Epiphytic Survival of *Pseudomonas syringae* pv. *tomato* on Tomato Transplants Shipped from Georgia

W. G. BONN, Research Station, Agriculture Canada, Harrow, Ontario N0R 1G0, R. D. GITAITIS, Department of Plant Pathology, University of Georgia, Tifton 31793, and B. H. MacNEILL, Department of Environmental Biology, University of Guelph, Guelph, Ontario N1G 2W1

ABSTRACT

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Survival of *Pseudomonas syringae* pv. *tomato* as an epiphyte occurred during shipment of symptomless tomato transplants from Georgia to Ontario, Canada. Disease occurred on these transplants in the field. Transplants inoculated in Georgia with 10^8 colony-forming units (cfu) per milliliter of *P. syringae* pv. *tomato* were assayed 1 hr postinoculation, 24 hr later in both Georgia and Ontario, after brief poststorage periods in Ontario, and finally after 7 days. Initial population levels of 10^5 cfu per leaf at 1 hr declined to 10^4 cfu per leaf in Ontario and 10^2 cfu per leaf in Georgia but then increased to 10^7 cfu per leaf in Ontario and 10^6 cfu per leaf in Georgia. Changes in epiphytic population were not influenced by cultivar or storage period. Consequently, until more effective control measures are developed, current control practices using streptomycin and copper compounds should be adhered to rigorously, even during the apparent absence of bacterial speck.

Southern Georgia is a major production area of certified tomato transplants grown for the northern areas of the United States and southern Canada. Although bacterial speck, caused by *Pseudomonas syringae* pv. *tomato* (Okabe) Young, Dye, & Wilkie, was reported in the early 1930s (4,12), it did not become an economically important disease in the tomato transplant industry in Georgia until 1978 (18). Major losses occurred in that year in Georgia and inadvertent shipment of infected transplants to northern production areas was reported (8). Bacterial speck not only lowers fruit quality (8,14) but also reduces yield if plants become infected early in the season (17,20).

The ecology and epidemiology of the bacterial speck pathogen have been studied recently (1,5,15,16,18,20). Epiphytic populations of *P. syringae* pv. *tomato* are important in the disease cycle (18). This paper reports observations on the survival of the bacterial speck organism on healthy tomato transplants shipped from Georgia to Ontario, Canada. A preliminary report has been published (2).

MATERIALS AND METHODS

The strain of *P. syringae* pv. *tomato* used in these studies was originally isolated from a diseased tomato plant growing in southern Ontario and subsequently selected for resistance to 500 μg of both rifampicin and nalidixic acid per milliliter (3). In 1981, the strain (designated G13) was grown for 48 hr at 20 C on plates of medium B of King et al (9) (KMB) for production of inoculum. Inoculum was prepared by washing the bacteria off the plates with sterile distilled water and adjusting the cell number to about 1×10^8 colony-forming units (cfu) per milliliter with a spectrophotometer (Spectronic 20, Bausch & Lomb). A concentration of 3.3×10^8 cfu/ml of inoculum was verified by dilution plating of the cell suspension on KMB agar plates.

In 1981, a crate containing about 1,000 Georgia-grown tomato transplants (cultivar New Yorker) was obtained from a grower and 100 disease-free plants were randomly selected for inoculation. Inoculum was applied as a mist with an aerosol chromatography sprayer. Plants were allowed to dry before tagging, then placed in the center of the crate of transplants. The crate was stored for 18 hr at 12 C, then shipped by air freight to Harrow, Ontario (imported under section 8 of the Canadian Plant Quarantine Regulations), where it was stored for 60 hr at 12 C. These storage conditions simulate those used by the industry; it is often necessary to store plants for various periods of time depending on weather conditions, labor availability, etc. Inoculated tomato transplants were subsequently planted in an isolated field on 4 May.

Epiphytic populations of *P. syringae* pv. *tomato* were studied by taking leaf samples at 1 and 28 hr (on arrival at Harrow) and 90 hr (after storage at 12 C) postinoculation. Fifty leaflets were selected and washed in 50 ml of sterile phosphate buffer (0.1 M, pH 7.0) for 1 hr at 200 rpm on a reciprocating shaker. The wash water was plated out in 1:10 dilutions onto plates of KMB containing 100 μg/ml each of rifampicin and nalidixic acid. The plates were incubated for 48 hr at 28 C and bacterial colonies counted. Colonies were streaked onto KMB and fluorescent bacteria were characterized by tests for oxidase (10), arginine dihydrolase (19), growth on KMB amended with rifampicin and nalidixic acid, utilization of tartrate and erythritol (11), and pathogenicity to tomato.

In 1982, three cultivars of tomato, Heinz 2653, Campbell 28, and Ontario 7710, were grown as transplants (plant spacing 1 cm in the row and 35 cm between rows in a four-row raised bed) at the University of Georgia Coastal Plain Experiment Station in Tift County. Plots were seeded on 31 March and cultivated to simulate a commercial transplant operation (7) until inoculation on 17 May. The inoculum was prepared by growing strain G13 of *P. syringae* pv. *tomato* in nutrient broth on a gyratory shaker for 24 hr at room temperature. Bacterial cells were collected by centrifugation at 2,000 rpm for 15 min and resuspended in sterile phosphate buffer (0.1 M, pH 7.0) containing 0.85% NaCl. The cell numbers were adjusted after photometry as described previously. A concentration of 3.2×10^8 cfu/ml of inoculum was determined by the dilution plate method.

The center two rows of each transplant bed (replicated four times) were sprayed gently with inoculum using a Burgess Home & Garden Sprayer Model 5 (Acme Burgess Inc., Grayslake, IL) on 17 May. Inoculation was done in the early evening when winds were light and the air temperature was 24 C. The control consisted of uninoculated plots of the susceptible cultivar Heinz 2653. About 500 transplants were pulled the next morning from the treated rows of each plot, bundled together, and placed in the centers of crates containing other tomato transplants. Contamination was reduced by wearing sterile surgical gloves and discarding them after harvesting each treatment. The crates were shipped the same day by air freight to Harrow, Ontario, where they were stored overnight (10 hr) at 12 C or for 58 hr at 12 C. After storage, the transplants were planted in the field at standard planting

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distances (30 cm in the row and 1.5 m between rows) in four-row plots.

The procedure for sampling transplants for *P. syringae* pv. *tomato* was similar to that outlined for 1981. Leaflet samples for assay were taken at 1, 24, and 28 hr (on arrival at Harrow), at 38 hr (after storage for 10 hr at 12 C), at 86 hr (after storage for 58 hr at 12 C), and on day 7 postinoculation. Lesions that developed on the transplants in the field were sampled and single representative colonies of bacteria were characterized by the diagnostic tests outlined before.

RESULTS

1981 Study. Bacterial populations of *P. syringae* pv. *tomato* strain G13 on leaves of plants were 7.5×10^2 cfu per leaf 1 hr after inoculation (Table 1). Leaf populations increased to 1.3×10^6 cfu per leaf after a storage period of 18 hr at 12 C in Tifton, GA, and an air freight shipment to Harrow, Ontario, of 10 hr. After an additional 60 hr of storage at 12 C in Canada, leaf populations increased to 3.6×10^7 cfu per leaf. Thus, during a 90-hr period, including the periods in Georgia and Ontario and the shipment to Canada, leaf populations increased from about 10^3 to 4×10^7 cfu per leaf.

Speck symptoms appeared 7 days after inoculation on the leaves of plants placed in the field. Uninoculated transplants from the same crate, planted at the same time, remained disease-free.

1982 Study. One hour after inoculation of tomato transplants with 10^8 cfu/ml of *P. syringae* pv. *tomato*, leaf populations of about 10^5 cfu per leaf were detected by leaf washings (Fig. 1 and Table 2). Low populations ($<10^1$ cfu per leaf) of G13 were detected on the uninoculated control (Table 2), probably as a result of the inoculum drifting from the treated plots because a light wind was noted at the time of inoculation.

Epiphytic populations of *P. syringae* pv. *tomato* were detected on leaves at 24 hr after inoculation on transplants remaining in Tifton and at 28 hr on plants transported to Harrow by air freight. Populations were about 10^2 and 10^4 cfu per leaf, respectively (Fig. 1).

During the next 6 days, populations of the marker strain increased to 10^6 and 10^5 cfu per leaf in Ontario and Georgia, respectively (Fig. 1). Transplants held in storage for 58 hr at 12 C before planting had epiphytic populations of *P. syringae* pv. *tomato* similar to those of transplants planted without additional storage on day 3 (Fig. 1).

Mean bacterial populations for the three cultivars for the five sampling periods did not differ by more than 10-fold (Table 2). After an initial drop in epiphytic bacteria, recovery occurred within 91 hr, and by day 7, the number of bacteria had surpassed the initial population of about 10^5 cfu per leaf. Disease symptoms, consisting of black specklike lesions surrounded by yellow halos, appeared on all cultivars by day 7 in both Ontario and Georgia. The bacteria isolated from typical speck lesions conformed to the characteristics of strain G13 of *P. syringae* pv. *tomato* used as inoculum. Speck symptoms that initially appeared on cultivar Ontario 7710 by day 7 could not be found 1 mo later even though the disease was apparent on both Heinz 2653 and Campbell 28.

Weather conditions in Tifton were markedly different from those in Harrow during this study. Tifton was hot (mean temperature of 24.5 C) and wet (28.4-mm rainfall), whereas Harrow was cool (mean temperature of 17 C) and dry (4.2-mm rainfall).

DISCUSSION

P. syringae pv. *tomato* survived as an epiphyte on transplants shipped from Georgia to Canada. Neither the shipment nor the subsequent storage of transplants adversely affected bacterial populations. The population increased slightly in storage and paralleled the populations detected on plant leaves in the field. Populations were not determined beyond the appearance of disease symptoms, because subsequent determinations could have reflected bacterial populations emerging from lesions and not those established by inoculation in Georgia.

Differences were not noted among the three tomato cultivars in their abilities to support epiphytic populations of *P. syringae* pv. *tomato*. Because resistant Ontario 7710 (13) and Campbell 28 (7) supported populations equally as well as speck-susceptible Heinz 2653, routine spraying with bactericides to reduce inoculum should be applied to all cultivars in a control program.

Smitley and McCarter (18) suggested that a preventative control program, such

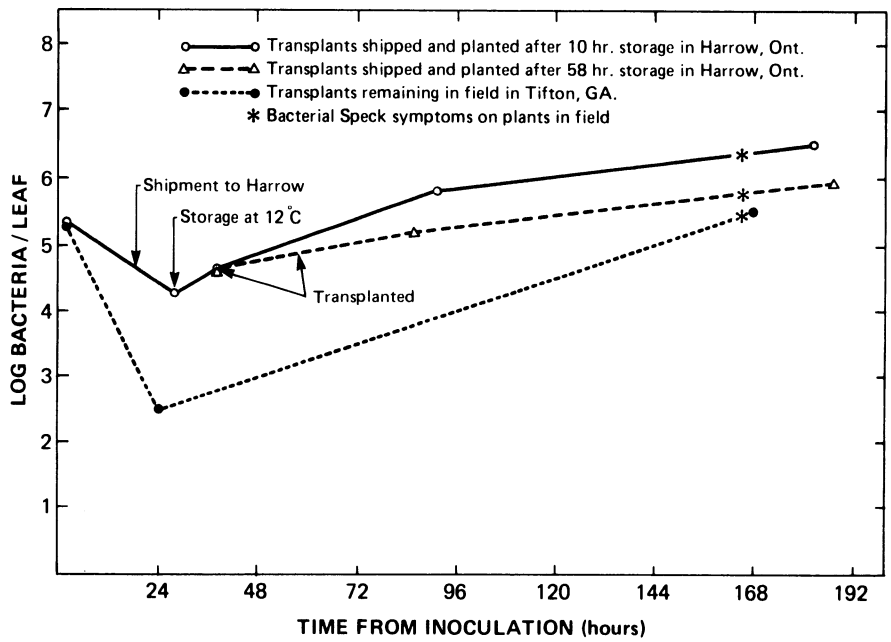

Fig. 1. Epiphytic survival of *Pseudomonas syringae* pv. *tomato* strain G13 on tomato transplants shipped from Tifton, GA, to Harrow, Ontario.

Table 1. Populations of *Pseudomonas syringae* pv. *tomato* strain G13 on cultivar New Yorker tomato transplants after inoculation with 3.3×10^8 colony-forming units (cfu) per milliliter and shipped from Tifton, GA, to Harrow, Ontario

Location	Hours after inoculation	Population (cfu/leaf)[a]
Tifton	1	7.5×10^2
Harrow	28	1.3×10^6
Harrow	90	3.6×10^7

[a] Each value is the mean of four replicates.

Table 2. Populations of *Pseudomonas syringae* pv. *tomato* strain G13 on tomato transplant cultivars after inoculation with 3.2×10^8 colony-forming units (cfu) per milliliter in Tifton, GA, and shipment to Harrow, Ontario

Cultivar	Population (cfu/leaf)[a] (hours after inoculation of transplants) 1	28	38	91	182
H2653 (uninoculated)	2.5×10^0	0	0	0	0
H2653	2.9×10^5	1.3×10^4	6.4×10^4	6.2×10^5	6.8×10^6
C28	1.8×10^5	2.2×10^4	2.3×10^4	8.5×10^5	1.3×10^6
Ontario 7710	1.2×10^5	1.6×10^4	3.4×10^4	2.8×10^5	8.8×10^5

[a] Each value is the mean of four replicates.

as that recommended by Conlin and McCarter (6) for Georgia tomato transplant fields, would control not only disease outbreaks but also epiphytic *P. syringae* pv. *tomato*. Because bacteria are able to survive on transplants, there is a need for a preventative control program even in the absence of detectable speck symptoms in the transplant fields. This is especially important in late May and early June, when hot weather interferes with symptom development and healthy-appearing transplants harboring epiphytic bacteria or incipient infections may be inadvertently shipped to northern growers. Because *P. syringae* pv. *tomato* spreads rapidly from inoculum sources and, once established, is often difficult to control, we suggest that disease control measures on Georgia transplants are a key factor in a total disease management program for the commercial tomato industry in the eastern United States and Canada.

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