Epiphytic populations of *Pseudomonas syringae* pv. *tomato* on tomato seedlings in a nursery

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Summary

Tomato seedlings, raised at a metropolitan nursery, were sampled at 4-weekly intervals between July and November to estimate the population of *Pseudomonas syringae* pv. *tomato* (PST), the causal organism of bacterial speck, on the surfaces of symptomless leaves. Plants which were sprayed with the chemicals commonly used to control the disease were included in the study to evaluate the effect of the chemicals on the epiphytic populations of PST.

The populations of PST were large (up to 1.04 × 10^8 colony-forming units per g fresh weight of leaves) in July and August. No PST were detectable at other sampling times with the exception of a sample in October. There was no difference between the numbers of PST on sprayed and unsprayed leaves. In fields at Wanneroo and Bunbury an assessment was made of bacterial speck in crops raised from seedlings purchased from the nursery in August. This was done to determine whether plants with large epiphytic populations on their leaves produce serious disease outbreaks in the field. This study showed that 73–80% of plants in the field crops were affected by the disease.

Introduction

Bacterial speck of tomato (*Lycopersicon esculentum* Mill.) caused by *Pseudomonas syringae* pv. *tomato* (Okabe) Young *et al.* (hereafter referred to as PST) has been considered to be an economically important disease of tomato for the last 10 years. Losses caused by the disease have been reported from many countries around the world (Pohronezny *et al.* 1979; Goode and Sasser 1980; Terrible *et al.* 1980; Leite and Mohan 1985). Lesions on fruit make it unfit for fresh market and processing (Goode and Sasser 1980). The worst case of loss caused by PST was that reported in U.S.A. (Pohronezny *et al.* 1979) in which there was a rejection of 160 hectares of the transplants. The disease can be severe in the nursery causing severe defoliation and can continue, though often with reduced severity, in transplants in the field.

PST, the causal organism, can survive as an epiphyte on seeds (Chambers and Merriman 1975), plant debris as trash or in soil (Chambers and Merriman 1975; Lindermann *et al* 1984). It was proved to survive on transplants which were stored at 1°C (Bonn *et al.* 1985). These plants have been considered to be sources of inoculum in the field. Even a low percentage of infected transplants may cause severe damage in a crop because of the high potential for spread of PST under conducive environmental conditions (Smitley and McCarter 1982). Little work on this bacterium has been done in Australia apart from that in Victoria by Chambers and Merriman (1975). In Western Australia, PST has been isolated frequently from plants, within field crops of tomatoes, which were believed to be affected by *Alternaria solani* (Ell. & G. Martin) Sor. (Sivasithamparam, unpublished). The leaf speck causes leaf blight and early defoliation and appears on plants which are symptomless at transplanting.

The aim of this experiment was to examine if PST exists epiphytically on leaf surfaces of symptomless seedlings in a nursery. The existence of a large population of PST on such plants would indicate that even healthy plants could act as a source of inoculum of the pathogen in the field.

Several chemicals are used in nurseries to prevent both bacterial and fungal diseases of tomato. These chemicals may affect the epiphytic populations of PST on the seedlings. The two hypotheses tested are that (*a*) the numbers of PST on symptomless leaves may vary depending on the time of the year when the sampling is made (July to November), and (*b*) that the chemicals applied will reduce the populations on leaf surfaces.

Materials and methods

Seeds of the tomato cultivar 'Duke' were planted directly in a mixture of pasteurized 85% pine bark and 15% peat moss. Soil temperature was maintained at approximately 21°C for 4 days to stimulate seed germination in winter. Seedlings were grown in the glasshouse for 2 weeks (in summer) to 4 weeks (in winter) and were then transferred to a shade-house to harden them before transplanting into the field. At transplanting, the seedlings were about 4–6 weeks old or at the two-leaf stage of growth.

Seedlings were supplied with liquid fertilizers applied through the irrigation system. During the period of germination on the heated bench, they were watered daily or less frequently depending on the weather.

Chemicals used in the nursery to prevent disease were (i) Kocide, cupric hydroxide (a.i. copper 500 g kg^{-1}, Shell Chemical Aust. Pty Ltd) used as a bactericide at 2 g l^{-1} of product in water, in addition to (ii) Mancozeb (a.i. Mancozeb, 800 g kg^{-1}, Rohm and Haas Aust. Pty Ltd) at 2 g l^{-1} of product in water. Plants were sprayed twice a week including the period when samples were collected.

Procedure

Sampling There were two treatments: one unsprayed and the other sprayed with chemicals. Sixty single plants (three from each of 10 trays per treatment) were collected at each sampling. An individual leaflet was taken from each replicate plant and placed in a plastic bag. Samples were carried in an insulated container to the laboratory and processed within 24 h. Samplings were done at 4-weekly intervals between July and November 1986.

Isolation of PST Each leaf sample was weighed, cut into small pieces with sterile scissors and placed into a 20-ml McCartney bottle containing 10 ml of sterile phosphate buffer (0.1M, pH 7.0). Bacteria were displaced from leaf samples by means of an ultrasonic cleaner operated at a frequency of 55 000 cycles sec^{-1} for 25 sec (Model Bransonic 221). The suspensions were serially diluted (10-fold) and 0.1 ml aliquots of each dilution spread on well-dried plates (3 plates for each dilution) of a modified King's medium B (Simon and Ridge 1974) at pH 7.2 and supplemented with cycloheximide (100 mg l^{-1}). Plates were incubated at 25°C and after 48 h fluorescent colonies were counted under 366 nm UV. light. Single colonies of fluorescent pseudomonads were picked at random and subcultured on to slants of King's medium B for further tests (Table 3).

Identification of PST A total of 100 isolates was picked randomly from those isolates which were considered to be PST because of their negative response to oxidase and arginine dihydrolase tests (Fahy and Lloyd 1983). *Pseudomonas aerugenosa* (Schroeter) Migula (M10848 provided by Dr B. J. Mee, Department of Microbiology, University of Western Australia), *P. syringae* ex tomato (C3485), and *P. syringae* ex plum (C3465) (provided by Mr P. McR. Wood, Western Australian Department of Agriculture) were used as comparisons for each test conducted. The bacteria were grown for 24–48 h on a slant of King's medium B before use. All tests were conducted at 25°C and were made at least twice on every isolate.

Kovac's oxidase test, tests for arginine dihydrolase, levan formation, and utilization of single-carbon sources were conducted using methods described in Lelliott *et al.* (1966); Misaghi and Grogan (1969); Fahy and Hayward (1983); and Jones *et al.* (1986).

Pathogenicity test Isolates were subjected to pathogenicity tests on tomato to aid identification of the pathovar (Fahy and Lloyd 1983). Tomato cv. 'Duke' seed used in the nursery were planted in plastic cups, one per cup in pasteurized soil. After 4 weeks each plant was inoculated with a single isolate of an oxidase and arginine dihydrolase negative fluorescent pseudomonad. Thirty-six isolates were tested and there were three replicate plants per

isolate. The inocula were prepared by flooding 72-h old cultures on sucrose-nutrient agar with sterile distilled water (Pohronezny *et al.* 1979). The plants were inoculated by gently rubbing a leaf with a cotton swab soaked with bacterial suspension. Each plant was covered with a moistened plastic bag and incubated at 25°C for 2 days when the bag was removed and incubation (at 25°C) continued for another 4–6 days.

Field assessment Properties at Wanneroo and Bunbury, with tomato crops grown from seedlings which left the nursery in August, were inspected for disease in October. The percentage of plants showing symptoms of bacterial speck was estimated in each crop. Symptomless leaf samples were checked for PST.

Results

Population of Pseudomonas syringae *pv.* tomato *on tomato seedling leaves in the nursery*

The testing of symptomless leaves of tomato seedlings for the presence of PST showed that with the exception of a sample in October the bacteria were recoverable only in July and August (Table 1). Large populations of PST were detected in August in both sprayed and unsprayed treatments. In this month a large number of the seedlings in the nurseries were infected with the disease which was misidentified, however, by the nurserymen as black spot caused by *Alternaria solani*.

The chemicals used in the nursery do not appear to affect the epiphytic populations of PST on tomato leaves since there was no significant difference between sprayed and unsprayed treatments in August (Table 2).

The results of biochemical and pathogenicity tests confirm that most of the fluorescent bacteria in leaf-washings were PST (Table 3).

Field assessment Eighty percent of the surveyed plants from Wanneroo and 73.8% of the surveyed plants from Bunbury were infected with bacterial speck. Isolations from symptomless leaves from these field crops indicated large populations of PST were present.

Table 1 Percentage of leaf samples positive for PST at five sampling times on tomato seedlings within the nursery

Treatment	% leaf samples positive for PST[A]				
	14 July	11 Aug.	8 Sept.	6 Oct.	3 Nov.
Sprayed: Kocide + Mancozeb	1.6	63	n.d.[B]	1.6	n.d.
Unsprayed	5	57	n.d.	n.d.	n.d.

[A]Means from 20 replicates each of three leaves.
[B]n.d., not detected.

Table 2 Numbers of PST (colony-forming units per g fresh weight) on symptomless tomato seedling leaves (4 weeks old) from a nursery
The transformed values (log n + 1) are in parentheses.

	Sprayed	Unsprayed
Mean[A]	1.04×10^8 (6.21)	9.55×10^7 (6.61)

LSD: 0.05 (log x + 1) = 1.92.
[A]From 20 replicates each of three leaves.

Table 3 Biochemical tests and pathogenicity of isolates from tomato seedling leaves suspected to be PST

Tests	No. isolates tested	No. isolates responding
Oxidase negative	100	100
Arginine dihydrolase negative	100	100
Levan formation	100	100
Fluorescence with sucrose	13	11
Non-fluorescent with erythritol	13	11
Non-fluorescent with DL lactate	36	36
Pathogenicity on tomato	36	36

Discussion

Under the conditions existing within the nursery glasshouse, the population of PST on leaves varied widely at each sampling time even within the same treatment (0 to 10^8 CFU per g of leaf fresh weight). Whenever PST was found the estimated population was high [10^8 colony-forming units (CFU) per g fresh weight of symptomless leaves]. This may be because the cool and humid, environmental conditions favour the multiplication of the bacterium on the leaf. In July and August daily temperatures (8 to 18°C) were cooler than in September and October (12 to 21°C), and the atmosphere more humid (79 to 82% RH. in the former and 61 to 69% in the latter period). Such an environment is known to suit PST (Gitaitis *et al.* 1985).

Large populations of PST were also found on leaves of the field-transplanted tomatoes raised from seed in this nursery. However, it cannot be assumed that they were the same strains of PST as those in the nursery because there were no tomatoes from other sources in those fields for comparison. It is possible that PST may also have been introduced from trash or non-susceptible crop and non-crop plants in the field.

PST in the nursery may have originated from diseased plant debris in the glasshouse. Chances of seed transmission is minimal as the nursery uses a technique of seed disinfection employing aerated steam. The apparatus was designed specifically for the purpose by Professor K. F. Baker.

To ensure that bacteria isolated were PST and not *P. syringae* pv. *syringae*, it was necessary to test their capacity to use single-carbon sources such as sucrose, erythritol and DL-lactate (Misaghi and Grogan 1969; Jones *et al.* 1986). Two of the isolates did not fluoresce in the medium with sucrose but did in the medium with erythritol. These isolates were, however, characteristic of PST in other tests.

Bacterial speck is a serious problem of tomato in Western Australia. It is evident from this study that the bacterium can occur in a well-managed nursery with clean, well ventilated and illuminated glasshouses. It is also possible for the pathogen to exist as an epiphyte on non-hosts. PST is cosmopolitan and difficult to eradicate. The fact that the bacteria were not detected on symptomless leaves between the months of September and November indicates that the populations may decline to non-detectable levels during certain months of the year. It also indicates that it is possible to reduce infection within the glasshouse by maintaining the environment within it as they exist in the months of September, October and November.

The lack of control using chemical sprays is alarming but confirms the observations made in several nurseries in Perth where such sprays were of little use in containing the disease in 1986. It is possible that the sprays may be effective only at low inoculum potentials. Copper-resistant strains of PST have been encountered in U.S.A. (Cooksey 1986) and it is possible that such a resistance may be present in the strains found in this nursery.

Further work comparing the extent of the disease in field crops raised from nurs-

ery seedlings with different levels of epiphytic populations will indicate the importance of such populations. Bacteriophages specific to strains of PST have been reported in North America (Cuppels 1984). Use of strain specific bacteriophages, bacteriocin (Vidaver and Buckner 1978) or sero-typing (De Boer *et al.* 1979) may eliminate the extensive labour involved in such studies and give a better indication of the source of pathogenic strains.

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