

Bacterial Wilt of *Perilla crispa*: New Host and New Transmission Method

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Abstract

Wilt of *Perilla crispa* was widespread in Kungkuan and Tunglo, Mioli county, Kuohsin, Nantou county, and Wufeng, Taichung county, Taiwan. Symptoms were first noticed after the top branches of *P. crispa* plants were clipped by a harvesting machine. The disease spread rapidly over the whole field in a pattern following the clipping direction. Stems were discoloured on the cut ends, and the lower leaves below the cut stems were epinastic. Infected plants turned brown, dried up, and died. The causal agent was identified as *Pseudomonas solanacearum*. Thirty-five strains isolated from *P. crispa* were race 1, biovar 3, and pathogenic to other solanaceous hosts and peanut. *P. solanacearum* strains from solanaceous hosts, peanut, and bird-of-paradise were not pathogenic to *P. crispa*. The pathogen was transmitted by scissors from diseased to healthy perilla plants. Stem inoculation caused 100% incidence, but disease incidence was lower after soil infestation. Symptomless plants may be an important inoculum source for the spread of the disease in the field. To our knowledge, this is the first record of bacterial wilt in *P. crispa*.

PERILLA CRISPA Tanaka (common name perilla), within the family Labiaceae, is an economic cash crop of importance in Taiwan for export to Japan as condiment or dye. It is also a Chinese herb and used as food in Japanese restaurants. Perilla plants are sown in spring and harvested from summer to fall. The shoot tops can be harvested 10 or more by harvesting machines during the growing period. An outbreak of a bacterial wilt of perilla was observed in the main cultivated area in recent years. In the field, the disease was first noticed after harvesting shoot tops. The objectives of the research reported here were to identify the causal agent, to investigate disease occurrence in the field, the host ranges and pathogenicity of the causal agent and to determine the mode of transmission of the new disease.

Materials and Methods

Isolation of bacteria

Strains of *P. solanacearum* were isolated from naturally-infected perilla plants growing at Kungkuan

and Tunglo, Mioli county, Kuohsin, Nantou county and Wufeng, Taichung county in Taiwan during 1987–1989. The pathogen was identified on the basis of colony morphology, biochemical and physiological properties and pathogenicity tests (Fahy and Hayward 1983; Hsu and Chen 1977).

Field observation, transmission tests and pathogenicity in greenhouse

Field investigations were done in perilla production areas between summer and fall from 1988 to 1990. The wilted plants were clipped one or five times by a pair of flamed scissors and the contaminated scissors then used to clip 40 or 90 healthy plants, respectively. To measure the population from a pair of contaminated scissors, they were immersed in 10 mL of sterile water in a 50 mL beaker. A shaken, tenfold dilution of the suspension was spread on TTC medium (Kelman 1954). For the pathogenicity test, infested soil and root dipping were used. Infested soil was prepared by mixing a bacterial suspension containing 10^8 cfu/mL at a ratio of 1:10 (v/w) with dry soil before transplanting 60-day-old seedlings. Seven replications were done with 10–25 plants in each. The root-dipping method was used to ensure rapid development of the disease. Plants were pulled from the pots and roots washed with tap water before cutting the lower roots with a pair of scissors.

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Cross inoculation test

Plants used in pathogenicity tests were perilla, tomato, tobacco, sweet pepper, eggplant, potato, peanut and bird-of-paradise. Thirty-five *P. solanacearum* strains from perilla were cross-inoculated into these plants by toothpick-stabbing.

Results and Discussion

Observation in the field

The disease first appeared in perilla fields after clipping the top branches of perilla plants by the harvesting machine (Fig. 1), and spread rapidly along the clipping direction (Fig. 2). Disease spread was also observed following manual clipping. The first visible symptom of the disease was the development of a dark discoloration on the cut wound and epinasty of the leaves which extended downward from the darkening cut wound. The affected plant subsequently turned brown, dried up, and finally the whole plant wilted and died. There was no sign of bacterial wilt in the nursery beds or fields before harvesting. The infection rate reached 100% in some of the diseased fields. The disease recurred in the same pattern in subsequent years. Bacterial wilt on perilla caused by *P. solanacearum* is reported as a new disease in Taiwan. It has not been found in other countries. According to the local farmers' association, the disease first occurred in perilla production areas in Kungkuan and Kuasing about 8 years ago.

Identification

Based on colony morphology, physiological and biochemical properties and pathogenicity to solana-



Fig. 1. Harvesting machine used by farmers to clip *Perilla crisper*.

ceous hosts, the causal agent of the disease was identified as *P. solanacearum*. Thirty-five *P. solanacearum* strains isolated from perilla in Kungkuan, Kuohsin and Wufeng were all race 1 and biovar 3.

Greenhouse cross inoculation and transmission tests

Strains of *P. solanacearum* from perilla were all pathogenic to solanaceous hosts and peanut, while strains from solanaceous hosts, peanut and bird of-paradise were not pathogenic to perilla (Table 1, Fig. 3).

The pathogen was transmitted successfully from the stem of a diseased plant to a number of healthy plants by a pair of scissors. Forty of 40 and 86 of 90 plants were diseased when they were clipped by



Fig. 2. Disease pattern resulting from machine harvesting.

Table 1. Pathogenicity to perilla and tomato of strains of *Pseudomonas solanacearum* from *Perilla crispa* and other host plants.

Strains	Hosts	Perilla	Tomato
35 strains	<i>Perilla crispa</i>	+	-
PS 21,70,75,95	Tomato	-	+
PS 27,31,T145	Tobacco	-	+
PS 86,91,96	Sweet pepper	-	+
PS 76,99,100	Eggplant	-	+
PS 60,92	Potato	-	+
PS 103,104,109	Peanut	-	+
BP 2,4,6	Bird-of-paradise	-	+

scissors that had been used to clip the tops of a diseased plants 1 and 5 times, respectively. From the five replications, a pair of scissors was found to carry 7.8×10^4 – 2.7×10^6 cfu of the bacterium after one clipping of a diseased stem. Stem inoculation of perilla with

Table 2. Percentage of *Perilla crispa* and tomato plants with bacterial wilt after inoculation by root dipping with an isolate of *Pseudomonas solanacearum* from *P. crispa*.

Plant	Weeks after inoculation (%)			
	1	2	3	4
<i>Perilla crispa</i> ^a	6	25	30	35
Tomato ^b	100	100	100	100
Check	0	0	0	0

^aThirty five 2-month-old *Perilla crispa* plants were inoculated.

^bFifteen 2-month-old tomato plants were inoculated.

P. solanacearum produced 100% wilt, but soil infestation and root-dipping inoculation induced disease wilting of only 0–30% and 35.43%, respectively (Table 2.)



Fig. 3. *Perilla crispa* plants inoculated into leaf axils with toothpicks. Strains from *P. crispa* gave typical wilt symptoms while strains from other hosts had no effect.

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Plenary Session Reports

Plenary Session

A.C. Hayward

WORK presented at this symposium has provided ample evidence of the range of variability of the bacterial wilt pathogen in genotype, phenotype and pathotype. The question should be asked to what extent the present formal and informal nomenclature successfully accommodates this range of variability and, if there is deficiency, what steps might be taken in the future to improve the situation.

There seems little doubt that at the highest level there will be further nomenclatural changes to reflect the genotypic diversity within the genus *Pseudomonas* as defined at present. It is 20 years since Palleroni et al. (1973) showed that *Pseudomonas* species were separable into 5 homology groups on the basis of DNA:rRNA homology (hybridisation); since then the phylogenetic heterogeneity of *Pseudomonas* has been confirmed by comparison of the total sequence of the 1540 nucleotides in the 16SrRNA of representative species of *Pseudomonas* (Woese et al. 1984). Representatives of rRNA group III have already been distributed into new genera; it is very likely that in the next 5 years or earlier that the species contained in rRNA group II, including *P. solanacearum*, will also be placed in new genera.

The sequence information presented at this symposium, together with the results of Palleroni et al. (1973) show that within this homology group there are two clusters of related species, one including *P. solanacearum* and *P. pickettii*, the other *P. cepacia*, *P. andropogonis* and some other species (Li et al. 1993). These two sub-homology groups are equivalent to genera established elsewhere on the basis of sequence similarity in the nucleotides of the 16SrRNA. However, it would be premature to make any taxonomic changes until additional sequence data are obtained for those species such as *P. syzygii*, the cause of Sumatra disease of cloves, the blood disease bacterium '*Pseudomonas celebense*', and some others, which show either significant levels of DNA:DNA hybridisation, reaction with probes in RFLP analysis, or similarity in phenotype to *P. solanacearum*.

The evidence from several complementary investigations has led to the belief that there may be the basis for the establishment of at least two subspecies in *P. solanacearum*. Cook et al. (1989) have shown that RFLP analysis separates all isolates of *P. solanacearum* into two major divisions, one primarily of Asian origin the other in the Americas. This suggests distant evolutionary dichotomy, an idea also supported by the data on sequence of nucleotides of the 16SrRNA in isolates of *P. solanacearum* representing biovars 1, 2, 3 and 4 (Li et al. these proceedings). However, any subspecific classification should be delayed until more detailed comparative studies have been made of larger numbers of isolates of wider geographical origin.

At the infrasubspecific level various informal systems of nomenclature have been used that are not always easy to relate to one another, and hence there has been some confusion. Differences in genotype are illustrated in the RFLP groupings of Cook et al. (1989) and in the DNA fingerprinting patterns obtained on restriction enzyme analysis (Gillings and Fahy, these proceedings). Differences in phenotype are shown in the biovar classification, and of pathogenicity and host range in races and pathotypes. It is clear that molecular methods of genotypic analysis allow far greater discrimination between strains (isolates) of *P. solanacearum* than do the phenotypic methods, and these powerful techniques find particular application in some epidemiological investigations and in plant quarantine. The traditional phenotypic methods, though simple and inexpensive to apply, often require days or weeks of incubation and the end result of the reaction may be difficult to interpret.

Biovar 2, which was previously considered to be a uniform phenotype, has now been shown to be differentiable into three sub-phenotypes (French et al., these proceedings). Two of these are metabolically less active, differ in a few phenotypic properties, and equate with race 3; they probably evolved at high elevation in the Andes as pathogens of potato and subsequently were distributed worldwide to the altitudinal and latitudinal limits of distribution of the species on latently infected planting material. The metabolically more active sub-phenotype of biovar 2 has so far been found only east of the Andes in Peru and Brazil and mainly at lower elevations. The nomenclature of these sub-phenotypes is in transition. French et al. (these proceedings) have used the term biovar 2-T for the tropical strains from Peru and Brazil, whereas others have used the term 'N-2' for the same sub-phenotype. The term biovar 2-A was given to the strains of Andean origin by French et al. (these proceedings). The latter is perhaps the best defined entity within the entire *P. solanacearum* species complex. All isolates are found in either of two closely-related RFLP groups, 26 and 27, and biovar 2-A (race 3) isolates from whatever geographical location have also been shown to be remarkably uniform in DNA fingerprinting pattern (Gillings and Fahy, these proceedings). This uniformity in genotype is matched by phenotypic homogeneity and degree of pathogenic specialisation. It seems that any future classification at the level of subspecies should recognise this major potato pathogen as a distinct and separate entity. One argument against the erection of subspecies for the two divisions of Cook et al. (1989) is that the potato pathogen would be submerged into a subspecies including pathogens specialised to other hosts such as banana. Although there is no immediate solution to this problem the objective should be an infrasubspecific classification that is universally understood and of maximum utility to plant pathologists, plant breeders and others, such as plant quarantine officers, who would make use of such a classification.

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Host Resistance

E.R. French

REPORTS were presented on breeding for resistance in tomato, tobacco, groundnut, brinjal (eggplant) and pepper. The work on potato was raised in discussions. For *Eucalyptus* spp., selection for resistance has begun for another 'new encounter' disease along the Amazon river in Brazil where strains of biovars 1, 2, and 3 are devastating newly introduced species (could the biovar 2 be similar to the tropical strain described for potato: Bv 2 - T?).

Common to most hosts is the unavailability of adequate resistance, making it necessary to develop integrated control programs. The available tolerance is usually overcome by high temperatures around 30°C or more, or by high inoculum potential (present at times as systemic latent infection during screening procedures). It would seem desirable to utilise recombinant DNA procedures to transform plants with potentially superior genes; however, this has proven elusive so far in work with potatoes. The use of hybrids in breeding for tobacco resistance is noteworthy.

Tomato breeding has seen much effort over several decades with only modest or temporary gains, because of unavailability of general resistance and linkage of resistance with small fruit character. It would seem that even more collaboration is needed between scientists and institutions if significant progress is to be attained in future. It is essential to widely test germplasm to detect, and subsequently accumulate, genes for most strains of the pathogen. Marker assisted selection using molecular techniques would accelerate this process.

The genetics of heritability of resistance in all the crops mentioned is not adequately known, though types of resistance have been described for tomato (and potato) and some genomic regions described for tomato for one strain.

There was general agreement that screening for resistance must be done, or at least culminated, in the field under natural conditions; although efficient prescreening under controlled conditions can be useful.

Resistance to bacterial wilt alone is inadequate. It must be combined with resistance to other diseases and pests (especially nematodes that interact with *P. solanacearum*), and with quality and yield characteristics.

The effectiveness of integrated control of bacterial wilt of potato was shown in Nepal, where mustard was shown to be undesirable in rotation. Similarly, the integrated control of wilt of groundnut in India using a resistance component which is also sought in Uganda, showed promise.

The importance of seed transmission of *P. solanacearum* on tomato was shown in Nepal.

Molecular Basis of Virulence and Pathogenicity

Fundamental research is often difficult to fund, its eventual usefulness not always being predictable, but it is the essence of progress. Results of progress were presented on pathogenicity genes (*hrp*), polygalacturonase as a component of disease, relation of exopolysaccharides to virulence and the reversibility of phenotype conversion.

Disease Management: Biological and Cultural Methods

The integrated management of tobacco wilt was presented for Australia, and for the U.S.A in the Host Resistance session.

New factors in the control of potato wilt in the warm tropics—soil amendments, antagonism by *P. cepacia*—were unveiled.

The importance of soil type in programs for integrated control in a tropical climate, as well as the colonisation of roots as a factor in biological control were covered for Guadeloupe, Taiwan and in a general review paper. Fundamental research continues to be needed in these areas. Induced resistance needs to be further explored.

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