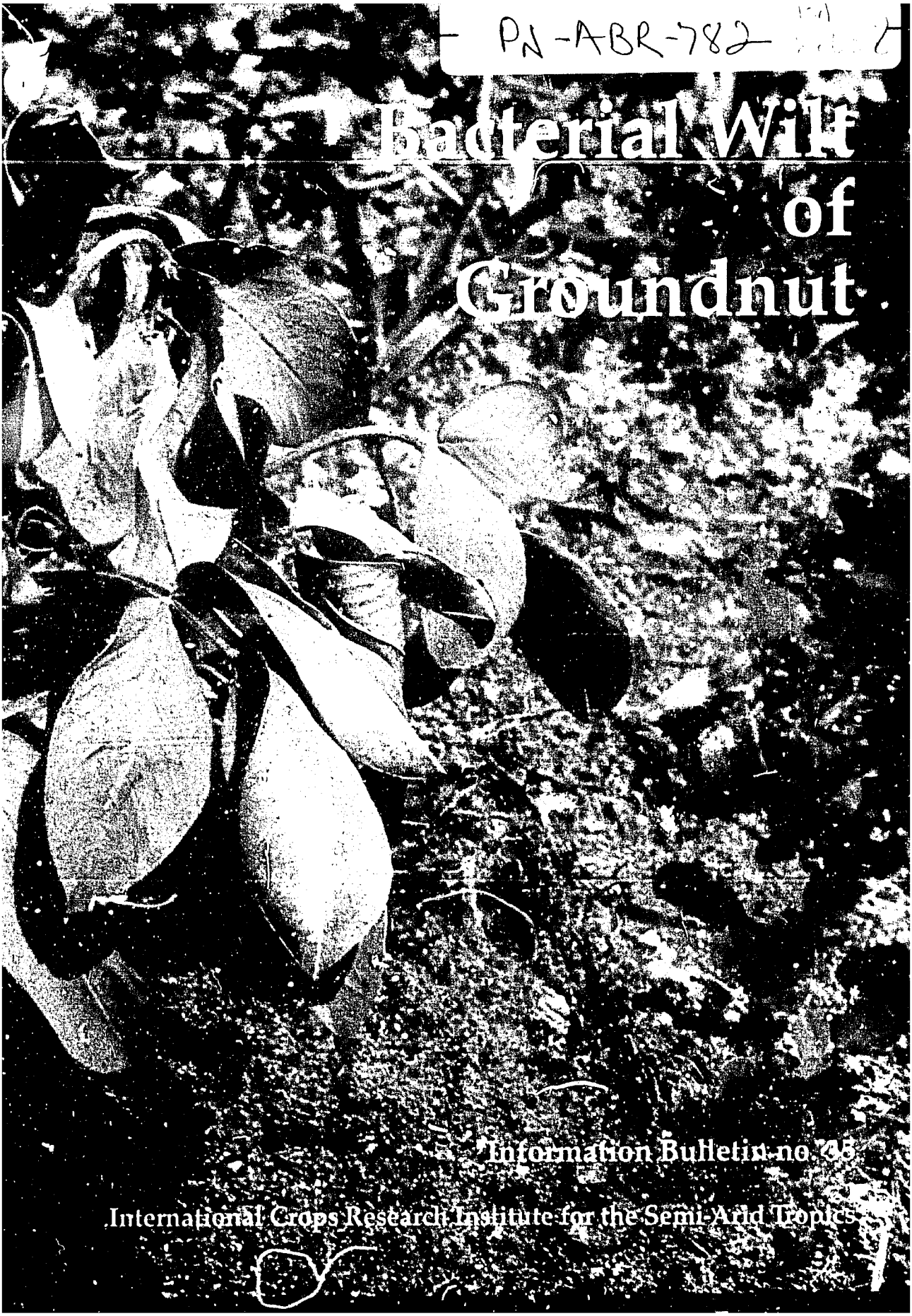
PN-ABR-782

Bacterial Wilt of Groundnut



Information Bulletin no. 45

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Abstract

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Bacterial wilt (*Pseudomonas solanacearum*) is a major constraint to groundnut production in several Asian and African countries. Although the disease is present in many countries, it is particularly important in China, Indonesia, Vietnam, Malaysia, and Uganda. Much progress has been made in understanding the disease epidemiology and the races and biovars of *P. solanacearum* that attack groundnut. Antisera have been produced for several *P. solanacearum* biovars and serological tests to detect the wilt pathogen in plant tissues and soil are available. Effective greenhouse- and field-screening techniques have been developed, resistance sources identified, and resistant cultivars developed. Various options for disease management are discussed, and an integrated approach is advocated.

Resumé

Le flétrissement bactérien de l'arachide. Le flétrissement bactérien (*Pseudomonas solanacearum*) représente une contrainte majeure à la production arachidière dans plusieurs pays asiatiques et africains. La maladie est particulièrement importante en Chine, en Indonésie, au Vietnam, en Malaisie et à l'Ouganda. Des progrès considérables ont été faits dans la connaissance de l'épidémiologie de la maladie, ainsi que des races et des 'biovars' de *P. solanacearum* qui attaquent l'arachide. Des antisérums ont été produits pour plusieurs 'biovars' de *P. solanacearum* et des tests sérologiques sont disponibles pour la détection de l'agent pathogène dans les tissus végétaux et le sol. Des techniques efficaces de criblage en serre et au champ ont été élaborées, des sources résistantes ont été identifiées et des cultivars résistants ont été mis au point. Des mesures de lutte différentes sont exposées et une approche intégrée est préconisée.

Cover: Groundnut plant showing primary symptoms of bacterial wilt.

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Bacterial Wilt of Groundnut

V.K. Mehan, B.S. Liao, Y.J. Tan,
A. Robinson-Smith, D. McDonald,
and A.C. Hayward

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About the Authors

V.K. Mehan: Scientist (Pathology), Crop Protection Division, ICRISAT, Patancheru, A.P. 502 324, India.

B.S. Liao: Groundnut Breeder, Oil Crops Research Institute, Wuhan, Hubei 430 062, China.

Y.J. Tan: Plant Pathologist, Oil Crops Research Institute, Wuhan, Hubei 430 062, China.

A. Robinson-Smith: Bacteriologist, Rothamsted Experimental Station, Harpenden, Hertfordshire AL5 2JQ, UK.

D. McDonald: Director, Crop Protection Division, ICRISAT, Patancheru, A.P. 502 324, India.

A.C. Hayward: Reader and Acting Head, Department of Microbiology, University of Queensland, Brisbane Qld 4072, Australia.

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Preface

International cooperative research on groundnut bacterial wilt had its origin in a meeting of Asian groundnut scientists at Malang, Indonesia, in Nov 1988. Research in Indonesia emphasized the importance of the problem, and the Australian Centre for International Agricultural Research (ACIAR) and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) cooperated in research coordination on groundnut bacterial wilt in the Asian region.

In 1990, a meeting to plan research was organized by ACIAR and ICRISAT, and co-sponsored by the Australian International Development Assistance Bureau (AIDAB) and the Peanut Collaborative Research Support Program (Peanut CRSP), USA. A formal Groundnut Bacterial Wilt Working Group (GBWWG) was established under the Asian Grain Legumes Network (now Cereals and Legumes Asia Network—CLAN) and this resulted in increased cooperation and exchange of materials and information between various national programs and international and regional research institutions. The national programs of China and Indonesia assumed the primary responsibility for research, and the task of coordinating the Group's activities was entrusted to ACIAR and ICRISAT.

A second meeting of the Working Group took place in Nov 1992 as a satellite to the International Symposium on Bacterial Wilt held in Taiwan. At this meeting, a recommendation was made that ICRISAT should produce an Information Bulletin on this disease.

This publication presents the current knowledge of groundnut bacterial wilt and its management to research and extension personnel who may not have ready access to specialist journals and reports. It should be of value to those concerned with research for the important production systems of southeastern and eastern Asia and the more restricted areas of eastern and southern Africa where groundnut bacterial wilt is a problem.

James G. Ryan
Director General

Introduction

Bacterial wilt caused by *Pseudomonas solanacearum* (Smith) Smith is a major constraint to groundnut (*Arachis hypogaea* L.) production over large areas of China, Indonesia, and Vietnam. Yield losses of 10 to 30% commonly occur, and can reach over 60% in heavily infested fields when wilt-susceptible cultivars are grown (Fig. 1). In China, annual losses in groundnut pod yield due to bacterial wilt are estimated at over 50 000 t. Severe losses also occur in some parts of Malaysia, Fiji, Papua New Guinea, and Uganda. The disease is present in various other countries of Asia, Africa, and North America, but the yield loss in groundnut is low compared with that of solanaceous crops attacked by the wilt pathogen. Bacterial wilt is a potential threat to groundnut production in several warm, humid areas of the world where groundnut cultivation is expanding, or where cultural practices are changing.

Geographical Distribution and Importance

The global distribution of groundnut bacterial wilt is shown in Figure 2a. In Indonesia, the disease is most severe in West Java, South Sulawesi, and South Sumatra (Fig. 2b). The disease is also important in central and eastern Java, Bali, and North Sulawesi.

In China, the disease occurs in 16 groundnut-growing provinces, ranging from 19 °N to 39 °N (Fig. 2c). It is most severe in the southern and central provinces, where it is estimated that over 200 000 ha of groundnut fields are infested with the wilt pathogen.

In North and South Vietnam, although the incidence and severity of bacterial wilt vary depending on locations and seasons, the disease can be severe in parts of several major groundnut-growing provinces (Fig. 2d).

In Malaysia, bacterial wilt is severe in Serdang and Kelantan areas (Fig. 2e) and less severe in the Kedah area.

Figure 1. Groundnut crop severely damaged by bacterial wilt in China.

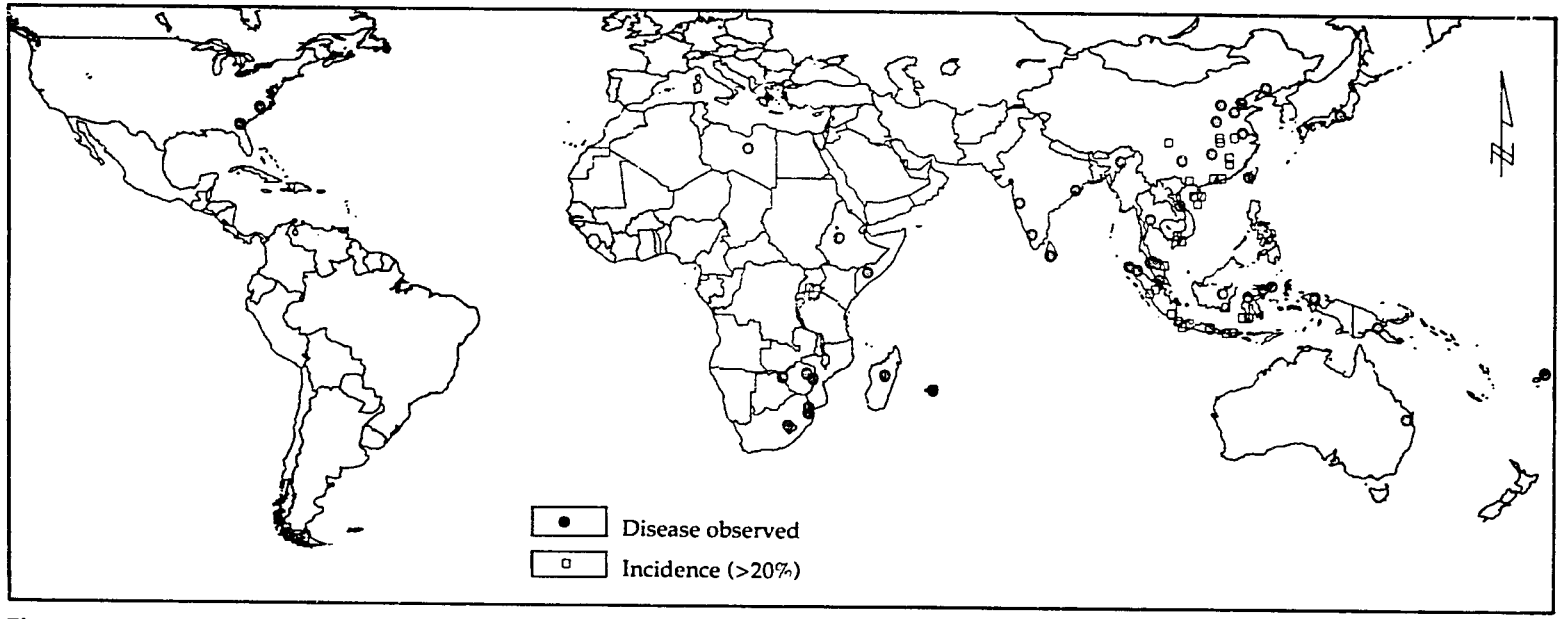

Figure 2a. Global distribution of groundnut bacterial wilt.

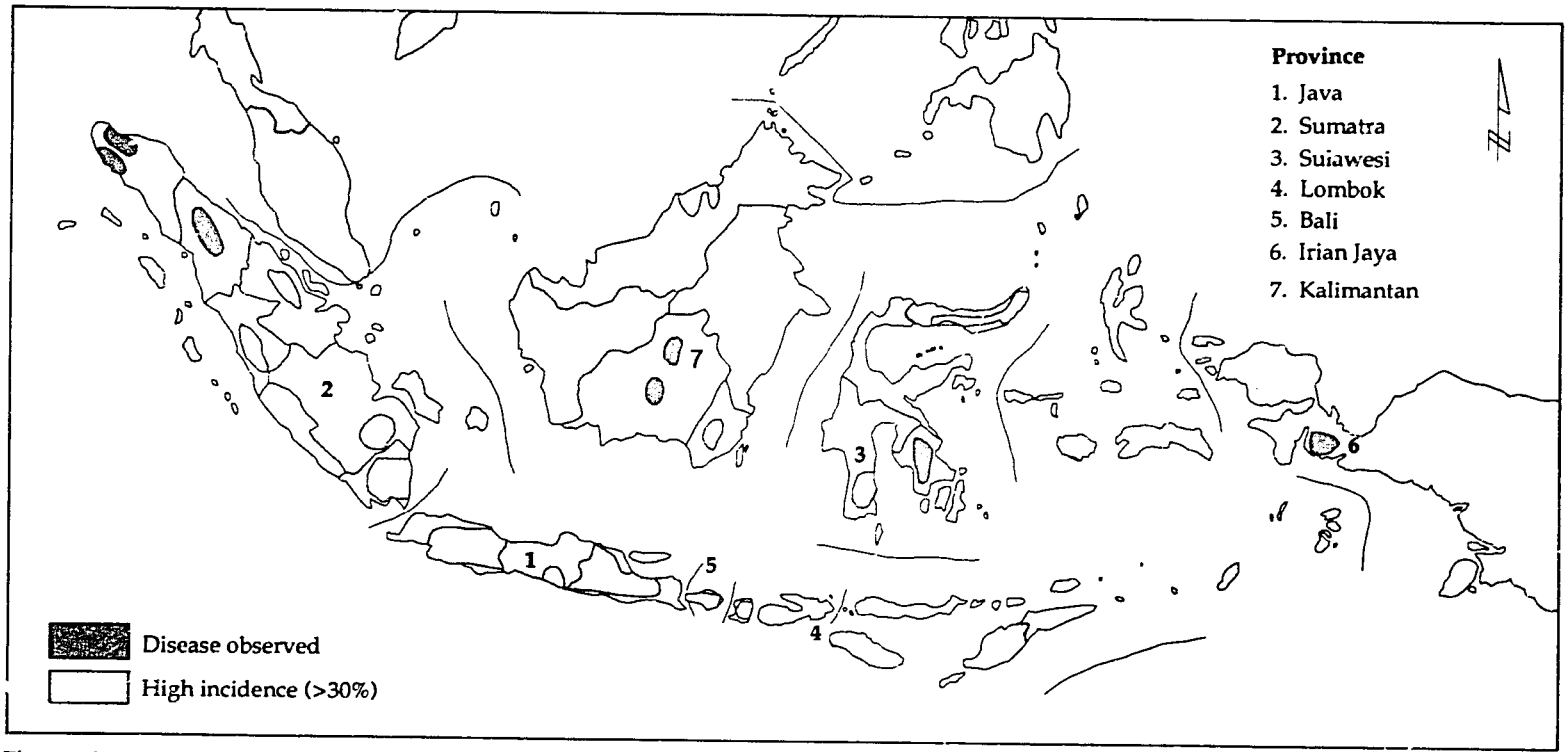

Figure 2b. Distribution and importance of bacterial wilt in Indonesia. (Based on information from surveys by Indonesian scientists, ACIAR, and ICRISAT.)

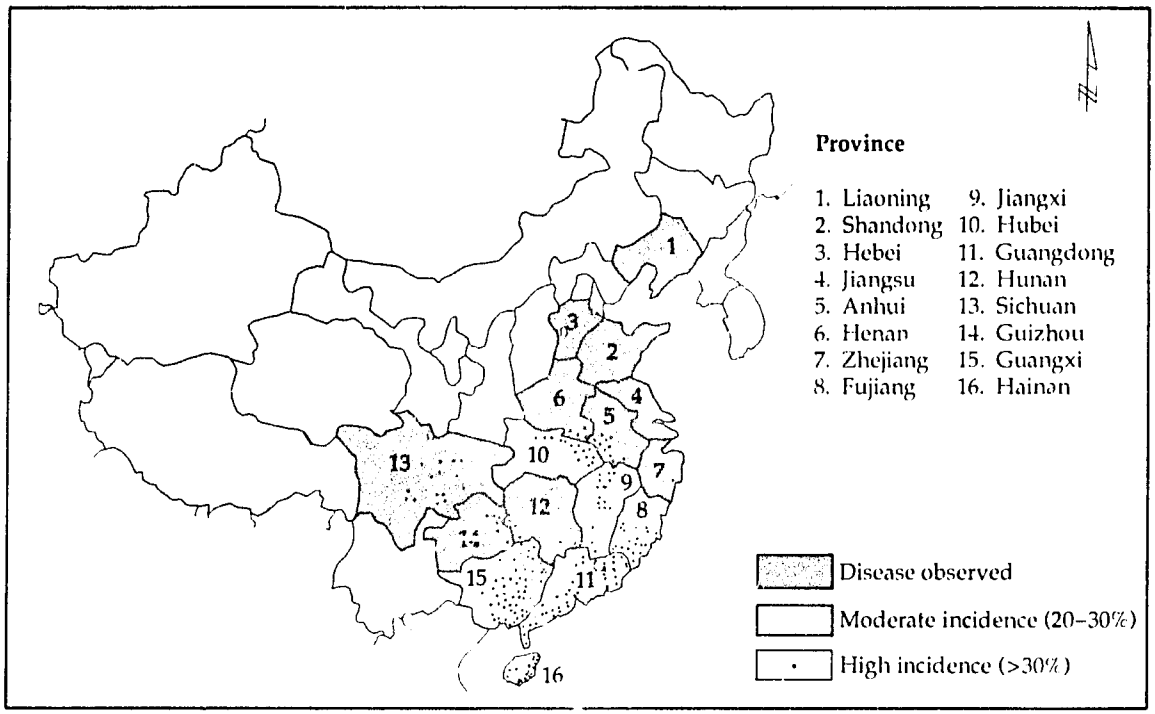

Figure 2c. Distribution and importance of bacterial wilt in China. (Based on information from provincial surveys.)

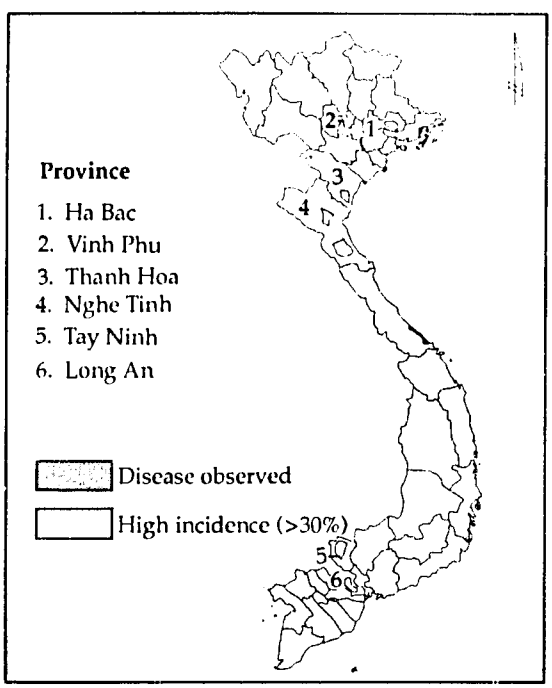

Figure 2d. Distribution and importance of bacterial wilt in Vietnam.

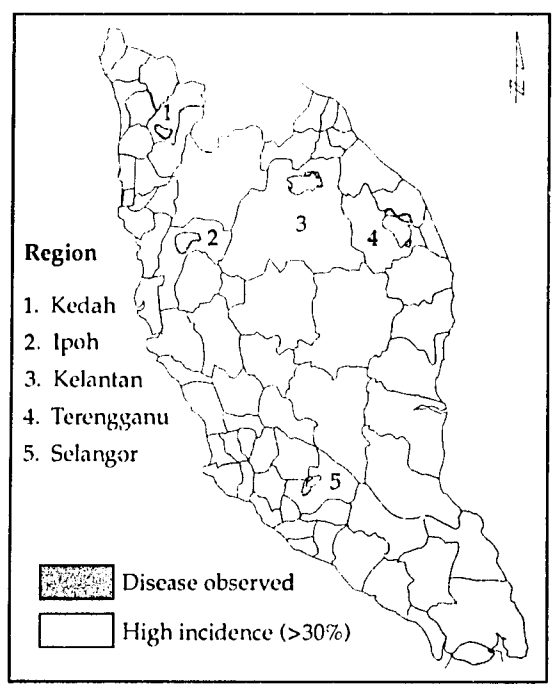

Figure 2e. Distribution and importance of bacterial wilt in Malaysia.

In Uganda, some central and north-western regions are reported to be heavily infested with *P. solanacearum*. The disease can cause 10–40% loss in crop yield (Opio and Busolo-Bulafu 1990).

Groundnut bacterial wilt was recently reported in the Philippines, Thailand, Sri Lanka, Papua New Guinea, and India (Hayward 1990), which were previously thought to be free from it. The apparent spread of the disease may be due to greater awareness about it, changes in cultural practices and varieties, or to movement of infected seed.

The disease has also been reported from Mauritius, Madagascar, Libya, Somalia, Ethiopia, Zimbabwe, South Africa, Swaziland, Taiwan, Japan, and Australia (Mehan et al. 1986), but information is sparse and the present status of the disease in these countries is unknown.

Groundnut bacterial wilt is currently of minor importance in USA, although it was reported to have caused heavy yield losses in Georgia and North Carolina in the 1930s (Gitaitis and Hammons 1984).

Symptoms

Infection of young plants results in rapid wilting of stems and foliage, but leaves remain green (Fig. 3). Wilt symptoms can be observed 2–3 weeks after sowing. The first sign of the disease is a slight drooping or curling of one or more leaves. In more advanced stages, the plants may bend over at the tip, appear dry, and eventually turn brown, wither, and die (Fig. 4). When

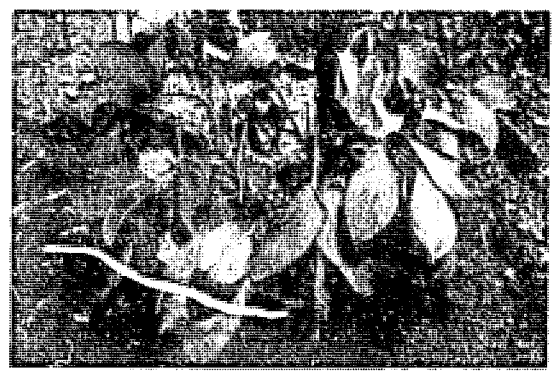

Figure 3. Groundnut plant showing primary symptoms of bacterial wilt.

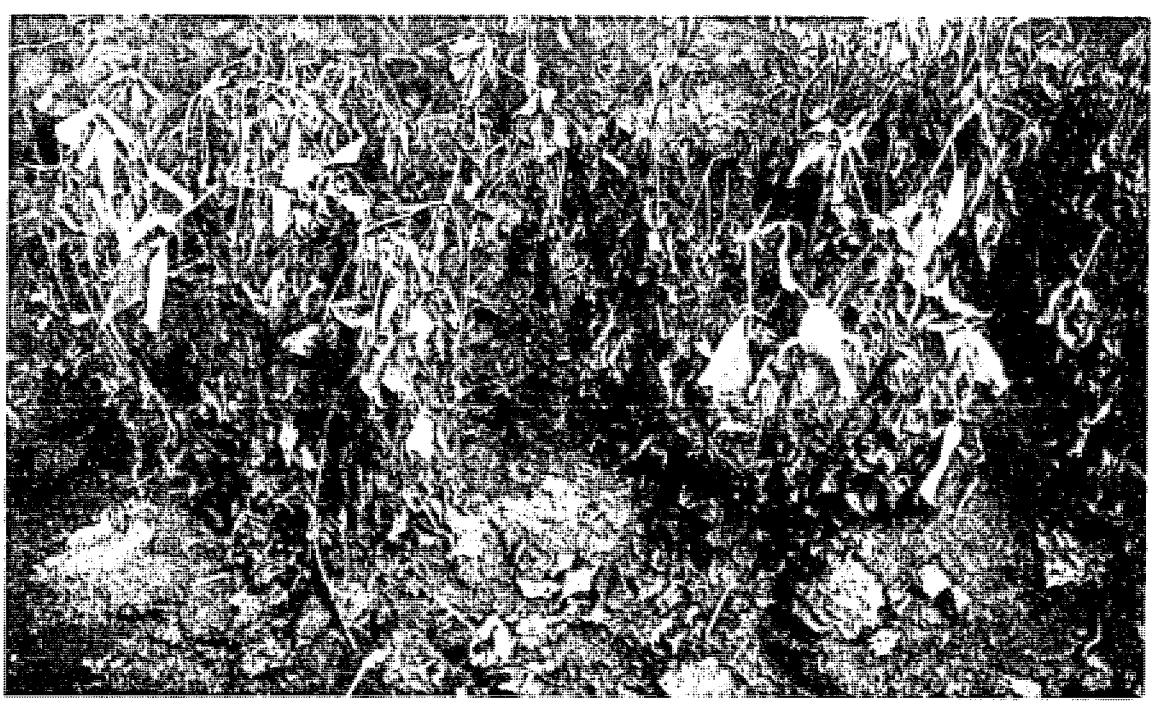

Figure 4. Groundnut plants showing advanced symptoms of bacterial wilt (complete wilt and death).

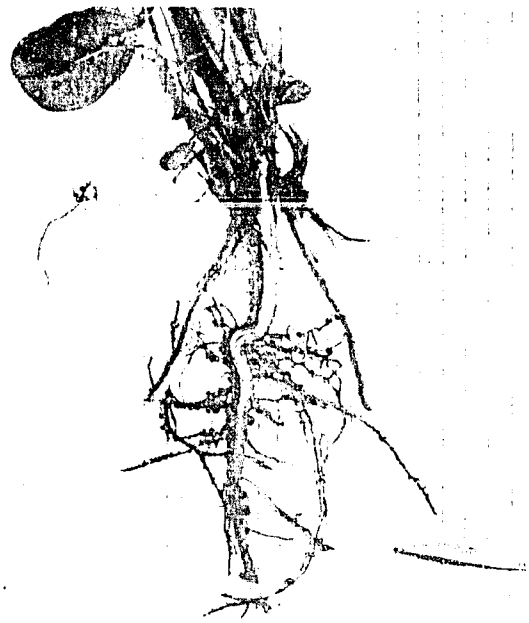

Figure 5. Infected groundnut plant showing characteristic dark brown discoloration in the root.

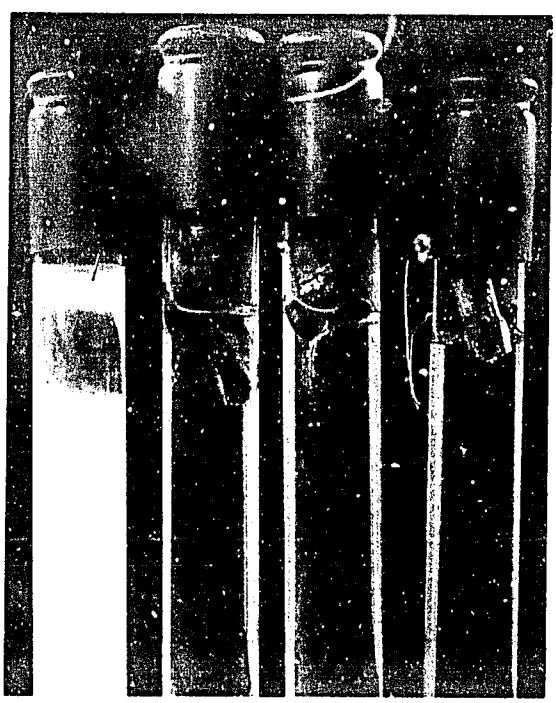

Figure 6. Bacterial wilt infected stem pieces showing bacterial ooze when dipped in water. (Note the thread-like bacterial masses oozing out from the cut ends of the stems in the two central tubes. The tube at extreme left shows milky-white water due to bacterial ooze and the tube at extreme right shows a healthy stem with no ooze).

older plants or cultivars that are not highly susceptible are infected, wilting proceeds gradually, usually starting with the lateral branches. Sometimes infected plants do not show obvious wilt symptoms, but rather a gradual decline. Wilt and death of a single branch or of the entire plant may follow. Infected plants have discolored and rotten roots and may have discolored or rotten pods. The diagnostic characteristics of this disease are the dark brown discoloration in the xylem and pith (Fig. 5), and the streaming of "bacterial ooze" (Fig. 6) it generates (see Disease Diagnosis).

Disease Diagnosis

Ooze Test

1. Cut a 3–4 cm long piece from an infected stem, preferably from the base of the plant.
2. Suspend it in clean water in a glass container; do not agitate the container. Within 2–3 min milky-white thread-like bacterial masses ooze out from the cut end of the stem.

Causal Organism

Pseudomonas solanacearum **(E.F. Smith)**
E.F. Smith
Bacillus solanacearum E.F. Smith
Bacterium solanacearum Chester
Phytomonas solanacearum (E.F. Smith) Bergey et al.
Xanthomonas solanacearum (E.F. Smith) Dowson

Pseudomonas solanacearum is an aerobic, non-spore-forming, rod-shaped gram-negative bacterium. The bacterial cells measure approximately 0.5 × 1.5 μ. Virulent isolates are mainly non-flagellate and non-motile. Avirulent isolates usually bear 1–4 polar flagellae and are highly motile. Common fimbriae are often present in both virulent and avirulent isolates.

The important physiological characteristics of *P. solanacearum* are summarized in Table 1.

Table 1. Physiological characteristics of *Pseudomonas solanacearum*.

Tests	Reaction[1]
Gelatin liquification	-
Starch hydrolysis	-
MR test	-
Indole production	-
Arginine utilization	-
H_2S production	+
Nitrite from nitrate	+
Gas from nitrate	+ (mainly isolates of biovars 3 and 4)
Tween 80 hydrolysis	+
Levan production	+
Litmus milk	Alkaline reaction
Oxidase	+
Catalase	+
Urease	+
Pectinase	+
Oxidation of acetate	+
Oxidation of citrate	+
Oxidation of malonate	+
Oxidation of gluconate	+
NaCl tolerance	
1%	+
2%	-

1. + = positive reaction or growth; - = negative reaction or no growth.
Source : Hayward (1964), He et al. (1983)

The bacterium accumulates poly-ß-hydroxybutyrate as a carbon reserve. Although it does not produce fluorescent pigments, it produces a brown diffusible pigment on a variety of agar media containing tyrosine. The bacterium can reduce nitrate to nitrite frequently producing gas, but it cannot hydrolyze starch. It liquifies gelatin only weakly or not at all. It cannot utilize either arginine or betaine as the sole carbon source.

Acid production from carbohydrates varies greatly between biovars/strains. Optimum temperature for growth varies between strains from 25 to 35 °C. *Pseudomonas solanacearum* usually shows a low level of salt tolerance. Isolates of *P. solanacearum* do not grow on media containing 2% NaCl and are often inhibited at 0.5%–1.5% NaCl.

Isolation and Identification of *Pseudomonas solanacearum*

The bacterium can be isolated from the infected stem or root of a wilt-affected plant. The infected stem is cut into 3–4 cm long pieces which are thoroughly washed in sterile water, dried on sterile blotting paper, and placed in test tubes containing 5 mL of sterile distilled water. Bacterial cells exude from the vascular bundles, and in a few minutes cloud the water so that it appears milky-white. (This is typical of bacterial infection of vascular bundles and differentiates it from wilts caused by *Fusarium* spp where the water does not cloud). After 3–5 min, a loopful of the suspension is streaked onto an agar medium—sucrose peptone agar (SPA) or tetrazolium agar (TZC) medium—in petri plates, and the plates are incubated at 28–30 °C. Bacterial colonies become visible after 48–72 h of incubation. The bacterium develops well on SPA, King's B agar (KBA) (King et al. 1954), and TZC medium (Kelman 1954). The potato dextrose agar commonly used for fungus cultures can also be used to culture *P. solanacearum*.

Cultural Characteristics

Cultural characteristics of colonies produced on tetrazolium agar medium are used to identify *P. solanacearum*. Virulent isolates of the bacterium form irregularly-round, fluidal, creamy-white colonies with light pink centers on this medium (Fig. 7) while avirulent colonies are round, butyrous, and uniformly deep red. Extracellular slime formation is a common attribute of all virulent isolates of *P. solanacearum*.

It is difficult to maintain virulence in cultures of *P. solanacearum* as highly motile, avirulent forms can rapidly dominate populations in culture media under laboratory conditions. Avirulent and virulent forms can be differentiated by growing the bacterium on TZC medium.

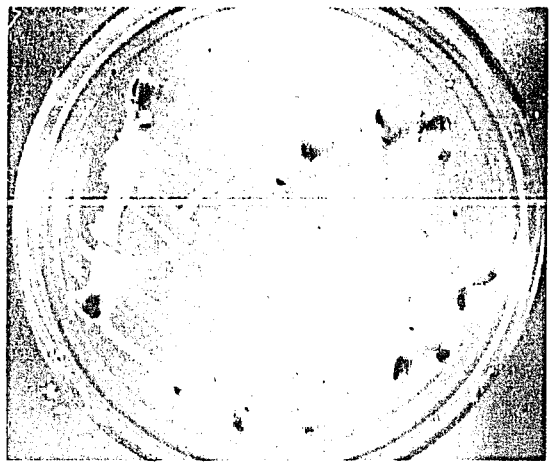

Figure 7. Virulent colonies with pink centers of *P. solanacearum* on the tetrazolium agar medium.

A simple method of preserving cultures of virulent *P. solanacearum* is to store them on agar slopes covered by sterile mineral oil (Kelman and Jensen 1951). The bacterium is grown on a standard culture medium slant, and then covered with sterile liquid paraffin oil to a depth of at least 10 mm above the top of the slant. These cultures are best stored at 4 °C, but even at ambient temperatures they remain viable for a long time.

When subculturing, some of the growth can be scraped off with a small loop which should be withdrawn carefully through the oil layer to ensure that the growth is not detached.

While this procedure is recommended for long-term storage, cultures can be preserved at 10–25 °C without loss of viability for 6–12 months as turbid suspensions of the bacterium in sterile distilled water. This method is best used as a source of working stock cultures.

Serological Techniques for the Identification of *Pseudomonas solanacearum*

The serology of *P. solanacearum* has been studied by several researchers (Morton et al. 1966, Schaad et al. 1978), but only recently have immunoenzymatic techniques been applied to detect the wilt pathogen.

Production of Polyclonal and Monoclonal Antibodies

Several polyclonal and monoclonal antibodies (McAbs) against *P. solanacearum* have been produced in laboratories at the University of Hawaii, USA, the Rothamsted Experimental Station, UK, the Institute of Plant Protection, China, and the International Potato Center, Peru (Alvarez et al. 1993, Robinson 1993, L.Y. He and H.M. El-Nashaar 1992, personal communication). Most of these antibodies showed cross-reaction to several closely related bacterial species—*P. syzygii*, *P. pickettii*, *P. cepacia*, and the blood disease bacterium (*P. celebensis*). Some highly specific McAbs produced at the University of Hawaii, and at the Rothamsted Experimental Station, do not show any cross-reaction to these bacterial species. However, they are not race- or biovar-specific. McAbs produced against *P. solanacearum* strains from groundnut and banana have shown identical specificities (Alvarez et al. 1993) and could detect over 98% of isolates of *P. solanacearum*.

Immunizing mice with whole bacterial cells with the immunosuppressant cyclophosphamide helps in producing McAbs that do not cross-react with related bacterial species (Robinson 1993). The monoclonals produced in this way were found selective in their reactions to *P. solanacearum* and were only able to detect isolates belonging to biovars N2, 3, and 4. No reaction to biovar 1, 2, or 5 was observed (Robinson 1993).

Procedure for Producing Polyclonal Antibodies

1. Prepare a suspension of 5×10^8 glutaraldehyde-fixed whole bacterial cells in 0.5 mL sterile saline (0.85% NaCl).
2. Emulsify the bacterial suspension with 0.5 mL of Freund's complete adjuvant.

3. Immunize female inbred rabbits (Dutch × Lop, New Zealand White, or other suitable inbred line) by injecting intramuscularly emulsified bacterial suspension at two or three sites in the inner thigh muscle.
4. After 2 weeks, immunize them again but with bacterial cells emulsified in Freund's incomplete adjuvant. Further intramuscular injection can be given 2 weeks later.
5. Starve the rabbits at least for a day before bleeding them.
6. Bleed (via the ear vein) 2 weeks after the last injection is given and collect the blood (up to 30 mL) into a sterile centrifuge tube.
7. Allow the blood to clot at room temperature for 3 h and then spin it at 2000 g for 15 min.
8. Carefully decant the supernatant (the sera fraction) from the tube. Store it at -20 °C. (At least 10 mL of serum can be obtained from 30 mL blood).
9. Test the titre of the antiserum by ELISA. If the titre is sufficiently high (>1:10 000) continue bleeding at weekly intervals.
10. If titre is poor, give another intramuscular injection as in step 4.
11. Continue bleeding up to 10 bleeds, or until the titre drops below that required. Rabbits can be bled up to 3 months at weekly intervals, but it is important to note that they should be starved before each bleeding.

Preparation of Buffers and Solutions

1. 0.05 M sodium carbonate buffer (carbonate coating buffer), pH 9.6, for plant assays: mix 6.36 g Na_2CO_3, 11.72 g $NaHCO_3$, and 2 g Na_2SO_3 in 1 L of distilled water.
2. 0.05 M sodium carbonate buffer, pH 9.6, for soil assays: mix 6.36 g Na_2CO_3, 11.72 g $NaHCO_3$, 83 g polyvinyl pyrrolidone (mol wt 44 000) and 40 g sodium cholate in 1 L of distilled water.
3. Phosphate buffer saline (PBS), pH 7.4: mix 8.0 g NaCl, 0.2 g KH_2PO_4, 0.14 g Na_2HPO_4, and 0.2 g KCl in 1 L of distilled water.
4. Phosphate buffer saline-Tween (PBS-Tween): add 5 mL of 10% Tween-20 to PBS.
5. Blocking buffer: mix 0.5 g full cream milk powder, 0.5 mL of 10% Tween-20, and 2 g polyvinyl pyrrolidone (mol wt 44 000) in 100 mL of PBS.
4. TMB substrate: mix 1 mg 3,3',5,5'-tetramethyl-benzidine, 2 µL of 30% H_2O_2, and 1 mL of 1 M sodium acetate, pH 5.8, in 10 mL of distilled water.

Enzyme-linked Immunosorbent Assay (ELISA)

Several ELISA procedures for detection of *P. solanacearum* have been standardized at the Rothamsted Experimental Station. The most sensitive is the indirect ELISA procedure (Robinson 1993), details of which are given below:

1. Using a micropipette, add 100 µL of a suspension of whole bacterial cells (1×10^8 cfu mL^{-1} in 0.05 M carbonate coating buffer, pH 9.6) to each well of a microtitre ELISA plate (Nunc polysorp plate or any other type with similar high affinity).

   N.B. This coating should be used for bacterial suspensions from plant tissues or cultures. Where bacterial suspensions are obtained from soil, extract bacteria from the soil particles using 0.05M carbonate coating buffer containing 8.3% polyvinyl pyrrolidone and 4% sodium cholate. Resuspend the soil sample in the carbonate buffer (1 g mL^{-1}) and shake vigorously for 2 min. Allow to settle and use the supernatant to coat the wells of the ELISA plate.
2. Incubate the ELISA plates for 1 h at 37 °C.
3. Wash the plates in three changes of PBS-Tween, taking 3 min for each wash.
4. Add 100 µL of appropriate dilution of either a polyclonal or monoclonal antibody diluted in blocking buffer to each plate well.
5. Incubate the plates for 1 h at 37 °C.
6. Wash the plates in PBS-Tween as in step 3.
7. Prepare suitable dilutions of horseradish peroxidase-conjugated goat anti-rabbit

antibody (e.g., 1:5000) or rabbit anti-mouse antibody (e.g., 1:2000) in blocking buffer. Dispense 100 µL into each well and incubate at 37 °C for 1 h.

8. Wash the plates in PBS-Tween as in step 3.
9. Add 100 µL of TMB substrate to each well, and incubate at room temperature for 10–20 min until blue color develops indicating a positive reaction.
10. Add 25 µL of 3 M sulfuric acid to each well to stop the reaction. A yellow color develops indicating the termination of the reaction.
11. Measure the absorbance at 450 nm, using a microtitre plate reader. This ELISA can detect 1×10^4 cfu mL^{-1} bacterial cells in either plant or soil samples.

N.B. Sodium azide should not be added to any of the buffer solutions when peroxidase is used.

Races, Biovars, and Strains of *Pseudomonas solanacearum*

Isolates of *P. solanacearum* are classified into five races (Buddenhagen and Kelman 1964, He et al. 1983) based on their host range, and five biovars (Hayward 1964, He et al. 1983) based on their biochemical characteristics (ability to utilize and/or oxidize several hexose alcohols and disaccharides). Races and biovars are informal groupings at the intra-subspecific level that are not governed by the Code of Nomenclature of Bacteria. These two parallel classification systems can be matched to a limited extent only. However, each system has contributed considerably towards understanding the complex pathogen behavior on various host plant species.

The term 'race' used to designate isolates of the pathogen of a particular origin and host range is obviously different from that used for such fungal pathogens as wheat rusts. Within each race there are several strains/pathotypes. The term 'strain' represents groups of isolates that have some common traits (geographical range, colony morphology, transmissibility by insects, temperature adaptation, etc.). The term 'pathotype' is used for strains specialized in pathogenicity/virulence on specific host cultivars.

Races

The five races of *P. solanacearum* are:

Race 1. Has a wide host range, affects solanaceous and other plants including leguminous plants, occurs mainly in the lowlands of the tropics and subtropics and includes biovars 1, 3, and 4.

Race 2. Affects bananas, *Heliconia* spp, and plantains, is indigenous to Central and South America, where its native host is *Heliconia*.

Race 3. Has a narrow host range, affects mainly potato and tomato and is found in the higher latitudes worldwide and the higher elevations of the tropics.

Race 4. Primarily affects ginger. Isolates of high virulence to ginger are obtained only from diseased ginger plants. It is mainly found in the Philippines and includes biovars 3 and 4.

Race 5. Primarily affects mulberry in China and includes biovar 5.

Biovars

The term 'biovar' is used for isolates of *P. solanacearum* differing in biochemical or physiological properties.

Five biovars can be identified based on their ability to oxidize three disaccharides

(cellobiose, lactose, and maltose) and three hexose alcohols (dulcitol, mannitol, and sorbitol) (Table 2).

Table 2. Physiological characteristics[1] of five biovars of *Pseudomonas solanacearum*.

	Biovars				
Tests	1	2	3	4	5
Oxidation of					
Cellobiose	-	+	+	-	+
Lactose	-	+	+	-	+
Maltose	-	+	+	-	+
Mannitol	-	-	+	+	+
Sorbitol	-	-	+	+	-
Dulcitol	-	-	+	+	-

1. + = positive reaction or growth; - = negative reaction or no growth.
Source : Hayward (1964), He et al. (1983)

Biovar 1. Does not oxidize either group of carbohydrates.

Biovar 2. Oxidizes disaccharides but not hexose alcohols.

Biovar 3. Oxidizes both groups of carbohydrates.

Biovar 4. Only utilizes hexose alcohols.

Biovar 5. Is unusual as it can oxidize lactose, maltose, cellobiose, and mannitol.

Biovars 1 and 2 are nutritionally less versatile than biovars 3 and 4, and also appear to be distinct from other biovars at the molecular level (Cook et al. 1989). There are marked differences in the geographical distribution of biovars indicating their separate evolutionary origins. In general, biovar 1 is predominant in the Americas and biovar 3 in Asia (Hayward 1990, 1991).

The relationship between host specialization and phenotype is evident only in race 3 and biovar 2. In general, race 3 and biovar 2 are equivalent.

Strains of *Pseudomonas solanacearum* Affecting Groundnut

Biovars 1, 3, and 4 are associated with groundnut, while biovars 2 and 5 have not been reported as groundnut pathogens. Groundnut bacterial wilt in USA is caused by biovar 1, which is indigenous to the Americas, whereas in other countries the disease is caused by biovar 3 or 4. Isolates of biovar 3 are more virulent to groundnut than isolates of biovar 1 or 4.

Isolates from groundnut in Africa and eastern and southeastern Asia belong mainly to race 1 and biovars 3 and 4. In Indonesia and China, most of the groundnut isolates belong to biovar 3. In general, groundnut isolates are reported to be more virulent on groundnut than isolates from other host crops, except in cases where some tomato isolates have been found to be more virulent (Hamidah and Lum 1993).

Strains of *P. solanacearum* differ greatly in their virulence on groundnut. Limited research in China has also demonstrated the existence of pathotypes. Thirty-six isolates of *P. solanacearum* from six provinces of China were classified into seven pathotypes based on their pathogenicity on six indicator-cultivars with different levels of wilt resistance (Xiekangqing, Taishan Sanlirou, Huangchuan Zhigan, Lukangqing, Fuhuasheng, and Ehua 1). All six cultivars were susceptible to pathotype 7, and moderately resistant to pathotype 6 (Tan et al. 1992). Generally, the strains from southern China are more virulent to groundnut than those from northern regions of China. Under field conditions, the bacterial strains appear to be mixtures of several pathotypes of which pathotypes 2 and 4 are dominant (Tan 1993, personal communication).

Disease Cycle

Bacterial wilt is a soilborne disease. Long-term survival of the wilt pathogen is favored by continuous cropping of a susceptible host

and/or an association with weed hosts that serve as alternate hosts. The important weed hosts that commonly grow in groundnut fields are *Ageratum conyzoides* Linn., *Crassocephalum crepidiodes* (Benth.) S. Moore, *Crotalaria juncea* Linn., *and Croton hirtus* Linn. Infected crop residues also serve as primary sources of inoculum.

The bacterium is mainly disseminated through infested soil and water. It invades the groundnut plant through wounds or natural openings in roots. It enters the water-conducting tissues and bacterial colonies grow in the lower stem. As the bacterium multiplies, the water-conducting tissues become blocked and adjoining tissues may be invaded. Infected plants may wilt partially or totally and die. When the infected root and stem decay, the pathogen is disseminated in soil through rain or irrigation water, and on farm implements.

Soil temperatures above 25 °C (at 5 cm soil depth), together with high soil moisture, favor the development of bacterial wilt (Wang et al. 1983). The pathogen survives best in wet but well-aerated soils, while it is adversely affected by soil desiccation, and by waterlogging which may reduce soil temperature and so slow down wilt development. The disease is severe in irrigated uplands, low tablelands, and on sandy soils along river banks (Yeh 1990). It develops most rapidly if the soil moisture is high during early crop growth. Young succulent plants develop critical wilt symptoms much more rapidly than do older plants, and the disease is generally most severe at peak flowering.

The disease peaks when the soil temperature is over 30 °C (air temperature over 25°C) for 10 days (Tan and Liao 1990). Optimum temperatures for wilt development range from 28 to 33 °C. In greenhouse studies, wilt severity has been shown to be most pronounced under diurnal temperature regimes of 35/30 and 30/25, and slight or absent under regimes of 25/20 and 20/15 °C (Subandiyah and Hayward 1990). Wilt symptoms appear rapidly if the diseased plants are subjected to a dry spell. If the weather remains continuously wet, the disease develops and spreads, but symptoms of severe wilt may not appear for some time. Late-infected plants may not develop severe wilt symptoms until subjected to hot, dry weather late in the season.

There are contradictory reports on the relationship between soil type and groundnut bacterial wilt incidence/severity. In Indonesia, the disease has been most prevalent and severe in heavy clay soils (Machmud 1986), whereas in China, it is predominant in sandy soils and relatively unknown in heavy clay or loam soils (He 1990). The disease also occurs in red lateritic soils. Abdullah et al. (1983) showed in greenhouse studies that the disease was more severe in heavy clay soil than in two types of sandy soils at the same moisture level. Disease severity increased 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). Heavy clay and silt soils with high water-holding capacity usually favor longer survival of the pathogen than sandy soils; however, wet, sandy soils can support high wilt incidence (Yeh 1990). In wet soils, continuous planting with susceptible cultivars leads to a rapid buildup of inoculum.

The disease is less prevalent in soils with high organic matter than in soils of low fertility. In general, a soil pH of 5.0 to 6.8 is favorable to the wilt pathogen. Some preliminary studies suggest that alkaline soils are wilt-suppressive (Yeh 1990). This is rather strange when one considers that in axenic cultures, low pH media inhibit *P. solanacearum* while high pH media favor its growth (Kelman 1953). Although soil type and pH appear to influence wilt significantly, the disease pattern is not consistent, and more research is needed to understand their effects. The soil population of *P. solanacearum* is directly related to bacterial wilt incidence and severity (Li et al. 1981). Root injury by soil insects and nematodes provides ports of entry for the bacterium, and may increase disease severity.

Seed Transmission

Pseudomonas solanacearum is transmitted through infected seed in groundnut (Machmud and Middleton 1990a). Seed transmission is reported to be at the rate of 5–8%, particularly with freshly harvested seed. Thus, infected seed is likely to provide a primary source of inoculum, particularly for disease-free areas. There is rapid loss of viability of the bacterium as groundnut seeds dry out to moisture content below 9% (Zhang et al. 1993). Seed transmission is of obvious quarantine significance and more research is needed to determine the degree to which *P. solanacearum* can be seed-transmitted in groundnut.

Disease Management

Cultural Measures

Crop Rotation

As groundnut bacterial wilt is mainly soil-borne, rotation of groundnut with crops that are immune or highly resistant to *P. solanacearum* and with nonhost crops such as rice, maize, soybean, and sugarcane are effective measures. Rotation of groundnut with rice or sugarcane for 2–3 years can greatly reduce wilt incidence and severity (Wang and Hou 1982, He 1990, Tan and Liao 1990, Machmud 1993). Groundnut-rice rotation systems are successfully used in several regions of China (He 1990). Crop rotation with wheat, sorghum, and cotton is also effective in reducing wilt incidence. In drylands, rotation of groundnut with maize and sorghum, or intercropping groundnut with maize are useful ways to contain the disease.

Although crop rotations for shorter periods with immune crops have proved effective in containing the disease, giving a gap of at least 3–4 years between groundnut crops is more effective, especially in soils that are heavily infested with the pathogen. Little is known as to how the various cropping systems affect soil microorganisms and the perpetuation/survival of the wilt pathogen.

Continuous cultivation of highly resistant groundnut cultivars should reduce the *P. solanucearum* pupulation.

Modification of Soil Moisture

Flooding groundnut fields for 15–30 days before sowing also reduces wilt incidence (Li et al. 1981, He 1990).

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

Improved soil drainage helps in reducing wilt incidence and severity.

Adjustment of Sowing Date

Depending upon the length of the growing season and cultivars grown, the sowing date can be adjusted to avoid periods of high temperature or ample soil moisture conditions that favor bacterial infection and disease development. A few attempts have been made to minimize crop losses by altering sowing dates but these have had limited success (Kelman 1953). In the Hubei Province of China, early sowing in mid-April results in much lower incidence/severity of bacterial wilt than sowing in June.

Crop Sanitation

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

Soil Amendments

Soil application of urea, mineral ash, and organic manure can be useful in reducing wilt incidence (Chang and Hsu 1988, Yeh 1990). These fertilizers are likely to stimulate soil microbial activity against the wilt pathogen, and some of their components may enhance host resistance. More research is required to elucidate the mechanisms by which soil amendments control wilt.

Chemical Control

Soil treatment with sulfur, lime, and other chemicals, including fungicides and antibiotics, has not proved useful in controlling groundnut bacterial wilt. Applying 300 kg ha^{-1} of chloropicrin 10 days before sowing can effectively control the disease (Wang and Hou 1982), but this treatment is expensive.

Plant Quarantine

Since the bacterium is seedborne, seed movement should be strictly controlled to avoid spreading the pathogen to disease-free areas.

Genetic Resistance

The use of resistant cultivars is the most effective and practical method to control bacterial wilt. Much progress has been made in developing screening techniques, identifying sources of resistance, and breeding resistant cultivars.

Screening Techniques

Evaluation of bacterial wilt resistance has been largely based on field experiments. Greenhouse tests for large-scale screening of germplasm lines, using pure cultures of the pathogen, have been limited, as it is difficult to maintain the virulence of cultures. However, such techniques are required for more precise studies (e.g., controlled soil temperature and moisture, and inoculum concentration and placement), especially when host or pathogen materials are in short supply.

Greenhouse Screening Techniques

Several greenhouse screening techniques have been developed, using plants at the seedling stage.

When virulent cultures and succulent host plants are used, successful inoculations with pure cultures of the bacterium can be made by several techniques. These include stem inoculation (stem puncture), hypodermic injection, and root inoculation (Kelman 1953). Soaking seed in bacterial suspension (6×10^8 cfu mL^{-1}) for 30 min is another useful inoculation technique (Li and Tan 1984). Infested soil placed in pots or other containers can also be used as a source of inoculum.

The root inoculation technique appears to be the best way to evaluate plants for resistance. Marked differences between resistant and susceptible genotypes can be identified by this technique. Inoculation by stem puncture usually produces higher wilt levels in resistant groundnut plants than does root inoculation (Winstead and Kelman 1952, Machmud and Middleton 1990b). However, stem inoculation has an advantage as it can identify lines with very high levels of resistance. Introducing the bacterium directly into the stem does not appear to completely overcome the inherent resistance of the more resistant genotypes. But, in extensive greenhouse screening, the exclusive use of the stem inoculation technique may eliminate certain lines which might have field resistance.

Stem Inoculation

1. Prepare bacterial inoculum from 2- to 3-day old cultures of highly virulent isolates grown on SPA slants.

2. Wash the growth on a slant in 5 mL sterile distilled water, mix well, and dilute in sterile distilled water to obtain a bacterial population of 1×10^8 cfu mL^{-1}.
3. Inoculate 2-week old plants by forcing a sharp needle into the stem through a droplet of bacterial suspension placed in the axil of the third fully expanded leaf below the stem apex. Alternatively, inject 100 µL of the bacterial suspension into the leaf axil with a hypodermic syringe.
4. Cover the inoculated plants with polythene for 24 h to prevent the droplet of suspension from drying.
5. Incubate the plants in a greenhouse or growth chamber under the following conditions: temperature 30–32 °C during the day and 25 °C at night, relative humidity (RH) >85%, with 12 h light and 12 h dark periods. It is important to grow the inoculated plants in high soil moisture conditions.
6. Observe the wilt symptoms from 5 to 21 days after inoculation.
7. Record disease ratings using the following scale:

   1 = no symptoms, 2 = one leaf wilted at the inoculation point, 3 = two to three leaves wilted, 4 = four or more leaves wilted, and 5 = whole plant wilted (dead plant).
8. Calculate the wilt intensity 21 days after inoculation, using the following formula (Winstead and Kelman 1952):

$$I = \frac{\Sigma(n_i \times v_i)}{V \times N} \times 100$$

   where I = wilt intensity (%); n_i = number of plants with respective disease rating; v_i = disease scale (1–5); V = the highest disease scale (5); and N = number of plants observed.

Root Inoculation

1. Prepare bacterial inoculum as in step 1 of the stem inoculation technique.
2. Uproot 2-week old plants (grown in sterilized soil) from pots and wash the root systems clean of soil.
3. Trim the roots with sterile scissors or a scalpel along one side of the plant, and pour 10 mL of bacterial suspension (1×10^8 cfu mL^{-1}) over the injured roots. Plant the inoculated plants in suitable containers with sterilized soil.
4. Grow the plants in a greenhouse or growth chamber as in step 5 of the stem inoculation technique.
5. Observe wilt symptoms from 5 to 21 days after inoculation. Calculate the wilt intensity using the same formula given for the stem inoculation technique.

Seed Inoculation

Seed inoculation is a uniform and simple artificial inoculation technique suitable for screening large numbers of genotypes for wilt resistance.

1. Prepare bacterial inoculum from 2- to 3-day old cultures of highly virulent isolates grown on SPA slants.
2. Wash the growth on a slant in 5 mL sterile distilled water, mix well, and dilute in sterile distilled water to obtain a bacterial population of 6×10^8 cfu mL^{-1}.
3. Soak seeds in the bacterial suspension for 30 min.
4. Sow the inoculated seeds in sterile soil in pots in the greenhouse or in the field. It is important to keep the soil wet to ensure disease development.
5. Incubate the pots with inoculated seed in a greenhouse or growth chamber under the following conditions: temperature 30–32 °C during the day and 25 °C at night, RH >85%, with 12 h light and 12 h dark periods.
6. Observe wilt symptoms 20 days after sowing. Calculate the wilt intensity using the same formula given for the stem inoculation technique. (In the field, disease incidence should be recorded).

Field Screening Techniques

Field screening under uniformly high disease pressure is a useful way to identify sources of resistance. It is desirable to use the same field each year to encourage the buildup of bacterial inoculum in the soil. Fields with heavy clay or sandy soils are suitable for resistance screening. Genotypes selected for screening should be sown in replicated plots with rows of a highly susceptible cultivar (Chico or J 11) arranged systematically throughout the trial (Sharma and Soekarno 1992). Good disease development is ensured by providing high soil moisture for up to 50 days after sowing.

A visual estimate of the percentage of wilted plants is an efficient evaluation method when testing large numbers of genotypes by this technique.

Identification of Sources of Resistance

Following extensive screening of about 5 000 germplasm and breeding lines in wilt-sick plots in China and Indonesia, many lines with varying levels of resistance have been reported (Duan et al. 1993, Yeh 1990, Liao et al. 1990, Machmud 1993, Sharma and Soekarno 1992). These wilt-resistant lines have approximately 80–95% plant survival compared with below 10% survival in susceptible cultivars in infested fields (Fig. 8). Lines showing wilt incidence up to 10% are considered highly resistant (Fig. 9), and those with 10–20% incidence resistant. Some exotic genotypes (PI 341885 and 341886) have shown resistance in Uganda (Simbwa-Bunnya 1972).

Disease reactions of some lines have been found to vary at different locations. For instance, the cultivars Gajah, Kidang, Macan, and Banteng found resistant in Indonesia were only moderately resistant in some areas of China (Yeh 1990). Such variations in wilt reactions may be due to inoculum pressure, pathogen virulence, environmental factors, and host × pathogen × environment interactions. In most cases, disease ratings in the field and in greenhouse tests do not correlate. Many of the reported sources of resistance have only field resistance and show substantial wilt incidence in greenhouse screening tests. A few lines (e.g., Schwarz 21) have been reported to be resistant in both field and greenhouse tests (Jenkins et al. 1966, Machmud and Middleton 1990b, Schwarz 1926, Yeh 1990).

Lines with high levels of resistance are described in Table 3. These lines have consistently shown resistance across several seasons in China or in Indonesia. Most of the sources of resistance identified in China belong to the Chinese 'dragon' type (*hirsuta* type ?, the taxonomic status of this type is not clear), and only a few to the *hypogaea* type.

High levels of resistance have also been found in several wild *Arachis* species (Yeh 1990): *A. duranensis*, *A. spegazzini*, *A. correntina*, *A. stenosperma*, *A. cardenasii*, *A. chacoense*, *A. villosa*, *A. appressipila*, *A. pusilla*, and *A. glabrata*. Resistance has also been identified in some interspecific hybrid derivatives (CS 7 and CS 30) (Yeh 1990) developed at ICRISAT.

Resistance Mechanisms

Very little is known about the mechanism(s) of resistance. Resistance appears to manifest itself mainly through host defence to disease development, though there may be differences among genotypes in their ability to resist invasion by *P. solanacearum*. Some infected plants do not show wilting symptoms, although they are invaded and colonized by the bacterium. Partial wilting can be observed in resistant genotypes, particularly at late stages in crop development. Resistance can be expressed through different components including latent period, degree of vascular browning, and wilting rate. Bunch-type groundnut genotypes appear to have a shorter latent period than runner-type genotypes. The expression of resistance is influenced by the genetic background of the host plant, inoculum level, virulence of the pathogen, and environmental

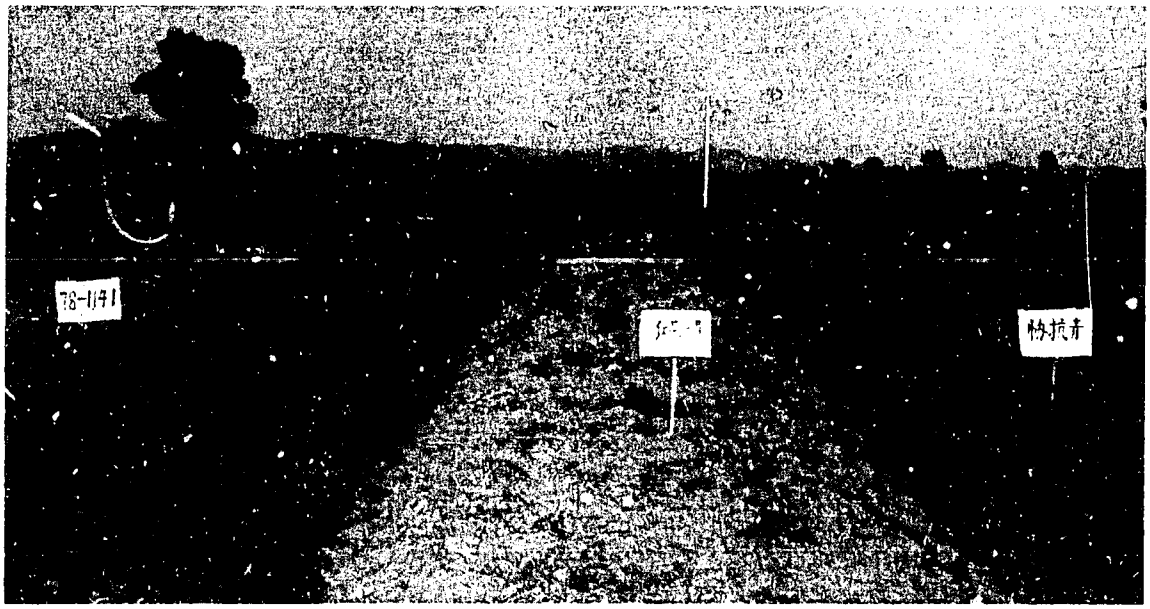

Figure 8. Field screening of groundnut genotypes for resistance to bacterial wilt at Hong An, Hubei, China. Left, 78-1141 (El Hua 5), an improved wilt-resistant cultivar; right, Xiekangqing, a resistant genotype; center, El Hua 4, a susceptible genotype.

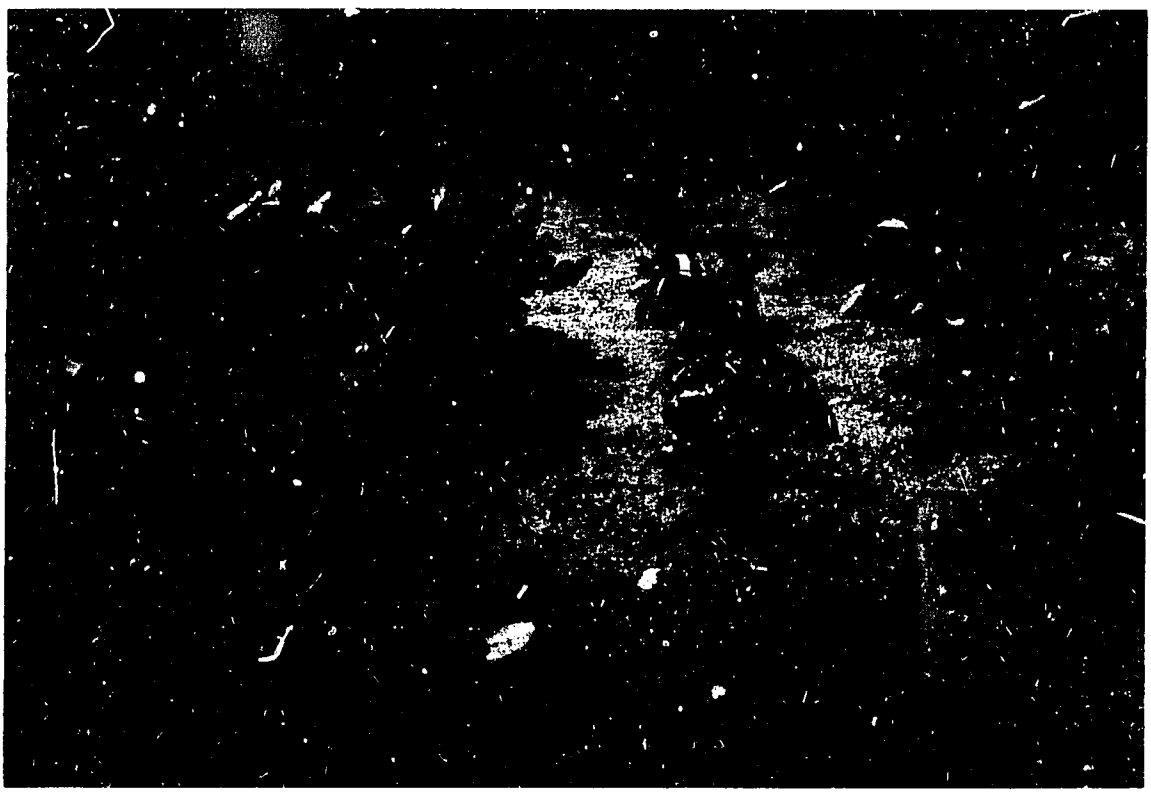

Figure 9. Wilt-resistant and susecptible groundnut cultivars, China. Left, 8818-17 (Xiekangqing), a highly resistant cultivar; right, 302 (Zhao 18), a highly susceptible cultivar.

Table 3. Groundnut genotypes resistant to bacterial wilt.

Genotype and origin	Other identity	Botanical type/variety
China		
Taishan Zhengzhu	Zhh[1] 0255	*vulgaris*
Youxuan 10015	Zhh 1775	*vulgaris*
Taizhu 148	Zhh 2097	*vulgaris*
Tiebanqing Huasheng	Zhh 1748	*vulgaris*
Xiekangqing	-	*vulgaris*
Zhong Hua 2	Zhh 1801	*vulgaris*
Zhong Hua 112	-	-
Zhong Hua 212	-	-
Lu Hua 3	-	*vulgaris*
Yue You 92	-	*vulgaris*
El Hua 5	-	*vulgaris*
Guiyou 28	-	*vulgaris*
Jinyou 3121	-	*vulgaris*
Taishan Sanlirou	Zhh 0305	*fastigiata*
Jiangtianzhong	Zhh 1319	*hypogaea*
Gouliaozhong	Zhh 1310	*hypogaea*
Bayuezhi	Zhh 0599	*dragon*[2]
Erhao Huasheng	Zhh 0610	*dragon*
Lengshui Damake	Zhh 0616	*dragon*
Yaoshan Xiaomake	Zhh 0619	*dragon*
Hexian Gaoxindou	Zhh 0639	*dragon*
Yongningdexiang Huasheng	Zhh 0649	*dragon*
Yongningshitang Dahuasheng	Zhh 0650	*dragon*
Ningming Zhixing	Zhh 0652	*dragon*
Tiandeng Dalong(2)	Zhh 0653	*dragon*
Mashanhequn Huasheng	Zhh 0655	*dragon*
Beiliu Yindou	Zhh 0660	*dragon*
Yulin Wadou	Zhh 0661	*dragon*
Yulin Qiudou	Zhh 0663	*dragon*
Bobai Gouyaodou	Zhh 0665	*dragon*
Bobai Baihuasheng	Zhh 0669	*dragon*
Rongxian Niujiaodou (1)	Zhh 0671	*dragon*
Quanzhoufenghuang Huasheng	Zhh 2330	*dragon*
Bunundi	Zhh 2331	*dragon*
Zhaoping Bayuedou	Zhh 2337	*dragon*
Qingmiaodou	Zhh 2346	*dragon*
Shikadou	Zhh 2348	*dragon*
Wazhai Dadou	Zhh 2349	*dragon*
Shibiemansheng Huasheng	Zhh 2355	*dragon*
Indonesia		
Schwarz 21	-	*vulgaris*
Gajah	-	*vulgaris*
Macan	-	*vulgaris*
Anoa	-	*vulgaris*
Rusa	-	*vulgaris*
Kidang	-	*vulgaris*
Banteng	-	*vulgaris*
Pelanduk	-	*vulgaris*
Tapir	-	*vulgaris*
Tupai	-	*vulgaris*
Local Tuban	-	*vulgaris*
Kacang Brudul	-	*vulgaris*
Uganda		
Bulundi[3]	ICG[5] 7502	-
Israel		
PI 341885[4]	-	-
PI 341886[4]	-	-

1. Zhh = Chinese groundnut germplasm accession number.
2. Chinese 'dragon' type (*hirsuta* type ?); taxonomic status of Chinese 'dragon' type is not clear.
3. Tested in Indonesia.
4. Tested in Uganda.
5. ICG = ICRISAT groundnut germplasm accession number.

factors. In almost all reported cases, wilt resistance is expressed in terms of survival percentage of plants in infested fields.

Breeding for Resistance

Use of Sources of Resistance

Efforts to breed groundnuts with resistance to bacterial wilt have been concentrated mainly in Indonesia and China. In Indonesia, Schwarz 21 or its derivatives (Gajah and Kidang) have been extensively used as resistance donors in breeding programs since the early 1950s. In China, two wilt-resistant lines with good general combining ability, Xiekangqing and Taishan Sanlirou, have been used as resistance donors (Liao et al. 1990). As most resistance donors used in breeding programs belong to subsp *fastigiata*, the genetic base of wilt resistance is narrow.

Development of Resistant Cultivars

In Indonesia, two wilt-resistant cultivars, Schwarz 21 released in 1925 and Gajah released in 1952, are widely grown in Java where bacterial wilt previously caused heavy yield losses in susceptible cultivars. In the 1950s, three wilt-resistant cultivars (Kidang, Banteng, and Macan) were developed from crosses between Schwarz 21 and some introductions from Japan, Israel, and USA. Such wilt-resistant cultivars as Anoa, Rusa, Pelanduk, Tupai, and Tapir, released in 1982/83 were developed from crosses between Gajah/Kidang and introductions from Honduras and USA. Some of these cultivars (e.g., Pelanduk and Tupai) are now grown by farmers in Indonesia.

In China, breeding efforts in the 1960s led to the release of two moderately resistant cultivars, Yue You 589 and Sueitien. Later, an exotic resistance source was crossed with a local cultivar and two important resistant and high-yielding cultivars, Yue You 92 and Yue You

256, were bred in Guangdong. In the last decade, several high-yielding wilt-resistant cultivars were released to farmers. These include Jinyou 3121, Guiyou 28, Lu Hua 3, El Hua 5, and Zhong Hua 2 (Liao et al. 1990). El Hua 5 is grown on some 18 000 ha of most of the wilt-affected areas in central China. Zhong Hua 2, an early maturing wilt-resistant cultivar with wide adaptation, is becoming popular with farmers in central China. Some recently bred cultivars that have multiple resistances to wilt and rust will be shortly released.

A modified pedigree method was used to select wilt-resistant cultivars (Liao et al. 1990). Since wilt resistance is controlled by additive genes, it is important to use highly resistant parents to obtain high levels of resistance in hybrid progenies. However, multi-directional crossing is also an effective method for using moderately resistant genotypes with desirable agronomic traits. Selection for wilt resistance and yield can be made at F_4 and F_5 generations (Liao et al. 1990).

Wilt-resistant groundnut cultivars released in Indonesia and China belong to the spanish (variety *vulgaris*) type. Wilt-resistant Chinese 'dragon' type and virginia type genotypes are now being used in breeding programs in China.

In recent years, combined resistance to bacterial wilt, rust, and leaf spots is emphasized, as these foliar fungal diseases are also severe in production systems where bacterial wilt is a serious problem. In breeding for wilt resistance, some parents with rust and late leaf spot resistance, and some (e.g., PI 414332) incorporating resistance to all three diseases, are being used by breeders.

Wilt resistance can easily be transferred through hybridization, which suggests that the genetic background for the resistance is simple. In general, there is no close genetic linkage between wilt resistance and undesirable characters in groundnut germplasm/cultivars. Resistance to bacterial wilt and foliar diseases appears to be independently inherited (Liao et al. 1990). Most groundnut germplasm accessions with resistance to rust and late leaf spot are of the *fastigiata* (valencia) type. Their resistance is closely associated with poor yield and with undesirable pod and seed characters. It is important to develop strategies to overcome these undesirable genetic linkages for successful multiple disease resistance breeding.

Inheritance of Resistance

Resistance is reported to be partially dominant, involving three pairs of major genes and some minor genes (Liao et al. 1986). Another report indicated that resistance is a recessive trait (Wang et al. 1985). There are also contrasting reports on inheritance of resistance to bacterial wilt in tomato where resistance is found to be dominant in some parents and recessive in others. The information on the genetics of wilt resistance is not conclusive. Recent preliminary results indicated that the genetics of resistance in Chinese 'dragon' types is different from that of spanish types (Liao, Wang, Tan, and Sun Darong 1993, unpublished).

Stability and Durability of Resistance

There is evidence that some reported wilt-resistant genotypes do not show stable resistance across different locations (Yeh 1990). There are also indications that the resistance of some groundnut cultivars has been overcome by the pathogen over a period of time (Machmud 1993), but this is not true for some cultivars (e.g., Gajah) that are extensively grown by farmers in Indonesia. A resistant cultivar has also consistently shown high resistance for over 10 years in one of the wilt-sick plots in the Hubei Province, China. Limited studies (Tan et al. 1992) have shown the existence of highly virulent strains/pathotypes in China. It is therefore important to study stability and durability of wilt resistance in areas where the presence of different strains/pathotypes is suspected. Durability of wilt resistance should be ensured by adopting

appropriate breeding strategies and by bringing in additional resistance genes from diverse sources.

Biological Control

Several antagonistic rhizobacteria and avirulent mutants of *P. solanacearum* have been found to delay the development, and reduce the incidence of bacterial wilt (McLaughlin and Sequeira 1988, Trigalet and Trigalet-Demery 1990). It appears that protection dependent on root surface colonization holds less promise than that based on the use of avirulent mutants of *P. solanacearum*. However, none of these approaches to biological control has reached the point of commercial application and more research needs to be done.

Integrated Control

An integrated approach to bacterial wilt control, involving wilt-resistant cultivars, rotation with nonhost crops, and crop sanitation, is advocated (Mehan et al. 1993). More research is needed to devise appropriate packages of these strategies for bacterial wilt endemic areas.

Conclusion

Specific monoclonal antisera have been produced, and researchers in several countries are now better able to diagnose the wilt pathogen in infected groundnut plants, seed, and soils. The availability of improved diagnostic tools should strengthen research on the wilt pathogen, and eventually result in improved disease control. Several high-yielding, wilt-resistant groundnut cultivars have been developed. Efforts to broaden the genetic base of wilt resistance, and combine resistances to bacterial wilt, rust, and leaf spot diseases are emphasized. Considerable progress has been made in elucidating the cropping systems/cultural practices that significantly reduce wilt incidence/severity, but more information is needed on how they affect the survival and perpetuation of the pathogen. An integrated approach to bacterial wilt mangement involving the use of resistant cultivars, appropriate rotation with nonhost crops, and crop sanitation is recommended.

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ICRISAT
International Crops Research Institute for the Semi-Arid Tropics
Patancheru, Andhra Pradesh 502 324, India

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