 absorbed with			
Duclos S	-	-	-
Ps 80	-	+ (c)	+
134 absorbed with			
Duclos S	-	+ (d)	-
Ps 80	-	-	-
Duclos absorbed with			
134	+ (e)	-	+

Table 6. O. Antigen - Serotyping (slow agglutination): Antigenic determinants of isolates of P. solanacearum as determined by mirror-cross absorption with formalized antigen

Strains		Races	Determinants		
			b	d	e
Mar to 1	(Digat) Martinique	1	+	+	+
Gua to 1	(Digat) Guadeloupe	1	+	+	-
Tri	(Digat) Trinidad	1	+	+	+
J_2	(Digat) Japan	1	+	+	-
69 914	(CNBP) Guadeloupe	1	+	+	+
69 715	(CNBP) Upper Volta	1	+	+	+
Gua to 4	(Digat) Guadeloupe	1	+	-	+
Duclos S	(Coleno) Guadeloupe	1	+	-	-
Gua to 1	(Digat) Guyane	1	+	-	+
Gua to 1	(Digat) Guadeloupe	1	-	+	+
	(Digat) Reunion	1	-	+	-
178	(Digat) Colombia	2	-	+	-
368	(Digat) Panama	2	-	+	-
Ps 80	(Kelman) Colombia	3	+	-	+
119	(Digat) Guadeloupe	3	+	-	+

Table 7. Frequency of b, c, d, and e antigenic determinants (percentage)

Antigen	Race		
	1	2	3
b	75	30	90
c	70	100	30
d	70	90	30
e	70	40	45

Table 8. Serotyping with polyosidic precipitating antigens. Precipitation between polyosidic antigen of representatives of the three strains of *P. solanacearum* and three type sera.

Strains	Races	145 (Kelman)	712 (Digat)	Au Reu (Digat)
135 (Kelman) Colombia	2	+	+	+
Heliconia (Digat)	2	+	+	+
707 (Digat) Tahiti	1	+	+	+
734 (Digat) Madagascar	3	+	+	+
119 (Kelman) Costa Rica	3	+	+	-
Duclos (Coleno) Guadeloupe	1	+	+	-
712 (Digat) Upper Volta	1	+	+	-
trito 3 (Digat) Trinidad	1	+	+	-
Jc (Digat) Japan	1	+	+	-
145 (Kelman) Australia	3	+	-	-
80 (Kelman) Colombia	3	+	-	-
81 (Kelman) Colombia	3	+	-	-
82 (Kelman) Colombia	3	+	-	-
138 (Kelman) Costa Rica	2	-	-	+
70 (Kelman) Costa Rica	2	-	-	+
Au Reu (Digat) Reunion	1	-	-	+
P. mallei (Dodin 3709)		+	-	-

LITERATURE CITED

BUDDENHAGEN, I. W., L. SEQUEIRA and A. KELMAN. 1962. Designation of races Pseudomonas solanacearum. Phytopathol. 52:726 (Abstr.)

CASTELLANI, A. 1902. Die agglutination genischter infectation und die Diagnose der Letzteren. Z. Hygiene 40:1.

CHAMBON, L. and J. FOURNIER. 1956. Contribution antigenique de Malleomyces pseudomallei. I. Caracteres immunologiques, culturaux, biochimiques et variations de type immunologique. Ann. Institut Pasteur 91:355-362. II. Demonstration de l'existence des antigenes M, K et O et etude de leurs proprietes immunologiques. Ann. Institut Pasteur 91:472-485.

COLENO, A., M. LENORMAND and M. R. BARZIC. 1971. A qualitative study on the antigens involved in the complement fixation reaction and some phytopathogenic pseudomonads. Proc. Third Int. Conf. on Plant Pathogenic Bacteria Wageningen 1971: PUDOC 143-150.

COLENO, A. 1973. Contribution a l'etude taxonomique de quelques Pseudomonas phytopathogenes. These Doct. es Sciences CNRS AO. 8556.

COLENO, A. and M. RIDE. 1973. Serology of phytopathogenic bacteria. Third Int. Congress of Plant Pathology. Abstract:323.

CRAVITS, L. and W. R. MILLER. 1950. Immunological studies with Malleomyces pseudomallei and Malleomyces mallei. J. Inf. Dis. 86:46-51; 56-62.

DIGAT, B. and A. ESCUDIE. 1967. Reconnaissance du fletrissement bacterien des Solannees aux Antilles francaises. Phytiatrie-Phytopharmacie 16:187-197.

ESCUDIE, A. and B. DIGAT. 1965. Diagnostic rapide du fletrissement bacterien des Solanees par le test de sero-agglutination. Agronomie Tropical 6-7:640-643.

FOURNIER, J. 1967. Les antigenes thermostables de Pseudomonas pseudomallei et de Malleomyces mallei et leurs communautes. Ann. Institut Pasteur (Paris) 112:93-104.

HARRISON, D. E. and M. FREEMAN. 1961. Bacterial wilt of potatoes. II. Ecological relationship of two strains of Pseudomonas solanacearum and a culture of Corynebacterium sepedonicum. Austr. J. Agr. Res. 12:872-877.

KAUFFMAN, F. 1950. The diagnosis of Salmonella types. C. Thomas, Springfield.

KELMAN, A. 1954. The relationship of pathogenicity in Pseudomonas solanacearum to colony appearance on a tetrazolium chloride medium. Phytopathology 44:693-695.

KOJIMA, E. S. and I. W. BUDDENHAGEN. 1969. Antigenic relationships of Pseudomonas solanacearum. Phytopathology 59:1035-1036 (Abstr.)

LUCAS, L. T. and R. G. GROGAN. 1969. Serological variation and identification of Pseudomonas lachrymans and other Pseudomonas nomenspecies. Phytopathology 59:1908-1912.

LUCAS, L. T. and R. G. GROGAN. 1969. Some properties of specific antigens of Pseudomonas lachrymans and the Pseudomonas nomenspecies. Phytopathology 59:1913-1917.

MORAES, A. 1947. Uma bacteriose vascular da batata (Bacterium solanacearum E.F. Smith). Agron. Lusit. 9:277-328.

MORTON, D. J., P. D. DUKES and S. F. JENKINS. 1965. Serological identification of Pseudomonas solanacearum in four solanaceous hosts. Phytopathology 55:1191-1193.

MORTON, D. J., P. D. DUKES and S. F. JENKINS. 1966. Serological relationship of races 1, 2 and 3 of Pseudomonas solanacearum. Plant Dis. Rept. 50:275-277.

NICHOLS, I. 1930. Cited by L. Chambon et J. Fournier (1956).

OKABE, N. and M. GOTO. 1954. Studies on Pseudomonas solanacearum. VI. A comparative study of the colony type organisms F and Op. Shizuoka Univ. Fac. Agr. Rept. 4:41-60.

OUCHTERLONY, O. and L. A. NILSSON. 1973. Immunodiffusion and immunolectrophoresis. In Handbook for Experimental Immunology. 19-39. D. M. Weir. Blackwell Scientif. Publication 19-1.

PEREZ, J. E. 1962. The use of Fuller's foramide method in the serological identification of Pseudomonas solanacearum. J. Agr. Univ. Puerto Rico 46:144-153.

QUINON, T. L., M. ARAGAKI and ISHII. 1964. Pathogenicity and serological relationship of three strains of Pseudomonas solanacearum in Hawaii. Phytopathology 54:1096-1099.

BACTERIOCIN PRODUCTION BY PSEUDOMONAS SOLANACEARUM

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Forty-eight Pseudomonas solanacearum isolates from 11 different hosts and nine different regions of the world produced bacteriocins that selectively inhibited other strains of the same species. These isolates were bacteriocinogenic for at least one of 53 indicator isolates. Seventeen potato isolates assayed against each other (A), as well as against 21 tomato-tobacco isolates (B) and 15 banana-plantain isolates (C), were bacteriocinogenic in 31% (A), 25% (B) and 31% (C) of the possible combinations. In contrast, the tomato-tobacco isolates were active in 57% (A), 57% (B) and 71% (C), and the banana-plantain isolates active in 42% (A), 58% (B) and 45% (C) of the combinations. Potato isolates belonging to biotypes II, III and IV (13) were weakly bacteriocinogenic, whereas biotype I isolates (4) were highly active. Initial data suggests that P. solanacearum isolates could be typed by bacteriocin production when the banana-plantain isolates are used as indicators. Only four of the 48 isolates could not be typed with these indicators. One typing pattern predominated which was characteristic of 13 different isolates; this pattern was not associated with a particular host or locality from which the isolate was obtained. However, there was a typing pattern which appeared to be characteristic of certain plantain strains of northern Peru.

Certain bacteriocin-sensitive strains produce resistant colonies in the inhibition zones formed following exposure to bacteriocin. The resistant colonies of one strain (#168) which was initially characterized as weakly bacteriocinogenic were found to be active producers of bacteriocin.

SELECTIVE MEDIUM FOR ISOLATION OF *PSEUDOMONAS SOLANACEARUM* FROM SOIL

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A selective medium was developed for studying survival of *Pseudomonas solanacearum* in soil, since media developed by others were unsatisfactory in our studies. The medium consisted of 4 g dextrose, 10 g peptone, 1 g casamino acid, 1 g yeast extract, 18 g agar, 1000 ml deionized water and a mineral stock solution containing: 1.18 g K_2HPO_4, .44 g KH_2PO_4, 1.32 g $(NH_4)_2SO_4$, .2 g $MgSO_4 \cdot 7H_2O$, .0015 g $MnSO_4 \cdot H_2O$, .0016 g $Zn\ SO_4$, .003 Ferric citrate. Fifty ppm bacitracin, 10 ppm vancomycin, 20 ppm tyrothracin, 5 ppm chloromycitin, 1 ppm penicillin G, 20 ppm pimaricin, 100 ppm polymixin B, 500 ppm benomyl, 100 ppm Dichloran, 30 ppm pentachloronitrobenzene, 50 ppm actidione and 100 ppm chloroneb were added as antimicrobial agents. Growth of other soil bacteria was reduced 99 percent, allowing quantification of *P. solanacearum* as low as 10^4 cells/g soil. Much lower populations were detectable, but could not be quantified apparently due to the highly inhibitory nature of the remaining soil microbes. Although fungi grew poorly on this medium without the fungicides, germinating spores were often inhibitory to *P. solanacearum* unless the fungicides were added. Isolate K-60 of *P. solanacearum* was used as the test bacterium in the development of the medium. Many other isolates grew well on the medium, while some were partially or completely inhibited. Detection of 84% of an initial population placed in nonsterile soil was possible with the medium. On this medium, *P. solanacearum* colonies were similar in appearance to those observed on Kelman's tetrazolium medium with virulent and avirulent colony types distinguishable.

FACTORS AFFECTING SURVIVAL OF PSEUDOMONAS SOLANACEARUM

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Vertical distribution and population changes of *Pseudomonas solanacearum* in naturally infested tobacco field soils and environmental factors affecting its survival were investigated by the dilution plate method, using a modified Drigalski's medium and by the indicator plant method using susceptible tobacco cultivar Hicks.

The distribution of the pathogen was observed in naturally infested field soils (sandy loam) of the 0-80 cm layer. High infestation levels were observed at 0-50 cm in soils immediately after harvesting. When fields were left fallow, population of the pathogen remained at a high level (10^2-10^5 cells/ml fresh soil) at all depths for one year. The pathogen had practically disappeared 2 years later. It increased rapidly in steam-sterilized water (pH 7.0), sterilized river water (pH 7.23) and in water-saturated, sterilized volcanic ash (Hatano) soil (pH 6.10) at 20°C or more and survived longer than 350 days. It disappeared in 100 days at 10°C. In nonsterilized Hatano soil, the pathogen disappeared in 70 days at 3°C. Air-dried on glass slide, it survived for less than 5 days at 30°C. In sterilized, air-dried, heavily fertilized soil (60 percent manure) kept at more than 40 percent moisture-holding capacity, the pathogen grew vigorously at first at 30°C and survived for longer than 50 days. This growth was more vigorous with a higher water content (80-100 percent), but under identical conditions no growth was observed in nonsterilized soil. In surface soil with high levels of organic matter and microbes, the population fell faster than in the subsoil with a lower content of these. Addition of manures to infested subsoil reduced considerably the population of *P. solanacearum* and wilt severity. However, the addition of chitin (10 g/10 l soil) did not appear to reduce disease development.

COMPARISON OF SURVIVAL OF TWO PSEUDOMONAS SOLANACEARUM STRAINS IN SOIL COLUMNS UNDER CONSTANT PERFUSION AND IN FIELD PLOTS DEVOID OF HOST COVER

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ABSTRACT

Changes in populations of Pseudomonas solanacearum biochemical type II and III with time were followed after inoculating cylindrical water-saturated soil cores (20 cm. x 5 cm.) with about 10^8 or 10^{11} cells/ml. in sterile distilled water. The cores were kept saturated and well aerated in the automatic perfusion apparatus described by Audus (Nature 158:419, 1946).

Populations of both types increased initially, reaching a peak in 3 to 4 days. This increase was not exponential and determinations of growth rates on assay plates showed that cells were entering the resting phase. Resting cells declined exponentially with type II having a far greater rate of decline than type III. Field experiments confirmed the latter observation. However, survival was enhanced in the field, under wet conditions, as compared to survival in the soil cores under constant perfusion. The reasons for differences in survival ability under laboratory and field conditions are discussed.

INTRODUCTION

Differences in soil survival abilities (longevity) among strains of Pseudomonas solanacearum may in part be due to differences in innate capacities to survive (4). The work reported here is an attempt at directly determining this fundamental property in two Kenya strains. Field data are included to ascertain laboratory results.

In the laboratory, survival was determined in soil columns under constant perfusion in an Audus apparatus (3). In this apparatus, a small volume of water rises from a reservoir to the top of the soil column, percolates through it and returns back into the reservoir. This process is continuous and automatic. The apparatus was chosen because it is designed to minimize biological variation between soil samples thus lending itself to reproducibility of results (3).

MATERIALS AND METHODS

Field experiments

Survival of biochemical type II (Race 3) of P. solanacearum (9, 5, 4) was determined in 72 3m x 6m plots which were used for two consecutive years (1964-1966) to screen potato clones for resistance to this bacterium (10). The soil could therefore be considered to be naturally infested. Survival of type III Race 1 was (9, 5, 4) determined on naturally infested land accidentally discovered in 1965 when a number of wilting Datura stramonium plants growing as weeds were found on the plot. Host range studies showed that type II isolates did not wilt Datura, but type III did (1). Land contaminated with type

III consisted of only eight 3m x 6m plots. These plots were about 4m away from the type II plots.

In 1967, the level of initial inoculum in each type II and III plot was determined by planting 100, 3-week old tomato seedlings (cultivar "Early Beauty") on each plot and recording the number of plants that had wilted 100 days later (2). Before planting, the soil in each plot was prepared to a fine tilth, care being taken to avoid cross-contamination. To supplement natural rainfall, seedlings were irrigated by means of overhead sprinklers which were turned on twice daily for 2 hours.

After initial inoculum determination, 36 type II plots, randomly chosen, were kept free of plants by constant hand weeding. Thirty-six plots were planted to Kikuyu grass (Pennisetum clandestinum Hochst. ex Chiov.) which became well established in 1 month. The type III plots were left bare.

Four indirect determinations of residual inoculum were made in nine type II plots and two type III plots 6, 12, 18 and 24 months.

Perfusion experiments

Perfusion units as described by Audus (3) were used. The soil column of each contained cylindrical cores 20 cm. x 5 cm. diameter taken next to each other in a type II plot after removing 1 cm. of top soil. Moisture holding capacity was determined on a third core.

Cores were perfused with 100 ml. sterile distilled water. The volume of water entering the cores was kept small by placing the air intake port just above the water level in the reservoir. Rate of perfusion was controlled by regulating the suction pressure which was provided by an aspirator.

Three days after perfusion, one of the cores was inoculated with type II bacteria and the other with type III bacteria, by momentarily stopping perfusion and adding 20 ml. of inoculum to the cores. Inocula were made by suspending bacteria from a 48-hour agar culture in sterile distilled water at 10^{10} or 10^{11} cells/ml.

Densities of P. solanacearum were followed from 1 day after inoculation, by small samples (about 1 gm.) from the top of cores. Exact wet weights were determined and converted to dry weights using a single moisture determination made eight days after inoculation.

Bacteriological methods

The types II and III cultures used in the perfusion experiments were isolated from a wilting potato and Capsicum plant obtained from a type II and III plot, respectively. Kelman's tetrazolium agar was used in all cases (7). Plates were incubated at 31°C.

Bacterial populations in cores under perfusion were determined as follows: each sample was shaken (for 2 min.) in 10 ml. sterile distilled water and six ten-fold serial dilutions were made of the supernatant fluid. Samples (0.01 ml.) from each of the 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} dilutions were spread on each of 10 agar plates. After incubation colony counts were made and converted to numbers/gm. dry soil.

Growth rate (number of doublings in 48 hours) of P. solanacearum on solid medium was determined by estimating number of cells/colony and

transforming to $\log_2$. Number of cells/colony were estimated from suspensions of 10 colonies in 10 ml. sterile distilled water.

To determine bacterial densities in field soil, 20 cm. x 20 cm. core samples were taken and diluted by the method of Johnson et al. (6). Colony counts were made as above using the 10^{-3}, 10^{-4} and 10^{-5} dilutions.

RESULTS

Field experiments

Average initial proportion of wilting to healthy plants in the 72 type II plots was $P_0 = 0.335$, with five plots having $P_0 = 0.04$ and seven plots having $P_0 = 0.60$. In the type III plots, $P_0 = 0.260$. After six months and 12 months, the final proportion of plants wilting (P) was correlated with P_0 (Fig. 1). This correlation was poor at 18 months and 24 months (Fig. 1).

Average log P/P_0 decreased linearly with time in months (Fig. 2). In the type II plots P/P_0 was >1 at 6 and 12 months and became <1 at 18 months and 24 months. In the type III plots P/P_0 was >1 even at 24 months. P/P_0 was >1 because of the increase in inoculum during P_0 determination, which occurred because of discharge of bacteria into soil from wilted plants.

Comparison of lines by covariance analysis showed that the type II grass plot and bare plot lines were similar (Fig. 2). The type III line differed in position but not in slope. The higher position of the type III line indicated that more type III inoculum survived after each period.

When P_0 was determined, P. solanacearum densities in soil under wilted plants varied from 1.39×10^8 to 2×10^9. The average of eight determinations (four from type II plots and four from type III plots) was 1.48×10^9. In soil away from wilted plants, only an occasional P. solanacearum colony appeared on the plates. A rough estimate of initial numbers under wilted plants was made as follows: an average of 1.48×10^9 cells/gm. dry soil occurred in 20 cm. x 20 cm. cylindrical cores taken from under wilted plants on plots with an average of 34 ($P_0 = 0.335$) wilting plants. Hence 5×10^{10} cells/gm. soil was contained in a total volume of 1.5×10^4 cm^3. On cultivation this volume was diluted to 3.6×10^6 cm^3 (6m x 3m x 20 cm. deep) giving a dilution factor of 4×10^{-3} and a final concentration of approximately 2×10^8 cells/gm. dry soil.

Perfusion experiments

Population trends of type II were determined in three separate experiments and that of type III in four separate experiments. Fresh cores were used in each experiment.

The moisture holding capacity of undisturbed soil was 75.1% at saturation and 39.8% at 0.1 atmospheres. Single moisture determinations at eight days after inoculation in the first three experiments were 73.4%, 73.9%, 71.6%, 69.2%, 72.1% and 69.6%. In experiment four, moisture of cores was determined at each sampling. From 2 to 18 days the moisture varied from 67% to 71% and at 66 days moisture content fell to 59%, but this was accompanied by slight compacting of the core. Accumulation of water at the top of cores did not occur.

At the beginning of perfusion the water in the reservoir became turbid but cleared in three days. On inoculation, the water became turbid again

but cleared a day later indicating that the inoculated bacteria had firmly settled in the soil. Bacterial density determinations were therefore begun a day after inoculation.

Density of saprophytes was 10^7 cells but never exceeded 10^8 cells/g. (Fig. 3). The effect of saprophytes on the appearance of P. solanacearum colonies was evident only when P. solanacearum densities dropped $<10^7$ cells. No antagonism was noticed. When P. solanacearum densities fell below 10^6, most probable number of estimates were made (8). Densities below 10^5 could not be estimated.

After inoculation of soil cores, densities of both types increased reaching a peak in three to five days (Fig. 3). The increase, which was not exponential, occurred even after residual growth in the inoculum was eliminated by leaving bacteria in suspension for 48 hours at 24°C (experiments 2, 3 and 4). After the initial increase, a sharp decline of type II occurred while the type III declined very slowly. Fluctuations of densities occurred during the decline phase and with type III this fluctuation tended to mask the slow decline. Experiment 4, in which moisture determinations were made at each sampling, showed that the fluctuations could not be explained by variations in water content.

To compare rates of decline, linear regressions of log. I/I_0 were calculated (Fig. 4). I_0 was the highest density reached and I was the subsequent density of survivors. Time was measured from the day I_0 was reached. The type II line was based on readings of three experiments and the type III line on four experiments. The lines were highly significant, indicating that the rates of decline of the two types were vastly different (Fig. 4).

In a solid medium, type II cells doubled 27 times in 48 hours and type III cells 31 times. Four to five days later the number of doublings on assay plates fell to 15.6 and 22.9, respectively. After this time no further reduction in growth rate occurred. The final growth rate of both types was comparable to resting cells in sterile distilled water.

DISCUSSION

Results of the population density studies in the Audus perfusion apparatus (3) clearly showed that biochemical type III, Race 1 (9, 5, 4) had greater soil survival ability than type II, Race 3 (9, 5, 4). An initial increase of both types occurred, but this increase reached a peak in three to five days and was not exponential. Determinations of doubling rates on solid media suggested that cells entered the resting phase within this time. Decline of these resting cells was exponential. It was not possible to determine whether extinction occurred but densities declined below that of saprophytes.

Results of field experiments confirmed the superior survival ability of type III. However, in the field, under wet conditions, both types survived much longer than in the water-saturated cores under constant perfusion. This enhanced longevity was not due to differences in initial densities, since a rough calculation showed that initial densities in field plots were comparable to those in soil cores under constant perfusion.

In the perfusion apparatus, decreased longevity could not be attributed to water saturation because there was adequate aeration. But artificial

culture, the method of inoculum preparation and the method of inoculation either minimized or abolished factors which normally contribute to enhancement of longevity in soil, such as variation in physiological ages of clones, aggregation of cells, and asynchronous inoculation. What was assayed in the soil cores under constant perfusion was the innate capacity for survival of the bacterium in the soil.

Literature cited

1. Annual Rept. Dept. Agric., Kenya. 1966. Vol. 11:14-20.

2. Annual Rept. Dept. Agric., Kenya. 1967. Vol. 11:17-20.

3. AUDUS, L. J. 1946. A new soil perfusion apparatus. Nature (London) 158:419.

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

5. HAYWARD, A. C. 1964. Characteristics of Pseudomonas solanacearum. J. Appl. Bact. 27:265-277.

6. JOHNSON, L. F., E. A. CURL, J. H. BOND and A. H. FRIBOURG. 1960. Methods For Studying Soil Microflora-Plant Disease Relationships. Burgess Publishing Company, Minneapolis, U.S.A. 178 P.

7. KELMAN, A. 1954. The relationship of pathogenicity of Pseudomonas solanacearum to colony appearance in a tetrazolium medium. Phytopathology 44:693-95.

8. MEYNELL, G. G. and E. MEYNELL. 1970. Theory and Practice in Experimental Bacteriology. Cambridge University Press, Cambridge. 346 P.

9. ROBINSON, R. A. and A. H. RAMOS. 1964. Bacterial wilt of potatoes in Kenya. E. Afr. Agric. For. J. 30:59-64.

10. ROBINSON, R.A. 1968. The concept of vertical and horizontal resistance as illustrated by bacterial wilt of potatoes. Commonwealth Mycological Institute, England. Phytopathological Papers, No. 10. 37 P.

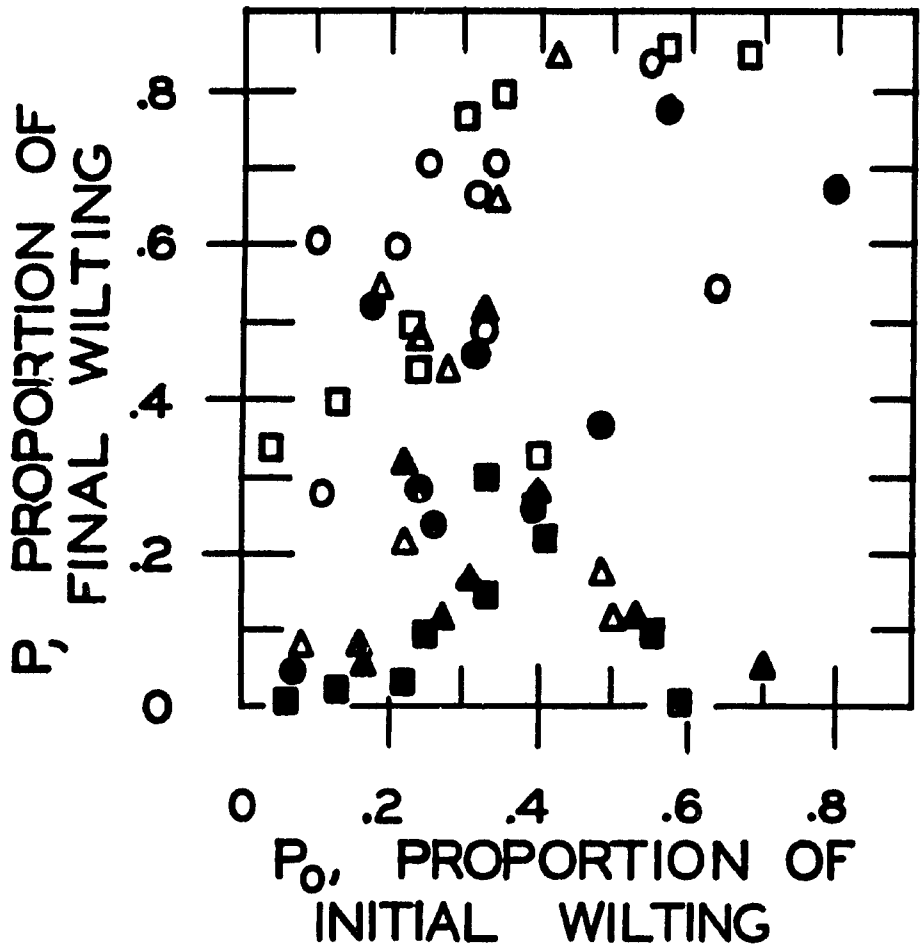

Fig. 1. Relationship between P_0 proportion wilting before and P proportion wilting after the bare fallow and grass fallow treatments.

Bare fallow 6 months □, P = 0.74
Bare fallow 24 months ■, P = 0.22
Kikuyu grass 6 months ○, P = 0.71
Kikuyu grass 12 months ●, P = 0.73
Kikuyu grass 18 months △, P = 0.41
Kikuyu grass 24 months ▲, P = 0.11

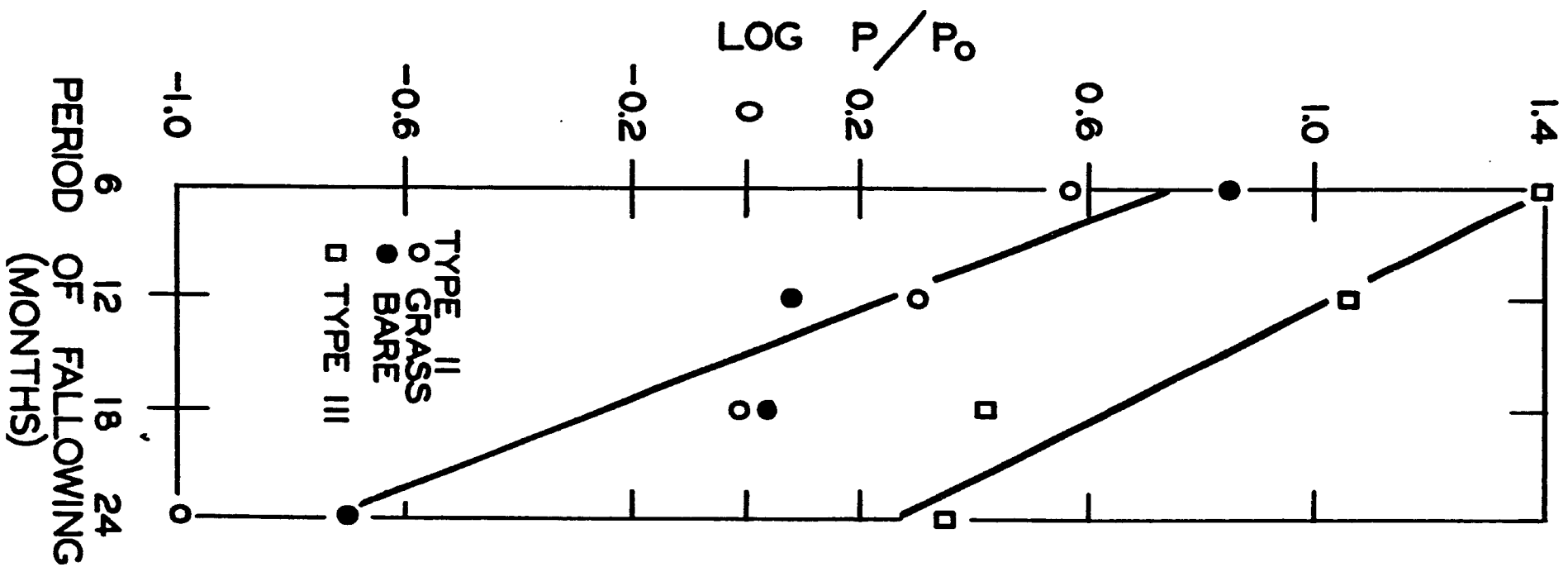

Fig. 2. The relationship between $\frac{P}{P_o}$ and period of fallowing in months.

P_o = proportion of initial wilting, P = proportion of final wilting.

Type II line grass, bare } 1.238 - 0.0826x

Type III line Y = 1.792 - 0.0645x

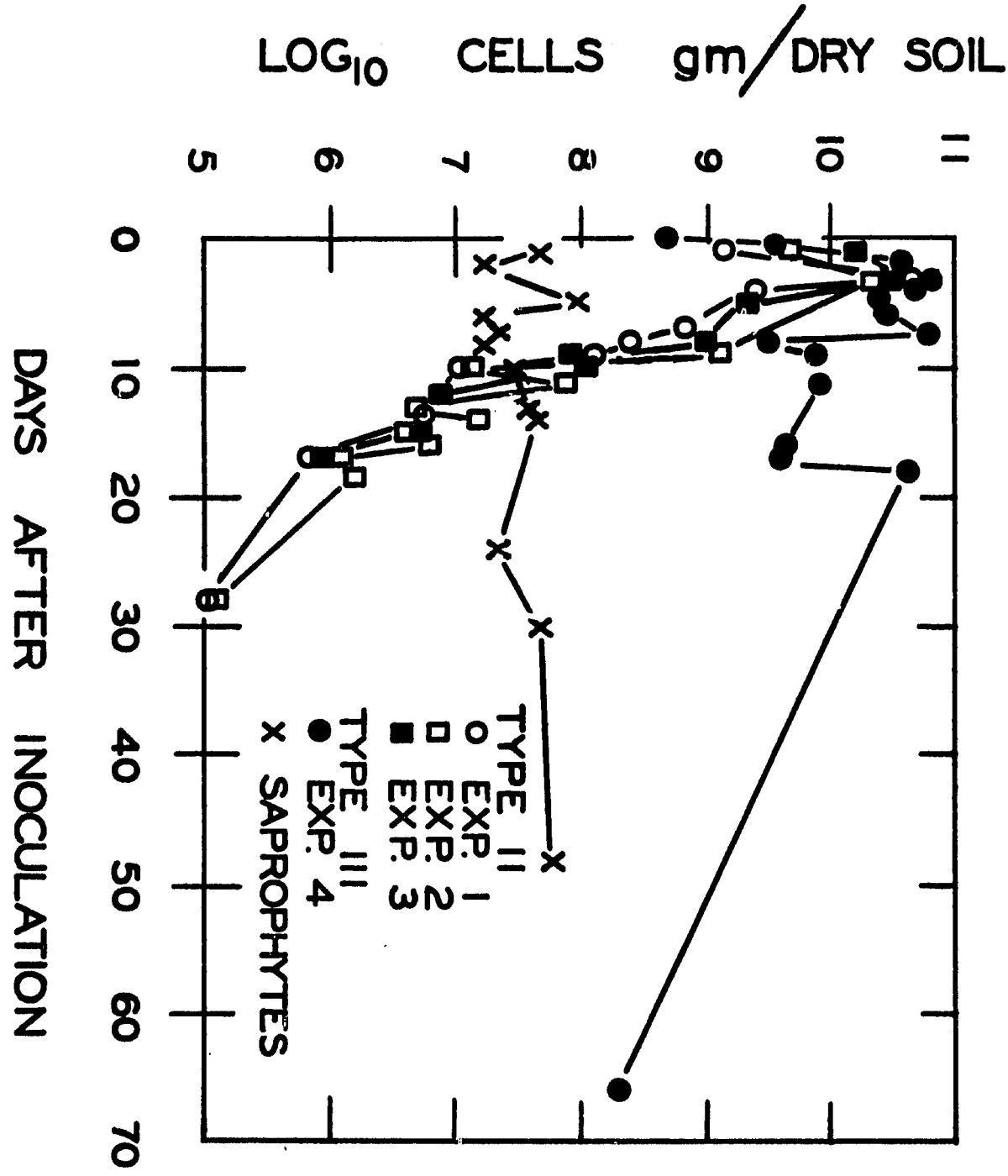

Fig. 3. Population density trends of P. solanacearum and of saprophytes in soil columns under constant perfusion.

Exp. 1
Exp. 2 Type II
Exp. 3
Exp. 4 Type III
Saprophytes

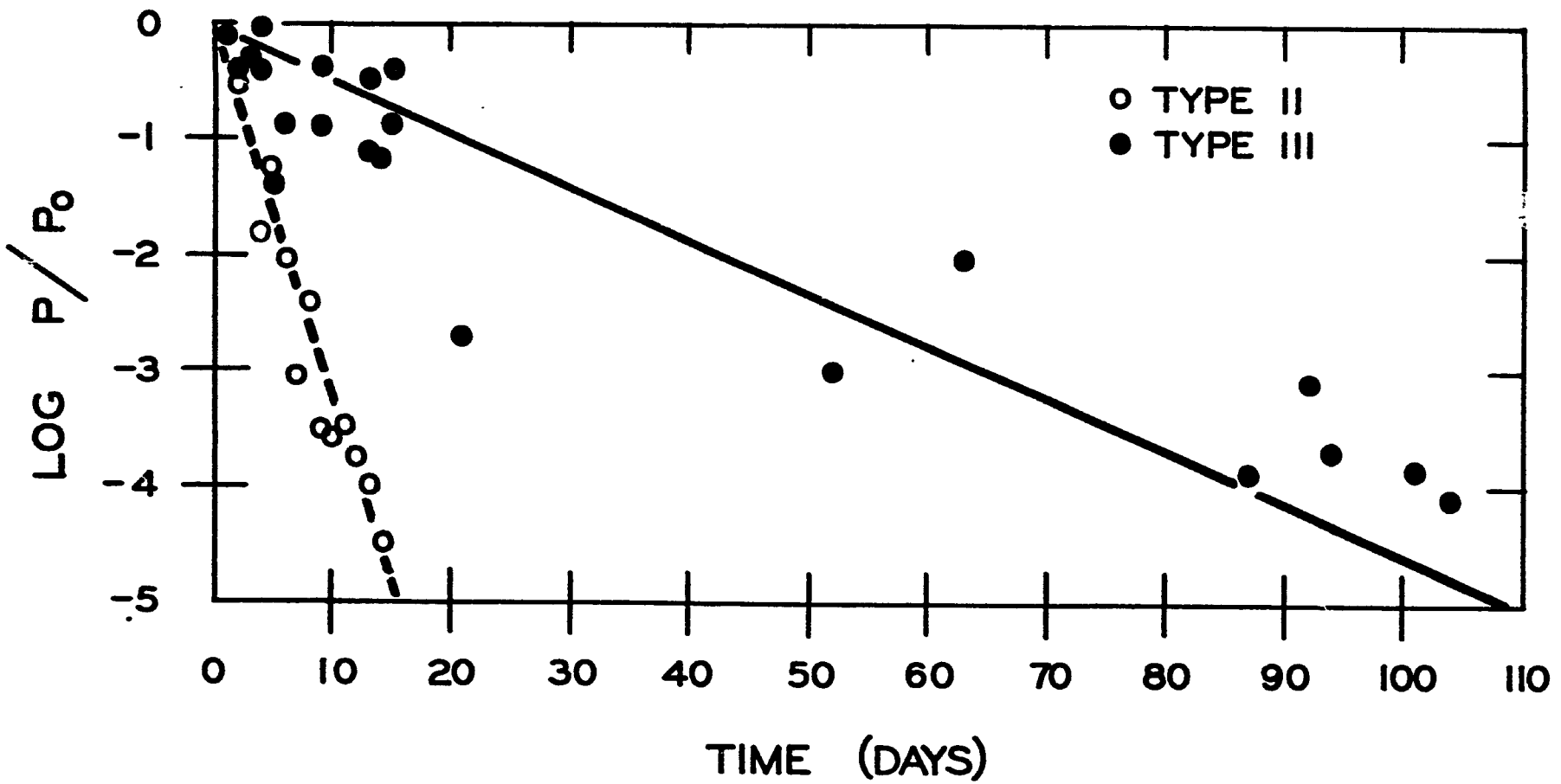

Fig. 4. Decline of P. solanacearum population densities in soil cores under constant perfusion.

o—o Type II Y = -0.324x

●—● Type III Y = -0.039x

BACTERIAL WILT OF TOMATO IN GEORGIA AND SURVIVAL OF *PSEUDOMONAS SOLANACEARUM* IN GEORGIA SOILS

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In Georgia, *Pseudomonas solanacearum* causes economic losses mainly on tomatoes, although several other solanaceous crops are grown. Bacterial wilt is important in field and greenhouse tomatoes cultured for fruit production and in tomato transplants produced in southern Georgia for shipment to more northern areas. The greatest threat of bacterial wilt is on the approximately 4,000 acres of field-grown transplants in which as many as 354 acres (1969) have been rejected for certification because of the disease. Presently, the primary control measures in transplant production are land selection to avoid infested fields and crop rotation. Various general purpose fumigants are currently being evaluated for control of bacterial and other soil-borne plant pathogens. The Venus and Saturn cultivars are planted by some home gardeners where other cultivars cannot be produced because of bacterial wilt.

Psuedomonas solanacearum persisted 4 years in two field soils artificially infested by clipping beds of young tomato plants with a contaminated mower followed by soil incorporation of the resulting diseased plants. The infestation remained high in one soil (Marlboro loamy sand) as indicated by 99.0, 91.0, 95.2, and 98.1% infection of Marion tomato plants during the 1972-75 test period. In the second soil (Appling sandy loam), 93.0% of Marion plants were infected the second year after the soil infestation, but infection percent dropped to 65.9, 60.8 and 63.1 during the following 3 years.

SURVIVAL IN THE SOIL AND VIRULENCE OF PSEUDOMONAS SOLANACEARUM

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The effect of 16 different types of soils and relation of their water-holding capacity, field capacity, percentage of sand, silt, clay, organic carbon, calcium carbonate, nitrogen, phosphorous, potash, electrical conductivity, pH, cation exchange capacity and population of bacteria, actinomycetes, penicillia and aspergilli of other fungi on survival of Pseudomonas solanacearum causing wilt in tomato was studied. Simple and multiple correlations were also studied. The data showed that all differences in pathogen population and disease indices in different soils were statistically significant. Wilt incidence was found to decrease with the decrease in pathogen population and vice versa.

Medium black soil of Jabalpur which was clay in texture, mixed red and black soil of Rewa (clay loam), shallow black soil of Seoni (clay loam), alluvial soil of Gwalior (sandy loam) gave highest (54.5-71.5) disease indices. Red and yellow soil of Balaghat (sandy clay loam), red and yellow soil of Nurabad (loamy sand), alluvial soil of Morena (loamy sand) and deep black soil of Pawarkheda (clay) gave lowest (25.0-36.5) disease indices.

Physical, chemical, physico-chemical and biological properties of soil affect the pathogen population and wilt incidence as evidenced by significant positive multiple correlations. Correlation studies indicated that the wilt incidence increases with the increase in water-holding capacity, silt, clay, electrical conductivity, cation exchange capacity, pH, organic carbon, and with the decrease in field capacity, sand, calcium carbonate and population of actinomycetes and penicillia of soils.

Nitrogen, phosphorous and potash content of soil and population of bacteria, aspergilli and other fungi in these soils had no effect on pathogen populaton and wilt incidence.

BACTERIAL WILT IN A COLD-TEMPERATURE CLIMATE OF AUSTRALIA

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Bacterial wilt, caused by Pseudomonas solanacearum, is an important disease in Australia of potato, tomato, ginger, eggplant, capsicum, and to a lesser extent, townsville stylo (Stylosanthes humilis) and tobacco. The disease is mainly confined to the tropical, sub-tropical and warm temperate regions of Australia, but surprisingly, does occur in potato crops grown in certain cool temperate regions such as parts of the Northern Tablelands of New South Wales (Dorrigo) where the disease is endemic (Conroy, 1969; Lloyd, 1975).

Bacterial wilt is endemic to the Dorrigo region of the Northern Tablelands mainly because some local growers continue to benefit financially from the practice of importing potato seeds for late sowing from sub-tropical regions where bacterial wilt is prevalent (Lloyd,1975). It is this intermittent introduction of diseased tubers which ensures the continual presence of bacterial wilt disease in some potato crops of the Dorrigo region.

From our field observations and field trials (Shamsuddin and Lloyd, unpublished data) it now appears that in such a cool temperate climate as that of Dorrigo, unlike a tropical climate, alternative weed hosts are of little consequence. Rather, the bacterium appears to survive in soil for up to 2-3 years, protected within previously colonized pieces of host debris. Presumably, cold soil temperatures delay microbial degradation of the pathogen's habitat, thus allowing the pathogen to survive for a long time within host-plant debris. This tentative hypothesis is based on the following observations:

a) The biotype present (biotype II, race 3) has a restricted host range with a few alternative weed hosts. Moreover, these alternative weed hosts do not survive in a vegetative state throughout the cold winter months when winter temperatures often fall below freezing point and snow is not uncommon for part of the winter.

b) In extensive field trials covering some 3 years (Shamsuddin and Lloyd, unpublished data), it has been shown that infested soil left bare-fallowed or weed-fallowed for 1½ years and then replanted to potatoes, produced a large number of diseased potato plants, and a few diseased plants after 2½ years (Table 1). This demonstrates that in a cool temperate region such as the Northern Tablelands of N.S.W., sufficient numbers of the pathogen can survive in soil between harvest of a diseased crop in summer and subsequent replanting to another susceptible crop during the following spring, a common practice on the Northern Tablelands.

c) When similarly infested experimental plots were sown to pasture for 1½ years and then resown to potatoes, only a few diseased plants were recorded at the edge of the experimental plots, and no diseased plants were recorded following 2½ years in pasture (Table 1). Cultivation of the infested soil to prepare a pasture seed bed and the increased microbial activity stimulated by the presence of grass roots, may have both contributed to microbial degradation of infected plant debris and thus hastened the destruction of the pathogen's habitat.

d) Some potato growers on the Northern Tablelands who did experience bacterial wilt in their potato crops during the 1960's, have successfully eliminated the disease from their property. These growers, with assistance from the Department of Agriculture, instigated the following practices: effective farm quarantine; purchase of certified potato tubers from crops known to be free from bacterial wilt disease; and resowing infested soil to pasture for several years.

e) Infested field soil containing infected plant debris, when placed in pots and kept moist in a greenhouse at temperatures simulating those of a more tropical climate (28°C day, 24°C night), produced healthy tomato plants within 20 weeks of soil storage. Conversely, infested soil left undisturbed in the field for 20 weeks and then potted and sown to tomatoes, produced diseased plants. Thus it appears that in a warm moist disturbed soil, the pathogen cannot survive in detectable numbers for more than about 20 weeks, probably due to enhanced microbial activity resulting in decomposition of the infected pieces of plant debris which may be the pathogen's habitat.

In summary, it appears that in a cool-temperate climate, unlike a tropical climate, alternative weed hosts are of little importance to survival of P. solanacearum biotype II. Instead, the pathogen probably survives in these cold soils between harvest and replanting of a susceptible crop the following season, within previously colonized pieces of host debris. Thus any practice which tends to enhance breakdown of plant debris, such as soil disturbance during cultivation, and the vigorous microbial activity associated with the roots of a grass pasture, should result in accelerated degradation of the pathogen's habitat, exposing the pathogen to the rigors of the soil environment and increased competition from the soil microflora. Under such adverse conditions, P. solanacearum may well be a poor competitor and would therefore soon vanish from the free soil.

References

Conroy, R. J. 1969. Bacterial wilt or brown rot of potatoes. Agric. Gaz., N.S.W. 80: 175-8.

Lloyd, A. B. 1975. Grower attitudes to bacterial wilt of potatoes. J. Australian Inst. Agric. Sc. 41: 215-216.

Table 1. Mean numbers of diseased plants in experimental plots either bare fallowed, weed fallowed or sown to pasture for 1½ or 2½ years, and then resown to potatoes

Treatment*	Length of treatment 1½ years	2½ years
Bare-fallowed soil	45	1
Weed-fallowed soil	56	4
Pasture	8	0

* Mean number of diseased plants per experimental plot prior to commencement of treatment was 68.

CONTROL OF BACTERIAL WILT OF POTATOES IN VICTORIA, AUSTRALIA BY EXCLUSION

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All local isolates of Pseudomonas solanacearum examined in the State of Victoria have been identified as Biotype II (Hayward) or Race 3 (Buddenhagen et al) and have been obtained from potatoes.

Early this century, the disease caused considerable damage in parts of the area known as Kooweerup Swamp, and by about 1950 it was found to be causing some concern in the hilly areas around Gembrook. In 1969, it was detected in a commercial (ware) crop at Thorpdale, one of the main seed production areas and, in the following three years, in ware crops in the Toolangi-Kinglake region, adjacent to another seed-growing district.

Because of the threat to the seed potato industry in Victoria, the major seed-producing state in Australia, and particularly in view of the success of the pathogen-tested seed scheme, an industry-supported eradication program was commenced in 1972. This was based on the local evidence of the dominant role of tuber transmission of the pathogen.

Once the disease was detected in a paddock, the grower was issued with a "Notice to Control Parasites" and the procedures to be adopted. These included a prohibition on growing potatoes in the affected land for 5 years, potatoes from the affected crop could not be used or disposed of for planting, and infected tubers had to be destroyed by deep burial or cooking for stock food.

The emphasis was on the prohibition of potato growing for 5 years. Thus, once growers realized that this would happen with each new outbreak, they adopted the suggested control measures, particularly the introduction of known bacterial wilt-free seed from areas where the disease does not occur.

Although it is still too early to assess the full impact of this program, it has clearly had a very great effect, not only in controlling the spread of the disease, but in apparently eradicating it from the areas into which it had more recently been introduced.

SOME TECHNIQUES OF IMPORTANCE IN THE IDENTIFICATION OF PSEUDOMONAS SOLANACEARUM

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1. Staining properties and extraction of poly-β-hydroxybutyrate

The observation that P. solanacearum in culture, or examined directly in ooze from affected plants, exhibits bipolar staining was made when the species was first described (11). It is now known that the lack of affinity for basic dyes which results in bipolar staining is caused by the presence in the cells of massive inclusions of poly-β-hydroxybutyrate (3). There are three complementary observations which indicate the presence of this substance: bipolar staining when heat fixed smears are stained with dilute basic fuchsin or a dilute solution of safranin; the appearance of refractile, phase bright inclusions, under the phase contrast microscope; and staining of the inclusions by a solution of Sudan black B according to the method of Burdon (2).

The following procedure for sudan staining may be applied to heat fixed smears of bacterial ooze expressed from the host plant or of cultures on an appropriate artificial medium.

Sudan black stain: Sudan black B powder, 0.3 g; 70 per cent ethyl alcohol, 100 ml. Shake thoroughly at intervals and stand overnight before use. Keep in a well stoppered bottle.

Procedure: 1) Make a film, dry in air and fix by flaming; 2) Cover the entire slide with Sudan black stain and leave at room temperature for 15 min; 3) Drain off excess stain, blot, and dry in air; 4) Rinse thoroughly with water under the tap and again blot dry; 5) Counterstain lightly by covering with 0.5 per cent aqueous safranine or dilute carbol fuchsin for 5-10 sec; rinse with tap water, blot and dry. Lipid inclusion granules are stained blue-black or blue-grey, while the bacterial cytoplasm is stained light pink. The method given above is that of Burdon (2), except that in step 4) rinsing is done with tap water instead of xylol.

Inclusions of poly-β-hydroxybutyrate are formed in greatest amount on media of high carbon-nitrogen ratio. The medium of Kelman (7) is suitable, or that of Hayward (3). It should be noted that inclusions of this type are formed by many aerobic, Gram-negative bacteria; however, there are few other bacteria causing a wilt in which this property is found (P. caryophylli is one example).

Since Sudan black B is a lipid stain of general use not specific for poly-β-hydroxybutyrate it may be necessary to confirm that the inclusions consist of this substance. Extraction may be achieved by the following method: 1) Collect about 250 mg of dried cells from a suitable agar or liquid medium. The cells should be centrifuged, washed once or twice in sterile distilled water, and dried to constant weight in a hot air oven at 110°C. Alternatively the cells may be dried in acetone and the acetone powder obtained used for extraction. 2) Grind the heat-dried cells to a powder with pestle and mortar. 3) Suspend the dried cells in 20-30 ml of chloroform and heat

over a hot plate until gentle boiling occurs. 4) Filter the hot chloroform into 3 volumes (60-90 ml) of diethyl ether. Note the appearance of a white flocculent precipitate. 5) Filter off the white flocculent precipitate and allow to dry in air.

CAUTION: steps 3 to 5 inclusive should be done in a fume hood. If fume hood facilities are not available the extraction by this method should not be attempted! On oxidation chloroform gives phosgene (carbonyl chloride, $COCl_2$) which is an extremely poisonous, suffocating gas. Diethyl ether is a mobile, highly inflammable liquid, and mixtures of ether and air are explosive.

An alternative method of isolation of the inclusions of poly-β-hydroxybutyrate involves the use of an alkaline solution of sodium hypochlorite (13). This method is safe and requires only the equivalent of ca. 10 mg dry weight of organisms.

On destructive distillation inclusions of poly-β-hydroxybutyrate yield crotonic acid, a substance with a distinctive odor. The white powdered extract is heated gently in a narrow glass tube (150 mm x 3-4 mm internal diameter) to boiling. The distilled extract recondenses as crotonic acid on the cooler parts of the tube. Melting point determinations may be used to finally confirm the identity of the breakdown product(3).

2. Kovacs' oxidase test

There are various methods for carrying out this test. There are oxidase strips available commercially which should be used according to the manufacturer's instructions. The following method follows closely that originally described by Kovacs (8).

Place a Whatman No. 1 filter paper in a petri dish and add 3-4 drops of a freshly prepared 1% aqueous solution of tetramethylparaphenylenediamine dihydrochloride on the center of the paper. A platinum loop heavily charged with growth from a 24-48 hr culture of P. solanacearum on Kelman's medium (7) or on nutrient agar is rubbed in a band about 1 cm long across the reagent impregnated paper. With P. solanacearum the change to a purple color is usually instantaneous. Any change within 10 sec of application of the culture may be regarded as a positive result. The reagent solution should be made up weekly or fortnightly and stored in a stoppered dark glass bottle at 4°C.

CAUTION: The reagent is an aromatic amine and a highly toxic substance; skin contact with the powder or solution should be avoided!

Choice of medium may be important for the proper execution of this test. False positive reactions may occur where nitrite present in the medium reacts at once with the reagent to give a purple color. If, therefore, the medium contains nitrite, or, more probably, if the medium contains nitrate and the species in question (e.g. P. solanacearum) is able to reduce it to nitrite, an apparently 'positive' reaction is likely to ensue.

There is evidence that the N,N-dimethyl-p-phenylenediamine reagent is to be preferred to the tetramethyl form as it is less sensitive to nitrites which may be formed in the culture medium (5).

The mechanism of this test is not fully understood; there is evidence that the presence of cytochrome c is correlated with a positive reaction. Examination of the absorption spectrum of several oxidase negative bacteria has shown that they possess cytochromes of type b, and not cytochrome c which is invariably present in oxidase-positive bacteria (9).

The oxidase reaction is negative in the Enterobacteriaceae and positive in Aeromonas and most pseudomonads and related bacteria. Within the genus Pseudomonas the test is of differential value. It is useful for distinguishing the complex of species related to P. syringae from P. cichorii, P. marginalis, and the saprophytic fluorescent pseudomonads. The oxidase reaction is also of differential value among the many plant pathogenic species which produce inclusions of poly-β-hydroxybutyrate (e.g. P. andropogonis gives a negative reaction; P. solanacearum gives a positive reaction).

The presence of a high concentration of a utilizable carbohydrate in the medium on which P. solanacearum is grown for the oxidase test does not appear to affect the result. With other oxidase-positive species, however, there is evidence that false negative results may occur in agar media containing a concentration of glucose greater than 0.5%. Inhibition of the reaction has been observed on media containing 2% glucose; this is believed to be a pH effect resulting when the pH of the medium falls to 5.5 - 5.0 or below.

3. Production of nitrite from nitrate; gas production from nitrate

P. solanacearum isolates vary in their effect on nitrite; some (biotype II) produce nitrite from nitrate only; others (biotype III and IV and some biotype I isolates) produce gas from nitrate, i.e., they denitrify. The reaction given by those which denitrify is not as strong as in, for example, P. aeruginosa, and may not be evident on certain media under some conditions. The following procedure has given good results: Medium: Difco bacto peptone, 10.0 g; NaCl, 5.0 g; KNO_3, 2.0 g; Difco bacto agar, 3.0 g; and distilled water, 1 liter. Adjust the pH to 7.0 with 40% sodium hydroxide solution; steam to dissolve the agar; dispense as 10 ml per 150 mm x 17 mm internal diameter tubes. Sterilize in an autoclave at 121°C for 30 min.

Melt the agar and cool to 40°C (i.e. just before the setting point). Inoculate duplicate tubes per isolate. Add a heavy loopful of growth to each tube and mix by rotation between palms before the agar sets. One of the two tubes is sealed with 2-3 ml of either 3% water agar or vaspar (a 50:50 mixture of paraffin wax and vaseline). Incubate both tubes at 28-32°C. After 3-7 days test the unsealed tube for the presence of nitrite by addition of starch-iodide reagents which are prepared according to Skerman (10) in the following manner.

Starch iodide solution: starch, 0.4 g; $ZnCl_2$, 2.0 g; H_2O, 100 ml. Dissolve the zinc chloride in 10 ml water. Boil and add the starch. Dilute to 100 ml, allow to stand for one week, and filter. Add an equal volume of a 0.2 percent solution of KI.

Hydrochloric acid: concentrated HCl, 16 ml; and water, 84 ml. Using clean glass dropping pipettes, place 3-4 drops of each reagent to each tube. A blue color indicates the presence of nitrite. The test depends on the formation of nitrous acid and its subsequent reaction with potassium iodide with the liberation of iodine, which turns the starch blue. The test is not entirely specific. Control tests should be made on a non-inoculated medium.

A negative reaction for nitrite may indicate either that the nitrate has not been reduced, or that the nitrite has been reduced beyond nitrite. In order to differentiate between these possibilities a speck of zinc dust is added to tubes in which no reaction for nitrite, or a weak reaction, has occurred. If the nitrate has not been reduced by the bacterium, there is a reaction after addition of the zinc dust; if there has been a weak reaction which does not intensify after addition of the zinc dust, this indicates that most of the nitrate has been reduced beyond nitrite. The occurrence of a weak reaction which intensifies after the addition of zinc dust indicates that a little of the nitrate has been reduced to nitrite.

The tube sealed with agar or vaspar should be observed for the presence of gas bubbles trapped in the medium or beneath the seal each day for 14 days. The reaction is sometimes weak and slow to appear. A stronger reaction may be obtained if the isolates are subcultured several times through a medium containing nitrate in order to enhance the activity of the enzyme nitrate reductase (12). False positives may sometimes be obtained if the medium is stabbed to inoculate when solid. Bubbles of "gas" may appear on the stab marks in the agar, which in fact are caused by the method of inoculation. A more subtle cause of error may arise when medium has been prepared and dispensed long before use and stored at 4°C. Gas is released into the medium when subsequently incubated at 28-32°C. This effect can be avoided if the medium is re-melted before use. This effect may result from the greater solubility of certain gases at 4°C than at higher temperatures; these gases being released into the medium on subsequent incubation.

4. Oxidation of carbohydrates (acid production)

The medium used by Hugh and Leifson (6) for determination of the oxidative or fermentative utilization of glucose by bacteria may not be suitable for oxidative organisms which produce much ammonia from peptone. The following medium has been devised (4) for plant pathogenic bacteria and is suitable for determination of biotypes in *P. solanacearum*. The medium is that of Ayers, Rupp and Johnson (1) supplemented with 0.1 per cent peptone and sufficient agar to make semi-solid.

Basal medium for oxidation/fermentation tests: Peptone (Difco Bacto), 1.0 g; $NH_4H_2PO_4$, 1.0 g; KCl, 0.2 g; $MgSO_4 \cdot 7H_2O$), 0.2 g; Agar (Difco Bacto), 3.0 g; Bromothymol blue powder, 0.08 g; and distilled water, 1 liter. The pH is adjusted to 7.0 to 7.1 (an olivaceous green color) by dropwise addition of 40% sodium hydroxide solution, steamed to melt the agar and dispensed as 90 ml per bottle or 18 ml per bottle as preferred. The medium is sterilized by autoclaving at 121°C for 30 min.

Preparation of carbohydrate solutions: All carbohydrates are sterilized separately as 10% solutions and not in the medium. The following sugars are used: D-glucose, mannitol, sorbitol, dulcitol, lactose, maltose, D(+) cellobiose. Glucose, mannitol, sorbitol and dulcitol are relatively heat stable and may be sterilized in the autoclave at 110°C for 20 min as 10 ml quantities of 10% solutions. The other carbohydrates are heat-labile and may decompose on heating; they should be sterilized by membrane filtration, Seitz filtration or some other filtration technique, and dispensed in 10 ml quantities into pre-sterilized containers. Dulcitol is poorly soluble at 20-30°C; solution may be achieved by heating in a water bath followed by cooling to make up to volume.

The basal medium is melted, cooled to about 60°C and sufficient carbohydrate solution added to give a final concentration of 1% in the medium (i.e. 10 ml of 10% solution to 90 ml basal medium or 2 ml to 18 ml quantities of the basal medium). The still molten medium, after mixing, is dispen as ca. 3.0 ml quantities of medium per previously sterilized 150 mm x 10 mm internal diameter test tube.

Inoculum is prepared by adding several loopfuls of 24-48 hr culture on suitable agar medium to 3-5 ml quantities of sterile distilled water to make a suspension of milky opacity. A Pasteur pipette is used to add 2-3 drops of this suspension of P. solanacearum to each tube. In addition to the seven carbohydrates a tube containing no added carbohydrate should also be inoculated to serve as a control. The tubes should be incubated at 28-32 C and examined at 3, 7 and 14 days for the occurrence of a change to acid pH from the top of the medium downwards. With glucose and the hexose alcohols a change to yellow (acid pH, less than pH 6.0) indicating oxidation of the carbohydrate occurs within 3-5 days; those biotypes capable of oxidizing the disaccharides may take a few days longer to give a clear positive result. The tubes should be matched with the control tube in order to observe pH changes; in some cases there may be a slight change to alkaline pH in some of the tubes containing carbohydrates which are not oxidized.

In order to confirm oxidative versus fermentative metabolism of carbohydrates, duplicate tubes of glucose medium are stab-inoculated to the base of the tube with a straight wire loaded with inoculum. One of the two tubes is sealed to a depth of 2 cm with molten vaspar or 3% agar. The tubes are examined after 3-4 days for observation of the results shown below.

Y = yellow, pH 6.0 or less
G = green, pH 7.1

Fermentative Reaction

e.g. in Erwinia or Escherichia coli
This reaction may occur with or without gas evolution which will be trapped as bubbles in the semi-solid agar or displace the seal up the tube.

Oxidative Reaction
e.g. in Pseudomonas
Xanthomonas
Agrobacterium
Rhizobium

Organisms with a fermentative metabolism of glucose are invariably strict or facultative anaerobes; acid is produced throughout the depth of the medium in both open and sealed tubes. Organisms with an oxidative (respiratory) metabolism of glucose produce acid only at the surface of the open tube where conditions are aerobic.

LITERATURE CITED

1. AYERS, S. H., P. RUPP and W. T. JOHNSON. 1919. A study of the alkali-forming bacteria in milk. U.S. Dept. Agr. Bull. 782.

2. BURDON, K. J. 1946. Fatty material in bacteria and fungi revealed by staining dried, fixed slide preparations. J. Bacteriol. 52:665-678.

3. HAYWARD, A. C. 1960. A method for characterizing Pseudomonas solanacearum. Nature 186:405-406.

4. HAYWARD, A. C. 1964. Characteristics of Pseudomonas solanacearum. J. Appl. Bacteriol. 27:265-277.

5. HILDEBRAND, D. C. and M. N. SCHROTH. 1972. Identification of the fluorescent pseudomonads. Pages 281-287 in H. P. Maas Geesteranus, ed. Proc. 3rd Int. Conf. Plant Pathogenic Bact., Wageningen, The Netherlands, 365 p.

6. HUGH, R. and E. LEIFSON. 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various Gram-negative bacteria. J. Bacteriol. 66:24-26.

7. KELMAN, A. 1954. The relationship of pathogenicity in Pseudomonas solanacearum to colony appearance on a tetrazolium medium. Phytopathology 44:693-695.

8. KOVACS, N. 1956. Identification of Pseudomonas pyocyanea by the oxidase reaction. Nature 178:703.

9. SANDS, D. C., F. H. GLEASON and D. C. HILDEBRAND. 1967. Cytochromes of Pseudomonas syringae. J. Bacteriol. 94:1785-1786.

10. SKERMAN, V. B. D. 1967. A guide to the identification of the genera of bacteria. 2nd ed. Williams and Wilkins. Baltimore, Maryland. 303 p.

11. SMITH, E. F. 1896. A bacterial disease of the tomato, eggplant and Irish potato (Bacillus solanacearum nov. sp.). U.S. Dept. Agr., Div. Veg. Phys. and Path., Bul. 12:1-28.

12. STANIER, R. Y., N. J. PALLERONI and M. DOUDOROFF. 1966. The aerobic pseudomonads: a taxonomic study. J. Gen Microbiol. 43:159-271.

13. WILLIAMSON, D. H. and J. F. WILKINSON. 1958. Isolation and estimation of the poly- β-hydroxybutyrate inclusions of Bacillus species. J. Gen. Microbiol. 19:198-209.

TECHNIQUES FOR THE STUDY OF PSEUDOMONAS SOLANACEARUM

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I. Isolation Procedures:

When possible, plants should be in an early stage of infection. Plants in an advanced stage of infection will often be invaded by secondary organisms many of which are antagonistic to P. solanacearum. A firm, non-collapsed stem is an indication of good isolation material.

Materials: Poured Petri dishes of tetrazolium chloride agar (see section on media), sharp scalpel, forceps, tubes of sterile tap water (approx. 5 ml per tube), Bunsen burner, and 2 mm wire loop.

Isolation procedure: 1. Remove leaves, root system, and petioles (discard); 2. Wash the entire piece of tissue thoroughly in running tap water; 3. Section, using alcohol-flame sterilized scalpel and forceps, from mid portion of the sample.

a. Longitudinal: cut away thin slices from epidermal layer, removing this possible source of secondary organisms; reflame scalpel and then cut 2 to 3 thin slices from discolored vascular area (about 1 cm in length); place these sections in a tube of sterile tap water; allow to stand at room temperature for 15-30 min.; shake carefully (should be slightly turbid if bacteria present); streak on TZC agar.

b. Cross-sections: cut stem in half about the middle point; cut 3 or more thin cross sections; place in a tube of sterile tap H_2O and follow remaining steps in (a).

II. Identification on TZC agar (Husain and Kelman, 1958. Phytopathology 48:155-165):

Virulent or "fluidal" colonies are slightly raised, slimy and appear a creamy white with pink-orange red centers. They are rarely truly round, more often being quite irregular in shape. The pink centers may show the pink color in a spiral form.

Avirulent colonies are truly round and butyrous in texture and are richly pigmented in red and blue-red colors. In isolations from lesions in the advanced stage of infection these avirulent forms may be present, but secondary organisms often are so similar in morphology that it is wiser not to attempt to separate the two.

Secondary organisms may form round, or oddly-shaped colonies. They are often much larger and come in many assorted hues (sometimes blue or green). Cultures are best examined by oblique lighting.

III. Storage of Cultures:

Sterile tap water (Kelman and Person, 1961. Phytopathology 51:158-161)

Cultures should be examined first on the TZC medium and well-isolated colonies restreaked on a medium without TZC.

Select single distinct colonies from a 48-72 hr old culture and suspend 1 large or 2 small loops of the bacteria into the water in screwcapped tubes (approx. 8 ml/tube).

Store at 20° C.

Suspensions should be turbid (10^7-10^8 cells/ml).

Slant cultures covered with sterile paraffin oil (Kelman and Jensen, 1951. Phytopathology 41:185-187)

Transfer a loop of bacteria from 48-72 hr plate culture to a fresh PDA slant; incubate 48 hr at 30° C; cover with sterile paraffin oil until oil level is about 1.5 cm above tip of slant; store at 20° C.

These two methods of storage, if properly done, are adequate for storing P. solanacearum for several years. Cultures have maintained viability for 8-10 years in water and under mineral oil. Routine checks should be made at 6 mos to 1 yr intervals on stored cultures by streaking them out on TZC agar to determine the stability of the virulent type. Mutants may develop due to changes in temperature, pH, etc., so a culture can easily shift to the mutant form. Pathogenicity may be lost if this happens; if a culture reaches as much as a 50% mutant level, it is wise to prepare a new stock of the virulent form.

IV. Preparing Inoculum:

A. Prepare a liquid medium using formula in Sect. VIIA. After dissolving ingredients, dispense in 800 ml aliquants in 2 liter flasks. Plug with cotton, cover with aluminum foil, and autoclave for 15 minutes at 15 lbs. pressure.

B. Sterilize calcium carbonate separately. Place 6 g of $CaCO_3$/200 ml of distilled H_2O in 300 ml flask. Plug this with cotton and cover with aluminum foil prior to autoclaving for 45 minutes.

C. Prepare enough solution (A & B) for the amount of inoculum needed. Both solutions (A & B) may be prepared several days prior to using.

D. Just prior to adding inoculum, add $CaCO_3$ to basal medium. Prepare turbid suspension 10^8 cells/ml from a 48-72 hr plate culture and add 2-3 ml/flask.

E. Place flasks with inoculum on a shaker in a 28° C incubation chamber for 96 hrs. At the end of 96 hrs. remove from chamber and combine all inoculum, increasing the volume of inoculum to the desired amount needed for inoculating plants by adding sterile tap H_2O. (Dilution--1 part bacterial inoculum to 4 parts H_2O.)

F. Wound roots by cutting soil with sharp knife between each row of plants. Pour 100 ml of diluted inoculum in space between each row in flat.

V. Preparation of Tomato Plants for Greenhouse Inoculations:

A. Sow seed on sterilized sand and cover with Vermiculite. When germinated and about 1½ in. high, with well-developed cotyledonary leaves, transplant to flats, 7 rows/flat with 7 plants/row. The soil in these flats should have 3-1-1 mixture, 3 soil (topsoil), 1 sand and 1 peat moss. The soil and sand should be sterilized.

B. One week after transplanting, fertilize with a 20-20-20 liquid fertilizer, at the rate of 800 g/3 gal. H_2O and apply with a "hose on" or apply with watering can at 1 tbs./gallon. Repeat fertilizer every 14 days or when needed.

C. Flats to be inoculated should be transplanted 4-5 weeks prior to inoculation, with 7 rows/flat of a given line (rows 1-2-3-6-7 to be treated). There should be a resistant check (row 4) and a susceptible check (row 5) in each flat.

The flat dimensions for planting are 14 in. x 20 in. x 2 3/4 inches.

VI. Inoculation of Tobacco and Tomato Plants (Winstead and Kelman, 1952. Phytopathology 42:628-634):

A. Use 4-5 week-old plants from transplanting (or about 6 inches high). Rutgers tomato and Hicks tobacco are good susceptible plants.

B. Prepare fairly turbid, sterile tap H_2O suspensions (10^7-10^8 cells/ml) of each isolate to be tested.

C. Place 1 drop of inoculum in the leaf axil (about the 3rd mature leaf from top) with sterile dropper.

D. Flame a needle, allow to cool for a few seconds, and then insert through center of drop down into center of stem about 1-2 cm. Do not go through to opposite side.

E. Stem inoculations give most rapid and thorough infection. However, root inoculations may be made by cutting roots and pouring a bacterial suspension on soil. Amounts depend on container, etc.

F. Tomato plants should wilt in 5-14 days, and tobacco plants in less than 30 days after stem inoculations.

VII. Field Inoculations: Preparing Plants for Field Plots (Moore et al., 1963. Tob. Sci. 7:17-20):

A. Transplant plants from seed flat to 2¼ inch peat pots, using the same 3-1-1 soil mixture. Use the same method of fertilizing. When plants are 6-8 weeks old from transplanting time, plant them in the field. Plant them in rows with 5 ft. centers and spaced 30 in. apart in row.

B. Inoculum for field plots:

1. Prepare inoculum the same way as for inoculating flats, except dilute it at the rate of 1 gal/50 gal of H_2O.

2. A 20-20-20 liquid fertilizer (2 lb) may be mixed with 50 gal of H_2O with inoculum.

3. Remove plants from peat pots just prior to planting, in order to injure the roots. By using a hand transplanter you can place a required amount of inoculum and fertilizer mixture (1 pint usually) around root system when planted. Pack soil around plant with your foot.

4. Also plant resistant and susceptible checks at several locations in the plot in short rows.

5. Normal cultivation procedures are required.

VIII. Media (Husain and Kelman, 1958. Phytopathology 48:155-165):

A. Semi-synthetic Medium:

Excellent growth of P. solanacearum can be obtained in a semi-synthetic medium with the following composition: Phosphate buffer solution, 10 ml; $(NH_4)_2SO_4$, 10 ml; $MgSO_4 \cdot 7\ H_2O$, 5 ml; $ZnSO_4 \cdot 7\ H_2O$, 1 ml; Ferric citrate, 1 ml; $MnSO_4 \cdot H_2O$, 1 ml; Sucrose, 20 g; Yeast extract (Difco), 1 g; Casamino acid (Difco), 1 g; Water, 800 ml. Stock Solutions: Phosphate buffer: K_2HPO_4, 117.7 g/l; KH_2PO_4, 44.1 g/l; $(NH_4)_2SO_4$, 66 g/500 ml; $MgSO_4 \cdot 7\ H_2O$, 20 g/500 ml; $ZnSO_4 \cdot 7\ H_2O$, 0.82g/500 ml: Ferric citrate: Ferric citrate, 1.497 g + citric acid, 0.958 g in 500 ml H_2O; $MnSO_4 \cdot H_2O$, 0.77 g/500 ml.

B. TZC agar (Kelman, 1954. Phytopathology 44:693-695):

The TZC medium contains: 10 g dextrose, 10 g peptone, 1 g casamino acids (casein hydrolysates), 18 g agar, in 1000 ml distilled H_2O.

Using approx. 3/4 of the water, heat agar in steamer (covered) for 35-45 min. Meanwhile weigh other ingredients and dissolve in remaining water. When agar is melted, add solution of other ingredients. Stir vigorously and dispense in 200 ml aliquots. Measurement of amount should be done carefully, filling to a little above 200 ml mark on pharmaceutical bottle. Autoclave for no more than 15-20 minutes.

Recent observations indicate that it is advantageous to reduce the level of glucose to 5 g/l and also to sterilize the glucose by filtration and add it separately. Glycerol (5 ml/l) can be substituted for glucose in which case it can be autoclaved with the medium.

Addition to TZC solution: Prepare a 1% (w/v) solution of 2,3,5-triphenyl tetrazolium chloride. Autoclave 7-8 min. in small flask. By means of sterile pipettes, add 1 ml of this solution to each 200 ml bottle of medium just prior to pouring plates. Store sterile TZC solution in refrigerator; cover flasks with aluminum foil to avoid exposure to light.

C. Crystal-Violet Pectate Medium (Cuppels and Kelman, 1974. Phytopathology 64:468-475)

This medium has been found to provide an excellent means for isolation of P. solanacearum from soil. Colonies of the wilt pathogen form very shallow pits in contrast to those formed by Erwinia carotovora and related soft rotting Erwinia. It is suggested that dilution platings be made on this medium initially with pure cultures of P. solanacearum in order for the investigator to become familiar with the specific colony type associated with the wilt pathogen. Plates are best examined under oblique lighting from below (See Cuppels and Kelman, 1974).

Procedure: Preheat 5-cup Waring Blendor with running hot tap water or rinse in boiling water. Place 300 ml boiling water (distilled) in the blendor. Add: a) 4.5 ml of 1 N NaOH, b) 3 ml of 10% $CaCl_2 \cdot 2H_2O$ (freshly made), c) 1.5 g of Difco agar, d) 1 g $NaNO_3$. Blend at high speed for 15 sec. Slowly add and blend 15 g sodium polypectate and 200 ml boiling distilled H_2O. Pour the medium into a 2-liter flask and cap the flask with aluminum foil, rather than a cotton plug. Autoclave 25 min. at 120° C and 15-lb pressure. Allow pressure to drop slowly to avoid bubbling over. Add 1 ml of 0.075% aq. crystal violet solution (w/v) (final crystal violet conc: 1.5 ppm) to the hot medium and pour the medium into plates as soon as possible. (This can be added before sterilization.) After the medium has hardened, dry the surface by storing the plates in a 36-37° C incubator for 24 hr. This is essential for good results.

D. Semi-Solid Motility Medium (Kelman and Hruschka, 1973. J. Gen. Microbiol. 76:177-188)

Virulent cultures of *P. solanacearum* are usually non-motile. Avirulent forms in cultures from solanaceous hosts are usually actively motile. These differences can be demonstrated by placing 1 drop (10^9 cells) in the center of a plate containing 20 ml of the medium described below: Medium components are: Tryptone, 0.1 gm; Glycerol, 0.1 ml; Phosphate buffer solution (pH 7.0), 10 ml; (KH_2PO_4 = 117.7 gm + K_2HPO_4 = 44.1 gm in 1 l. distilled H_2O); Agar, 3.5 gm; in 1 liter distilled H_2O. Pour petri plates with 18-20 ml/plate.

MEDIA FOR ESTIMATING A STRAIN OF PSEUDOMONAS SOLANACEARUM IN KENYAN SOILS BY THE DILUTION PLATE TECHNIQUE

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Plant pathologists working with P. solanacearum are familiar with the tetrazolium medium devised by Kelman (1954) for improved differentiation of virulent and avirulent colony forms. The microbial inhibitor triphenyl tetrazolium chloride (TTC) confers a measure of selectivity (Weinberg, 1953) which also makes this medium useful for estimating the pathogen in soils by the dilution method. However, this selectivity is limited and ca. 10^6 cells/ml was the lowest concentration measurable in Georgian soils (Jenkins et al., 1967).

In a study of a strain of P. solanacearum causing wilt of potato and tomato in Kenya, it was found possible to improve progressively the selectivity of Kelman's medium (plus 100 ppm actidione to suppress fungi) in soil dilutions by increasing the concentration of TTC up to 4000 ppm. This increase in TTC levels had little or no effect on recovery of the pathogen. In this high tetrazolium medium both the numbers and size of background colonies were markedly reduced. Colonies of P. solanacearum were slightly smaller than on the unmodified base but their morphology remained highly distinctive. Colonies were irregularly round, raised, mucoid, white at first but becoming red through the deposition of formazan in a characteristic spiral fashion. By contrast, most saprophytic colonies were intensely red throughout and none had spiral patterns.

This high tetrazolium medium thus has the combined advantages of eliminating a large proportion of background organisms, restricting the colony size of those that do grow, and permitting the direct identification of colonies of P. solanacearum.

The selectivity of the medium for field soils is slightly enhanced by incorporating the highest non-inhibitory concentrations of neomycin (200 ppm), streptomycin (100 ppm), polymyxin (80 ppm) and tyrothricin (25 ppm). However, the first two antibiotics were very useful for inhibiting specific TTC tolerant bacteria which were sometimes numerous in glasshouse compost soils. When inhibitory concentrations of these antibiotics were used, or on very crowded plates containing non-inhibitory concentrations, the colony morphology of the pathogen was modified to a round, flat, non-mucoid, pink form with an eccentric clear zone. These colonies were also quite distinctive but their occurrence was associated with a reduced recovery rate of the pathogen.

When Kelman's base with 4000 ppm TTC, 100 ppm actidione and 200 ppm neomycin was used to estimate P. solanacearum in naturally infested field soils, it was found to be up to 20 times more selective than the unmodified medium. Depending on the soil source total counts were usually reduced by more than 80% and frequently by more than 95% compared with nutrient agar, and it was possible to detect the pathogen even when outnumbered up to 3000 to 1 by other microorganisms. The pathogen was detected in soils at even smaller ratios. In surface soils numbers as low as 10^3/g soil were counted, whereas at lower horizons where microbial activity was much reduced 10^2/g soil were counted. The medium was even more selective with glasshouse compost soils. Direct counting using this medium proved more sensitive, accurate

and convenient for following population trends of P. solanacearum than methods involving indicator plants.

Three isolates of a second Kenyan strain and five exotic isolates from tomato, tobacco, potato and banana grew on the high tetrazolium medium with and without neomycin although not as well as the Kenyan potato/tomato strain. Media of this type may therefore have a wide application in the study of P. solanacearum in soil.

References

JENKINS, S. F., D. J. MORTON and P. D. DUKES. 1967. Comparison of techniques for detection of Pseudomonas solanacearum in artificially infested soils. Phytopathology 57:25-27.

KELMAN, A. 1954. The relationship of pathogenicity of Pseudomonas solanacearum to colony appearance in a tetrazolium medium. Phytopathology 44:693-695.

WEINBERG, E. D. 1953. Selective inhibition of microbial growth by the incorporation of triphenyl tetrazolium chloride in culture media. J. Bacteriol. 66:240-242.

TASK FORCE COMMITTEE REPORT

BREEDING FOR RESISTANCE TO BACTERIAL WILT

1. Introduction

Pseudomonas solanacearum is primarily a soil-borne bacterial plant pathogen found in countries that fall within latitudes 45° N and S. It has recently been found overwintering in Sweden at 59° latitude and has been found in Peru at altitudes as high as 10,000 ft. As early as the turn of the century it was recognized in the Southeastern United States, Java, Australia, India and Indonesia. It has since been reported in Portugal, Italy, Central America, the Caribbean Islands, some Pacific Islands, several countries in Africa, Ceylon, and several of the South American countries. The bacterium is now spreading to other tropical and subtropical countries. For example, it has been found recently in potato growing areas of Mexico, Uruguay and Peru and the banana strain is spreading in Brazil and the Philippines.

Pseudomonas solanacearum is a variable bacterium that is of economic and international importance because it attacks many plants in over 25 separate botanical families. The losses caused by the bacterium are incalculable. The host plants include ornamentals and important food and fiber plants such as: potato, pepper, tobacco, peanut, plantain, tomato, eggplant, banana, ginger, strawberry, castor bean and abaca.

In the Caribbean Islands, Central and South America, the moko disease of plantain and banana affects a major food supply and a principal export crop of the region.

In many parts of the world including the Southeastern United States, farmers have abandoned the production of cash crops such as potatoes, tomatoes, tobacco and bananas to grow less remunerative crops that are resistant to the bacterium. Attempts to expand the range of many valuable adapted crops, such as potatoes in the Philippines, have failed because of *P. solanacearum*. This shift from high to low value cash crops has altered the socio-economic structure of affected communities.

Although *P. solanacearum* has been recognized as an important bacterial pathogen since the turn of the century, its distribution and the diseases it causes are poorly understood. The pathogenic variation within the species is enormous. Little is known of the ecological factors that affect the bacterium's longevity in soil or its disease producing potential in different soils. The control of the disease by such practices as crop rotation, manipulation of cultural practices, and chemical treatment of infested soils has been only partially successful or prohibitively expensive. The most successful control has been through the development of resistant varieties--tobacco in the U.S. and Japan, and peanuts in Indonesia. Adequate resistance has been discovered in wild potatoes and tomatoes, but acceptable commercial varieties of these crops are not available in most countries.

2. Present status of research on resistance to *Pseudomonas solanacearum*

For only two crops--peanuts and tobacco--does resistance seem to be adequate on a worldwide basis. Because of the potential for change in *P. solanacearum*, complacency about maintaining thir resistance is not desirable. The

main source of resistance in tobacco appears to be due to multiple recessive genes and thus is polygenic.

There are sources of resistance in pepper and eggplant, although reports of susceptibility of these varieties do occasionally occur.

Almost all bananas are susceptible to race 2, but sanitation and cultural methods of control are adequate to control the disease except with the "Bluggoe"-type plantain which is susceptible to the "SFR" strain of race 2. The variety Pelipita (ABB) is promising as a disease-resistant substitute for the "Bluggoe" type, however. The inherent difficulties in banana breeding make prospects for real progress in the development of resistant varieties necessarily long term.

The major challenges and the major opportunities for progress are in breeding for resistance to P. solanacearum in tomatoes and potatoes.

Resistance in potatoes, derived from S. phureja, does not seem to be adequate in all areas, and the need to produce locally-adapted varieties with many different characteristics complicates breeding efforts. The genetics of resistance is highly complicated, with 10 dominant genes for resistance identified to date. Thus, breeding with S. phureja as a source of resistance will be long-term, expensive and difficult. If the magnitude and importance of the problem are considered worldwide, present efforts are not adequate to produce rapid results. Additional sources of resistance need to be identified and utilized.

Resistance to P. solanacearum has been found in tomatoes by several workers, but this resistance does not appear to be adequate at the hot temperatures found at altitudes below 2000 feet in the tropics. No commercial variety is available today for these severe conditions. Many small programs are presently working toward this goal around the world, but without real coordination. A major effort to coordinate these efforts, exchange material, provide adequate standardized testing, and methodically test all available germplasm in the world has real potential and should produce adequate resistance in a relatively short time.

3. The need for research on resistance to bacterial wilt

Bacterial wilt continues to be one of the contributors to poverty and malnutrition in Africa, Asia, Central America, South America, the Caribbean and in the South Pacific. It is impossible to calculate the millions of hectares invaded by P. solanacearum, both cultivated and virgin land, which are now useless for the cultivation of these high value crops.

In the tropics the acreage devoted to vegetable crops such as tomato, potato, eggplant and pepper is increasing each year. In 1973, there were 680,000 hectares devoted to the cultivation of tomatoes in Africa, Asia and South America. The demand for vegetables is increasing so dramatically that it is not uncommon to see fresh tomatoes sold at a prohibitively high price. The consumption of processed vegetables, principally tomato products, is also increasing rapidly. At present almost all processed tomato products are imported into many tropical countries. The development of a viable processing industry is essential and depends entirely upon the consistent ability to grow quality tomatoes at low cost.

In the less developed countries vegetables are important components of the human diet, providing large amounts of carbohydrate, protein, vitamin A, vitamin C and numerous other vitamins and minerals. Not only are these vegetables nutritionally important, but as a source of income they are also contributing toward an improved quality of life for the rural population.

The persistence of this organism in the soil and its wide host range hamper cultural practices for control of this disease. In fact, reports that recently cleared virgin land or land that had been planted for 16 years to a nonsusceptible crop, is severely infested by the bacterium are not uncommon. In banana, proper management of cultural practices has limited the disease. Seed certification of brown rot-free potatoes is another approach which appears to have good potential in some tropical and subtropical areas. Chemical control has seldom been used effectively on high value crops such as tobacco and tomato, however. Obviously chemical control is not practical for the small farmer.

Because of the ability of the bacterium to persist in the soil and because of the lack of adequate cultural control measures, alternative control measures must be sought. The use of disease resistant varieties offers the best control for wilt diseases incited by P. solanacearum. Successful varieties of tobacco and peanut have long been in use. However bacterial wilt resistant varieties of tomato, eggplant, pepper and potato of acceptable horticultural types are not presently available. The levels of resistance in tomato are low and not adequate in some geographical areas. At this time there is no concerted worldwide effort to cooperate or coordinate efforts in the study of bacterial wilt resistance of vegetable crops. It is obvious that there is a need for such cooperative efforts in the development of multiple disease resistance in such crops for the lowland tropics and subtropics.

4. Objectives

1. To locate and utilize adequate sources of resistance to bacterial wilt of tomato, potato and other vegetable crops.
2. To study the genetic and environmental factors that control and affect the expression of resistance.
3. To develop standardized procedures for testing and evaluating resistance under laboratory and field conditions.
4. To establish a network which will include:
   a. A central institution for project coordination, and
   b. Testing locations at various tropical and subtropical regions.

5. Procedures

A. Tomato-Breeding for resistance to bacterial wilt

Despite efforts made in various parts of the world, so far no commercial tomato varieties have been released which combine good agronomic characteristics and an adequate level of resistance to P. solanacearum.

The materials now available do not remain resistant under conditions leading to severe disease development; these conditions commonly occur throughout the tropics. In addition, varieties that behave as resistant in some regions of the world have been shown to be susceptible in others, thus indicating that naturally occurring strains of the bacterium may overcome resistance.

In many instances resistance is linked to undesirable agronomic features, such as: poor fruit quality and poor fruit setting at high temperature regimes.

The lack of uniform behavior of the available sources of resistance in different parts of the world suggests the existence of distinct genetic mechanisms that control resistance. Thus, it is probable that a broader genetic basis for resistance can be obtained by combining the genetic factors which are present in different breeding materials. It is also possible that better sources of resistance exist in tomato germplasm collections which have not been properly screened for that specific purpose so far.

Indications are that lack of coordination between the various programs that exist represents the most important limiting factor in breeding of tomatoes for wilt resistance. It seems evident that resistance detected by each of these local programs has not been utilized in the most adequate way. Hence, the challenge for the obtention of successful wilt-resistant commercial varieties resides in the establishment of a coordinated effort to tackle the problem on an international cooperative basis.

The following procedures are considered essential for this project:

1. Standardization of testing procedures to evaluate resistance, including: inoculum dosage, methods of inoculation, age of testing plants, incubation conditions, etc.
2. Establishment of a centralized, extensive collection of P. solanacearum isolates including representative strains prevailing in the various parts of the world where the disease occurs on tomato.
3. Maintenance of a tomato germplasm collection, including all materials known to have wilt resistance in different geographical locations.
4. Systematic screening of the germplasm for resistance to the various strains of the organism.
5. The use of standard progeny analysis methods to elucidate the genetic mechanisms that control resistance, and of standard breeding methods to increase the levels of resistance by combining different resistance genes in a single genotype.
6. Field testing of advanced genetic materials after wilt resistance has been incorporated into tomato lines having resistance to other important diseases. The field testing should be carried out in different geographical locations following a standard design and standard evaluation procedures.
7. Support of local breeding programs toward the final goal of releasing wilt-resistant commercial varieties for the various parts of the world where the disease is a problem.

B. Potato—Breeding for disease resistance to bacterial wilt

Although sources for resistance to bacterial wilt of potato had been sought for decades, both in cultivated and wild tuber-bearing species of Solanum, it was not until the late 1960's that a useful source became available. Thurston and Lozano reported that certain clones of Solanum phureja had high levels of resistance to race 3 isolates of Pseudomonas solanacearum. Open-pollinated material was sent to Wisconsin and this material yielded several highly resistant clones (1386 series). These were used in crosses with "haploid" (2n = 24) lines of S. tuberosum; standard backcrossing and outcrossing methods have been used to produce a number of clones that have adequate yields and combine resistance to wilt and late blight. The variety "Caxamarca" was released in Peru in 1975 out of this program and other varieties are planned for release in the near future in Nigeria and other countries.

Although this program has had a measure of success, it is evident that if will not be adequate to resolve the problem throughout the potato-producing areas of the world. There are various reasons for this. In particular, the resistance from S. phureja is adequate only against some strains of the bacterium. Studies on genetics of resistance indicate that several, independent genes control resistance against each of the three isolates that have been examined genetically. It is apparent that numerous genes will have to be identified and incorporated into each specific clone in order to widen its spectrum of resistance. This will require a major effort; success will depend on the volume of material that can be processed. It is estimated that present efforts will prove inadequate.

A second, important problem is the relative instability of the genes for resistance presently available. The S. phureja x S. tuberosum hybrids are not resistant at high temperatures and thus cannot be adapted to low elevations in the tropics where bacterial wilt is a major problem. It is evident that a systematic search for resistance in other wild and cultivated potatoes should be made to locate better sources of resistance. There are known sources of resistance within S. phureja which must be utilized. However, available programs and resources are inadequate to process and utilize this material.

The following procedures are considered essential for this project:

The project involves breeding and initial selection of resistant potato hybrids at a center where expertise in these two aspects of the project exist. In general, the procedure would involve:

a. A search for additional sources of resistance in the tuber-bearing Solanums (in particular, S. phureja).

b. The use of standard procedures for crossing these lines with S. tuberosum at the appropriate ploidy level.

c. Preliminary selection of resistant material from hybrid progenies (resistant to both late blight and wilt).

d. Initial increase of the resistant clones to produce at least 10 tubers per clone per testing site.

e. Distribution of resistant material to collaborators at different testing sites, and

f. Field testing of the material following standard design and evaluation procedures. The evaluation criteria should include both i) time to wilting, and ii) number of infected tubers after harvest.

6. Proposed organization

No existing institution or entity, national or international, presently embodies all of the characteristics that an ideal organization needs to carry out the objectives of this proposal. Ideally, a center or centers should be established with the following characteristics:

1. Where in-depth experience and expertise exist in breeding and evaluating materials for resistance to P. solanacearum.

2. Where a worldwide collection of isolates can be maintained, characterized, and distributed.

3. Where a complete and up-to-date information center on P. solanacearum exists.

4. Where isolates from all over the world can be used in the field and greenhouse on a wide variety of hosts.

5. Where adequate field, laboratory, library and administrative support services are available.

It is recognized that no one location can combine all of these characteristics (for example a place where work on bananas and potatoes could be done together). It is proposed, therefore, that a worldwide network of cooperating institutions be established. For funding, fiscal disbursement and administrative purposes it is necessary that a single entity take overall responsibility to manage and administer the project. Inasmuch as possible the center should be an integral working part of the research and not just serve an administrative role.

The headquarters or center need not necessarily require a new physical institution, but can utilize existing institutions for this purpose. To build an effective network with cooperating institutions all over the world, the center will need personnel who can visit cooperators on a regular basis and spend the time necessary to help them with their research, fiscal, administrative and political problems.

The center institution, in addition to its research program, must be prepared to: a) disseminate information, isolates, and special equipment, b) obtain, maintain and disseminate germplasm, and c) establish cooperative projects and standardize procedures for uniform testing. Of most importance, it must have the flexibility to award grants or contracts to encourage outstanding research and make the network more than a "paper tiger."

A worldwide field testing network is essential to the success of the project and will be a major part of the project's effort.

Although the major emphasis in the program will be plant pathological, and testing and evaluation the major thrusts, it is envisioned that the center institution would identify the best breeder available, one who already has an active breeding program, long experience, contacts, and a germplasm collection. Through a contract, he would initiate the program for breeding for resistance to P. solanacearum using all worthwhile sources of resistance. If the experience, personnel and facilities exist at his institution initial screening for resistance could be made there. If not, the center institution or other qualified institutions could do the initial screening.

Once promising germplasm with high levels of resistance has been identified these materials should be distributed to several key network centers around the world.

For examples, in a program involving tomatoes, testing centers might be established as follows:

Pacific Islands -- Hawaii.
Latin America -- Costa Rica, Brazil and/or Colombia.
Caribbean Islands -- Guadeloupe and/or Trinidad.
Africa -- Mauritius, Nigeria, Kenya and/or Uganda.
Asia -- Philippines and/or Taiwan.

The materials generated by the project would be available to any qualified investigator in the world. Interchange of results, germplasm, information, techniques, personnel, and consultants plus regional workshops would be used to help make the network function in a cooperative manner.

Submitted by H. D. Thurston, Chairman

REPORT OF THE TASK FORCE COMMITTEE ON BIOVAR DIFFERENTIATION AND VARIABILITY

Proposal I. Establishment of an International Data Center and Culture Collection for *Pseudomonas solanacearum*

The committee considered that the proposed center should have the following functions:

a) Promotion of research effort and of collaboration and coordination among research workers in the field and provision of technical services.

b) Distribution of a quarterly News Bulletin.

c) Maintenance and preservation of a representative collection of isolates of *P. solanacearum* and of their bacteriophages for research and routine use.

d) Collection of a master file of all published literature on bacterial wilt and provision of a translation service where possible into a common language such as English.

e) Preparation of a revised Host List to be published in the News Bulletin and periodically made up to date with supplements.

f) Preparation of a list of research workers engaged in any aspect of research or extension concerning bacterial wilt.

g) Preparation of a loose-leaf laboratory manual of methods recommended for the identification of pathovars, biovars, serovars, etc., of *P. solanacearum*, and for maintenance and preservation of the pathogen and bacteriophages.

h) Provision of facilities for the training of graduate students and technical staff.

i) Preparation of a standard data sheet for the recording of new cultivated and weed hosts, and for provision of data on all isolates sent to the data center and culture collection. For the recording of new hosts the data sheet should contain information on the following: territory; location (State, district, county, etc.); altitude and latitude; host (with information on cultivar for commercial crops); soil type including information on humus content, organic matter, pH, salinity, electrical conductivity and classification; rainfall data; temperature data; previous crop history; nature of plant cover; description of symptoms whether latent or overt. For the deposition of isolates of *P. solanacearum* in the culture collection information should be provided on the following: host inoculation studies; pathovar determination; biovar determination and the results of biochemical tests and cultural observations.

The data sheet should contain space for the worker submitting the information to indicate whether permission is given for the record to be published without restriction.

j) Recommendation of standard methods and terminology in the study of pathovars and biovars.

Proposal II. Initiation of Comparative Studies on Pathovars *in vivo*

The committee considered that high priority should be given to the following investigations:

a) Selection of a standard range of hosts as differentials for the evaluation of pathovars. Where possible the same cultivars should be used by all workers and the environmental conditions under which such tests are to be carried out, e.g., photoperiod, temperature regime, and light intensity, should be defined and standardized.

b) Initiation of studies on the temperature relations for disease expression of different pathovars and biovars in growth chambers with particular emphasis on the strains of *P. solanacearum* affecting potato.

Proposal III. Initiation of Applied and Fundamental Studies *in vitro*

The committee considered that further information and research was required in the following areas:

a) Determination of the optimum, minimum and maximum temperatures for growth of representative pathovars and biovars using a temperature gradient incubator with particular emphasis on the biotype II (race 3) isolates affecting potato.

b) Initiation of studies on the physiology of virulent and avirulent strains of *P. solanacearum*.

c) Biochemical studies on the specificity of the enzymes involved in hexose alcohol and disaccharide utilization and/or oxidation by biotypes of *P. solanacearum*.

d) Standardization of media and methods for the cultural and physiological studies necessary for biovar determination, e.g., for detection of gas production from nitrate.

e) Preparation and evaluation of diagnostic antisera for diagnosis of pathovars.

f) Further evaluation of bacteriocin typing of epidemic pathovars of *P. solanacearum*. Bacteriocin production by biotype II (race 3) isolates may need to be re-examined once optimum temperature requirements for growth of these organisms has been determined according to III a) above.

g) Evaluation of the use of phage-typing for the identification of epidemic strains, and of the use of phage for the detection of *P. solanacearum* in diseased material. There is also a need for the isolation and characterization of a wide range of phages from diseased material; these phages may be of potential value in projected genetic studies on *P. solanacearum*. For example, flagellum-specific phages may be of use in the elimination of avirulent mutants from mixed populations of virulent and avirulent cells.

h) Initiation of the first major comprehensive taxonomic study of *P. solanacearum* isolates representing the full spectrum of hosts, territories, climatic zones, soil types, etc., with a view to the extraction of diagnostically

useful information and the identification of pathovars and biovars. This study would make use of the computer and the concepts of numerical taxonomy. The number of strains to be used should be within the range of 100-500.

Proposal IV. Genetic Studies on the Bases of Virulence in P. solanacearum

a) Genetic analysis of virulent and avirulent strains and of the mechanisms of variability. Examination of P. solanacearum for the occurrence of transduction, transformation and conjugation.

b) Investigation of the occurrence of recombination within the host plant.

c) Studies on the genetics and cytology of the interaction between avirulent strains and the hosts which resist them.

d) Investigation of the genetic determinants of virulence, pathogenicity and host range.

The committee considered that the genetic investigations listed above would be helped by the following approach. In order to define the various steps in the infective process it is necessary to make many random isolations of non-pathogenic or non-virulent mutants of P. solanacearum; this may be achieved by means of an infectivity assay which gives a high percentage of response. It is then proposed to investigate the behavior of these mutants in the host plant: their rate of multiplication in different parts of the plant and the cytological aspects of the infection and of the reaction of the host.

The search for means of genetical analysis of the functions which control pathogenicity and virulence should place particular emphasis on conjugation. The goal is to map and number the functional genes; a possible application would be to obtain hybrid strains in order to study interactions between the products of those genes. The naturally occurring system of genetic transfer discovered in the K60 strain of P. solanacearum will be exploited. It may also be possible to build up a conjugation system based on the use of such self transmissible R factors as RP_4. A peripheral aspect would be to examine for the occurrence of bacterial recombination inside the host plant.

Submitted by A. C. Hayward, Chairman

REPORT OF THE TASK FORCE COMMITTEE ON ECOLOGY AND CONTROL

A. General Recommendations:

a) A twice yearly newsletter be initiated
b) A review be published on the ecology and control of diseases caused by Pseudomonas solanacearum.
c) A listing be prepared of all persons having an interest in research projects in the area of ecology and control.
d) Individuals in all areas of research exchange reprints.

Benefit. Improved contact and communications among workers will facilitate more rapid research development.

B. Specific research proposals in the area of ecology and control.

1. a) Collect existing data relevant to the ecology of bacterial wilt from as many regions as possible where bacterial wilt is a problem. In particular:

1) Soil descriptions, physical and chemical characteristics.
2) Preparation of as complete a description as possible of the strains present.
3) Climatic conditions and cropping patterns.
4) Hosts affected, including weeds.

b) Analyze the data and distribute findings to all interested parties.

Benefit. A pooling of all present knowledge on ecology may provide the basis of some valuable new research guidelines.

2. Determine the principal factors which influence the survival of P. solanacearum between epidemics as a basis for developing effective control procedures. Specifically, to study the soil borne phase of the pathogen's life history in relation to (in order of priority):

a) The effect of physical and chemical factors, particularly soil moisture and temperature.
b) The effect of nematodes and other soil organisms on the incidence and development of bacterial wilt.
c) The precise location of bacteria surviving or multiplying in soil or on the living root systems of higher plants.
d) The basis of the conducive/suppressive soils phenomenon.
e) The importance of the virulent to avirulent transformation and possible reversal.

Benefit. The studies will provide the basis for an understanding of factors accelerating the destruction of soil borne inoculum and their application to control.

3. Develop and improve methods for studying P. solanacearum in soil and in association with living plant roots and in seed material. Specific examples could include the following:

a) Selective media and/or enrichment.
b) Serological methods.
c) Host-test methods.
d) Phage and other techniques.

Benefit. To facilitate studies outlined above.

4. Develop crop rotation sequences for control of soil borne disease on a world-wide basis using potato as the primary crop and model system. Rotation experiments would be standardized as far as possible and would be fostered through a co-ordinating advisory group.

Benefit. Provide information on which locally applicable control recommendations can be based.

5. Determine the means for predicting the susceptibility to bacterial wilt of various crops in areas differing in climate, strains, soils and other conditions through a systematic study of the interaction of all these factors in determining the course of disease development and yield losses.

Benefit. Improve ability to assess the danger of exposing new crops to bacterial wilt, or existing crops to new strains.

Submitted by D. C. Harris, Chairman

Recommendations of the Conference Steering Committee

1. A working group committee under ISPP should be established to continue the program recommended at the conference pending acceptance of the appointments and approval by the ISPP Executive Committee. The Chairman and committee members were appointed as follows:

Chairman:	Alan C. Hayward
Committee Members:	Masao Goto
	A. H. Ramos
	B. Digat
	Edward R. French
	Luis C. Gonzalez
	Arthur Kelman

2. A discussion session on Pseudomonas solanacearum should be held at the post-Congress meeting on Phytobacteriology scheduled at Angers in 1978.

3. The following sub-committees were appointed and assigned responsibility for possible implementation of specific task force recommendations:

I. Biotype Differentiation and Variability

Chairman:	Alan C. Hayward
Committee Members:	Luis Sequeira
	B. Digat
	R. A. Lelliott
	Pierre Boistard

II. Breeding for Disease Resistance

Chairman:	H. David Thurston
Committee Members:	P. R. Rowe
	Jon C. Watterson
	Ralph H. Phelps
	M. Vereebhadra Rao
	G. M. Lallmahomed

III. Ecology and Cultural Control Methods

Chairman:	A. B. Lloyd
Committee Members:	A. H. Ramos
	States M. McCarter
	A. Coleno
	Leopold Fucikovsky
	W. C. Nesmith

PROGRAM

PLANNING CONFERENCE AND WORKSHOP ON THE ECOLOGY AND CONTROL OF BACTERIAL WILT

Sunday, July 18

A.M.	9:30-11:30	Planning Session for Task Force Committee Chairmen: 2515 Gardner Hall, Plant Pathology Conference Room.
P.M.	2:00- 8:00	Registration: John Yancey Motor Hotel, 2200 Hillsborough St.

Monday, July 19

A.M.	9:00- 9:30	General Session: 3533 Gardner Hall, Seminar Room. Introduction and Greetings. S. F. Jenkins, Chairman. R. Aycock, Head, Dept. of Plant Pathology, N.C. State University J. L. Apple, Associate Director, N.C. State Agricultural Experiment Station.
	9:30-10:15	"Conference Mission". A. Kelman, Univ. of Wisconsin.
	10:30-11:30	Discussion Session: "Systematics and Relationships of *Pseudomonas solanacearum*". A. C. Hayward, Univ. of Queensland, Brisbane, Australia.
	11:30- 1:00	Lunch: N. C. State University Student Center. (Group)
P.M.	1:00- 4:00	Plating of Cultures, Biochemical Tests, Selective Media, Isolations from Soils: Microbiology Laboratory, 4524 Gardner Hall.
	4:00- 5:00	Strain Evaluations and Inoculations: Plant Pathology Greenhouses.
	7:30- 9:30	Task Force Committee Meetings: 2000 Gardner, 2515 Gardner, 3515 Gardner Hall.

Tuesday, July 20

A.M.	8:30- 9:00	Paper Session I. L. Sequeira, Chairman. 3533 Gardner Hall. "The History of Granville Wilt in North Carolina". H. W. Spurr, Jr., Tobacco Research Laboratory (ARS-USDA), Oxford, N.C.
	9:00-10:15	Short Papers: 1. El-Goorani, M. A., Plant Pathology Dept., Faculty of Agriculture, Alexandria University, Alexandria, Egypt: "Current Status of Potato Brown Rot Research in Egypt."

2. Erinle, I. D., Dept. of Plant Protection, Ahmadu Bello University, Zaria, Nigeria: "Bacterial Wilt in Northern States of Nigeria."

3. Garcia, Stella and C. Moscardi, Centro de Investigaciones Agricolas, Alberto Boerger, Estacion Experimental "Las Brujas", Uruguay: "Status of Potato Brown Rot in Uruguay."

4. Harris, D., East Malling Research Station, England (formerly National Agricultural Laboratories, Nairobi, Kenya): "Bacterial Wilt in Kenya with Reference to Potatoes."

10:30-11:30 Paper Session I (continued)

5. Shekawat, G. S., Simla Potato Research Institute, Simla, India: "Present Status of Research on Bacterial Wilt of Potato in India."

6. Gonzalez, L., University of Costa Rica, San Jose, Costa Rica: "Bacterial Wilt of Potato in Costa Rica."

7. Granada, G. A., Instituto Colombiano Agropecuario, Cali, Colombia: "Current Status of Bacterial Wilt Research In Colombia."

8. Seneviratne, S. N. de S., Central Agricultural Research Institute, Peradeniya, Sri Lanka: "Bacterial Wilt in Solanaceous Crops Grown In Rice Fields."

P.M. 1:00- 3:00 Tour of Phytotron and Department of Plant Pathology - Gardner Hall.

3:15- 3:45 P. Boistard, Station Centrale de Pathologie Vegetale, Versailles, France: "Approaches to a Genetical Study of Interactions Between *Pseudomonas solanacearum* and its Hosts."

3:45- 4:45 Special Topics.

7:30- 9:30 Task Force Committee Meetings: 2000 Gardner, 2515 Gardner, 3515 Gardner.

Wednesday, July 21

A.M. 8:30- 9:30 Discussion Session: 3533 Gardner Hall.

Ercolani, G., Istituto di Patologia Vegetale, Bari, Italy: "Assessment of Plant Resistance by Infectivity Titration."

9:30-11:00 Short Papers: 3533 Gardner.

1. Goto, M., Dept. of Plant Pathology, Shizuoka Univ., Shizuoka, Japan: "Bacterial Wilt of Strawberry in Japan."

2. Quimio, A., Dept. of Plant Pathology, Univ. of Philippines, Los Banos, The Philippines: "The Bacterial Wilt Problem in The Philippines."

3. Agboola, S. D., Moor Plantation, Ibadan, Nigeria: "Pseudomonas solanacearum - Induced Wilt Diseases in Nigeria."

4. Lallmahomed, G. M., Plant Pathology Division, Ministry of Agriculture, Reduit, Mauritius: "Strains of Pseudomonas solanacearum in Mauritius."

5. Olsson, K., National Institute for Plant Protection, Solna, Sweden: "Hosts, Temperature Data and Over-Wintering in Sweden."

11:00- 6:00 Field Trip, Eastern North Carolina.

A. Extension Research on Wheels, F. A. Todd, Tobacco Field Plot Demonstrations.

B. Clinton Experiment Station, S. F. Jenkins. Eggplant and Tomato Plantings.

Return to Raleigh at 10:00 P.M.

Thursday, July 22

A.M. 8:30-10:15 Individual Task Force Divisional Meetings - Preparation of Project Proposals: 2000 Gardner, 2515 Gardner, 3515 Gardner.

10:30-11:30 Discussion Session: 3533 Gardner Hall.

Digat, B., Station de Pathologie Vegetale-Phytobacteriologie, Angers, France: "Specificity of Antigens of Pseudomonas solanacearum and Application of Serology in the Study of Bacterial Wilt."

P.M. 1:00- 3:00 General Laboratory - Examination of Cultures, Physiological Tests, Serological Techniques: Microbiology Laboratory, 4524 Gardner Hall.

3:15- 5:00 Selective Media Comparisons; Inoculation and Isolation Techniques: Microbiology Laboratory, 4524 Gardner Hall.

7:30- 9:30 Paper Session II. A. Kelman, Chairman: 3533 Gardner.

1. Coleno, A., L. Hingand and B. Rat, Station de Pathologie Vegetale, Centre de Recherche Agronomiques des Antilles et de la Guyane, Petit-Bourg, Guadeloupe: "Serological Studies on Pseudomonas solanacearum."

2. Cuppels, D., A. Kelman and R. Hansen, Depts of Plant Pathology and Bacteriology, Univ. of Wisconsin, Madison, Wisconsin: "Bacteriocin Production by Pseudomonas solanacearum."

3. Nesmith, W. C., and S. F. Jenkins, Dept. of Plant Pathology, N.C. State Univ., Raleigh, N.C.: "Selective Medium for Isolation of Pseudomonas solanacearum from Soil."

4. Tanaka, Y., Kagoshima Tobacco Experiment Station, Kagoshima, Japan: "Factors Affecting Survival of Pseudomonas solanacearum."

5. Ramos, A. H., National Agricultural Laboratories, Nairobi, Kenya: "Comparison of Survival of Two Pseudomonas solanacearum Strains in Soil Columns under Constant Perfusion and in Field Plots Devoid of Soil Cover."

6. Lelliott, R. A., Plant Pathology Laboratory, Harpenden, England: "The Detection and Assay of Pseudomonas solanacearum Populations in Soil."

7. Lloyd, A. B., Dept. of Microbiology and Genetics, Univ. of New England, Armidale, N.S.W., Australia: "Bacterial Wilt in a Cold-Temperature Climate of Australia."

Friday, July 23

A.M.	8:30- 9:30	Examination of Inoculated Plants. Plant Pathology Greenhouse
	9:30-11:30	Discussion Sessions: 3533 Gardner Hall.
		Thurston, D., Cornell University, Ithaca, New York: "Breeding for Disease Resistance in Potato and Tomato."
		Sequeira, L., Univ. of Wisconsin, Madison, Wisconsin: "Specificity in the Infection of Plant Hosts by Pseudomonas solanacearum."
P.M.	1:00- 2:30	Review of Project Proposals by Task Force Committees: 2000 Gardner, 2515 Gardner, 3515 Gardner.
	3:00- 5:00	Presentation and General Discussion of Proposals, Future Plans and Recommendations: General Session, 3533 Gardner Hall.
	6:00- 7:00	Social Hour: Hilton Inn - Participants, Faculty and Graduate Students of the Department of Plant Pathology, North Carolina State University.
	7:00- 9:00	Banquet: Hilton Inn, Hillsborough Street.

Saturday, July 24

A.M. 9:00-11:00 Meeting of Task Force Committee Chairmen and Conference Discussion Leaders: Conference Room, Gardner Hall.

DISCUSSION AND PROGRAM PLANNING AREAS

Task Force Committees

1. Breeding for Disease Resistance. Leader, H. D. Thurston.

2. Biotype Differentiation and Variability. Leader, A. C. Hayward.

3. Ecology and Cultural Control Methods. Leader, S. F. Jenkins.

DISCUSSION LEADERS AND SUBJECT AREAS

A. C. Hayward -- Taxonomy and Biotypes

P. Boistard -- Genetics

B. Digat -- Serology

H. D. Thurston -- Disease Resistance

L. Sequeira -- Disease Physiology

G. L. Ercolani -- Assessment of Plant Resistance