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BACTERIAL WILT OF GROUNDNUT

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Bacterial Wilt of Groundnut

Proceedings of an ACIAR/ICRISAT collaborative research planning meeting held at Genting Highlands, Malaysia 18-19 March, 1990.

K.J. Middleton and A.C. Hayward, *Editors*

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Preface

In November 1988, Asian groundnut scientists met at Malang, Indonesia to discuss groundnut problems in the region, identify important constraints, and recommend opportunities for collaboration at national and international levels. That meeting was coordinated by ICRISAT. One high priority recommendation was for ACIAR's peanut (groundnut) project in Indonesia to coordinate groundnut bacterial wilt research.

An Australian Special Purpose Grant to CGIAR Centres (ICRISAT) was approved to support a meeting with the following objectives:

(a) to bring together scientists from the Asia-Pacific region working on the bacterial wilt disease of groundnut, caused by *Pseudomonas solanacearum*

(b) to review and share existing knowledge on *P. solanacearum*, and on breeding groundnuts with resistance to this disease, and

(c) to identify the needs for collaborative links in research, training and information exchange.

In addition, some ICRISAT funds were assigned to assist with the meeting expenses, and the Peanut Collaborative Research Support Program promised to support the attendance of project staff.

The meeting was arranged in association with the 3rd International Conference on Plant Protection in the Tropics, organised by the Malaysian Plant Protection Society (MAPPS) to allow delegates planning to come to the MAPPS conference to also participate in the bacterial wilt planning meeting; conversely, those scientists brought to the bacterial wilt meeting also had the opportunity to attend the MAPPS Conference, which has become the premier conference on tropical crop protection. The organising committee for MAPPS Conference, especially the chairman Dr K.Y. Lum, took care of all local arrangements for the bacterial wilt meeting.

The organisers are confident that the meeting was most successful, and will be remembered as a milestone in progress towards control of this important disease.

K.J. Middleton *A.C. Hayward*

Summary of Discussion and Recommendations

GROUP discussions were held on the topics of host plant resistance, pathogen detection and disease diagnosis, and disease management; brief discussion group reports follow. A combined set of recommendations formulated and agreed to at a subsequent planning session are printed in full.

Group Discussion Reports

(a) the **Host Plant Resistance** discussion group felt that more research was needed in evaluation of resistance, especially over a range of geographic regions to assess variation in host-pathogen interaction under different environments. The presence of such variation could lead to modification of existing procedures for selection and breeding for resistance. The group felt strongly that screening for resistance should be conducted under field conditions.

The group suggested that a network be established to address specific problems associated with bacterial wilt resistance in groundnut. The network could coordinate the establishment of an international bacterial wilt nursery set for testing at multiple locations, and could foster the exchange of resistant germplasm, especially that with multiple resistances. The network could also assist participants with technical assistance, information exchange etc. Potential participants in the network were identified, to be confirmed by contact with appropriate authorities. A future workshop to help establish the technical activities of the network (disease surveys, standardisation of resistance evaluation procedures etc) was proposed.

Further studies on inheritance of resistance appear warranted, encompassing a wider array of resistant material than has been studied to date.

The group suggested that scientists from Indonesia and Peoples' Republic of China should continue screening germplasm for resistance to bacterial wilt, and exchange germplasm for further study.

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(b) **Detection and Diagnosis** proved to be the area which created most discussion. Practical field control should be the goal of study in these areas.

After some debate, it was accepted that micropipetting is an appropriate method of inoculation for comparisons of isolates, but that it produced a severe challenge to the plant. Simulated natural root infection was likely to involve too many variables, and seed inoculation has not produced satisfactory results to date.

The group cautioned against the definition of specific inoculation procedures, but suggested that procedures found satisfactory should be offered as candidates for a standard procedure. At present, pathogenicity ratings are usually made on a scale of 1-5. Inoculation conditions (volume and concentration of inoculum, temperature condition, site of inoculation, stage of development of the host at inoculation, etc) should always be reported.

As far as facilities allow, standard susceptible reference plants should always be inoculated under the same conditions as test plants. It was felt that workers should use a standard set of peanut varieties, to minimise variability of reference hosts. ICRISAT'S collection could be a source of such material, but the most appropriate varieties might come from elsewhere.

The group felt that a reference collection of *P. solanacearum* strains should be maintained, including materials involved in research where new taxa are characterised. These should be maintained in a way that minimises confusion of data storage and identity, and minimises the influence of cultural history and maintenance method on characteristics. Possible sites for this collection suggested by the group included CIP, CMI and DSIR. Private collections should offer samples for this collection, as well as to other workers. Lists of holdings in these collections should be published in the ACIAR Bacterial Wilt Newsletter. Dr. Eden-Green undertook to coordinate all lists of private and institutional collections of *P. solanacearum*, and to resolve any problems of synonymy.

The group felt that new technologies offered much to assist in pathogen classification, and although they were not available widely, the results of their use was of benefit to all. The various methods of strain characterisation need to be compared.

Seed transmission is potentially significant, and requires verification urgently. Similarly, the significance of weed hosts is also high, and research into both of these areas could benefit from the use of new technologies for pathogen detection currently being developed.

(c) It was felt that **Disease Management** can make a significant impact on bacterial wilt severity, especially if used in an integrated way with other control methods. Crop management practices can influence both the pathogen and the host, as well as beneficial organisms, and theoretically should be able to provide low cost disease control if chosen wisely. However, much of this information is at best poorly understood; its application on a broad scale will depend on improving this situation. The role of research is to provide an understanding of the influence of crop management practices on disease incidence, to enable groundnut growers in future to change their practices and reduce losses due to disease.

The role of other plants (e.g. weeds and companion crops) on the size, activity and specificity of the *P. solanacearum* population in a groundnut field remains unknown, but might provide a key to control of this disease. Similarly, broader aspects of those cropping systems which include groundnuts such as bare fallowing (or the lack of it), the method, frequency, timing and quantity of irrigation, and crop nutrition could influence host susceptibility, pathogen survival and aggressiveness and the effects of other microorganisms on

infection rate. Soil type and pH appear to play some part in manipulating infection, but the pattern is not consistent, and more research is needed to allow us to understand these effects.

Environmental factors, especially temperature, may easily influence the infection of groundnuts by *P. solanacearum*, but changing these factors often conflicts with other management decisions. Injury to roots, a result of soil cracks caused by wetting and drying cycles, mechanical tillage operations in the field, or the activity of biotic agents such as nematodes and soil insects, could easily predispose the crop to infection if the only barrier to invasion depends on an intact root surface.

Seed transmission in either groundnuts or weeds could explain the appearance of the disease in fields where it had not been observed before. Seed transmission in groundnuts justifies close examination to ensure that simple methods of avoiding pathogen dissemination are utilised wherever available. This aspect of disease management is particularly important in relation to the international movement of seed.

Recommendations

The ACIAR/ICRISAT Collaborative Research Planning Meeting for bacterial wilt of groundnut, having reached consensus, commends these recommendations to the governments and science administrators of those countries where bacterial wilt of groundnut is recognised as a present or potential problem, and recommends:

1. Coordination of research on *Pseudomonas solanacearum*

Given the recent developments in characterisation of *Pseudomonas solanacearum*, and the significance of these findings for research on epidemiology and control of bacterial wilt, (i) a coordinator should be nominated to reconcile characterisation schemes and to promote the development of a unified infrasubspecific classification scheme for isolates of *P. solanacearum*, irrespective of host, (ii) funds and facilities should be sought for this work as recommended by the coordinator, (iii) the coordinator would be empowered to appoint his successor, and (iv) Dr. A.C. Hayward be requested to act as coordinator. Dr. Hayward accepted this responsibility.

2. Characterisation of *P. solanacearum*

a. Characterisation schemes should avoid reliance on technology available only to specialist research groups or commercial interests. Standardised techniques for detection and diagnosis which are readily accessible to the scientific domain should be adopted and published at the earliest possible opportunity. Reference samples of all described material, including the minimum number of isolates necessary to represent private collections, should be passed on to an international collection.

b. Characterisation schemes should take account of bacteriocin production and phage sensitivities. A reference panel of bacteriocin and phage-sensitive strains would be useful.

c. A single international reference collection of strains of *P. solanacearum* should be established, and necessary funding sought to achieve this. Stability and security of material and its ready availability to bona fide researchers in this field are important.

d. Lists of material in *P. solanacearum* collections should be provided and published in the Bacterial Wilt Newsletter, and the isolates should be made available to other researchers (at cost if necessary).

3. Host range and strain differentiation

a. The host range and aggressiveness of apparently groundnut-adapted strains should receive more attention. Virulence of strains should be assessed using the technique of infectivity titration under standard conditions.

b. International 'bacterial wilt nurseries' should be established, to use a defined set of germplasm to test pathogen variability and the effects of environment on disease incidence and severity.

4. Epidemiology and survival

a. Weed hosts should be identified, and their importance in survival and perennation of *P. solanacearum* quantified. Latently infected weeds should be included.

b. Consideration should be given to the possible role of nematodes and soil insects as predisposing factors in groundnut bacterial wilt epidemics.

c. The impact of crop sanitation practices on the pathogen and the disease needs to be established.

d. Appropriate new technologies should be used to monitor the survival and persistence of *P. solanacearum* in the soil and rhizosphere. The same methods should be used when appropriate to study the effects of cropping systems, soil type, cultural practices (including rotation), climatic conditions and soil solarisation on pathogen populations and disease severity.

e. The development of biological control systems should be encouraged. In this context (and in the context of recommendations 5a and 5b, below) the monoclonal antibody techniques developed by Dr A. Alvarez of the University of Hawaii, and by Dr S. Eden-Green of Overseas Development Natural Resources Institute appear to have particular application.

5. Detection of latent infections and seed transmission

a. The need for techniques to detect latent infection should be recognised, especially when vegetative propagation is used.

b. Studies of seed transmission, including the method of seed infection and transmission to groundnut, should be emphasised. The effect of storage on seed transmission appears important. These studies could be extended to other crops and weed species.

c. As the potential for seed transmission exists, caution must be exercised on the movement of groundnut seed, to minimise dissemination of *P. solanacearum* to disease-free areas.

6. Host plant resistance

a. Host plant resistance is recognised as having a most important role in control of groundnut bacterial wilt.

b. Greater effort is needed towards understanding the mechanisms of host plant resistance, to assist breeding for resistance.

c. Germplasm putatively resistant to bacterial wilt should be freely exchanged. Germplasm with multiple resistances is especially important.

7. Collaborative research network for bacterial wilt of groundnut

A collaborative network should be established to address needs related to groundnut bacterial wilt, i.e.

— to facilitate exchange of resistant germplasm
— to test resistant germplasm over a range of environments, and
— to provide technical assistance, define common procedures, disseminate information to participants, etc.

Potential network participants (subject to in-country approval) are:
AGLN Coordinator, ICRISAT (Administrative coordinator)
A.C. Hayward, Uni. of Qld. (Technical coordinator)
Liao Boshou, OCRI, Wuhan (China)
M. Machmud, BORIF, Bogor (Indonesia)
C.M. Busolo-Bulafu (Uganda)
V.K. Mehan (ICRISAT)
B.L. Ho (Malaysia)
M.P. Natural (Philippines)
K.W. Jayasena (Sri Lanka)
P. Surin (Thailand)

8. Research responsibility and support funds

a. Primary responsibility for research in particular areas lies with:

Chinese Academy of Agricultural Science, China - for effects of crop rotation, soil solarisation and biological control.

Agency for Agricultural Research and Development, Indonesia - for effects of crop rotation, seed transmission and crop sanitation.

b. National programs, particularly in Indonesia and China, should receive funding to assist research on disease management, as this research can only be conducted where the disease is endemic.

9. Training

Training is particularly needed in the areas of pathogen detection, disease diagnosis and disease management. Training should be provided by scientists within and without the region for others actively involved in groundnut bacterial wilt research.

10. Bacterial Wilt Newsletter

ACIAR should continue to support the publication of the Bacterial Wilt Newsletter, to a level equal to or greater than that provided to date.

Diagnosis, Distribution and Status of Groundnut Bacterial Wilt

A.C. Hayward*

Abstract

Most work on bacterial wilt of groundnut has been done in China and Indonesia, the two countries which are most seriously affected by the disease, but much of the published work has not been readily accessible. Bacterial wilt of groundnut in the southeastern United States is caused by *P. solanacearum* biovar 1, whereas in Indonesia and China and most other countries it is caused by biovar 3 or by biovar 4. Biovars 2 and 5 have never been reported on groundnut. A recent advance has been the application of restriction fragment length polymorphism (RFLP) analysis of DNA to *P. solanacearum*. The use of nine probes to the chromosomal DNA has led to the recognition of 28 groups, however, application of this methodology to isolates of *P. solanacearum* from groundnut is limited to date. There is evidence of differences in virulence among strains; for example, strains from southern China are more virulent than those from the north, but there is no clear indication of strains specialised to particular groundnut cultivars. Several studies have shown that the disease is most prevalent and severe in heavy clay soils, although the disease has also been recorded in red lateritic and light sandy loam soils. High soil temperatures ($\geq$25°C) and high day and night air temperature regimes profoundly increase disease incidence in susceptible cultivars. Continuous planting with susceptible cultivars, particularly in wet soils, rapidly leads to increase of disease. Many aspects of disease biology require further investigation, including a more critical examination of the possibility of transmission of the disease by/in seed. There is also a need for greater collaboration and coordination of effort; improved mechanisms for the exchange of promising resistant germplasm and breeding lines; and improved dissemination of information in English language publications.

Bacterial wilt of groundnut (*Arachis hypogaea*) caused by *Pseudomonas solanacearum* (Smith 1896) Smith 1914 was first reported in Indonesia as long ago as 1905, and since then has been reported in many regions throughout the world (Mehan et al. 1986). However, the amount of readily available information on the disease is very much less than that for the disease on solanaceous hosts such as tomato, potato, eggplant and tobacco. Groundnut is probably less susceptible than the solanaceous hosts, except where high intensity of cultivation under environmental conditions conducive to the disease is combined with the presence of specific strains of *P. solanacearum*. For many parts of the world these conditions do not obtain, so that the disease has either been considered of minor importance or is of only sporadic occurrence. This has meant a lesser volume of published work in English language periodicals. Not surprisingly, most of the research effort has emanated from China and Indonesia, the two countries which are most seriously affected. However, much of the valuable work which has been done in these countries has not been readily accessible and until recently remained less well known. Bacterial wilt is regarded as a potential threat to groundnut production in several warm humid areas in the world (Mehan et al. 1986) as production expands into new areas or cultural practices change.

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Geographical Distribution and Economic Importance

Bacterial wilt of groundnut is the cause of major losses in Indonesia, China and parts of Uganda (Table 1); however, there are many other countries where the disease is either relatively unimportant or where the status of the disease is uncertain (Table 2). In Fiji, for example, it is reported that in some areas groundnuts are severely attacked but that in general the loss is low compared to other crops (Iqbal and Kumar 1986). It is noteworthy that the disease has recently been reported in the Philippines (Natural et al. 1988), Sri Lanka (Jayasena et al. 1988) and Papua New Guinea (Tomlinson and Mogistein 1989), countries previously thought to be free of the disease (Hayward 1986; Tomlinson and Gunther 1986; Vellupillai 1986). This may reflect greater awareness of the disease as well as greater diligence in searching for it, or a real change in incidence/severity as a result of changing cultural practices.

Table 1: Worldwide distribution of bacterial wilt of groundnut

Countries in which the disease is of major importance:

Country	Authority
Indonesia	Machmud (1986)
Peoples' Republic of China	Boshou and Yujun (pers. comm.)
Uganda	Simbwa-Bunnya (1972)

Table 2: Worldwide distribution of bacterial wilt of groundnut

Countries in which disease is of minor importance or of uncertain status:

Country*	Authority
Fiji	Iqbal and Kumar (1986)
Malaysia	Abdullah et al. (1983)
Papua New Guinea	Tomlinson and Mogistein (1989)
Philippines	Natural et al (1988)
South Africa	Engelbrecht and Hattingh (1989)
Sri Lanka	Jayasena et al. (1988)
Thailand	Wongkaew (pers. comm.)
United States	Jenkins et al. (1966)
? Vietnam	Pham Xuan Tung (1986)

* Other records include Mauritius, Libya, Somalia, Ethiopia, Madagascar, Japan (Mehan et al. 1986), Zimbabwe and Australia (Northern Territory) (Hayward 1986).

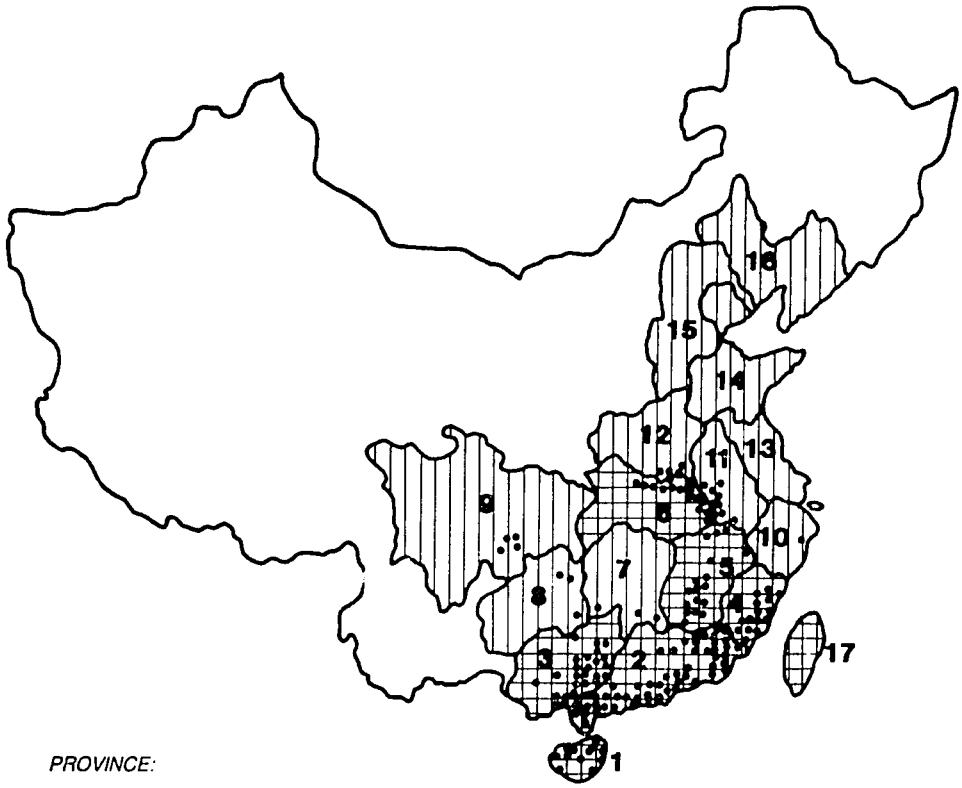
In China, the disease is most severe in central and southern production areas where it is estimated that more than 200 000 ha of groundnut fields are infested with *P. solanacearum* (Fig. 1). While the disease becomes more serious from north to south, within any region there are local variations in severity in response to soil type, presence of soil antagonists, climatic factors and host plant density. In recent decades, in spite of the adoption of control measures, the disease has spread further to Zhejiang and Guizhou provinces where the disease was not previously reported.

Bacterial wilt of groundnut is widely distributed in Indonesia. Generally the disease is more severe in South Sumatra, West Java and South Sulawesi than in other areas such as Central and East Java, Bali and Northern Sulawesi. Under conditions favourable for the disease, wilt incidence is very high and economic losses occur. The published record of bacterial wilt research in Indonesia, on all crops affected by *P. solanacearum*, starts in 1892 and for groundnut in 1905 (Kelman 1953). The literature reporting the results of research on bacterial wilt in Indonesia, which is more extensive than from any other source, has been well reviewed by Machmud (1986). Some central regions of Uganda are reported to be heavily wilt infested (Simbwa-Bunnya 1972), but the information is sparse and the present status uncertain. However the disease is of sufficient importance for a breeding program specifically directed against bacterial wilt to have been recently reinstituted (Louwaars, pers. comm.).

Table 3: Classification of strains of *Pseudomonas solanacearum* from groundnut according to biovar and RFLP group

Country	Biovar(s)	RFLP Analysis Division	Group(s)	Author(s)
Indonesia	3	I	ND*	Machmud (1986)
Papua New Guinea	3	I	ND	Tomlinson and Mogistein (1989)
Philippines	3	I	ND	Natural et al. (1988)
P.R. China	3	I	ND	Cook et al. (1989)
P.R. China	4	I	11 & 17	Cook et al. (1989)
Sri Lanka	3	I	ND	Jayasena et al. (1988)
Uganda	3	I	ND	Simbwa-Bunnya (1972)
Uganda	4	I	ND	Simbwa-Bunnya (1972)
United States	1	II	ND	Hayward (1964)

* ND not determined, no data available



PROVINCE:

1. HAINAN	7. HUNAN	13. JIANGSU
2. GUANGDONG	8. GUIZHOU	14. SHANDONG
3. GUANGXI	9. SICHUAN	15. HEBEI
4. FUJIAN	10. ZHEJIANG	16. LIACHING
5. JIANGXI	11. ANHUI	17. TAIWAN
6. HUBEI	12. HEBEI	

PROVINCES WHERE BACTERIAL WILT HAS BEEN REPORTED

PROVINCES WHERE BACTERIAL WILT IS GENERALLY IMPORTANT

REGIONS MOST SERIOUSLY AFFECTED BY THE DISEASE

Presented at the Planning Meeting to discuss future collaborative research on Bacterial Wilt of Groundnut, 18-19 March 1990, Genting Resort Malaysia, in association with the Third Conference on Plant Protection in the Tropics organised by the Malaysian Plant Protection Society.

Figure 1: Distribution of Groundnut Bacterial Wilt in China (based on data provided by Liao Boshou and Tan Yujun, Oil Crops Research Institute, CAAS, Wuhan, Hubei, Peoples' Republic of China).

Table 4: Classification of *Pseudomonas solanacearum* on the basis of restriction fragment length polymorphism involving nine DNA probes to chromosomal DNA into 28 groups (Cook et al. 1989)

	Division I	Division II
Biovars included in each Division*	Biovars 3, 4 & 5	Biovars 1 & 2
No. of groups	16	12
Similarity coefficients within division	78% 9%	62% 19%
Similarity coefficients between divisions	13.5%	

* Representatives of race 1 (Cook et al. 1989) are found in divisions I and II, while races 2 and 3 are found in division II.

Strains of *P. solanacearum* Affecting Groundnut

P. solanacearum is divisible into five biovars, three of which have been associated with bacterial wilt of groundnut (Table 3), on the basis of differences in utilisation and oxidation of certain hexose alcohols and disaccharides (Hayward 1986). Biovar 2, which is almost equivalent to race 3, the potato race, and biovar 5, have never been reported as pathogens of groundnut. The relatively minor disease on groundnut in the United States is caused exclusively by biovar 1, whereas in all other countries for which there is published information bacterial wilt of groundnut is caused by biovar 3 or biovar 4 (Table 3).

The definition of strains has been greatly advanced by the recent application of DNA analysis to classification of *P. solanacearum*. Cook et al. (1989) have used restriction fragment length polymorphism (RFLP) analysis to differentiate *P. solanacearum* into 28 groups, using nine probes to the chromosomal DNA. Similarity coefficients for all pairwise combinations of RFLP groups revealed two major divisions into which biovars and races could be allocated (Table 4). Similarity coefficients were high within each division and low between divisions. The present nomenclature does not reflect this fundamental difference at the genetic level. It is conceivable that, with expansion and confirmation of this work, divisions I and II will be separated at the level of subspecies. It is of considerable interest, and quarantine significance, that strains of *P. solanacearum* pathogenic for groundnut are found in both divisions, biovar 1 of division II in the southeastern United States, and biovars 3 and 4 of division I elsewhere. However, application of this methodology to isolates of *P. solanacearum* has so far been limited and should be extended. Cook et al. (1989) have concluded that all members of division II may have originated in the Americas. It is notable that strains of biovar 1 of division II are absent from many parts of Asia and Australasia (Hayward 1964 1975; He et al. 1983). Although conclusions about the evolutionary origin of biovars 3,4 and 5 of division I are greatly complicated by movement of *P. solanacearum* on vegetative propagating material of potato, ginger and banana, for example, it can also be concluded that division I is primarily of Asian origin. In this context it is interesting that the groundnut is of South American origin and that biovars 3 and 4 are apparently of much greater virulence to this host than isolates of biovar 1 which are indigenous to the Americas.

Variation in Virulence and Pathogenicity

There is ample evidence of differences in virulence among strains of *P. solanacearum* on groundnut. For example. Kelman and Person (1961) showed that isolates of *P. solanacearum* biovar 1 from tobacco, groundnut, tomato, eggplant and Irish potato from North Carolina, South Carolina, Georgia and Florida were uniformly and highly pathogenic on Irish potato, tomato and eggplant. However, there were marked differences among isolates from tomato in pathogenicity on tobacco and groundnut. Certain isolates that were avirulent on tobacco were highly virulent on groundnut, but the reverse was true of other isolates. Thus in the southeastern United States there are strains of *P. solanacearum* that differ in pathogenicity to hosts such as tobacco and groundnut.

Comparison of the pathogenicity of 26 isolates of *P. solanacearum* of Chinese origin on six host plants showed that groundnut was generally much less susceptible than eggplant, potato and tomato to isolates from a variety of hosts. Only 12 of the 26 isolates gave a high or moderate disease rating on groundnut and of the seven isolates giving a high rating five were from groundnut (Table 5).

Although bacterial wilt of groundnut has never been reported in Australia in production areas, this probably reflects the fact that

Table 5: Pathogenicity rating of 26 isolates of *P. solanacearum* from China on six host plants. Data from He et al. (1983)

Host Plant	Pathogenicity Rating* for 26 Isolates** High (H)	Moderate (M)	(H+M)	Low	Zero
Eggplant	21	3	(24)	2	0
Potato	19	1	(20)	6	0
Tomato	9	12	(21)	3	2
Pepper	5	6	(11)	4	11
Groundnut	7***	5	(12)	6	8
Tobacco	2	0	(2)	0	24

* Results based on average disease indices of 5-10 plants 21 days after inoculation; H = high (4.1-5.0), M = medium (2.6-4.0), low (1.1-2.5), and zero = none (1.0).

** Isolates came from the following hosts: tobacco, olive (3), sweet potato (2), groundnut (7), ginger (2), sesame, potato (2), tomato (2), urtica, pepper, *Casuarina* sp., eggplant and mulberry (2).

*** Including five isolates from groundnut and one each from eggplant and *Casuarina* sp.

groundnuts are not cultivated in areas of Queensland where soils are heavily infested with *P. solanacearum* biovar 3. Using the axillary inoculation technique, Subandiyah (pers. comm.) has shown that strains of *P. solanacearum* biovar 3 from various economic and weed hosts in Queensland and New South Wales are pathogenic for groundnut, but there are marked differences in virulence between isolates. She found that the most virulent isolates were from a part of southeast Queensland (Nambour) of high wilt intensity and incidence on other wilt-susceptible hosts.

Although there is a marked difference in pathogenicity and virulence of *P. solanacearum* isolates from different localities there is no clear indication of strains of the pathogen specialised to particular groundnut cultivars (Boshou pers. comm.).

Effect of Environment on Disease Expression

Several studies have shown that bacterial wilt of groundnut is most prevalent and severe in heavy clay soils. Despite this, the disease has also been recorded in red lateritic and light sandy loam soils (Mehan et al. 1986), and in China most of the naturally infested fields are of sandy soil (Boshou and Yujun pers. comm.). Abdullah et al. (1983) showed in greenhouse studies that the disease was most severe in soil of high clay content compared with two more sandy soils at the same moisture content. Soil types, alone and in combination with varying soil moisture levels, had a significant effect on the severity of bacterial wilt of groundnut. Disease severity was shown to increase significantly with increase in soil moisture from slightly above wilting point (-1.5 MPa) to slightly below saturation point (-0.03 MPa) for each of three different soils (Abdullah et al. 1983).

High soil temperature early in the growing season favours the development of bacterial wilt on young groundnut plants. Stable soil temperatures above 25°C at 5 cm depth, together with high soil moisture favour disease development (Boshou pers. comm.). Subandiyah (pers. comm.) has shown in experiments conducted in controlled environment glasshouses that the severity of wilt caused by *P. solanacearum* biovar 3 strains was most pronounced at diurnal temperature regimes of 35/30° and 30/25° and slight or absent at regimes of 25/20° and 20/15°C. Particularly in wet soils, continuous planting with susceptible cultivars leads to a rapid build up of disease. These heavily infested fields are very useful for screening of germplasm and breeding lines for resistance (Boshou pers. comm.).

Future Priorities

There are many aspects of disease biology which require further investigation. In particular, in view of the implications for local and international quarantine measures, the possibility of transmission on seed requires more critical investigation. Much more work is required on the host range and aggressiveness of strains and their geographical distribution. Are there strains of *P. solanacearum* specialised for different groundnut cultivars, and do the strains of *P. solanacearum* affecting groundnut possess specific virulence determinants? Other aspects requiring attention include: disease severity in relation to temperature regime, soil type, soil biological factors and cropping systems; the significance of weed hosts including those latently affected; the occurrence and significance of the pathogen in the rhizosphere of non- hosts; the importance of nematodes and soil insects in infection of groundnuts. There is also a need for a more systematic evaluation of crop rotation as a disease management aid.

Apart from more well directed research projects which address the most significant questions, there is a general need for greater collaboration and coordination of effort. Recently developed technologies must be

applied to bacterial wilt of groundnut. There must be more efficient mechanisms for the exchange of promising resistant germplasm. The flow of information needs to be greatly improved. For example, much of the important work which has been conducted in China is not readily accessible to workers outside China, is slow to penetrate into English language abstracting journals and then appears only in a highly truncated form. There is no doubt that the flow of information needs to be improved in the opposite direction as well.

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Host Plant Resistance to *Pseudomonas solanacearum*

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Abstract

Probably no other bacterial plant pathogen causes more economic loss to a more diverse range of food crops than the bacterial wilt pathogen. Because of the destructive and persistent nature of the pathogen, profitable production of susceptible crops becomes impossible in many areas of the world once certain strains are established in the soil. This persistence and its wide host range often limit the effectiveness of cultural and chemical control practices. With the exception of banana and plantain, where proper management, good sanitation and crop rotation can contain the disease, control of bacterial wilt in other crops has been achieved primarily through the use of host resistance. However, it is not uncommon that cultivars developed with high levels of resistance at a given location do not perform as well or survive in other geographic areas. This is apparently due to the presence of specific strains of the pathogen or because resistance is not expressed under certain environmental situations. Lack of information on the many different strains that apparently exist throughout the tropics and subtropics has undoubtedly hampered the effective use of genetic resistance in the control of bacterial wilt. Information on the intrinsic complexities of pathogenicity differences, and their geographic distribution are essential to the proper implementation of programs designed to test, distribute and utilise host germplasm. Improved communication between the scientists involved and the adoption of standardised methods in the description of pathotypes and the testing of host materials are important prerequisites. Molecular biology now provides us with techniques to better understand the genetics of pathogenicity of the bacterial wilt pathogen. As these new approaches continue to generate new and useful information, current approaches used in collaborative germplasm exchange and distribution programs should be constantly reviewed to ensure the cost-effective exploitation of the resources available.

Bacterial wilt caused by *Pseudomonas solanacearum* is responsible for economic loss to a more diverse range of food crops than any other bacterial plant pathogen (Kelman 1953). The persistence of the pathogen in soil, especially in the semi-tropical and tropical regions of the world, and its wide host range has rather limited the effectiveness of cultural and chemical control strategies. With the exception of banana and plantain, where proper management including good sanitation practices and crop rotation can contain the disease, control of bacterial wilt in other crops has primarily relied on host resistance. However, although progress has been made in the development and utilisation of host resistance in various parts of the world, the lack of uniform behaviour of available sources of resistance to bacterial wilt remains a reality. The observation that cultivars selected for genetic resistance to the pathogen do not behave consistently under different environmental situations may be attributed to a number of reasons. These include (a) the existence of different strains of the

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pathogens in different parts of the world, (b) the influence of environmental factors favouring severe development of disease on the genetic factors conferring resistance, and (c) differences in the procedures adopted in the evaluation of the performance of the cultivars in question. Compared to the other crop hosts, bacterial wilt of groundnuts appears to occur in an isolated fashion (Mehan et al. 1986). Although many countries in the tropics are plagued by the disease, its economic importance on groundnuts seems to be restricted to countries such as China (He 1986), Indonesia (Machmud 1986) and Uganda (Simbwa-Bunnya 1972). The severity of the disease varies according to soil and climatic factors that prevail, but remains important in these countries despite the use of resistant cultivars. In China, where groundnut represents an important cash crop, control of bacterial wilt emphasises the integrated management of the disease combining the use of host resistance and crop rotation systems.

Pathogenicity of *P. solanacearum* Affecting Groundnuts

Differences between strains in terms of their pathogenicity to groundnuts and other hosts were reported as early as 1924, when van der Goot (1924) observed that the strain of *P. solanacearum* attacking potatoes in Java was distinct from that affecting groundnut. This conclusion was reinforced by the findings of Schwarz (1926) that the strain which attacked groundnut, tomato and tobacco in Indonesia was different from that attacking potato and eggplant. In extensive cross-inoculation tests in the U.S., Kelman and Person (1961) identified strains which were avirulent to tobacco but highly virulent to groundnuts, as well as isolates which showed the reverse reactions.

Development of Resistant Cultivars

The subject of host resistance to *P. solanacearum* has been reviewed by Thurston (1976). Although the search for resistance continues (Hayward et al. 1987; Liao et al. 1986; Machmud and Middleton 1987 and Sankar et al. 1987), bacterial wilt remains important on all major host crops, suggesting that breeding for resistance has not been wholly successful, and much remains to be done (Buddenhagen 1986). It must be emphasised that the observed performance of a cultivar is the end product of complex interactions between the genetic potential of the host, the environmental conditions that prevail and the nature of the strains that exist. In other words, disease management strategies can only be developed out of sufficient knowledge of the host, the pathogen and the environment. The efforts of collaborative programs working towards the development, identification and dissemination of resistant germplasm, such as those supported by ICRISAT, AVRDC and ACIAR are commendable. But, with the limited number of good breeding programs where genetic materials are evaluated for performance under conditions which reflect the local pathogen situation and environmental condition, it is all the more important that collaborative networks be strengthened and operated in a manner that allows maximum exploitation of the efforts expended in evaluating germplasm. As we are all too aware, the limitations of collaborating countries in the developing world in terms of manpower and support facilities demand that maximum information be extracted from the trials conducted. To this end, the following considerations are suggested:

(a) collaborative trials should preferably be set up at different test localities where the disease is important and where different strains are suspected.

(b) protocols for screening host material should incorporate procedures which allow for some description of the strains of the pathogen to which the host materials have been subjected.

(c) such description should take a form that relates to the performance of the host materials and allow for comparison of results between collaborating individuals. The incorporation of a set of differentials has traditionally been the approach.

(d) the question of natural vs. artificial infection in evaluation programs should be reviewed. For example, Kloos and Fernandez (1986) reported that under artificial infection, all accessions of potato exhibited 10% infection.

A standard collaborative bacterial wilt disease nursery would conceivably have as its main objective the establishment of an international set of uniform trials to test accessions and breeding lines. This is achieved through the setting up of standard field nursery beds, multiplication of inoculum and the testing of materials distributed by the coordinating agency over a period of years. The incorporation of local resistant/susceptible checks serves to provide for comparison of performance of test

lines between trials. What is lacking in this system of assessment is the absence of any component which is able to match the resistance or susceptibility with a description of the prevailing strains in a particular locality. The reactions of the resistant and susceptible checks provides nothing more than a measure of performance of the test lines relative to the checks, and an indirect comparison with the performance of lines tested in other assessment trials. To maximise the utility of results gathered in these collaborative trials, to be able to generate a record of the characteristics of strains prevailing under the condition of the particular test, and to be able to match this information with that of host performance, other approaches may have to be considered.

It has been said that resistance breeding for crops cannot be well executed without an understanding of the pathogen and its range of variability The Task Force Committee on biovar differentiation and variability at the Planning Conference and Workshop on the Ecology and Control of Bacterial Wilt held at North Carolina in July 1976 considered that high priority should be given to '...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 standardised'. The Task Force Committee on Breeding for Resistance at the same meeting made the following recommendations:

(a) because of the potential for change in the pathogen, complacency about maintaining the somewhat adequate resistance in tobacco and groundnuts is undesirable;

(b) a concerted worldwide effort to cooperate or co-ordinate efforts in the study of bacterial wilt resistance in vegetable crops is lacking;

(c) resistances detected by individual local programs have not been utilised in the most adequate manner;

(d) there is a need to develop standardised procedures for testing and evaluating resistance under laboratory and field conditions.

Infectivity Titration

Infectivity titration has been proposed by Ercolani (1976) as a means to study the plant-microbe interaction. In the context of host plant resistance, reliable estimates of the relative resistance of the host rather than the pathogenicity of the microorganism are usually easier to obtain, because microbial populations can be preserved, at least temporarily, from changes in factors that determine virulence.

Infectivity titration is apt to provide estimates of the relative resistance of two or more hosts to a given bacterium either in terms of differences in the doses of the bacterium that induce the same indicating effect, or differences in the indicating effect induced by equal doses of the bacterium. Conversely, estimates of the relative virulence of two or more bacteria for a given host may be expressed either in terms of differences in the doses of each bacterium that produce the same indicating effect, or differences in the indicating effect induced by equal doses of each bacterium. Both host and pathogen effects can be distinguished into quantal (all or none) and quantitative responses. Infectivity titrations with quantal responses (e.g. turgid or wilted, dead or alive) have found greater applications with plant pathogenic bacteria.

A common finding of infectivity titration experiments is that the rate of increase of response with increasing dose may differ for different hostpathogen combinations. The relationship between dosage and response is best assessed over those portions of the dose-response curve where equal increments of dose cause comparatively greater increases in response. Because earlier investigators realised that quantal effects show this trend in the region of 50% response, the median effective dose (ED_{50}), i.e. the dose that causes the indicating effect to appear in 50% of the subjects, has become one of the standard measures of bacterial virulence. This value can, conversely, be used in comparisons of the relative resistances of host to the same bacterium. Because of the different rate of increase of response with increasing dose mentioned above, the ED_{50} is usually found to lie in the middle of an S-shaped curve when the cumulative percentage of response is plotted against the dose on a arithmetic scale. Probit transformation (Finney 1971) changes this into a straight line and allows the possibility of expressing the relation between dosage and response with just two parameters of the dose-response curve, i.e. the ED_{50} and the corresponding slope of the straight line fitted to the data points. Therefore, if two bacteria generate log dose-probit response

curves with the same slope on a given population of hosts, their relative virulence can be determined directly by the ratio of their ED_{50}'s, obtained using the same bacterium as inoculum.

Concluding Remarks

Host resistance continues to play a crucial role in the management of bacterial wilt. Besides the need to broaden the genetic base in existing resistance breeding programs, it is equally important to maximise the usefulness of the genetic materials that have been and are being generated by various breeding programs. The need to get the most out of current screening activities initiated through inter-country and inter-regional collaborative programs cannot be over-emphasised. If current protocols for the evaluation of germplasm material can be improved further, whether through better sets of differential lines or other means, the collaborative network systems offer the best mechanism for us to better understand the complexities of pathogenicity and resistance in the bacterial wilt disease.

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Control of Bacterial Wilt of Groundnut in China with Emphasis on Cultural and Biological Methods

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Abstract

Groundnut is one of the most important industrial crops in China. The area under cultivation is 3.3 million hectares. Bacterial wilt on groundnut is distributed over 17 provinces or regions of the country. Ten to 30 percent of wilted plants were observed in dryland areas. In China, in contrast with some other countries, bacterial wilt of groundnut is prevalent in sandy soils, particularly in poor gritty soil, but not in heavy clay or loam soils. Strains of *Pseudomonas solanacearum* on groundnut collected from different geographical locations were identified as biovars 3 and 4 of race 1. Control of bacterial wilt on groundnut depends on use of host resistance, cultural measures, biological approaches, use of chemicals, etc. as well as implementation of plant quarantine. Of these, use of resistant cultivars and suitable crop rotation systems are essential and have proved to be the most effective means of reducing damage caused by the disease. Several varieties, such as Luhua No. 3, Ehua No. 5, Zhonghua No. 2, Guiio No. 2 and Yueio 92 possessing resistance to bacterial wilt and good agronomic characters, have been released in recent years and are grown over a large acreage in production areas. Rotation of groundnut with rice demonstrated excellent effectiveness in reducing bacterial wilt. A variety of groundnut-rice rotation schemes suitable for local cultural systems are widely applied by farmers in irrigated areas of Guangdong, Fujian, Hubei and Shandong provinces. Dryland rotation for a relatively longer period (4 years and more) with immune crops, such as sugarcane, wheat, barley, maize, sorghum, etc., gave a significant reduction in bacterial wilt incidence. A preliminary study on utilisation of spontaneous avirulent strains of *P. solanacearum* for treatment of groundnut seeds showed reduction of 77.6% in disease incidence under greenhouse conditions. Application of chloropicrin (300 kg per hectare) in soil ten days before planting was proven to reduce populations of the pathogen. None of the pesticides tested so far showed potential for practical utilisation. Groundnut seeds or pods are suspected to be a potential route of dissemination of inoculum although 1699 seeds harvested from diseased plants were tested and no infection was observed. Nevertheless, the integrated management of this disease should include strict control of seed movement to avoid any possibility of spread of inoculum.

GROUNDNUT is a very important industrial crop grown on approximately 3.3 million hectares in China. Bacterial wilt (BW) on this host was first reported in China in the middle of the 1950s, although it was observed by local farmers 1 to 2 decades earlier than this date (Ma and Gao 1956; Li 1958). Before the middle of the 1960s, this disease was limited to a few provinces of southern China. But now it is observed in 17 provinces or regions, distributed from latitudes 19° N to 39° N (Wang et al. 1983). According to incomplete estimations the BW infected acreage of groundnut growing fields is more than 0.2 million hectares. The disease incidence ranges from 1 to 5% in rice cultivation areas and from 10 to 30% in drylands. However, more than 50% wilting plants were observed in severely infected fields (Fan et al. 1960; OCRI of Hubei 1977;

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LYAI 1976). Many studies on dissemination, epidemiology, strain identification and control methods for bacterial wilt of groundnut were conducted during the past 35 years, particularly in the period 1974-1983. This paper presents a review of various control measures for bacterial wilt of groundnut, with the emphasis on cultural and biological methods. Disease epidemiology and strains of the pathogen are also briefly discussed.

Some Environmental Factors Affecting BW Epidemiology

In contrast with many other countries or regions of the world, in China bacterial wilt of groundnut is prevalent in sandy soil, particularly in gritty soil, but not in heavy clay or loam (Ma and Gao 1956; Li 1958; OCRI of CAAS 1977b). A well designed study of the relationship between sand content of soil and BW incidence indicated that the higher the percentage of sand in soil the more groundnut plants wilted. For instance, in pure sand (100%) BW incidence of groundnut plants was 92%, while in heavy yellow loam the BW incidence was zero. Also, the BW incidence reached 21.8% in the treatment 60% sand plus 40% yellow loam, whereas the BW incidence was 4.2% in the treatment 60% yellow loam plus 40% sand (Hou and Wang 1980). The authors concluded that the higher incidence in sandy soil was attributable to its greater air content which was of benefit to multiplication of the bacteria.

Bacterial wilt of groundnut is known as a disease of warm and wet regions. High temperature and moisture favoured its development (Zhou and Liu 1962; Li et al. 1981). According to observations in Linyi, Shandong province, when the air temperature was higher than 20°C and the 5cm depth soil temperature was kept stable at 22°C or higher, the disease symptoms appeared in 10 days. When the soil temperature exceeded 25°C accompanied by a certain amount of precipitation, bacterial wilt developed rapidly (Wang et al. 1983). Precipitation or soil moisture affected wilt development less than temperature, although they were necessary factors. Large amounts of precipitation or high moisture usually reduced soil temperature, which might slow down wilt development (Li et al. 1981; Wang et al. 1983).

Strains of *Pseudomonas solanacearum*

A number of isolates were collected from different geographical locations and identified as biovars 3 and 4 by Hayward's scheme (He 1986; Ren et al. 1981). Examination of their pathogenicity on different host plants showed that all isolates tested belonged to race 1 based on Buddenhagen and Kelman's classification system with some variation in pathogenicity on pepper, common tobacco and sesame (LYAI 1979; Chen et al. 1981; He 1986; He et al. 1983). The virulence of strains from Guangdong and Guangxi markedly decreased when they were tested in the northern provinces Hubei, Jiangxi and Shandong, indicating an environmental effect (Xu et al. 1980).

Utilisation of Resistant Cultivars

Following the observation of differences among groundnut cultivars in response to bacterial wilt in the field, Chinese scientists started selection of local germplasm and evaluation for wilt resistance in the late 1950s. Several resistant cultivars, such as Taishan Sanlirou, Taishan Zhenzhu, Tianjing, Xiekangqui, Huengchuan Zhigan and Yueio 589 were released for practical use in the 1960s and 1970s (Zhou and Liu 1962, IPP of GAAS 1976; OCRI of CAAS 1977a; Xu et al. 1980; Wang et al. 1983). In recent years, some new resistant varieties with high productivity and good quality, such as Luhua No. 3, Ehua No. 5, Zhonghua No. 2, Guio No. 2 and Yueio 92 were certified and introduced into groundnut production. Luhua No. 3 gave on average 2.99 t per hectare from 15 sites in infected areas and 3.37 t per hectare on average from 4 sites in disease-free areas, which was 167.3% and 10.8% higher than the local cultivated variety Beisha 1016. Moreover, this variety has good resistance to drought and tolerance to poor soil and was suitable not only for Shandong province but also some southern cropping regions (Hou 1985).

Cultural Control

A variety of cultural measures, including application of lime, plant ash and manure, addition of sludge and clay to sandy soil, and drainage, etc., were examined for control of bacterial wilt of groundnut (Ma and Gao 1956; Li 1958; Fan et al. 1960; Zhou and Liu 1962; IPP of GAAS 1976). These measures had a variable effect in reducing BW incidence, but were not applicable on a large scale. Rotation

with non-host crops, especially with rice, was proven to be the most effective method. Various groundnut-rice rotation systems are applied in different regions. One or two years of rice cultivation could markedly reduce disease incidence in groundnut fields. Rotation with rice for a period of three or more years could minimise and even eradicate bacterial wilt. Results from field experiments in Linyi, Shandong province, indicated that to grow groundnut in a previously diseased field after three years rice cultivation reduced bacterial wilt incidence from 83.4% to 1.5% (Wang and Hou 1982). Crop rotation in drylands may be quite effective if a longer period (four years or more) of growing alternative immune crops is adopted or if the rotation takes place before the disease incidence in a groundnut field becomes high. In most cases, sugarcane, barley, wheat, maize, sorghum and cotton are suitable alternative crops in drylands (OCRI of CAAS 1977b; Li 1958). Sweet potato has been used in groundnut rotation systems for a long time and is still utilised in current production in some regions. However, recent reports from the Fujian Academy of Agricultural Sciences indicate that strains from sweet potato were able to cross infect groundnut and vice versa under artificial inoculation conditions (Lu et al. unpublished; Zhong et al. unpublished). More work is needed to find out whether both crops are naturally infected by the same strains. Flooding of groundnut fields for 15 days before planting was considered to effectively reduce bacterial wilt incidence (IPP of GAAS 1976; OCRI of CAAS 1977b).

Chemical Control

A number of chemicals, including fungicides, antibiotics and fertilisers were examined for control of groundnut bacterial wilt in the 1960s-1970s. Few of them were shown to be effective or available in practice (DPPM of OCRI 1975; IPP of GAAS 1976; Wang and Hou 1982). Application of 300 kg/ha of chloropicrin 10 days prior to planting gave 97.5% disease control and 62.6% yield increase (Wang and Hou 1982). But this chemical seems not to be promising over large areas of groundnut because of its high cost.

Biological Control

Attempts were made to use antagonistic actinomycetes for control of groundnut bacterial wilt (Meng and Zhou 1963; IPP of GAAS 1976). Several strains of *Streptomyces* were artificially cultured in cottonseed residue cakes and examined on field plots. Unfortunately, they appeared not so effective or not stably effective for wilt control.

A tentative study on utilisation of avirulent strains of *P. solanacearum* for control of groundnut bacterial wilt was carried out in our laboratory. Significant reduction of disease index was observed. The avirulent strain AP7 showed 70-80% control efficiency in the greenhouse and appeared effective to a certain extent until 60 days after planting (He and Kang 1986). Avirulent strains of *P. solanacearum* and antagonistic rhizobacteria were examined for control of bacterial wilt of tomato, potato and tobacco (Ren et al. 1988; Kempe and Sequeira 1983; Chen and Echandi 1984; Aspiras and Cruz 1986). The efforts probably should be aimed at increasing control effects in fields and, therefore, much basic work has to be done on the interaction of control agents and soil microorganisms and related environmental factors.

Plant Quarantine

One thousand six hundred and ninety nine seeds harvested from BW diseased plants of groundnut were planted in sterile soil for examination of seed-borne infection but no wilt infection was observed (Zhou and Liu 1962). Similar results were obtained by the Oil Crop Research Institute (OCRI of Hubei 1977). The question arises: by what means did BW of groundnut spread so rapidly from one or two locations to so many regions within 35 years? It is still not clear whether the strains of *P. solanacearum* on groundnut in different regions are indigenous or introduced.

The bacterial wilt pathogen is considered to be potentially seed-borne on groundnut (Mehan et al. 1986). Accordingly, strict control of seed movement to avoid the spread of inoculum to disease-free areas must be included in the integrated management of this disease.

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Monoclonal Antibodies for the Identification of Plant Pathogenic Bacteria: Potential Applications to *Pseudomonas solanacearum*

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Abstract

Monoclonal antibodies (mAbs) have been used to identify plant pathogenic bacteria, trace the movement of strains and subpopulations in the field, and aid in classification of strains of a pathogen. Taxon-specific epitopes have been identified with a series of mAbs in *Xanthomonas albilineans*, pathovars of *X. campestris* (*begoniae*, *campestris*, *citri*, *citrumelo*, *dieffenbachiae*, *oryzae*, *oryzicola*, *pelargonii*, and *vesicatoria*), *Erwinia* spp., *Pseudomonas solanacearum*, and *Clavibacter michiganensis* subsp. *michiganensis*. Depending on the pathovar, the mAbs detect various epitopes associated with cell surfaces, such as proteins, lipopolysaccharides, and extracellular polysaccharides. Examples of the utility of the mAbs are as follows: 1) A *X.c. oryzae*-specific mAb (*Xco*-1) reacted with all 178 tested strains of the bacterial leaf blight pathogen of rice, whereas another mAb (*Xco*-5) reacted only with a subpopulation of strains isolated from a recent blight outbreak in the United States (Texas and Louisiana); 2) A panel of mAbs delineated pathotypes of *X.c. citri* associated with various forms of citrus bacterial canker (CBC-A, CBC-B, and CBC-C); 3) mAbs specific for the citrus leaf spot pathogen, *X.c. citrumelo* have been produced, and the serological reaction patterns of selected strains corresponded closely with their genomic fingerprints determined by RFLP analysis; 4) mAbs specific for *X.c. campestris* were associated with strains having various degrees of virulence. The generation of pathovar and pathotype-specific mAbs in *X. campestris* suggests that certain surface antigens may be related to host specificity. In general, plant pathogens that infect a single host were detected by a specific mAb to a unique surface antigen, whereas a panel composed of several specific mAbs was needed to detect all strains of a pathogen that has a broader host range. Attempts to find surface antigens of *P. solanacearum* related to host specificity, biovar, or RFLP groupings have been initiated.

The reliability of monoclonal antibodies (mAbs) for identification of specific plant pathogenic bacteria and the usefulness of mAbs for the analysis of subpopulations in epidemiological studies has been demonstrated in a few cases (Alvarez et al. 1985; Benedict et al. 1989; Benedict et al. 1990; Yuen et al. 1987). In view of these examples, the potential of mAbs for the identification and characterisation of the groundnut strains of *Pseudomonas solanacearum* and the possible delineation of other strains of the species can be examined.

Monoclonal antibodies have been generated for a variety of plant pathogenic bacteria (Table 1). Principally these are pathogens in the genus *Xanthomonas* that affect a variety of hosts. In addition, specific mAbs have been produced to identify the tomato canker pathogen, and studies have begun on some *Erwinia* species, and two pseudomonads, *P. fuscovaginae* and *P. avenae*. Recently an antibody that appears to be specific for *P. solanacearum* has been generated, and another that delineates a banana strain of *P. solanacearum* from other

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pseudomonads is being tested with additional strains.

Table 1: Some examples of monoclonal antibodies produced for phytopathogenic and other bacteria.

Genus species pathovar	Disease	Host
Xanthomonas campestris	leaf spot	crucifers
pv. *armoraciae*		
pv. *campestris*	black rot	crucifers
pv. *dieffenbachiae*	blight	aroids
pv. *vesicatoria*	leaf spot	tomato, pepper
pv. *citri*	canker	citrus
pv. *citrumelo*	leaf spot	citrus
pv. *oryzae*	blight	rice
pv. *oryzicola*	leaf streak	rice
pv. *pelargonii*	blight	geranium
pv. *begoniae*	blight	begonia
X. albilineans	leaf scald	sugar cane
X. maltophilia	—	—
Pseudomonas solanacearum	wilt	many
Erwinia carotovora subsp. *carotovora*	soft rot	many
E. chrysanthemi	rot, wilt	many
Clavibacter michiganensis subsp. *michiganensis*	canker	tomato

The mAbs produced for plant pathogenic bacteria have different characteristics that eventually become important when designing rapid diagnostic tests. They vary in their isotypes, their affinities, and in their capacity to agglutinate antigens. Working dilutions vary from 1:1000 to 1:32 000 as determined from a binding curve.

Table 2: Taxon-specific monoclonal antibodies produced for phytopathogenic bacteria

Genus species pathovar	mAb(s)	No. strains tested
Xanthomonas campestris	X1, X11	>2000
pv. *campestris*	X9, X13, X17, X21	>1000
pv. *citri*	A1, B1, B2, C1, F1	284
pv. *oryzae*	Xco-1	178
pv. *pelargonii*	Xcp-1	76
pv. *begoniae*	Xcb-1	26
Clavibacter michiganensis subsp. *michiganensis*	Cm-1	88

The surface antigens to which these mAbs react are of varying chemical composition. Many of the antibodies generated to cell surface antigens react with macromolecules that appear to be lipopolysaccharide (Benedict et al. 1989 and 1990). Some antigens are extracellular polysaccharides, and some are proteins. The antigens may be located in the cell envelope or in some cases, on the flagella. The outer membrane of gram negative bacteria is complex and has a variety of potentially antigenic molecules which may be detected by mAbs.

The electronmicrograph (Fig. 1) shows an immunogold stain of a *X.c. citri* cell when reacted with a *citri*-specific mAb, A2. The antibodies conjugated to the gold particles are attached to the flagellum which has been detached from the cell. Figure 2 shows a cell of *X.c. pelargonii* in which the gold is attached to what was shown by extraction procedures and Western blots to be lipopolysaccharide. In the case of *C.m. michiganense*, the gold particles appeared to be associated with extracellular polysaccharide (EPS) (figure not shown). Avirulent strains of this organism lacked the EPS and failed to react with the mAb.

Figure 1: Immunoelectron micrograph of *Xanthomonas campestris* pv. *citri* reacted with a 1:10 dilution of mAb A2.

In order to generate antibodies with the desired specificities it is necessary to test large numbers of target and nontarget strains of other bacterial genera, species and pathovars (Alvarez and Benedict 1990). The numbers of strains of *X. campestris* and of *C.m. michiganense* that were tested in order to verify the specificities of the mAbs generated to various pathogens are shown in Table 2. For example, the genus specific mAbs X1 and X11 have been tested with over 2000 strains of *Xanthomonas* as well as a large number of non-xanthomonads. The pathovar-specific mAbs for *X.c. campestris* have been tested with over 1000 strains, and large numbers of strains of the other *X. campestris* pathovars (*dieffenbachiae, citri,*

Figure 2: Immunoelectron micrograph of *Xanthomonas campestris pv. pelargonii* reacted with a 1:10 dilution of mAb Xpel-1.

oryzae, *pelargonii*, and *begoniae*) also were tested to verify the specificities of their respective antibodies.

In studying the serological groups of bacterial plant pathogens, the *Erwinia* species, *E. carotovora* and *E. chrysanthemi*, were found to be serologically heterogeneous, confirming earlier studies with polyclonal antisera (Dickey et al. 1984). Pathovars of *X. campestris* (*campestris*, *citri*, *dieffenbachiae* [Alvarez et al. 1985; Bonner R.L. 1988; Bonner et al. 1987; and unpublished data] and *vesicatoria*) have several serological subgroups. In contrast, other pathovars (*pelargonii*, *begoniae*, *oryzae*, *oryzicola*, and *C.m. michiganense*) appear to be relatively uniform (Benedict et al. 1989, 1990 and unpublished data).

The homogeneity and heterogeneity of bacterial pathogens with respect to surface antigens is indicated by the numbers of the mAbs that are required to identify a particular taxon. The pathovars *X.c. pelargonii*, *begoniae*, *oryzae*, *oryzicola*, and the tomato canker pathogen, *C.m. michiganense*, all can be detected by a single mAb, indicating that the strains of the pathovar share a common epitope. In contrast, other pathovars of *X. campestris* (*campestris*, *dieffenbachiae*, *citri*, and *vesicatoria*) and the species, *X. albilineans*, do not share a single epitope, and a panel of mAbs must be generated to detect all strains of the taxon.

Those pathovars that are detected by a single epitope in general are those that also infect a single host. For example, *X.c. pelargonii* is quite specific to its host, pelargonium; pathovar *begoniae* affects only begonia; and pathovars *oryzae* and *oryzicola*, principally affect rice. These pathovars also share a single epitope that identifies the taxon. In contrast, *X.c. campestris* causes disease on a wide range of cruciferous hosts, indicating variation in the genetic profile of the pathovar, and mAbs indicate that the pathovar is serologically heterogeneous. Likewise, *X.c. citri*, which affects a wide range of citrus species, also varies with respect to surface antigens (Benedict et al. 1985).

The variability of strains of *P. solanacearum* permits us to predict that serological heterogeneity will be found when mAbs are generated to strains of this species. First, the bacterial wilt pathogen has a wide host range, and strains differ in their host specificities as indicated by the races that have been described for the species (Buddenhagen and Kelman 1964). Strains have been grouped into biovars to account for the biochemical variability, into phage-types that vary in bacteriophage sensitivity, and into restriction fragment length polymorphism (RFLP) groups that provide a measure of their genetic variability (Buddenhagen and Kelman 1964; Cook et al. 1989; Hayward 1964). Given this information, we would expect to find mAbs that will group the strains with respect to their surface antigens. In initial studies, we already have detected antibodies that separate several race 1 strains and a groundnut strain from strains of race 2 (the banana race), but extensive screening is required to verify these specificities. Perhaps of greater significance are two mAbs that in initial studies have reacted predominantly with strains which by RFLP analysis of Cook et al. (1989) are in Division II.

What does the analysis of surface antigens indicate about the inherent variability of a bacterial plant pathogen? Using highly specific mAbs that react with a single epitope much information is gained that was previously obscured by analysis with polyclonal antisera. Analysis with a panel of mAbs might provide data on a) the existence of subgroups (serotypes), b) the homogeneity or heterogeneity of a bacterial population in a given area, c) the geographical distribution of serotypes, d) the possible origin of inoculum in a disease outbreak, e) the spread of serotypes in a field plot, f) the epidemiological fitness of a subpopulation, g) the potential relationships between serotype and virulence, h) the relationships of surface antigens to genetic characteristics (as determined by RFLP analysis). Finally, when

all this information is combined, one may be able to speculate as to the possible geographic centres of disease, asking where the diseases originated and what determines their spread.

Three pathovars of *X. campestris* (*campestris*, *citri*, and *oryzae*) serve as illustrations of the above applications, because they represent the various situations that have been encountered when generating mAbs to bacterial plant pathogens.

The first, *X.c. campestris*, is a heterogeneous pathogen that cannot be detected with a single antibody; rather, three pathovar-specific antibodies were needed to encompass the range of strains that make up the pathovar (Alvarez et al. 1985). Later an additional mAb was added to the panel (Alvarez et al. 1987). That is, a positive reaction with one or more of the four pathovar-specific mAbs (X9, X13, X17, X21) is used to identify 98% of the 1000 strains of *X.c. campestris* tested. In the first study of 200 strains the pathovar was separated into six serogroups based on reactivity to selected mAbs. The geographical distribution of these serogroups also varied. The largest percentage of type 5 strains was encountered among strains originating in the states of Georgia and North Carolina, whereas type 1 and 2 strains were prevalent among strains isolated from California, Japan, and Hawaii on the island of Maui.

Monoclonal antibodies also provided insight into the geographical variation among strains of *X.c. oryzae*, the cause of bacterial leaf blight of rice. In contrast to *X.c. campestris*, all tested strains of *X.c. oryzae* shared a single epitope (antigenic determinant) that could be identified by a single mAb (*Xco*-1). Typical blight strains, prevalent throughout Asia, all reacted with *Xco*-1, and most reacted with a second mAb, *Xco*-2. In 1987, an unusual leaf blight of rice was observed on rice cultivar Lemont in Texas and Louisiana. All the pathogenic *X.c. oryzae* strains associated with atypical blight reacted with *Xco*-1, and some reacted with *Xco*-2. However, a new mAb (*Xco*-5) was found that reacted only with the U.S. strains and clearly distinguished them from the typical Asiatic leaf blight strains.

For *X.c. citri*, again a different situation occurs; for this pathogen, various pathotypes exist that can be identified by reactions on various citrus hosts as well as by bacteriophage sensitivities, plasmid profiles, and restriction fragment length polymorphisms. In the case of citrus bacterial canker, several forms of the disease occur. The typical citrus bacterial canker type A strains cause a severe disease on citrus world-wide and are detected by two antibodies, A1 and A2, that do not react with the mild strains. Citrus canker B, found principally in Argentina on lemon and grapefruit, is a milder disease caused by strains that react with several 'B' antibodies. Strains causing citrus canker C, a mild disease found on *Citrus aurantifolia* (key lime) in Brazil, are detected by mAbs C1 and B2, indicating that the B and C strains are serologically related.

Citrus bacterial spot, originally attributed to a possible variant of *X.c. citri*, and now called *X.c. citrumelo*, is serologically distinct from all the citrus canker strains and can be identified by a different panel of mAbs. Finally, avirulent xanthomonads found on leaf surfaces of citrus and other hosts reacted with none of the *citri*- or *citrumelo*-specific mAbs but did react with the genus-specific mAbs, X1 and X11. The occurrence and potential significance of such xanthomonads can be studied further with these detection tools.

In comparing RFLP patterns, host plant reactions, and serological groupings, interesting correlations were apparent. In the case of citrus bacterial spot RFLP patterns separated the aggressive strains from moderately and non-aggressive strains, and the RFLP groupings corresponded to those formed by serological analysis with mAbs. Similar correlations were found through RFLP and serological analysis of *X. campestris* strains having various levels of virulence on cabbage.

Following the above examples, a serological analysis of the groundnut strains of *P. solanacearum* using mAbs in combination with other methods of characterisation may generate some interesting and useful information. Since mAbs may be carefully selected to reveal either a unique epitope of a particular strain or common epitopes among groups of strains, a panel of mAbs may be constructed and manipulated to determine the serological and biological relatedness of a subpopulation. In this manner, the groundnut strains may be compared to race 1 strains and other strains within the species.

Key aspects of a detailed serological analysis are the collection of strains and the confirmation of pathogenicity. Large numbers of *P. solanacearum* strains collected from groundnut in China, Indonesia and other geographical

regions, representing the range of races and biovars, would be needed to make the appropriate comparisons. Other genera and species as well as epiphytes from groundnut, representative of the biological niche, should be included in the screening procedures to verify the specificity of the selected mAbs.

Procedures for generating and selection of genus and pathovar-specific mAbs for bacterial plant pathogens have been well-documented. This involves immunisation, fusion of mouse spleen cells with mouse myeloma cells to generate hybridomas, screening, cloning, rescreening, production of ascites, and rescreening with large numbers of strains to confirm the desired specificity. Finally, the analysis of the antigens and the development of rapid diagnostic tests will facilitate detection, identification, and epidemiological studies. In view of the information already generated for other bacterial plant pathogens, a study of the groundnut strains of *P. solanacearum* appears to be quite promising.

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Molecular Biology and Research on *Pseudomonas solanacearum*

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Abstract

Pseudomonas solanacearum is a Gram-negative bacterial pathogen which can cause wilting of many plants. Techniques of genetic manipulation have been used to study and elucidate the pathogenesis of wilt induction. With the application of transposon (Tn5) mutagenesis, site-directed mutagenesis, and construction of genomic libraries and gene cloning, a number of *P. solanacearum* genes involved in pathogenesis were identified and their functions studied. Genetic evidence for the role of EPS in the wilting process was contradictory, but it seems that EPS production alone is not sufficient for pathogenesis. Genes encoding endopolygalacturonase (*pglA*) and a-1,4-endoglucanase (*egl*) have been cloned, however their function in pathogenicity appears only to enhance the wilting process instead of being absolutely necessary. Progress has been made in the cloning of genes controlling pathogenicity and hypersensitivity in *P. solanacearum*. Two types of genes; (*hrp* genes required for HR induction, and *dsp* genes modulating aggressiveness), were cloned in a 25 kb region of plasmid pVir2. The DNA sequences in the insert of pVir2 were found to be present and to show a RFLP pattern in many *P. solanacearum* strains. Experimental data suggest that there are independent positive factors determining host range in *P. solanacearum* rather than an avirulence gene system. Genes involved in host specificity were cloned in a 12.8 kb *Eco*R1 fragment in plasmid pGX1252, which appeared to be unique to *P. solanacearum*.

All results indicate that there must be a considerable number of genes in *P. solanacearum* which are involved in the disease process. It is helpful to look at how these genes are regulated and interact with corresponding genes in plants.

Bacterial wilt caused by *P. solanacearum* is one of the most important, widespread and lethal bacterial diseases of plants. In tropical and subtropical areas this bacterial pathogen is destructive to many crop plants, such as potato, tobacco, tomato, groundnut and banana (Buddenhagen and Kelman 1964). Many wilt producing factors have been proposed to elucidate the mechanisms of pathogenesis. These factors include exopolysaccharide (EPS), IAA, pectic and cellulolytic enzymes etc. (Wallis and Truter 1978; Beckman et al. 1962; Husain and Kelman 1958a; Kelman and Cowling 1965). Since these data were mainly based on phenotypic changes related to wilt pathogenesis the precise nature of factors causing plant wilt is largely unclear.

The advent of molecular genetics has led to approaches that allow the investigation of specific mechanisms of pathogenesis. Genetic manipulations with Gram-negative bacteria are well developed (Maniatis et al. 1982). *P. solanacearum* is a Gram negative bacterial pathogen which can be easily cultured in simple, defined laboratory media. The DNA isolation procedure used for *E. coli* can be used for *P. solanacearum* with the slight modification of reducing the amount, and removal by washing, of the extracellular polysaccharide. Small and large scale isolations of plasmid DNA from *P. solanacearum* are also easily performed

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(Boucher et al. 1985; Ma et al. 1988). Foreign genes (up to a size of 40 kb) can be introduced into *P. solanacearum* by transformation at a frequency of 10^{-5} to 10^{-6} for a single marker, or by conjugation for plasmid vectors with mobilisation (Mob) function by a triparental mating method (Boucher et al. 1985; Ma et al. 1988; Xu et al. 1988). Recently electroporation was tested and shown to be able to introduce plasmid DNA into *P. solanacearum* with a frequency of 10^{-4}-10^{-5} transformants per μg DNA (Cheng, unpublished data). The stability of foreign plasmids in *P. solanacearum* varies from strain to strain. It seems that P group broad host range vectors pLAFR1 and pLAFR3 are fairly stable in *P. solanacearum* recipient cells (Ma et al. 1988; Xu et al. 1988) although an unstable situation was reported (Boucher et al. 1987).

Mutations may be induced by chemicals, UV radiation or by transposon insertions. Because the transposon-induced mutations have advantages for subsequent analysis, transposon mutagenesis has been widely used in *P. solanacearum* genetic studies.

Transposons are discrete short DNA segments that determine resistance to one or more antibiotics and have the property of being able to insert into new sites of DNA replicons in the absence of the *recA* gene function (Kleckner et al. 1977). Many transposons can be inserted into a large number of sites in the bacterial genome. Some transposons, such as Tn7, appear to have 'hot spots' in the whole genome at which insertion events have occurred frequently; however, for some transposons such as Tn5, the distribution of insertion sites is more random (Berg 1977). In mutations caused by transposon insertions, the continuity of the affected gene is disrupted. Therefore, insertion mutations by transposons usually result in complete loss of the function encoded by the blocked gene. Since transposons carry drug-resistance determinants, any mutations caused by transposon insertions will result not only in the mutant phenotype but also in the drug-resistance phenotype specified by the inserted transposon. These two phenotypes are thus completely linked. They are transferred together in genetic crosses and lost together in reversion events. Transposons not only inactivate the gene into which they insert but also have a strong polar effect on the expression of genes in the same operon which are located downstream of the insertion site. Therefore, insertion mutations may be used to determine the extent of transcription units in uncharacterised gene clusters.

It is of particular importance that transposon mutagenesis provides a means to isolate mutants which have no readily scoreable phenotype (e.g. nodulation in *Rhizobium* and pathogenicity in plant pathogens). These insertion mutants are generally single mutations and will lead to a complete loss of the gene function in question. In addition, a scoreable phenotype (drug-resistance) will be associated with the mutation, which can be used to map the mutation genetically or physically.

There are a number of ways to deliver transposons into *P. solanacearum*. Beringer et al. (1978) developed a general method for introducing transposon Tn5 into the genome of a wide variety of Gram-negative bacteria, in which a broad host-range plasmid (p1 group) carrying both phage Mu and a transposon Tn5 is conjugated from *E. coli* into the recipient. Because the Mu-containing plasmid fails to replicate in the recipient, transposition events of the transposon from the Mu-containing 'suicide plasmid' into the recipient genome can be directly selected. Similar 'suicide' behaviour has been used to construct transposon Tn5-delivering plasmids pSUP1011 and pSUP2021 (Simon et al. 1983) which can successfully generate Tn5 insertion mutants of *P. solanacearum* (Boucher et al. 1985; Denny et al. 1988; Xu et al. 1988). However, experience shows that different pathogens, or even different strains of a single species vary greatly in the ease with which Tn5 insertion mutants can be isolated. Sometimes the frequency of transposition events is too low to be identified (Turner et al. 1984).

The value of Tn5 mutagenesis lies also in the possibility of identifying and cloning genes which cause alteration in pathogenicity, but without obvious defects in growth in vitro. After Tn5 mutagenesis large numbers of colonies of mutagenised bacteria can be tested on plants, and Path⁻ mutants can be selected; flanking genomic DNA sequences can be excised and cloned from the mutants and used as hybridisation probes to locate corresponding clones in a DNA library (Boucher et al. 1987).

Site-directed mutagenesis (Ruvkun and Ausubel 1981) is another important use for transposons. This method relies upon transposition events in *E. coli* into the desired region

of a segment of introduced DNA, followed by its return to the original host through a procedure called marker exchange; that is, by selecting for recombinants involving double homologous exchanges of the inserted and wild type DNA segments. After mutagenesis, insertion mutants in the original genome of bacteria result, and the function of the inserted region can be tested by observable phenotypic changes. This is particularly useful in providing genetic evidence for the role of suspected pathogenicity factors since a parallel observation of a phenotypic change and pathogenicity defect is not always a real reflection of their relationship (Denny et al. 1988; Roberts et al. 1988).

Construction of a gene library has become a routine task in gene isolation. In studies of *P. solanacearum*, cosmid vectors with a broad host range, such as pLAFR1 and pLAFR3 (Friedman et al. 1982) are widely used (Boucher et al. 1987; Ma et al. 1988; Xu et al. 1988). Cosmid libraries are maintained in *E. coli* strains and each library consists of, in general, several thousand clones each of which contains a 20-30 kb piece of *P. solanacearum* DNA joined to a vector. Genes of interest in these 20-30 kb DNA segments can be screened by hybridising with existing probes or by specially designed procedures, depending on the gene expression situation (Xu et al. 1988; Boucher et al. 1987). Sometimes the whole library is conjugated into a desired recipient either en masse with pooled library cultures, or through large numbers of matings, each with an individual clone (Ma et al. 1988). The resultant transconjugants are screened by special assays or by tests on plants.

Molecular Genetic Studies towards the Understanding of Pathogenicity

Exopolysaccharide (EPS) and Lipopolysacharide (LPS)

In culture, wild type *P. solanacearum* strains produce copious amounts of EPS a viscous, high molecular weight, neutral compound mainly composed of galactosamine (Buddenhagen and Kelman 1964; Sequeira 1985). Spontaneous avirulent mutants can be easily recognised by their red non-slimy colonies on a tetrazolium chloride glucose rich medium whereas on the same medium the wild-type strain produces white to pink slimy colonies (Kelman 1954). It has been believed that EPS produced by *P. solanacearum* may play a key role in pathogenesis by interfering with water movement through vessels or pit membranes and result in the typical wilt symptoms (Buddenhagen and Kelman 1964; Van Alfen 1982; Husain and Kelman 1958b). However, evidence from genetic studies on involvement of EPS in pathogenesis is contradictory. Staskawicz et al.(1983) obtained an EPS-deficient red mutant of strain S-82 by IS50 insertion and found that it was no longer virulent on potato. But this mutation is pleiotropic, therefore the loss of EPS production was not completely responsible for the change in virulence, although the result suggested that EPS is important in pathogenesis. Using Tn5 mutagenesis Boucher et al. (1987) and Xu et al. (1988) obtained non-pathogenic (Vir−) mutants of the strains GMI1000 and K60 respectively. All of the Vir− mutants of GMI1000 and six out of eight Vir− mutants of K60 produced normal amounts of EPS, indicating that EPS production alone is not sufficient for pathogenesis. In addition, mutant types of both strains were identified that were deficient in EPS production when grown on agar medium but still able to wilt host plants, suggesting that EPS may not be the major factor in wilt pathogenesis (Xu et al. 1988; Boucher et al. 1987).

Denny et al. (1988) divided EPS mutants into EPS^{l} (impaired in EPS production) and EPS− (EPS deficient), and found that the amount of EPS produced in planta by the EPS^{l} mutants of strain AW1 was correlated with the severity of wilt symptoms. Their study showed that great care should be taken in characterising EPS-deficient mutants of *P. solanacearum* both in culture and in planta; they concluded that EPS production in tomato plants is required for typical wilt symptoms. However, Xu et al. (1988) reported that *P. solanacearum* K60 mutants that retained virulence to eggplant and tobacco, but produced no EPS either in culture or in planta, were obtained after Tn5 mutagenesis.

Less attention has been given to LPS, one of the peripheral components of the surface envelope of *P. solanacearum* (Sequeira 1985). Drigues et al. (1985) compared the composition of *P. solanacearum* lipopolysaccharide (LPS) of wild-type and rough mutant strains and found that both contained the same component sugars in their polysaccharide moieties, but differed greatly in the relative amount of each sugar. Since the mutation to rough phenotype is pleiotropic the nature of the genetic alteration is unknown.

So far, several clones containing *P. solanacearum* DNA sequences involved in EPS production have been obtained, yet no detailed information about the function of these DNA sequences has been reported. More studies are needed to clear up the controversy over EPS involvement in pathogenesis.

Cell Wall Degrading Enzymes (Polygalacturonase and Endoglucanase)

In plants wilted by *P. solanacearum* degradation of the vascular system occurs, indicating that cell wall-degrading enzymes may be involved in pathogenesis (Husain and Kelman 1958a; Kelman and Cowling 1965). Schell et al. (1988) purified a major endopolygalacturonase (52kDa) excreted by *P. solanacearum* and cloned the gene encoding this enzyme (*pglA*) from a genomic library. Then the *pglA* gene was inactivated in vitro and used to mutate the chromosomal *pglA* gene of *P. solanacearum* by marker exchange. They found that the resulting mutant strain was deficient in production of the 52kDa polygalacturonase, and that the time required to wilt and kill tomato plants was doubled, indicating that the *pglA* gene is important, but not absolutely necessary, for pathogenesis.

Similar results were obtained for the *P. solanacearum egl* gene encoding the β-1,4-endoglucanase that cleaves soluble cellulose. Roberts et al. (1988) cloned the *egl* gene of *P. solanacearum* on a 2.7 kb *Xho*I-*Sal*I DNA fragment, which was mutagenised by Tn5 and used to mutate the chromosomal *egl* gene of *P. solanacearum* by site-directed mutagenesis. The mutant strain produced much less endoglucanase, but was still capable of killing tomato plants, albeit after a prolonged period of time.

Genes Controlling Pathogenicity and Hypersensitivity

In the molecular genetic studies of *P. solanacearum* the most exciting advance has been in the cloning of genes controlling both pathogenicity (on tomato) and hypersensitivity (on tobacco) by Boucher et al. (1987). In an early study they isolated 12 prototrophic avirulent mutants out of 8250 clones tested after Tn5 mutagenesis (Boucher et al. 1985). Among these mutants nine were Hrp$^-$ mutations of *P. solanacearum*, the majority of which were mapped on a megaplasmid in a 25kb region cloned in plasmid pVir2. After localised mutagenesis two types of genes were found to be carried in this region: *hrp* genes required for HR induction and located in the middle and left part of the 25kb insert of pVir2, genes which were also required for pathogenicity on tomato; and, *dsp* genes located on the right end of the *hrp* cluster which modulated aggressiveness on tomato.

Subsequently Boucher et al. (1988) tested 52 strains of *P. solanacearum* representing different races, biovars and geographical origins, and found that all pathogenic strains carried DNA sequences homologous to *hrp* genes present in pVir2, indicating that these *hrp* genes are necessary for pathogenicity in all the strains.

When plasmid pVir2 was digested with restriction enzyme *Eco*R1, six insert bands (8.1, 6.0, 4.0, 2.8, 1.7, 1.5 kb) were generated. *Eco*R1 bands of the same size were also identified in *P. solanacearum* groundnut strain T2005 when probed with ^{32}P-labelled pVir2. However, when these six *Eco*R1 fragments were individually ^{32}P-labelled and separately hybridised with total DNAs of a number of *P. solanacearum* strains, in one potato strain T2003, no homologous DNA to the 4.0 and 8.0 kb probes located in the left part of pVir2 was detected (Feng, unpublished data). This indicates that strain difference for *hrp* genes may exist, or that *hrp* genes in the middle part of pVir2 are more common in *P. solanacearum*.

Functions encoded by the pathogenicity genes located in the pVir2 region are still unknown. It is interesting to note that there is a structural homology of the *hrp* cluster of pVir2 with many pathovars of *Xanthomonas campestris* (Boucher et al. 1987). In *X. campestris* pv. *campestris* strain 8004 this homologous DNA fragment to pVir2 has been cloned (Daniels pers. comm.)

Recently, a second cluster of genes that specify pathogenicity has been identified (Huang et al. 1990). However, the DNA encoding these genes showed no homology with those in pVir2, indicating that a rather complex pattern exists in determining the pathogenicity of *P. solanacearum*.

Genes Involved in Host Specificity

Many of the concepts concerning the genetics of plant-pathogen interactions arose from attempts to breed disease-resistant plants. One of the basic concepts is Flor's gene-for-gene hypothesis (Flor 1955) which is based on research on the interaction of flax (*Linum*

usitatissimum) and the rust fungus *Melampsora lini*. Flor postulated that for every gene in the pathogen determining virulence or avirulence there was a corresponding gene in the host determining resistance or susceptibility. It is believed that the avirulence genes interact with the resistance allele, then induce plant defence mechanisms. The search for *avr* genes in bacterial plant pathogens has been successful. Several *avr* genes were isolated from *P. syringae* pv. *glycinea*, *X. campestris* pv. *malvacearum* and *X.c.* pv. *vesicatoria* (Staskawicz et al. 1984, 1987; Gabriel et al. 1986; Swanson et al. 1988). Some avirulence genes have been completely sequenced (Napoli and Staskawicz 1987; Ronald and Staskawicz 1988; Tamaki et al. 1988). Surprisingly, no significant sequence homology at the nucleic acid or amino acid level was detected (Bonas et al. 1989). Although the identification of these genes is a great step forward in plant pathogen genetics, their functions and mode of interaction with plant genes are not clear.

In all cases where *avr* genes were isolated the race-cultivar interaction patterns were well established. In *P. solanacearum* the host range covered by a certain race may involve not one plant species, but many plant genera and families. Nevertheless attempts were made to search for the putative *avr* genes in *P. solanacearum*. Ma et al. (1988) chose two *P. solanacearum* strains T2003 and T2005. T2003 is pathogenic to potato, but nonpathogenic to groundnut, and T2005 pathogenic to groundnut. Nearly 3000 clones of strain T2003 gene library were individually conjugated into the T2005 derivative recipient. No transconjugants were obtained which altered the pathogenic character of the T2005 recipient, indicating that the presumptive *avr* genes may not be present.

However, transfering clones from the T2005 gene library to T2003 recipient resulted in the identification of a cloned 12.8kb DNA which contained genes to extend the host range of *P. solanacearum* T2003 transconjugant to groundnut (Ma et al. 1988). This suggests that there are independent positive factors determining host range in *P. solanacearum* rather than an avirulence gene system. Evidence of such positive factors has also been obtained with *X.c.* pv. *translucens* (Mellano and Cooksey 1988).

A 12.8kb piece of DNA was cloned in plasmid pGX1252. No hybridisation was found between pGX1252 and pVir2, indicating that two different DNA sequences were involved.

In *Rhizobium* host-specific nodulation genes with a similar positive function have been characterised. These genes are not conserved, since alleles from different *Rhizobium* strains cannot substitute for each other on different host plants (Long 1989). It is not clear if genes contained in the 12.8kb DNA fragment have the same property as these host-specific nodulation genes. But it is interesting to find that this 12.8kb DNA sequence pattern appears to be present only in *P. solanacearum* strains which can cause wilt on groundnut, and not in strains which are not pathogenic on groundnut. This sequence is also not detected in many other strains of *Pseudomonas*, *Xanthomonas*, *Agrobacterium*, *Rhizobium*, *Erwinia* and *Corynebacterium* tested (Feng, unpublished data).

Concluding Remarks

There has been only a short period for phytopathologists to do genetic work or for geneticists to choose bacterial plant pathogens as research material. However the information obtained so far is rather impressive although some results are fragmentary and even contradictory. In general, *P. solanacearum* is amenable to genetic analyses. With the progress in recombinant DNA technology it is possible to identify individual genes, their products and the way they are regulated. More and more evidence shows that pathogenesis is a complex interaction of two partners, bacterial pathogen and host plant; there must be a considerable number of genes from both partners, which are directly involved in the disease process each of which has only a small influence. Undoubtedly new information about these identified genes will be coming out with the application of gene fusion, DNA sequencing and RFLP techniques. It may be helpful to put emphasis on the regulation of pathogenesis. In *Xanthomonas campestris* pv. *campestris*, genes involved in regulation of pathogenesis have been cloned (Tang 1989). Blocking of such regulatory genes resulted in the abolition of several cell wall degrading enzymes in the pathogen at the same time, and the pathogen was no longer pathogenic. It is conceivable that such regulatory genes may be more important in understanding the plant-pathogen interaction than previously realised.

Also the plant partner should be considered and studied when we are trying to explain the function of genes in plant pathogens.

Before there is a clear understanding and elucidation of the wilt process as a result of molecular genetic studies on *P. solanacearum* there may be practical applications. Boucher et al. (1988) used pVir2 as a molecular probe for hybridisation tests and demonstrated that a kind of restriction fragment length polymorphism did exist in *P. solanacearum* strains. Similar results were obtained for pGX1252, which also showed RFLP differences among *P. solanacearum* strains (Feng, unpublished data). This may be used for the classification of strains or for field identification.

It is known that non-pathogenic mutants are able to induce protection against the wild-type pathogenic strains (Trigalet and Demery 1986). Perhaps non-pathogenic deletion mutants are good candidates to carry on this work. Progress has been made in the construction of such mutants of groundnut and tobacco strains of *P. solanacearum* (Feng, pers. comm.).

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Genetic and Breeding Aspects of Resistance to Bacterial Wilt in Groundnut

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Abstract

Although the genetic basis of resistance in groundnut to bacterial wilt (BW), and its underlying mechanism, are at present poorly understood, considerable progress has been made in China in the development of resistant germplasm. Extensive and intensive studies including disease surveys and screening for host plant resistance were initiated in the 1950s. Some desirable resistant germplasm lines including Xiekangqing were identified in 1974 and since then breeding for resistant cultivars has been successfully conducted at many research stations. In the past two decades several resistant cultivars with high yield traits have been released and these are playing an important role in controlling disease and increasing production in the affected areas. In China almost all of the groundnut lines identified as being resistant to BW were collected in the south where the disease is generally most serious. In general there is no obvious genetic linkage between resistance to BW and undesirable characters. Resistance to BW and rust (*Puccinia arachidis*) are independently inherited and some groundnut lines resistant to BW are also resistant to rust and Cercospora leaf spots. However, most BW resistant groundnut lines show poor resistance to drought while no groundnut cultivars with good tolerance to drought are found to be resistant to the disease. The directions which future research should take in order to improve yield potential, level of resistance and stability, food quality, and incorporation of resistance to other diseases, are indicated.

Bacterial wilt of groundnut (*Arachis hypogaea*) caused by *Pseudomonas solanacearum* is the most important bacterial disease of this crop throughout the world. This disease has been reported in many countries and regions among which central and south China, Indonesia and Uganda are most seriously diseased areas (Mehan et al. 1985; Liao Boshou et al. 1986). The disease is also believed to be a potential threat to groundnut production in several humid areas in the world (Mehan et al. 1985). In China, there are more than 200 000 ha of groundnut fields naturally infested with *P. solanacearum* where yield losses of 10-30% are commonplace. More serious damage, even to the extent of total loss of crop on heavily diseased fields, is often experienced when susceptible cultivars are grown. Extensive efforts for controlling groundnut bacterial wilt have established well that the most effective and practical way to control this disease is to breed and plant resistant cultivars.

The first groundnut cultivar resistant to bacterial wilt, Schwarz 21, was released in Indonesia in the 1920s and its resistance is still useful. Some resistant cultivars were also released in the United States in the 1930s. In China, extensive and intensive studies including disease survey and screening for host plant resistance to groundnut bacterial wilt were initiated in the 1950s. Some desirable resistant germ-plasm lines including Xiekangqing were identified in 1974 and since then breeding for resistant cultivars has been successfully conducted at many research stations in the

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country (Sun Darong et al. 1978; Wang Yuying et al. 1989). In the past two decades much attention has been paid to breeding; several resistant groundnut cultivars with high yield have been released which are playing an important role in controlling disease and increasing production in the affected areas. Groundnut yield losses due to bacterial wilt in the major disease areas in China have so far been markedly reduced to less than 10% by planting resistant cultivars. Meanwhile, research progress has also been made on the pathological aspects of groundnut bacterial wilt, resistance screening and genetics of host plant resistance. This paper reviews the work done in China on the genetics and breeding for resistance to bacterial wilt in groundnut.

Genetics of Resistance to Bacterial Wilt in Groundnut

The inheritance of resistance in groundnut to bacterial wilt was not systematically studied until recent years. There has not yet been any authentic research on the mechanism of resistance in groundnut to bacterial wilt. The expression of the resistance is always influenced by the genetic background of the host plant, the population level and the pathogenicity of *P. solanacearum*, and environmental factors. Furthermore, the reaction of groundnut plants to *P. solanacearum* may be different at different growth stages, which might complicate research on mechanism and inheritance of the resistance. However, resistance may be mainly through resistance to disease development, even though there might be differences among groundnut cultivars in their ability to resist invasion by *P. solanacearum*. Not all the infected groundnut plants will display wilting symptoms, and hypersensitive partial wilting symptoms could be observed in resistant genotypes under relatively stable conditions (Liao Boshou et al. 1986).

Many studies on groundnut germplasm screening have confirmed the existence and genetic diversity of host plant resistance to bacterial wilt in cultivated groundnut (Jenkins et al. 1966; Sun Darong et al. 1981; Duan Laixiong et al. 1987; Wang Yuying et al. 1989). Genetic resistance to bacterial wilt has also been found in some accessions of wild *Arachis* species (Wang Yuying et al. 1989). With reference to China, almost all of the groundnut lines identified as being resistant to bacterial wilt were collected from south China where the disease is generally more serious (Sun Darong et al. 1981; Wang Yuying et al. 1989). In the laboratory, partial resistance has been induced in susceptible groundnut by exposure to an avirulent mutant strain of *P. solanacearum* (Kang Raowei 1986). These results might suggest a possible close relationship between the origin or evolution of resistance to bacterial wilt in groundnut and exposure to and selection by the disease pressure under natural conditions. However, in order to satisfactorily explain the origin and evolution of resistance, further studies are still needed on; first, the differences in disease reaction among different botanical accessions of *Arachis hypogaea*, and secondly, the disease or resistance status of the related wild *Arachis* species, and thirdly more detailed information on the influence of environmental factors on the disease development and on the expression of host resistance.

Experience in breeding has shown that the resistance of groundnut to bacterial wilt could be easily transfered from one genotype to another through hybridisation which suggests that the genetic background for the resistance is simple. From results obtained through breeding in a natural disease nursery, Wang Yuying et al. (1985) indicated that in most crosses the resistance level in F_1 hybrids was lower than the average of their parents, which means that the resistance was controlled by recessive genes.

A complete diallel-cross design consisting of three resistant lines and one susceptible cultivar was conducted to explore the inheritance of resistance in groundnut to bacterial wilt (Liao Boshou et al. 1986). Artificial inoculation was conducted in the greenhouse to screen the resistance in F_1, F_2, BC_1 hybrid progenies and their parents. A 1-5 point scale was used to estimate the relative differences among the infected plants with different partial symptoms. However, the results showed the resistance was partially dominant when the relative differences of partial resistance were involved in analysis, but in most crosses the dominance indexes were not high. No significant difference in resistance values between F_1 hybrid and the average of its parents was observed.

Some other results from genetic studies also proved that the resistance of groundnut to bacterial wilt was just slightly dominant or recessive. The resistance levels of hybrid populations were largely related to the average

of their corresponding parents. Both general combining ability and special combining ability were important for the resistance, while the general combining ability was more important, which meant that the resistance was controlled mainly by additive genes. From the segregation in F_2 and BC_1, the resistance was thought to be controlled by three major genes located in the nucleus (Table 1). There might be other minor modifying genes relevant to the resistance (Liao Boshou et al. 1986).

Table 1: Proposed genotypes for resistance to bacterial wilt (*P. solanacearum*) in some groundnut lines

Line	Resistance Value (%)	Reaction Type*	Proposed Genotype
Xiekangqing	88.6	R	G1G1G2G2g3g3
Taishan Sanlirou	83.1	R	G1G1G2G2g3g3
Taishan Zhengzhu	73.7	MR	G1G1g2g2g3g3
Hong Hua 2	3.6	S	g1g1g2g2G3G3

* R resistant, MR moderately resistant and S susceptible

Xiekangqing, Taishan Sanlirou and Taishan Zhengzhu might possess similar genetic backgrounds for their resistance to bacterial wilt. The general combining ability of Xiekangqing and Taishan Sanlirou were useful and several resistant cultivars have been released by using these two lines as resistance donors (Wang Yuying et al. 1989).

Generally there is no obvious close genetic linkage between resistance to bacterial wilt and other undesirable characters in groundnut cultivars or germplasm lines. The resistance to bacterial wilt and the resistance to rust (*Puccinia arachidis*) are independently inherited and some groundnut lines resistant to bacterial wilt are also resistant to rust and *Cercospora* leafspots (Wang Yuying et al. 1989). However, the response of most bacterial wilt-resistant groundnut lines to drought is relatively poor, while no groundnut cultivars with good tolerance to drought are found to be resistant to bacterial wilt, which could be related to the ability of the roots of groundnut plants to absorb moisture (Wang Yuying et al. 1989).

Although there have been some research results indicating considerable differences in pathogenicity among different strains of *P. solanacearum* affecting *A. hypogaea* (Li Wenrong et al. 1987), there is no clear evidence of specialisation of pathogen strains to groundnut cultivars. There is a need to conduct more genetic research on resistance in groundnut to bacterial wilt in different genotypes, which could help to explore the origin of resistance and utilise the resistance resources more effectively.

Breeding Groundnut for Resistance to Bacterial Wilt

From 1974 to 1978, a nation wide screening of groundnut germplasm for resistance to bacterial wilt was carried out effectively through the collaboration of many research units in China and from that screening some useful resistant lines were identified (Sun Darong et al. 1981). After purifying these resistant lines, the Oil Crops Research Institute of CAAS initiated in 1975 a breeding program for resistance to bacterial wilt using the resistance sources identified and the natural disease nurseries established in Hong'An. Since 1975, more than 200 hybridisation crosses have been made and many breeding parents have been involved. Most of the trials to identify and select for both resistance and yield were conducted in the natural disease nurseries. Materials with high yield traits in advanced generations were also subjected to artificial inoculation. Up to now, some resistant lines with high yield and/or good food quality have been obtained, and two resistant cultivars, El Hua 5 and Zhong Hua 2 have been released to farmers.

El Hua 5 (78-1141) was selected from the progenies of Xiekangqing x Yueyou 589 made in 1975 using a medified pedigree method. This cultivar was released in 1985. It combines high resistance to bacterial wilt with high yield. Its resistance was shown to be quite stable probably because both of its parents were resistant. El Hua 5 was shown in several different places to outyield by 10-30% Taishan Sanlirou, the cultivar which was once planted in diseased areas. El Hua 5 now covers most of the bacterial wilt affected areas in central China along the Yangtze River Valley.

Zhong Hua 2 (85-007) was selected from the progenies of El Hua 4 x Taishan Sanlirou made in 1979. It was also bred through a modified pedigree method and was released in 1988. In the multilocational trials, this cultivar was shown to be as highly resistant to bacterial wilt as Xiekangqing and El Hua 5. It could outyield El Hua 5 and Xiekangqing by 20% and 50% respectively. The seed protein content of Zhong Hua 2 is up to 30% and, like El Hua 4, it is an early maturing cultivar with wide adaptation.

Since 1980 some new groundnut cultivars resistant to bacterial wilt have also been released and extensively cultivated in Guangdong, Guangxi, Fujian, and Shandong provinces, such as Yueyou 92, Guiyou 28, Jinyou 3121, and Lu Hua 3. The resistance performance of some newly-released groundnut cultivars in Hubei Province is shown in Table 2.

In breeding for resistance to bacterial wilt, some parents resistant to rust have been used and some breeding materials with resistance to both diseases have been obtained, but their yield characters need to be improved. However, most of the rust-resistant groundnut accessions are of Valencia type from Peru, and their resistance to rust shows close linkage with some undesirable pod and/or seed characters and poor yield. Overcoming this undesirable genetic linkage is the key to breeding for multiple resistances in the future. In breeding for resistance to bacterial wilt many resistant lines with high protein content (above 28%) have been obtained.

Table 2: Resistance (%) of some groundnut cultivars (1989)

Cultivar	Natural disease nursery (Hong'An, Hubei)				
	1	2	3	4	Mean
Yueyou 92	85.5	80.2	87.5	77.9	82.8
Guiyou 28	74.7	81.7	75.8	74.1	76.6
84-2117	83.8	83.5	81.8	62.1	77.8
El Hua 5	95.0	93.5	96.7	96.2	95.4
Zhong Hua	86.1	95.9	85.8	96.3	89.9
Schwarz 21	82.2	88.3	87.2	89.3	86.7
Hong Hua 1 (Susceptible check)	5.0	2.5	3.7	4.0	3.8
	Artificial inoculation (Wuhan, Hubei)				
	1	2	3	4	Mean
Yueyou 92	ND	ND	ND	ND	ND
Guiyou 28	ND	ND	ND	ND	ND
84-2117	65.1	72.7	64.0	65.0	66.7
El Hua 5	81.5	82.2	90.0	83.3	84.3
Zhong Hua 2	84.0	80.0	88.2	84.4	84.2
Schwarz 21	79.2	78.8	89.4	83.3	82.7
Hong Hua 1 (Susceptible check)	0.0	0.0	3.2	0.0	0.8

It is necessary to use highly resistant materials as crossing parents in order to obtain good resistant lines in hybrid progenies because the resistance is controlled by additive genes. However, multi-directional crossing is also an effective method in terms of utilising germplasm materials with only medium-level resistance to bacterial wilt but with more desirable agronomic traits. In breeding, yield selection and identification could be done from F_{4-5} where the resistance level of selected families could be quite stable.

Generally, the yield potentials of most released groundnut cultivars resistant to bacterial wilt are relatively lower than those of the susceptible cultivars, and most bacterial wilt-resistant cultivars are also generally sensitive to water deficiency, which might be due to the narrow genetic background of the resistance donors used. More recently, some 40 groundnut lines of *hirsuta* identified as resistant to bacterial wilt have been collected from south China (Duan Laixiong et al. 1987). With these materials, the drought tolerance and poor seed dormancy of the present bacterial wilt-resistant groundnut cultivars might be improved.

Future Research Priorities

Notable progress has been made in breeding groundnut for resistance to bacterial wilt and the disease has been substantially controlled by planting resistant cultivars in China. However, in order to improve the yield potential, resistance level and stability, food quality and other disease resistances of the released bacterial wilt-resistant groundnut cultivars, further research efforts are needed on many aspects. There needs to be a more detailed study of the disease status of groundnut bacterial wilt and variation in the *P. solanacearum* strains affecting groundnut throughout the country. Further, more extensive screening of germplasm for resistance to bacterial wilt, including determination of the disease reaction of all the wild *Arachis* species to find better resistant germplasm resources with a higher level of resistance, or with better agronomic traits, or with desirable resistances to other production constraints, should be carried out. There is a need for genetic evaluation of bacterial wilt-resistant germplasm accessions for differences in genetic background of resistance, genetic relationship of bacterial wilt-resistance with other disease resistances, drought tolerance and other agronomic traits. The mechanism and components of resistance in groundnut to bacterial wilt need further research to improve techniques for disease rating and resistance identification. These initiatives will lead to better control strategies and the development of groundnut cultivars with enhanced resistance.

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General Aspects of Groundnut Bacterial Wilt in China

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Abstract

Although bacterial wilt of groundnut is known to affect more than 200 000 hectares of land in parts of southern, central and northern China, there is a need for a more systematic and detailed disease survey to determine disease distribution and to make loss assessment. There is some evidence that strains of *P. solanacearum* affecting groundnut differ in their pathogenicity for the same groundnut cultivar in different parts of China. In general strains from the north of China were less virulent to groundnut than those from the south. It appears that the strains of *P. solanacearum* affecting groundnut are quite complex, and further research efforts, including the use of molecular and serological techniques, are needed to understand strain variation. The infection process and disease severity are particularly influenced by high soil and air temperatures and by rainfall, as well as by soil type, populations of *P. solanacearum* in the soil and host plant resistance. It has been well established that rotation with paddy rice can significantly reduce pathogen populations in soil and effectively decrease bacterial wilt incidence. Flooding of soil for 30 days prior to planting can also markedly reduce disease incidence. Progress in control of groundnut bacterial wilt depends on a better understanding both of the disease and of host plant resistance, including the mechanism of resistance and its functional components. The search for new sources of resistant groundnut germplasm and its evaluation on an international basis should continue.

GROUNDNUT (*Arachis hypogaea*) is an important oil crop and also an important cash crop widely cultivated in China. In recent years the annual sowing area for groundnut has been about three million hectares. Of this, more than 90% is concentrated in the main production areas including northern, central, and southern zones. Although the agroclimatic conditions vary considerably in the different zones, the warm weather and plentiful rainfall during the period of maximum plant growth in most groundnut production zones favour the development of bacterial wilt on this crop. In some places continuous cropping of groundnut on diseased fields has also increased disease incidence. In China identification and survey for groundnut bacterial wilt was initiated in the 1950s while more intensive research on control methods has been carried out since 1970. In the past twenty years much work has been done on the pathogen, epidemiology and comprehensive control measures for groundnut bacterial wilt. This paper briefly reviews the research work done in China on groundnut bacterial wilt.

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Distribution and Importance

Bacterial wilt of groundnut caused by *Pseudomonas solanacearum* is widely distributed in Guangdong, Guangxi, Hainan, Fujian, Jiangxi, Hubei, Hunan, Anhui, Jiangsu, Sichuan, Shandong, Henan, Hebei and Liaoning provinces (Fan Huaizhong et al. 1960; IPP of GAAS 1976; Luo Daxin 1956; Mehan et al. 1986; Meng Xianchen 1957, 1964; Anon. 1979) and this disease is especially serious in the southern part of the country. There are more than 200 000ha of groundnut fields naturally infested with *P. solanacearum* in China; however more survey work is still needed to obtain more information about disease distribution and severity. In China losses from groundnut bacterial wilt of 10-30% in moderately affected fields and of more

than 50% in heavily infested fields are often experienced. The annual yield losses of groundnut (pods) due to bacterial wilt are estimated to be 45-65 thousand tonnes.

Strains of *P. solanacearum* Affecting Groundnut

Cheng et al. (1981) reported that *P. solanacearum* isolates from diseased groundnut plants were highly virulent to tomato, eggplant, bean and chilli but such isolates could not cause wilting of sesame, sunflower, castor or soybean. These isolates could also infect *Nicotiana glutinosa* but were avirulent on *Nicotinia tabacum*. The strains isolated from groundnut may be different in pathogenicity from those isolated from *N. tabacum* (Cheng et al. 1981; Ren Xinzhen et al. 1981).

Hu Baoyu et al. (1981) reported that a groundnut cultivar may show differences in its reaction to bacterial wilt in different geographical locations. For instance, the incidence (% plants killed) of Shuikou Yazi was 61.7% in Rongchen, Shandong; but in Lunan and Wendeng in the same province, the incidence was just 0.5-6.5% (Hou Xuyou et al. 1980). The pathogenicity to groundnut of *P. solanacearum* strains from different places may differ (Xu Zeyong et al. 1980).

Li Wenrong and Duan Laixiong (1987) confirmed that *P. solanacearum* strains affecting groundnut from different regions differ in their pathogenicity even to the same cultivar. In general, the strains from the north of China were less virulent to groundnut than those from the south. A groundnut cultivar may be resistant when planted in diseased fields in northern China but be highly susceptible to the disease when it was planted in southern China. Thirty-six strains collected from various parts of China were inoculated into six groundnut cultivars, with different resistance levels previously identified in Hubei, and used as indicator cultivars to investigate the pathogenicity differences among isolates of the pathogen affecting groundnut. The results suggested that these 36 strains could be divided into five pathogenicity groups among which groups II and IV were prevalent in China (Li Wenrong and Duan Laixiong 1987).

Hayward (1964) divided *P. solanacearum* into four biovars based on various physiological characters. Hua Jinyue et al. (1984) reported that *P. solanacearum* isolates affecting groundnut belonged to biovars 3 and 4. They studied seventeen isolates among which six were of biovar 3 and eleven were of biovar 4. Of ten isolates collected in Guangxi, six were of biovar 3 and four were of biovar 4. The isolates collected from Hubei were all of biovar 4 (Liao Bihui et al. 1984).

The strains of *P. solanacearum* affecting groundnut in China are quite complex, and further research efforts including the use of serological and molecular techniques are needed to understand them better.

Life Cycle and Modes of Dispersal

P. solanacearum overwinters in soil, and infested soil is the most important primary inoculum source. However, crop residues and organic manure infested with the pathogen may also serve as primary inoculum. The pathogen is mainly disseminated through infested soil and water. Farm implements and machinery, cultural practices and even animals and insects can also aid the dissemination of the pathogen. Dissemination of the pathogen through seed appears to be less likely (IPP of GAAS 1976; Li Wenrong et al. 1981) because *P. solanacearum* is highly sensitive to desiccation.

The pathogen invades the groundnut plant through wounds or natural openings in roots. Infected plants express wilting symptoms when the environmental conditions are favourable. After the infected plants have wilted the bacteria are returned to the soil and can infect adjacent plants. Groundnut plants artificially inoculated by soaking seeds before planting in a suspension of the pathogen show rapid onset of wilt and bacteria from the diseased plants could kill uninfected plants nearby within the same growth period. The pathogen can move as much as one metre through soil without the aid of water movement in one growth period.

Epidemiology

The establishment, development and disease severity of groundnut bacterial wilt are generally influenced by the climatic factors, especially temperature and rainfall, soil type, pathogen population in soil, and host plant resistance.

When the daily air temperature is over 20°C and the soil temperature in the top 5 cm layer is over 25°C for one week, symptoms of wilt in infested fields will occur. When the air temperature is over 25°C and soil temperature over 30°C, the infection of the pathogen and wilt symptoms will reach their peak. In China the peak disease period for bacterial wilt of

groundnut is late June to late July in the north and in June in central China. In the south the peak disease period is May and June for spring-sown groundnut and in late September to late October for the autumn-sown crop.

Rainy days and rainfall can influence the severity of groundnut bacterial wilt. If soil and air temperatures are optimal for disease development, heavy rainfall after drought, or sudden hot weather after heavy rain, or short intervals between fine and rainy days, will favour the disease and result in serious wilting. By contrast if rainfall is prolonged disease development could be slow.

The incidence of groundnut bacterial wilt is also related to soil type. In general, the disease is less prevalent in soils with a high organic matter content and more prevalent in soils of low fertility and with poor water retention capacity. The disease incidence is positively related to the ratio of sandy particles in soil. Hou Xuyou et al. (1980) reported that there was a significant negative correlation between disease incidence and soil bulk density while the correlation between disease incidence and non-capillary porosity and percentage of air in soil was positive.

The population of *P. solanacearum* in soil influences bacterial wilt disease severity. In experiments in sterilised soils it was shown that different densities of inoculum suspension of *P. solanacearum* resulted in different disease incidence. When the concentration of inoculum was 10^7 cfu mL^{-1}, the disease incidence was 10%; when 6×10^7 cfu mL^{-1} and 1.5×10^8 cfu mL^{-1} were used, the disease incidence was 21.4% and over 50%, respectively (Li Wenrong et al. 1981).

Control

Genetic manipulation of host plant resistance

It has been well established that the use of resistant groundnut cultivars is the most effective and practical way to control bacterial wilt. Preliminary screening of groundnut germplasm for resistance to bacterial wilt was conducted in the 1950's in China. From 1960 to 1964 some 500 groundnut lines were evaluated for their reaction to bacterial wilt in Guangdong, from which some 30 lines with resistance including Taishan Zhengzhu, Suixi Dali, Shuikou Yazi, Shenghai Badou and runner Tientsin were found (IPP of GAAS 1976). Since 1972 more extensive screening for resistance to bacterial wilt through nation-wide cooperation has been successfully conducted in China, and several more desirable resistant groundnut lines including Xiekangqing, Taishan Sanlirou, Lukangqing 1, Yueyou 589, Huangchuan Zhili, and some lines in *A. hypogaea* var. *hirsuta*, have been identified. With the help of these resistant germplasm materials, some new groundnut cultivars with good resistance to bacterial wilt and high yield characters have been released in recent years and they are making an important contribution to production. The genetics of host plant resistance has also been studied (Liao Boshou et al. 1986; Wang Yuying et al 1985). Further research is under way to combine resistance to bacterial wilt with resistances to other groundnut diseases (Li Wenrong and Tan Yujun 1983).

Crop rotation

It has been well established that rotation of groundnut with paddy rice can significantly reduce the pathogen population in soil which in turn can effectively decrease bacterial wilt incidence. In Dianbai County of Guangdong Province the bacterial wilt incidence on groundnut was reduced from 50-70 % to 5% through a single rotation of groundnut with rice. If practicable flooding of diseased fields for 30 days before sowing groundnut could also reduce bacterial wilt incidence markedly (Li Wenrong et al. 1981; Li Wenrong and Tan Yujun 1984). In uplands, rotation of groundnut with other non-host crops such as sugarcane, maize, sorghum, and wheat for 2-5 years substantially reduces groundnut bacterial wilt.

Field management

Greater application of organic fertilizer, improvement of groundnut growth, crop sanitation and removal of susceptible weed hosts in fields all help in the control of groundnut bacterial wilt.

Chemical Control

Since the early 1950s experiments on the use of chemicals in control of groundnut bacterial wilt have been carried out in Guangdong, Guangxi and Hubei Provinces, but no chemical was found to be effective. During 1973-1975, nine bacteriocides were tested. Of these, only 2-amino-1,3,4-thiadiazole ($C_2H_4N_4S_2$) could reduce bacterial wilt disease incidence by 50% (Anon 1974), but its use was prohibited because of toxicity to human beings.

Future Research Priorities

In order to control groundnut bacterial wilt more effectively and breed better resistant cultivars, a better understanding of both the disease and the host plant resistance is needed. A more detailed knowledge of the distribution of groundnut bacterial wilt in China should be based on a more comprehensive disease survey. Further research efforts are also needed on the characteristics of *P. solanacearum* strains affecting groundnut and their differences in pathogenicity, and the influence of the pathogen on the origin and evolution of host plant resistance. It is necessary to make some preparation for those areas where groundnut bacterial wilt is not an important problem today but might become important in the future.

With regard to host resistance, it is necessary to screen better resistant groundnut germplasm materials, and this should be conducted on an international basis. More genetic evaluation for bacterial wilt-resistance in groundnut is needed in order to devise better strategies for the use of resistance sources in breeding. The mechanism and components of the resistance should be studied for they might be important in many ways. These goals and objectives will surely result in better control measures in the future.

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A Review of Bacterial Wilt on Groundnut in Guangdong Province, Peoples' Republic of China

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Abstract

Bacterial wilt of groundnut caused by *Pseudomonas solanacearum* is severe and widespread in Guangdong, and yield losses ranging from 15-20% are common in diseased fields. In general, rotation of groundnut with rice in lowland areas or in upland areas equipped with an irrigation system, or rotation with a non-host coupled with the use of resistant groundnut cultivars and the use of herbicides in irrigated areas, are important and effective means of reducing disease incidence in infested soils. Although the epidemiology of the disease in Guangdong is similar to that in other tropical countries, the climate and cropping systems are more complex. In recent years, plant breeders have produced some new high yielding and good quality lines and cultivars with high resistance to bacterial wilt and other diseases. The parents of these new lines and cultivars were obtained from exotic sources with high level resistance to wilt and other diseases. It seems that some exotic sources of wilt resistance, especially wild *Arachis* species, provide a useful gene pool for combining with resistance genes to foliar diseases in Guangdong.

GUANGDONG province in the southern part of China is a principal groundnut production area located in the subtropics, with warm temperatures and high precipitation during the period of vegetative plant growth. Bacterial wilt caused by *P. solanacearum* is one of the important diseases of groundnut. It is widespread and severe throughout the province. Yield losses in fields affected by the disease are commonly in the range of 15 to 25%. The disease is more severe in irrigated uplands, low tablelands and on the sandy river banks upstream, than on plains or river delta regions in all districts. In general, diseased fields are widespread in the main groundnut producing areas of Zhangjiang, and Jiangmen districts and to a lesser extent in Shaoguang, Zhaoging, Foshan, Huizhoua and Shantou.

Epidemiology

Field observations and the experience of farmers have shown that soil type and fertilizer use have a profound affect on disease incidence. The disease is clearly more severe in poor quality sandy soils or in fine sand than in sticky clay or sandy loam soils, and lesser in extent on fertile loam or alluvial loam soils. Poor quality soils continuously fertilized with organic manure have a lower incidence of wilt than those receiving chemical fertilizers.

In general, a soil pH of 5.0 to 6.8 is favourable for the pathogen. Some preliminary observations suggest that alkaline soils may be suppressive. A purple soil with a pH ranging from 8 to more than 9, widely distributed in the north of Nanxiong county and in Scian county, is continuously cropped with groundnut and tobacco, another host of bacterial wilt, and yet the incidence of wilt is low and usually less than 3%. Tobacco isolates were shown in cross inoculation experiments to be capable of producing a wilt in groundnut. In tests on potted plants with soil pH adjusted to 8.2 and 9.0, the incidence of bacterial wilt was 5 and 0% respectively.

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Field observations suggest that flood irrigation, or cultivation of rice paddy for one or two seasons followed by groundnut, is effective in reducing wilt to less than 3 and 1%, respectively. However, the wilt incidence could be as high as 15-20% in fields which are irrigated during the period of vegetative plant growth, or under rainy conditions even where groundnut is rotated with non-hosts for 2-3 years. Soils of low moisture content tend to give a somewhat lower incidence of wilt. Similarly, wilt incidence is higher in spring crops than in the autumn crop, probably because there is less rain at that time and soil moisture is also reduced by drying winds. Inoculation experiments in the field tend to confirm this observation on the effect of soil moisture. The disease was readily reproduced in soils with moisture content of 60% of waterholding capacity, whereas inoculations sometimes failed in soils of low moisture content. These observations show clearly that flood irrigation and related cultural practices can greatly reduce disease incidence through reduction in soil populations of the pathogen, whereas, by contrast, high soil moisture content contributes to disease dispersal and multiplication, and disease tends to be limited by soils of low moisture content.

What is the role of alternate hosts and of non-hosts in disease epidemiology? The main alternate hosts to groundnut in Guangdong are tomato, eggplant, pepper, potato, tobacco and a medicinal crop, *Agastache rugosa*. *Casuarina equisetifolia* has been shown to be susceptible to local isolates by branch puncture inoculation and by soaking seedlings in a bacterial suspension. The most important alternate weed host is *Ageratum conyzoides* which occasionally shows symptoms of wilt in groundnut fields, is widely distributed even in poor quality acidic soils in waste land, and can survive and overwinter in wet lands. Isolates from weeds in groundnut fields were shown to produce symptoms on inoculation to groundnut, tomato and potato, but not tobacco. However, a tobacco isolate from Nanxiong county was shown to infect groundnut. The most important non-host crops in groundnut cultivation areas are rice, corn, wheat, sorghum, sugarcane, sweet potato, soybean, black bean, mung bean and *Phaseolus angularis*. Rice is a more important non-host for use in crop rotations than dryland crops.

In recent years the wilt resistant cultivars Yie-you 92 and Yie-you 256 have been planted in diseased fields, resulting in a reduction in disease incidence from 45-60% to less than 8%. Even greater control may be anticipated if non-host crops are used in rotation with resistant cultivars.

Seed transmission is another possible factor in disease epidemiology. However, during 1959-1961 many thousands of seeds from wilted plants were sown in sterilised soil, without any showing symptoms of wilt. These observations provide no evidence in support of the occurrence of seed transmission. Freshly voided manure from oxen fed on diseased plants used as a fertilizer apparently can serve as a source of infection. Underground insects and nematodes cause root injury and may contribute to disease dispersal.

Sources of Resistance and Breeding of Resistant Cultivars

For the purpose of obtaining isolates of high and stable virulence for use in resistance screening, 14 selected isolates from different locations in Guangdong and three from other provinces, were tested on 12 groundnut cultivars in order to demonstrate differences in pathogenicity. The results clearly identified three isolates, of which two were from Guangdong and one from Shandong, with high and stable virulence. However, the race and biovar of these isolates was not determined.

The first screening of wilt resistant groundnuts was made in the 1950s with continuation in the early 1970s. The results showed that among the wilt resistant plants 25 were of the runner type, four were of Spanish type and one was of Virginia type. Runner types are more disease resistant than other types, which has been confirmed by field observations.

Screening for resistance to the systemic wilt and major foliar disease pathogens was begun in 1978 with the introduction of numerous exotic germplasm sources from ICRISAT and the USDA, most of which originated in Latin America. Testing for resistance to foliar diseases was either carried out in the field under natural disease pressure or in the glasshouse, and screening for resistance to bacterial wilt in the field by stem puncture inoculation, or in the glasshouse by immersion of seed in a bacterial suspension or transplantation of seedlings into infested soil. The results of the screening of selected cultivars and species for resistance to bacterial wilt and foliar diseases may be summarised as follows. Screening of selected lines from exotic interspecific hybrids showed

Table 1. Reaction of some groundnut genotypes to wilt and foliar diseases in Guangdong.

Identity	Wilt Incidence	Disease Reaction B.W.*	Rust	LLS*	Botanical Type	Country of Origin
NCAc 17127	5.0	R	MR	R	Valencia	Peru
PI 393531	1.3	R	R	MR	"	"
PI 393641	7.6	R	R	R	"	"
NCAc 17124	7.3	R	MR	MR	"	"
ICG 1073	0	R	R	S	Runner	Brazil
TCG 5346	0.3	R	MR	S	"	"
NCAc 17129	3.7	R	MR	MR	Valencia	Peru
NCAc 17130	3.0	R	MR	MR	"	"
PI 414332	11.4	R	MR	S	Virginia	Honduras
PI 393528-B	1.4	R	MR	S	"	Peru
NCAc 17142	21.4	MR	R	MR	Valencia	"
PI 390595	32.9	MR	R	R	"	"
PI 407454	32.9	MR	MR	S	"	Ecuador
RMP-91**	85.7	S	S	MR	Virginia	Upper Volta
RMP-12**	66.7	S	S	MR	"	"
Robut 33-1	93.8	S	S	S	"	Ghana
Schwarz 21	3.6	R	S	S	Spanish	Indonesia
7343***	1.2	R	S	S	"	"
8632***	6.7	R	S	S	"	"
8647***	2.5	R	S	S	"	"
Gajah	21.5	MR	S	S	"	"
Macan	27.7	MR	S	S	"	"
Kidang	28.0	MR	S	S	"	"
Bentang	39.8	MR	S	S	"	"

* B.W. = bacterial wilt, LLS = late leafspot, ** Schwarz 21 derivative, *** Rosette resistant

that CS 30 and CS 7 possessed both bacterial wilt resistance and either resistance or moderate resistance to foliar diseases. Furthermore, screening of cultivated species showed that germplasm with these levels of resistance generally originated in Latin America (Table 1). Germplasm originating in Indonesia with resistance or moderate resistance to bacterial wilt was susceptible to rust and late leaf spot. It was also of interest that germplasm incorporating resistance to Witches Broom and rosette virus, and some sources with resistance to *Aspergillus flavus* were susceptible to bacterial wilt.

In addition, some species of four sections representing some wild *Arachis* species were screened in the glasshouse for resistance to bacterial wilt and foliar diseases. The results showed that representative entries in all sections possessed immunity to rust and high levels of resistance to bacterial wilt and leaf spots. It is of interest that *monticola* species of series Amphiploides in the Arachis section was identified as wilt and foliar disease susceptible, whereas *stenosperma* species of the *Perennue* series of the *Arachis* section combined high wilt and foliar disease resistance with high protein content in each of the three accessions examined. It was concluded that wild Arachis species are potential sources of high level resistance to both bacterial wilt and foliar diseases. They greatly enhance the available gene pool in groundnut for purposes of breeding resistant cultivars (Table 2).

In order to solve the problem of bacterial wilt disease and foliar diseases on irrigated land, the first attempts at breeding wilt resistant cultivars were made in the late 1960's. Two moderately resistant cultivars, Yie-you 589 and Suii-tien were identified in the early 1970's. Later an exotic source of wilt resistance was crossed with a local cultivar, and as a result two more resistant cultivars, Yie-you 92 and Yieyou 256, were released. More recently Yie-you 92 was included in quantitative tests at different localities in diseased fields in southern China. The results show conclusively that Yie-you 92 maintains its resistance to bacterial wilt with greater pod yield, and that this cultivar is suitable for cultivation in diseased fields. At the same time an exotic runner type was crossed with a local cultivar, and a cultivar combining bacterial wilt resistance with moderate resist-

Table 2. Reaction of wild Arachis species to four groundnut diseases in Guangdong

Section	Species	ICG/PI No.	Disease Reaction			
			Rust	ELS*	LLS*	B.W.*
Arachis	batizocoi	8124	HR/I	HR	HR	R/MS
	duranensis	8123	HR	HR	N**	N**
	spegazzinii	8139(LL)	HR	R	N	N
	correntina		I	R	HR	N
	stenosperma	8126	HR	HR	N	N
	"	8137	HR	HR	N	N
	"	8125	HR	HR	N	N
	cardenasii	8216	I	HR	N	N
	chacoense	4983	I	N	N	N
	villosa-1	PI 210555	I	R	R	N
	villosa-2	PI 210555	HR	R	R	R
	monticola	8135	S	S	S	S
	"	8198	S	S	S	S
Erectoides	appressipila	8129	I	HR	N	N
	rigonii	8186	I	HR	N	N
	Arachis sp.	8128	I	HR	R	R
Triseminalae	pusilla	8131	I	R	R	N
	"	PI 298628	HR	R	HR	N
	"	PI 331189	HR	R	HR	N
Rhizomatosae	glabrata	8178	I	HR	N	N
	"	8925	I	HR	N	N
	Arachis sp. HLKHe565-60	8160	I	HR	R	N
	Arachis sp.	PI 338297	I	HR	N	N

* ELS = early leaf spot, LLS = late leaf spot, B.W. = Bacterial wilt, ** N = No sporulation or no symptom

ance to rust was released. However, quantitative tests showed that this cultivar was unstable in yield in different seasons and also showed undesirable runner traits. This problem was overcome by back crossing, and a new cultivar combining bacterial wilt resistance with high yield has been obtained and is being evaluated in different localities, with encouraging results in preliminary tests.

Progress in improving the range of sources of bacterial wilt resistance has been good, but success has been only partial in combining this resistance with resistance to rust, partly because of the low yield character of the exotic parents providing rust resistance. Nevertheless two institutions in Guangdong have released three high yielding cultivars with rust resistance derived from exotic parents by the composite cross method. The rust reaction was identified as moderate resistance. Preliminary test results have been encouraging.

Present Status of Groundnut Bacterial Wilt Research in Sri Lanka

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Abstract

A severe wilt disease of groundnut (cv. Red Spanish) in fields at the Regional Agricultural Research Station, Angunakolapelessa, Sri Lanka, was shown to be caused by *Pseudomonas solanacearum* biovar 3. Most of the groundnut lines cultivars tested from ICRISAT were resistant to bacterial wilt.

GROUNDNUT (*Arachis hypogaea*) is mainly grown in the dry zone of Sri Lanka with an annual rainfall of 1250 mm to 1875 mm and is used for local consumption (confectionary). At present 8 000 ha are being cultivated. In the 1985-86 Maha season (September to February), we observed a few groundnut (cv. Red Spanish) plants wilted (incidence less than 2%) in fields at the Regional Agricultural Research Station, Angunakolapelessa. In the subsequent Maha season (1986-87), the incidence was 40-45% in the same field. This unusually high occurrence lead us to examine the wilted plants, to identify the causal pathogen and determine the varietal susceptibility.

Disease Symptoms

The leaves become flaccid, followed by drooping and wilting; finally the plants die off. Thin sections taken through infected stems and roots show brown discolouration in the vascular system.

Table 1: Bacterial colony appearance on different artificial media (after 48 h. at 30 C)

Medium	Cultural Characteristics
Triphenyl tetrazolium chloride (TZC)*	Fluidal white colonies with light pink center
Sucrose Peptone Agar (SPA)**	Cream coloured round colonies
King's Medium B Agar***	White fluidal non-fluorescent colonies

* Kelman 1954 ** Hayward 1960 *** King et al. 1954

Table 2: Response of different host plants to inoculation of bacteria isolated from groundnut

Host	Reaction (Symptoms) 14-21 days after inoculation
Groundnut cv Red Spanish[1]	+
Tomato cv 'Local'	+
Tobacco cv White Burley	+
Egg plant cv Jaffna Purple	+
Pepper MI-2	+
Control (distilled water)	—

[1] Wilting of plants (observed) 4-6 days after inoculation.

Identification of the Causal Agent

Isolation from the infected roots and stems consistently produced cream coloured rounded colonies on sucrose peptone agar (SPA) (Table 1). Isolates from groundnut were able to produce acid from lactose, maltose, cellobiose, mannitol, sorbitol and dulcitol; after four days at 30°C nitrite and gas were produced from nitrate. Pathogenicity test was performed by stem pin prick inoculation of threeweek-old seedlings using a 48 h culture of the bacterium grown on SPA medium. The wilt symptoms were observed 4-6 days after inoculation on groundnut and 14-21 days after inoculation on pepper, tobacco, tomato and egg plants (Table 2). These tests indicate that the causal organism of

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groundnut wilt is *Pseudomonas solanacearum* biovar 3.

Table 3: Incidence of wilt caused by *P. solanacearum* on introduced lines and cultivars from ICRISAT

Entries	Wilt intensity (%)
ICG (FDRS) 21, 28, 29, 30, 31	10
34, 38, 41, 43, 44, 47; ICG 7887, ICG 4746, ICG 7895, ICG 1705, Robut 33-1 and NCAC 17096, ICG (FDRS) 16, 27; ICG 7885	20
ICG (FDRS) 19, JL 24	30
No. 45; 280/20	40
Red Spanish	90

Screening for Resistance

Groundnut lines cultivars received from ICRISAT were evaluated for resistance to bacterial wilt disease in the glasshouse. Results are presented in Table 3. Most of the lines tested showed resistance to bacterial wilt disease. Virginia type cv. Red Spanish was the most susceptible variety followed by No. 45 and 280/20.

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Status of Bacterial Wilt on Groundnut in Uganda

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Abstract

Bacterial wilt of groundnut was first reported in Uganda in 1938 at Bukalasa farm and severe losses have been reported in some localities in the Lake Crescent area. Some introduced groundnut entries show a high level of resistance to the disease. A germplasm collection has been made from within Uganda for screening and for subsequent hybridisation work. There is also a need for a more extensive disease survey and for investigation of the mode of transmission of the disease on groundnuts.

GROUNDNUT (*Arachis hypogaea*) is the second most important pulse crop in Uganda, after beans. It is cultivated mainly in the Eastern and some parts of the Western regions of the country. The crop serves as an important cheap source of protein, and is also being emphasised as one of the crops for export in barter trade.

Bacterial wilt caused by *Pseudomonas solanacearum* is one of the important diseases of groundnut in Uganda. Other important diseases are the rosette viruses, early leaf spot (*Cercospora arachidicola*) and late leaf spot (*Cercosporidium personatum*) (Simbwa-Bunnya 1972).

Wilt of groundnut was first diagnosed and recorded in Uganda in 1938 by Hansford (Uganda Department of Agriculture files) at Bukalasa farm. At the time, the disease caused 10% loss of the crop. Bukalasa farm is 48 kilometres north of Kampala.

In 1963 an outbreak occurred at the same farm, causing more than 40 percent loss of crop (Simbwa-Bunnya 1972). In 1968, 80% of the groundnuts grown at Kawanda were infected by wilt, and in 1976 the disease resulted in 24% loss of the groundnuts which had been grown at Kabanyolo University farm.

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Research work on this disease in Uganda has mainly emphasised screening for resistance to the disease. The Principal of Bukalasa Agricultural College initiated a screening trial at Bukalasa farm in 1964. The results of the trial indicated that four numbered Indonesian varieties were highly resistant to the disease. In 1969 and 1970 Simbwa-Bunnya (1972) screened 23 groundnut varieties for resistance to *P. solanacearum* under field conditions at Bukalasa farm and Kawanda Research Station. He found that one entry from Brazil and the United States Department of Agriculture accessions PI 341884, PI 341885 and PI 341886 were highly resistant to the disease, while local commercial varieties Roxo and Red Beauty were susceptible. It was suggested by Simbwa-Bunnya (1972) that commercially acceptable varieties resistant to bacterial wilt at Kawanda and Bukalasa be developed from the varieties screened. Some crosses were made in 1974 between the Brazilian entry and both Roxo and Red Beauty. From these crosses, several resistant lines were developed by single plant selections and family selections. These lines were evaluated at Kawanda between 1979-1981 (Kayiwa-Male 1981).

At the same time, screening work continued at Kawanda with addition of thirty introductions from ICRISAT (Kayiwa-Male 1981). Nineteen of the ICRISAT lines showed resistance but these lines, together with the crosses and all the breeding material, were lost because of the problems at the station between 1982-1986. Breeding work was reactivated at Namu-

longe Research Station in 1987. A germplasm collection has now been made from within the country for screening and for subsequent hybridisation work.

Biotype identification has also been done in some parts of the country. Biotype 3 and 4 have been identified affecting groundnuts in the Lake Crescent part of the country (Leakey 1963; Opio 1988; Simbwa-Bunnya 1972).

The distribution of bacterial wilt on groundnut in Uganda is not known since no survey has been done. The disease has been reported from the Lake Crescent area in Uganda (Leakey 1963; Opio 1988; Simbwa-Bunnya 1972). Observations and reports have been received from farmers' fields and on all the research stations in this area.

There is therefore need to carry out an extensive survey to determine the distribution of the disease and the extent to which farmers' varieties are affected. Breeding for resistance to the disease needs to be emphasised. In addition, the mode of disease transmission needs to be investigated on groundnuts in Uganda.

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The Influence of Temperature Regime on the Interaction of Some Isolates of *Pseudomonas solanacearum* with Peanut (*Arachis hypogaea* L.)

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INFECTIVITY titration was used to study the influence of temperature regime on the interaction of some isolates of *Pseudomonas solanacearum* with groundnut cv. Chico. Six isolates of biovar 3 and one isolate of aberrant biovar 2 originating from different host plants and different areas in Northern Territory (NT), Queensland (Qld), and New South Wales (NSW), Australia, were tested.

The inoculum was suspended in sterile distilled water at a concentration ranging from 10^3 to 10^{10} cfu mL^{-1}. Three week old groundnut seedlings were inoculated at the third leaf axil from the top using sterile microtips containing 20 μL of inoculum for each seedling. The inoculated seedlings were moved into controlled environmental glasshouses with day/night temperatures of 20/15, 25/20, 30/25, or 35/30°C. The experiments were done twice, once in summer and once in winter.

All of the isolates were able to infect groundnut and caused pronounced symptoms at the temperature regimes of 30/25 and 35/30°C. At the temperature regimes of 20/15 and 25/20°C the symptoms were slight. The isolate 0732 (Tomato, NT) and isolate 01017S (*Solanum nigrum*, NSW) caused symptoms only at 30/25 and 35/30°C, while the other isolates could produce symptoms at all the temperature regimes. Each isolate behaved significantly differently when the regimes of 20/15 or 25/20°C were compared with 30/25 or 35/30°C. Most of the isolates did not behave significantly differently when the temperature regime of 30/25° was compared with 35/30° except isolate 001; however, all of the isolates developed better at 35/30°C. Disease progress curves showed that all isolates caused less severe symptoms in winter than in summer. High temperature regimes supported the development of all isolates on groundnut but only isolates 0171 (*Solanum melongena*, Qld), 0234 (*Pultenaea villosa*, Qld), 0190 *Xanthium pungens* (Qld), 001 Tomato, Qld), and 0369A (Tomato, NSW) could infect groundnut at a low temperature regime.

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Seed Infection and Transmission of *Pseudomonas solanacearum* on Groundnut

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EIGHT Indonesian groundnut cultivars with varying degrees of resistance to bacterial wilt were grown at Cikeumeuh Experimental Farm, a site known to be heavily infested with *Pseudomonas solanacearum*, the bacterial wilt pathogen. Many plants were wilted and killed at an early stage of growth, while many others which were either late or slowly infected by the bacterium could survive and produce seeds, although they were wilted. Seeds were harvested from the wilted plants and brought to the laboratory for further tests. Observations based on symptoms or abnormalities were made on the seeds. The presence of the bacterium on or in the seeds was determined through isolation on either SPA or TZC medium. Samples of seeds from the infected plants were also grown in sterile soil to determine the presence of wilted plants originating from the infected seeds.

Some of the harvested pods from the infected plants showed discolouration of the shells and sometimes rot, while others looked healthy. Discolouration was also found on the seedcoat, cotyledon, and rarely on the embryo. Bacterial colonies were isolated from the different parts of the infected seeds. Seedlings grown from seeds of infected plants showed wilting within 2-4 weeks after sowing with intensities ranging from 5 to 8%. This result provided further evidence that *P. solanacearum* was able to be transmitted through groundnut seeds.

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