Survival of *Pseudomonas syringae* pv. *tomato* in Association with Tomato Seed, Soil, Host Tissue, and Epiphytic Weed Hosts in Georgia

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ABSTRACT

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Pseudomonas syringae pv. *tomato* (PST) was recovered from 19 of 1,566 samples of commercial tomato seed lots used for transplant production in southern Georgia in 1979–1982. The bacterium was also recovered from a diverse group of symptomless weed species growing in tomato fields near Athens, GA, during 1980–1981, but not from 48 weed or volunteer crop plant species collected from tomato transplant fields near Tifton in 1979–1981. Results of an Athens test indicated that PST is disseminated to spring-seeded tomatoes after overwintering on native weeds. PST declined rapidly in both natural soil and buried host debris. In one test, it was not detected in artificially-infested (10^9 cfu/g) soil or buried host tissue when assayed 15 and 24 days after being placed in buried pots in fields at Tifton and Athens, respectively. Low populations were detected in tissue left on the soil surface for 15 and 24 days, but not when second assays were run after 45 days at Tifton and 60 days at Athens. In another test at Tifton, PST did not survive from June until the following March in diseased host tissue left on the surface or incorporated 15–20 cm deep. In temperature tanks, PST was recovered for <1 mo at 33–38 C whether free in the soil or in host tissue, but survived at least 81 days in host tissue buried in natural soil at 18 C. Our results suggest that seed and weed hosts are likely sources of primary inoculum in tomato fields. Survival of PST in either soil or host debris is highly unlikely under high soil temperature conditions in southern Georgia but is possible where temperatures are lower.

Additional key words: bacterial speck, ecology, epidemiology, *Lycopersicon esculentum*.

Growers in southern Georgia produce about 650 million certified tomato (*Lycopersicon esculentum* Mill.) transplants annually on 950 hectares for shipment to the northern United States and Canada. Although bacterial diseases have been a threat to the southern transplant industry since its beginning in the early 1900s (22,25,33), bacterial speck caused by *Pseudomonas syringae* pv. *tomato* (Okabe) Young et al (hereafter designated PST) was not identified on tomato transplants until 1978, when the disease resulted in rejection of 160 hectares for certification by the Georgia Department of Agriculture. This first outbreak of bacterial speck on transplants coincided with increased incidence of the disease worldwide (9). Bacterial speck, once considered to be of little economic importance (1,34), is now recognized as a significant disease of tomato capable of causing both quality and yield reductions (14,29,31,36). The disease is of serious concern in the transplant industry as infected transplants may serve as inoculum sources for spread in northern fruit-producing areas where transplants are shipped (14).

Little was known about the ecology of PST in transplant fields when the first outbreak occurred in 1978. Although the pattern of disease outbreaks in the field suggested seed dissemination, there was no direct evidence, and the available literature from other areas was inconclusive (2,5,14,27). PST survived epiphytically on a diverse group of weed hosts in California (27) and in soil and host debris in Australia (5), but the importance of these survival sites under Georgia conditions was not known. No studies have been reported on the field and greenhouse survival of PST in the southeastern United States.

The present work was conducted to study the ecology of PST under Georgia conditions with emphasis on the roles of seed, soil, host tissue, and epiphytic weed hosts in survival.

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MATERIALS AND METHODS

Occurrence of PST in seed. Seeds used for tomato transplant production in southern Georgia were obtained from the Georgia Department of Agriculture and assayed for PST. A total of 1,566 seed lots were tested: 277, 206, 573, and 510 in the years from 1979–1982, respectively. Each sample was assayed by plating 100 seeds in petri plates on King's medium B (KMB) (15) and by planting 100 in 10-cm-diameter clay pots filled with a methyl bromide-fumigated soil mix. Pelleted seeds were tested only by planting. Seeded pots were placed in lighted (12 hr, intensity 400 μeinsteins/m^2/sec) growth chambers at 18–20 C. When plants were in the first true-leaf stage and again when plants were 6–7 cm tall (except in 1979), pots were covered separately with a clear polyethylene bag to provide high moisture conditions. Pots were either equipped with splash guards (polyethylene-coated cylinders, 35 cm tall) to prevent cross contamination or seed lots were replanted one or more times to confirm initial positive readings.

Suspect fluorescent colonies that developed from plated seed were restreaked on KMB for purification and tested for oxidase reaction (17), arginine dihydrolase activity (32), and tobacco hypersensitive reaction (16). Isolates that gave results typical of pathogenic types (23) were further tested by inoculating Chico III tomato plants. In other studies (12) conducted in 1980–1981 we found that *P. syringae* pv. *syringae* (PSS) was also associated with lesions on tomato that could be confused with those caused by PST. Consequently, tests used to separate PSS and PST (11) were also run on all pathogenic isolates from seed in 1981 and 1982: erythritol and DL-lactate utilization as described by Misaghi and Grogan (23) and ice nucleation activity as described by Lindow (21) with slight modification (12). In the planting tests, bacteria were isolated from lesions and identified as described above.

Occurrence and survival of PST in association with weeds, soil, and host plant material in Georgia fields. Most studies were conducted at two locations (Tifton and Athens) to determine any influence of soil and environmental factors on occurrence and

survival. The Tifton site, which is located in the coastal plain where tomato transplants are produced, has sandy soils, high summer temperatures, and short, mild winters. At Athens, which is located in the piedmont section, the soils have a high clay content and temperatures are lower than at Tifton during both summer and winter.

Occurrence of PST in tomato fields at Tifton and Athens, GA. Weeds, tomato plants, and soil from fields where recent outbreaks of bacterial speck had occurred were assayed to determine if they were survival sites for PST. Forty-eight plant species, primarily weeds, representing 21 plant families were collected in and around tomato transplant fields near Tifton during 1979–1981 or from tomato fields near Athens during 1980–1981 and assayed for PST. Foliage and root samples of at least four plants of each species were collected from each field except in a few cases where it was only practical to collect foliage samples.

In 1979, weeds were collected three times in March or April near Tifton from seven transplant fields in which outbreaks of bacterial speck had occurred in 1978. Tomato transplants were growing in the fields at the time of sampling, but no bacterial speck was present. Tomato seedlings were collected from each field and assayed for PST when the weeds were collected. Twenty 2.5-cm soil cores were also taken at random from each field with a soil sampling tube, the soil was mixed thoroughly, and a composite sample was collected for assay. Plant and soil samples were placed in a cooler over ice, transported to Athens, and processed within 24 hr. In the laboratory, shoots and roots were processed separately. Roots were shaken free of excess soil. Shoots and roots were cut into 5-cm lengths, placed in flasks, and covered with sterile distilled water. Prepared samples were shaken for 20 min on a wrist-action shaker and serial dilutions were plated on KMB. Soil samples (25 g) were mixed for 3 min in a Waring blender in 250 ml of sterile distilled water, the mixture was shaken for 20 min, and plated on KMB. After 3 days at 25 C, representative fluorescent colonies resembling PST were transferred and tested for oxidase reaction and arginine dihydrolase activity. Strains that tested negative in both tests were tested for tobacco hypersensitivity and for pathogenicity on Chico III tomato.

Additional collections of weeds were made from eight transplant fields near Tifton from March–June in 1980 and 1981. Six fields were sampled both years, and two others were sampled in one of the two years. A total of 72 samples representing 39 plant species were collected and assayed.

In March and April of 1980–1981, weed samples were collected from tomato fields near Athens where PST was first introduced in 1979 and occurred on tomato each year thereafter. Collections included *Stellaria media* (L.) Cyrillo, *Gnaphalium* sp., *Arabidopsis thaliana* (L.) Heynh., *Lamium amplexicaule* L., *Secale cereale* L., *Festuca* sp., and *Oenothera* sp. Three samples of *S. media* were also collected from fields where no tomatoes had grown previously.

Samples collected at Athens and Tifton during 1980–1981 were handled as in 1979, except that a vacuum infiltration assay procedure (13) was used because it proved to be more efficient than standard plating methods for detecting low populations of PST in its natural habitats (13). Populations as low as 10 cfu per gram of soil or per milliliter of leaf washings could be detected by using the procedure. The assay was performed by infiltrating Chico III tomato plants (15–18 cm tall) under vacuum with washings from foliage or roots or with soil extracts. Five plants were infiltrated with each sample and were placed in a growth chamber at 18–20 C or on a greenhouse bench to allow disease development. Bacteria were isolated from developing lesions and identified as described previously.

Survival of PST in association with weeds, soil, and host material. Survival of PST in the field was studied in three separate tests conducted from 1980–1982: a test near Athens and Tifton in 1980 in which survival in infested soil and host tissue in buried containers was determined; a test at Tifton in 1981–1982 in which survival in host debris (surface applied or incorporated by rototilling) and on native weeds was studied; and a third test at Athens in 1981–1982 in which the role of weeds in the epidemiology of PST was emphasized. Treatments were designed to simulate various survival situations in tomato transplant fields and to study the influence of different soil and environmental conditions on survival.

Survival in soil and host tissue in buried containers at Tifton and Athens in 1980.—Soils artificially infested (10^9 cfu/g) with PST and diseased tomato tissue were placed in bottomless, 19-L polyethylene pots buried in field plots near Tifton and Athens. The soil at Tifton was a Tifton loamy sand and at Athens a Cecil sandy loam. Four treatments were used at each location: on-site soil infested with PST; off-site soil (Athens soil at Tifton and Tifton soil at Athens) infested with PST; diseased tomato tissue placed on the surface; and diseased tomato tissue buried 20 cm deep. Cells of PST for soil infestation were produced on plates of KMB for 48 hr at 25 C. A bacterial suspension was atomized into the soil rotating in a cement mixer. Diseased tissue was produced by inoculating greenhouse-grown Chico III tomato plants (15–18 cm tall). Plants with numerous lesions were cut at soil level, placed in pouches made of fine-mesh (1-mm pores) nylon netting, and placed on the soil surface or buried in the pots. Four replications of each treatment were placed in the field on 30 May at Tifton and 24 July at Athens. Survival of PST was determined by periodic sampling of the soil and tissue in containers and assaying by the vacuum infiltration method described earlier.

Survival of PST at Tifton in 1981–1982.—The survival of PST in association with host tissue and native weeds was studied. Plots 3.9 × 6.1 m separated by 9.2 m plant-free alleys were established at two separate locations on research farms near Tifton in June, 1981. Three treatments were used at each location: bacterial speck-infected tomato tissue applied (0.8 kg green tissue per square meter) to the surface of freshly tilled plots; diseased tissue applied to the soil surface and incorporated 20 cm deep with a rotary tiller; and diseased plants transplanted into tilled rows (0.46 m) in the center of nontilled plots in which a natural growth of weeds was permitted. Diseased plants and tissue for the test were produced in field plots near Athens. Plants were harvested by hand, transported to Tifton with roots in water, and planted 30 cm apart. Diseased tissue for surface application and soil incorporation was produced by mowing a field of severely-diseased Chico III plants (18–25 cm tall) with a rotary mower and collecting the tissue. The tissue was applied to the plots by hand.

Survival of PST in the plots from June until March was determined by assaying a composite soil sample (20 2.5-cm cores collected from each plot and mixed) by vacuum infiltration and by planting PST-free Chico III seed both directly in the plots and in soil collected from the plots and placed in 15-cm-diameter pots. The soil samples for assay by vacuum infiltration or pot culture were collected 24 March. Seeded pots with splash guards were placed in a growth chamber under optimum conditions for disease development (18 C and frequent periods of high moisture). The field plots were hand-seeded 10 April with seed of Chico III. Routine cultural practices were used except that care was taken to prevent contamination between plots. Plants in the pot and field tests were examined frequently for the presence of bacterial speck as an indication of survival.

Survival of PST at Athens in 1981–1982.— The main objective of this test was to determine whether PST could survive epiphytically on indigenous weeds and be disseminated to spring-seeded tomatoes. The role of soil and host tissue in survival was also studied. An 18 × 60-m plot located on the University of Georgia Plant Sciences Farm was direct-seeded (60 seeds per meter of row in rows 20 cm apart) with Chico III tomato in April of 1981. Routine cultural practices and sprinkler irrigation were used. The bacterium was introduced by transplanting infected plants from the greenhouse into the plots at 5-m spacing. Disease was severe and uniformly distributed when treatments were established on 22 June. Twenty-four 1.8 × 3.1-m plots separated 4.3 m were established. The alleys were kept free of vegetation throughout the test by cultivation and spot application of paraquat. Six treatments with four replications were established in a randomized complete-block design: diseased tomato plants pulled by hand, soil tilled 15 cm deep with a rotary tiller, and fumigated with methyl bromide (0.2 kg/m^2); tomato plants pulled by hand, soil tilled immediately

and also later as needed to keep plots fallow; plants clipped at ground level with a sickle bar mower and diseased tissue left on the surface; plants clipped at ground level and incorporated into the top 15–20 cm of soil with a rotary tiller; tomato plants pulled and weeds allowed to develop; and plants left undisturbed and weeds allowed to develop. Plots receiving tomato tissue (surface or incorporated) and those fumigated with methyl bromide were treated as needed with paraquat herbicide to eliminate weeds. Plots were maintained as described above throughout the remainder of the 1981 growing season and until planting in April of 1982. The plots treated with methyl bromide in 1981 were refumigated 7 days before planting on 9 April in 1982. All plots were planted by hand with Chico III seed free of PST. Three rows spaced 25 cm apart were prepared and seeded (180 seeds per meter of row) in the middle of each plot, and the remainder of the plot was maintained as originally established in 1981. Plots maintained without weeds were kept clean by hand weeding or application of paraquat. Equipment used for land preparation and cultivation was cleaned and sterilized between plots. Chlorothalonil (tetrachloroisophthalonitrile, Bravo 500) and carbaryl (1-napthylmethylcarbamate, Sevin 80%) were applied at recommended rates at 7-day intervals for fungal foliar disease and insect control.

Three times (once in March before seeding and twice during July–August), native weeds were collected from plots in which they were allowed to develop and were assayed for PST by vacuum infiltration of foliage and root washings into Chico III tomato plants as described previously. Collections included one or more samples of *Ambrosia artemisiifolia* L., *Chenopodium album* L., *Digitaria sanquinalis* (L.) Scop., *Erigeron canadensis* L., *Lamium amplexicaule*, *Lepidium virginicum* L., *Plantago lanceolata* L., *Stellaria media*, and *Xanthium pennsylvanicum* Wallr. Foliage and roots of symptomless tomato plants and soil were collected from weed-infested plots and assayed. Soil collected between plots was assayed as a control. Bacterial speck severity was recorded in all plots at 7-day intervals on a 0 to 5 scale in which 0 = no disease, 1 = few scattered lesions, and 5 = numerous lesions on most plants. The study was terminated in June when contamination among plots occurred.

Effect of temperature on survival of PST in soil and host tissue. The effect of temperature on survival of PST in soil and host tissue was determined in greenhouse studies. Five constant temperatures from 18 to 38 C at 5 C intervals were maintained in water-bath tanks. Two soils were used: a Tifton loamy sand and a Cecil sandy loam, the same soils used in previous studies. The soils were untreated except that they were screened to remove clods and large pieces of debris. Two treatments with four replications were used for each soil-temperature combination: soil infested uniformly with 10^9 cfu of PST per gram of soil; and diseased tomato foliage buried 1 cm deep, giving a total of 20 treatment combinations. Infested soil and diseased tissue (12 leaflets held in porous nylon pouches) were placed separately in 4-L plastic containers. The isolate of PST and procedures used for soil infestation and production of diseased tissue were the same as described previously. Prepared pots were watered to field capacity when the experiment was started and were checked at least twice daily thereafter to maintain uniform moisture levels. Pots were sampled after 7, 27, 54, and 81 days by removing three 2.5-cm-diameter soil cores with a cork borer from the top 10 cm or a pouch with tissue, and samples were assayed by the vacuum infiltration procedure already described.

RESULTS

Association of PST with seed. The 1,465 seed samples plated on KMB were mostly free of both bacteria and fungi. A few samples had moderate to high numbers of nonfluorescent bacteria, primarily *Bacillus* spp. and some other unidentified saprophytes. Fluorescent colonies grew from 3% of the samples tested. All of the fluorescent bacteria except two strains (one in 1979 and one in 1981) were oxidase positive and were considered to be saprophytes. The two strains that were oxidase negative were also negative for arginine dihydrolase, positive for the tobacco HR reaction, and produced typical bacterial speck symptoms on Chico III tomato. Both isolates were identified as PST in laboratory tests.

In the planting tests, plants from most seed samples were free of foliar lesions. Seventeen of the samples tested (four, zero, four, and nine in 1978, 1980, 1981, and 1982, respectively) developed typical bacterial speck symptoms. Seedlings from some seed lots had bacterial speck lesions on cotyledons and the first true leaves within 7 days after emergence whereas others showed symptoms only after the plants were enclosed in polyethylene bags to provide high humidity. Lesion numbers varied from a few to 60 per pot, suggesting different levels of seed contamination. Strains of bacteria isolated from typical lesions from positive samples were identified as PST.

Occurrence and survival of PST in association with weeds, soil, and host material. *Occurrence in tomato fields at Tifton and Athens.* PST was not isolated from any of the weed species, symptomless tomato plants, or soil collected during 1979 from commercial tomato transplant fields near Tifton, although bacterial speck had occurred in the fields the previous year. Fluorescent pseudomonads were often a major component of the bacterial microflora appearing on KMB plates prepared from weed or soil samples. Some weed species, especially root samples, had a characteristic bacterial population easily distinguishable as saprophytes by their rapid growth rate, yellow fluorescent hue, and slimy growth. These colonies were consistently oxidase- and arginine dihydrolase positive and were discarded as probable saprophytes. Other fluorescent colonies also resembled those in the group with *P. syringae*; these were transferred for further testing. Although certain strains from tomato, evening primrose (*Oenothera laciniata* Hill), rye (*S. cereale*), and wild lettuce (*Lactuca* sp.) were oxidase- and arginine dihydrolase negative and tobacco HR positive, none produced bacterial speck symptoms on tomato in greenhouse tests.

Washings from some of the foliage or root samples of weeds collected at Tifton during 1980 and 1981 caused necrotic spots when infiltrated into Chico III plants. However, with one exception, the spots did not have yellow halos typical of bacterial speck. Results of isolations and subsequent laboratory and greenhouse pathogenicity tests showed that none was caused by PST. Washings from the roots or foliage of four of 12 rye samples produced a few lesions indistinguishable from those caused by PST when infiltrated into tomato plants. However, the isolates were identified as *P. syringae* pv. *coronafaciens* based on other differential tests (11).

Washings from foliage or roots of five weed species growing in

TABLE 1. Severity of bacterial speck on Chico III tomato plants in 1982 when grown in plots that received various treatments in 1981

Treatment in 1981, maintained until planting in 1982[a]		Sampling date			
Diseased plants	Weeds	12 May	26 May	1 June	9 June
Removed	Killed (MeBr fumigation)[b]	0[c]	0	0.2	2.3
Removed	Removed	0	0	T[b]	1.4
On soil surface	Killed (herbicide)	0	0	T	1.6
Rototilled into soil	Killed (herbicide)	0	T	0.2	1.3
Removed	Allowed to develop	0.2	1.2	1.8	2.4
Allowed to develop	Allowed to develop	0.5	1.7	2.5	2.9

[a] All plots had severely diseased tomato plants before treatments were applied.

[b] Plots were fumigated a second time with methyl bromide before planting in 1982.

[c] Plants were rated on 0–5 scale where 0 = no disease, 1 = scattered lesions, and 5 = many lesions on most plants. T = trace or few scattered lesions in entire plot.

tomato fields near Athens in 1981 or 1982 caused typical bacterial speck symptoms when infiltrated into tomato foliage. PST was recovered from the roots and foliage of chickweed (*S. media*), henbit (*L. amplexicaule*), mouse ear cress (*A. thaliana*), and from the foliage of cudweed (*Gnaphalium* sp.) and evening primrose (*Oenothera* sp.). The highest population was recovered from chickweed, with some infiltrated plants having 800 or more lesions. PST was consistently isolated from chickweed collected from a field in which bacterial speck had occurred on tomato the previous year but not from chickweed collected the same day from nearby sites where tomatoes had never been grown.

Survival of PST in association with weeds, soil, and host material. Survival in soil and host tissue in buried containers at Athens and Tifton in 1980.—PST survived poorly in both artificially infested soil and diseased host tissue placed in pots buried in the field at Athens and Tifton during the summer of 1980. The bacterium was not recovered from either a Tifton loamy sand or a Cecil sandy loam (both originally infested with 10^9 cfu/g) when first assayed 15 and 24 days after the experiment was established at Tifton and Athens, respectively. In the same assays it was also not detected in tomato tissue buried 20 cm deep in the indigenous soils at the two locations. A low population of the bacterium was recovered in the first assay from all tissue samples placed on the soil surface at Tifton and Athens but not from any tissue samples when second assays were made at 45 days at Tifton and 60 days at Athens.

Survival at Tifton in 1981–1982.—PST apparently failed to survive from June until the following March at Tifton in the 1981–1982 field test in which diseased tissue was either placed on the surface, incorporated into the soil, or diseased plants were transplanted into plots with a natural weed population. PST was not detected in either soil or on mature weeds (*Oenothera laciniata, Rumex crispus* L., *Richardia scabra* L., and *Gnaphalium* sp.) or volunteer rye plants collected from the plots in March of 1982. Washings from the leaves and roots of rye and Florida purslane (*R. scabra*) produced brown necrotic spots without halos on infiltrated plants. Fluorescent bacteria isolated from these lesions were identified as PSS rather than PST. Bacterial speck also did not appear when the field plots were seeded with Chico III tomato during the spring of 1982, although there were periods when weather conditions were favorable for disease development. Bacterial speck developed rapidly in nearby fields. A foliar disease did appear, but the causal agent was determined to be PSS.

Survival at Athens in 1981–1982.—PST apparently overwintered on several weed species growing in experimental plots near Athens. In March of 1982, before planting in April, PST was recovered from the leaves of common chickweed (*S. media*), lambsquarters (*C. album*), and buckhorn plantain (*P. lanceolata*) growing in plots where weeds or weeds and tomatoes were allowed to remain in 1981. After seeding, bacterial speck appeared first and eventually became most severe in plots where weeds or weeds and tomatoes were left in 1981 (Table 1). The bacterium survived well on weeds in the absence of tomatoes. In the weed plots disease severity was highest in plots where the greatest number and variety of weeds were present. Plots kept free of vegetation from late June 1981 through planting in 1982 were generally free of bacterial speck during the early growing season, even when some plots with weeds already had a high incidence of disease (Table 1). Some disease appeared in all plots by early June and thereafter treatment effects were less obvious. Patterns of disease development indicated that cross contamination, probably caused by windblown rain during severe storms and not indigenous inoculum, was responsible for the disease spread in early June. PST persisted on weeds throughout the growing season, even after temperatures were too high for disease development on tomato. PST was recovered from the roots of common cocklebur (*X. pennsylvanicum*), foliage of pepperweed (*L. virginicum*) and large crabgrass (*D. sanquinalis*), and the roots and foliage of horseweed (*E. canadensis*) collected from plots in late July or early August. It was also recovered from the leaves and roots of symptomless tomato plants and from tomato rhizosphere soil collected at the same time as the weeds, but not from soil collected from alleys between plots.

Effect of temperature on survival of PST in soil and host tissue. In the temperature tank studies, PST survived better at low than at high temperatures in both soil and host tissue regardless of the soil type (Table 2). However, the bacterium survived for only short periods when it was free in the soil regardless of temperature. The bacterial population was either low (at low temperature) or nondetectable (at high temperature) after 7 days in both soil types. It was not detected in either soil at any temperature when soils were assayed after 27 days. The bacterium survived longer in buried host debris than when free in the soil but was still sensitive to high temperatures. It was not recovered after 27 days at 33 and 38 C. However, low populations were detected in three of four samples after 81 days at 18 C in a Tifton loamy sand. The assays were discontinued at that time because all tissue samples had been used.

DISCUSSION

Seed transmission of PST has been considered in several previous studies (2,3,6,14,27). Schneider and Grogan (27) failed to detect the bacterium in suspect commercial seed lots and considered seed to be unimportant in the epidemiology of the disease in California. However, Kim (14) isolated PST from both commercial seed lots and seed harvested from infected fruits. Chambers and Merriman (5) also isolated PST from seed taken from infected fruit but not from similar seed extracted by the fermentation or acid processes. PST was found on two commercial seed lots in Israel (2) but not after surface sterilization. Devash et al (6) showed that PST survived on artificially-inoculated seed and produced disease after 6 mo of storage at room temperature.

TABLE 2. Recovery of *Pseudomonas syringae* pv. *tomato* (PST) from two infested soils and buried host tissue held for various periods at five temperatures

Soil temp. (C)	Soil type[a]	Days after soil infestation or tissue burial: 7 Soil[b]	7 Tissue[b]	27 Soil	27 Tissue	54 Soil	54 Tissue	81 Soil	81 Tissue
18	Cecil sandy loam	4 (96)	4 (CN)	0	4 (75)	0	2 (<1)	0	0
	Tifton loamy sand	4 (8)	4 (CN)	0	4 (268)	0	4 (2)	0	3 (<1)
23	Cecil sandy loam	4 (14)	4 (CN)	0	4 (8)	0	0	0	0
	Tifton loamy sand	2 (5)	4 (CN)	0	3 (8)	0	0	0	
28	Cecil sandy loam	2 (<1)	4 (>1000)	0	0	0	0	0	
	Tifton loamy sand	1 (<1)	4 (>1000)	0	1 (<1)	0	0	0	
33	Cecil sandy loam	2 (<1)	4 (7)	0	0	0	0	0	
	Tifton loamy sand	0	4 (135)	0	0	0	0	0	
38	Cecil sandy loam	0	4 (7)	0	0	0	0	0	
	Tifton loamy sand	0	4 (3)	0	0	0	0	0	

[a] The soils came from Athens and Tifton, Georgia.

[b] Soil and tissue samples were assayed by vacuum infiltrating water extracts into Chico III tomato plants. Values shown are the numbers of replications positive for PST and mean number of lesions per infiltrated assay plant (in parentheses). Lesion counts are means of actual counts below 100 and estimates when more than 100 lesions occurred on infiltrated plants. CN = complete or major necrosis resulting from high populations of PST so that lesion counts were not possible.

Recently, Bashan et al (3) reported that PST survived for 20 yr on dried tomato seed in Israel.

Our tests show that PST is present, although infrequently, on seeds used for transplant production in southern Georgia. Planting seed under conditions conducive for disease development was a more reliable assay than plating on KMB. Our detection percentages are probably conservative since low numbers of seed of each sample were assayed because a large number of samples were involved. Even a low infection rate is a significant threat to the transplant industry since PST spreads rapidly (31). A few seed infected with bacteria have caused epidemics in the field (26,35), eg, 12 bean seeds per acre infected with *Pseudomonas syringae* pv. *phaseolicola* and five cabbage seeds infected with *Xanthomonas campestris* pv. *campestris* among a total of 10^4 seeds resulted in epidemics of halo blight (35) and black rot (26), respectively.

Our results suggest that contaminated seed are important in the epidemiology of PST in tomato transplant fields and may have been responsible for the first epidemic in 1978. Tomato transplants have been produced commercially in southern Georgia since 1914 (33). It is highly probable that bacterial speck would have occurred before 1978 had the bacterium been present in the ubiquitous association with soil or weeds that occurs in some locations (27). Circumstantial evidence suggests the recent introduction of PST into transplant fields, probably on commercial seed.

The importance attributed to soil and host debris in the overseasoning of PST has varied with location (2,4,5,8). In Australia, these were considered the main sources of primary inoculum after tests indicated that PST survived 25–30 wk in diseased tissue either on the soil surface or buried in nylon mesh bags (5). PST was readily detected in soils from fields with diseased plants in Israel (2) and in the same fields 1 yr later. However, the latter results are difficult to interpret as the fields were planted in wheat, a known epiphytic host of pathogenic fluorescent pseudomonads (18,30) including PST (27). PST survived for 6 mo in autoclaved soil (6), but this apparently is a poor measure of survival under natural conditions. For example, PST survived for 120 days in some of 11 artificially infested autoclaved soils in Switzerland but only from 1 to 30 days in the same soils in which the normal microflora was maintained (4). Bosshard-Heer and Vogelsanger (4) concluded that PST was so sensitive to soil microbial and environmental factors that survival in field soil for extended periods is unlikely and suggested that weeds and seed were more probable sources of primary inoculum. Pohronezny et al (24) doubted whether PST could overseason under the warm conditions in Florida and suggested a closer evaluation of the role of seed transmission.

Our temperature tank and field studies suggest a short survival period for PST in both soil and host tissue under high temperature conditions in tomato transplant fields in southern Georgia and the unlikelihood of persistence under such conditions. Tomato transplants are produced during March–June when temperature and moisture conditions may be favorable for bacterial speck development (28,31). However, summer soil temperatures may reach 45 C and average 39 C (*unpublished*) for extended periods at a 5-cm depth, which is highly unfavorable for survival of PST. We failed to isolate PST from soils or tomato plants collected in 1979 from transplant fields in which epidemics of bacterial speck occurred in 1978. Results of our temperature studies suggest extended survival of PST in host tissue under low temperature conditions, which may explain the overwintering of the bacterium in tomato leaf tissue at various soil depths in Michigan that led Getz et al (8) to conclude that overwintered diseased tissue is a source of primary inoculum there. Differences in soil temperature and other soil factors at various locations may explain the divergent conclusions relative to the role of soil and host debris in the epidemiology of PST.

The epidemiological role of resident (19) populations of bacterial plant pathogens on both host and nonhost plants has received increased attention in recent years (10,18,19,30), and pathovars of *P. syringae* have been studied most (18). In California, the leaves or roots of several weed and crop species harbored resident populations of PST (27). The presence of PST on plants from fields never planted to tomatoes led Schneider and Grogan (27) to conclude that the bacterium is ubiquitous in certain areas and that epiphytic populations on weeds are the primary inoculum for infection of tomato. We have shown that a taxonomically diverse group of weed species, different from those already reported (27), also support a resident population of PST in northern Georgia. PST not only occurred on several weeds but spread from them to nearby spring-seeded tomato. The role of native weeds in the epidemiology of PST in certain areas may be similar to that of hairy vetch in the development of brown spot of bean caused by *P. syringae* pv. *syringae* (PSS) (7). PSS strains pathogenic to sour cherry were recovered from a wide range of grass and broadleaf species under cherry trees in Michigan, but their epidemiological role was not determined (20).

We do not believe that PST is a native resident organism on weeds in Georgia but that it originated from diseased tomato plants or contaminated seed planted into disease-free locations. In our tests, the consistent association of PST with chickweed collected from a tomato field with a history of bacterial speck and its absence on similar plants from nearby fields without tomatoes support this conclusion. Although native weeds apparently play a major role in the survival of PST in certain areas, their role in southern Georgia is still in doubt because we were unable to recover the bacterium from numerous species collected from tomato transplant fields. Detection is limited by the sensitivity of assay methods available. We feel that the vacuum infiltration procedure that we used is the most sensitive assay currently available for detecting PST in its natural habitats.

LITERATURE CITED

1. Barksdale, T. H., Good, J. M., and Danielson, L. L. 1972. Tomato diseases and their control. U.S. Dept. Agric., Agric. Handb. 203. 109 pp.
2. Bashan, Y., Okon, Y., and Henis, Y. 1978. Infection studies of *Pseudomonas tomato*, causal agent of bacterial speck of tomato. Phytoparasitica 6:135-144.
3. Bashan, Y., Okon, Y., and Henis, Y. 1982. Long-term survival of *Pseudomonas syringae* pv. *tomato* and *Xanthomonas campestris* pv. *vesicatoria* in tomato and pepper seeds. Phytopathology 72:1143-1144.
4. Bosshard-Heer, E., and Vogelsanger, J. 1977. Überlebensfahigkeit von *Pseudomonas tomato* (Okabe) Alstatt in verschiedenen Boden. Phytopathol. Z. 90:193-202.
5. Chambers, S. C., and Merriman, P. R. 1975. Perennation and control of *Pseudomonas tomato* in Victoria. Aust. J. Agric. Res. 26:657-663.
6. Devash, Y., Okon, Y., and Henis, Y. 1980. Survival of *Pseudomonas tomato* in soil and seeds. Phytopathol. Z. 99:175-185.
7. Ercolani, G. L., Hagedorn, D. J., Kelman, A., and Rand, R. E. 1974. Epiphytic survival of *Pseudomonas syringae* on hairy vetch in relation to epidemiology of bacterial brown spot of bean in Wisconsin. Phytopathology 64:1330-1339.
8. Getz, S., Stephens, C. T., and Fulbright, D. W. 1981. Winter survival of *Pseudomonas tomato* in Michigan. (Abstr.) Phytopathology 71:218.
9. Goode, M. J., and Sasser, M. 1980. Prevention—The key to controlling bacterial spot and bacterial speck of tomato. Plant Dis. 64:831-834.
10. Goto, M. 1972. The significance of the vegetation for the survival of plant pathogenic bacteria. Pages 39–53 in: Proc. Third Int. Conf. on Plant Pathogenic Bact., H. P. Maas Geesteranus, ed. Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands.
11. Hildebrand, D. C., and Schroth, M. N. 1972. Identification of the fluorescent pseudomonads. Pages 281–287 in: Proc. Third Int. Conf. on Plant Pathogenic Bact., H. P. Maas Geesteranus, ed. Centre for Agricultural Publishing and Documentation, Wageningen, The Netherlands.
12. Jones, J. B., McCarter, S. M., and Gitaitis, R. D. 1981. Association of *Pseudomonas syringae* pv. *syringae* with a leaf spot disease of tomato transplants in southern Georgia. Phytopathology 71:1281-1285.
13. Jones, J. B., McCarter, S. M., and Smitley, D. R. 1981. A vacuum infiltration inoculation technique for detecting *Pseudomonas tomato* in soil and plant tissue. Phytopathology 71:1187-1190.
14. Kim, S. H. 1979. Dissemination of seed-borne *Pseudomonas tomato* by transplants. (Abstr.) Phytopathology 69:535.
15. King, E. O., Ward, M. K., and Raney, D. E. 1954. Two simple media for the demonstration of pyocyanin and fluorescin. J. Lab. Clin. Med. 44:301-307.
16. Klement, Z., Farkas, G. L., and Lovrekovich, L. 1964. Hypersensitive

reaction induced by phytopathogenic bacteria in the tobacco leaf. Phytopathology 54:474-477.
17. Kovacs, N. 1956. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature 178:703.
18. Leben, C. 1974. Survival of plant pathogenic bacteria. Spec. Circ. 100. Ohio Agric. Res. and Dev. Center, Wooster. 21 pp.
19. Leben, C. 1981. How plant-pathogenic bacteria survive. Plant Dis. 61:633-637.
20. Latorre, B. A., and Jones, A. L. 1979. Evaluation of weeds and plant refuse as potential sources of inoculum of *Pseudomonas syringae* in bacterial canker of cherry. Phytopathology 69:1122-1124.
21. Lindow, S. E., Arny, D. C., and Upper, C. D. 1978. *Erwinia herbicola*: A bacterial ice nucleus active in increasing frost injury to corn. Phytopathology 68:523-527.
22. McCarter, S. M., and Ratcliffe, T. J. 1977. Incidence of major diseases on tomato transplants produced in Georgia. Plant Dis. Rep. 61:129-131.
23. Misaghi, I., and Grogan, R. G. 1969. Nutritional and biochemical comparisons of plant-pathogenic and saprophytic fluorescent pseudomonads. Phytopathology 59:1436-1450.
24. Pohronezny, K., Volin, R. B., and Stall, R. E. 1979. An outbreak of bacterial speck on fresh-market tomatoes in south Florida. Plant Dis. Rep. 63:13-17.
25. Ratcliffe, T. J., and Morton, D. J. 1963. Diseases affecting production of certified tomato transplants in Georgia during 1942–1963. Plant Dis. Rep. 47:1065-1066.
26. Schaad, N. W., Sitterly, W. R., and Humaydan, H. 1980. Relationship of incidence of seedborne *Xanthomonas campestris* to black rot of crucifers. Plant Dis. 64:91-92.
27. Schneider, R. W., and Grogan, R. G. 1977. Bacterial speck of tomato: Sources of inoculum and establishment of a resident population. Phytopathology 67:388-394.
28. Schneider, R. W., and Grogan, R. G. 1978. Influence of temperature on bacterial speck of tomato. (Abstr.) Phytopathol. News 12:204.
29. Schneider, R. W., Hall, D. H., and Grogan, R. G. 1975. Effect of bacterial speck on tomato yield and maturity. (Abstr.) Proc. Am. Phytopathol. Soc. 2:118.
30. Schuster, M. L., and Coyne, D. P. 1974. Survival mechanisms of phytopathogenic bacteria. Annu. Rev. Phytopathol. 12:199-221.
31. Smitley, D. R., and McCarter, S. M. 1982. Spread of *Pseudomonas syringae* pv. *tomato* and the role of epiphytic populations and environmental conditions in disease development. Plant Dis. 66:713-717.
32. Thornley, M. J. 1960. The differentiation of *Pseudomonas* from other Gram-negative bacteria on the basis of arginine metabolism. J. Appl. Bacteriol. 23:37-52.
33. Van Haltern, F. 1935. Control of tomato seedbed diseases of southern plants. Ga. Agric. Exp. Stn. Bull. 187. 39 pp.
34. Walker, J. C. 1952. Diseases of Vegetable Crops. McGraw-Hill, New York. 529 pp.
35. Walker, J. C., and Patel, P. N. 1964. Splash dispersal and wind as factors in epidemiology of halo blight of bean. Phytopathology 54:140-141.
36. Yunis, H., Bashan, Y., Okon, Y., and Henis, Y. 1980. Weather dependence, yield losses, and control of bacterial speck of tomato caused by *Pseudomonas tomato*. Plant Dis. 64:937-939.