Potential Sources of Initial Inoculum for Bacterial Speck in Early Planted Tomato Crops in Michigan: Debris and Volunteers from Previous Crops

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ABSTRACT

Jardine, D. J., Stephens, C. T., and Fulbright, D. W. 1988. Potential sources of initial inoculum for bacterial speck in early planted tomato crops in Michigan: Debris and volunteers from previous crops. Plant Disease 72: 246-249.

Colonies of genetically marked *Pseudomonas syringae* pv. *tomato* were recovered from diseased tomato leaf tissue contained in nylon mesh bags and left to overwinter for 26 wk on the soil surface or buried to depths of 8 or 18 cm. A rifampicin-resistant strain of *P. s.* pv. *tomato* used to inoculate a field of tomato plants during the 1983 growing season was recovered from tomato surface debris in April 1984. In 1984 and 1985, however, the organism could not be isolated from the leaves, roots, or rhizosphere soil of 23 different weed species sampled from fields in which the pathogen was present the preceding year. In addition, the pathogen has been consistently isolated from volunteer tomatoes in the spring. Thus, infested crop debris and volunteer tomato plants, rather than weeds, appear to be a source of initial inoculum in northern U.S. tomato fields. The ability of overwintered *P. s.* pv. *tomato* to serve as a source of initial inoculum was affected by tillage system used and time of year of the tillage. Leaf infection by *P. s.* pv. *tomato* occurred when tomato seedlings were planted in a previously infested field that was either spring-plowed or left untilled. Plants did not become diseased when planted into areas that were fall-plowed.

Pseudomonas syringae pv. *tomato* (Okabe) Young et al, cause of bacterial speck of tomato (*Lycopersicon esculentum* Mill.), is a major concern to growers in southwestern Ontario, Canada, and in Michigan, Ohio, Indiana, and other parts of the United States. The disease spreads rapidly from point inoculum sources (23) and is difficult to control once established. Chemical spray programs must be strictly preventive if control of bacterial speck is to be successful. The organism can survive on tomato seeds for as long as 20 yr (2), which may represent one source of infection. Attempts to prevent introduction of the organism on southern-grown transplants are difficult because the organism can survive epiphytically on leaf surfaces of plants for long periods without symptom development (22,23). Epiphytic *P. s.* pv. *tomato* survived shipment from Georgia to Ontario and produced symptoms after transplanting (13). Although Michigan growers have begun to produce their own transplants in greenhouses in an attempt to reduce the chance that transplants will be infested, outbreaks of the disease persist both in the greenhouse and in the field (9). In California (22), Georgia (23), and Israel (8), *P. s.* pv. *tomato* survived in soil and in association with the rhizosphere and leaves of nonhost plants. Possible sources of infection in northern climates have not been investigated. The purpose of this research was to locate potential overwintering sites of *P. s.* pv. *tomato* in Michigan and to determine whether overwintered *P. s.* pv. *tomato* cells can serve as initial inoculum for tomatoes grown in Michigan. Preliminary data have been reported elsewhere (10).

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Michigan Agriculture Experiment Station Journal Article 12437.

Accepted for publication 29 October 1987 (submitted for electronic processing).

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MATERIALS AND METHODS

Pathogen survival. A pathogenic, rifampicin-resistant mutant of *P. s.* pv. *tomato* (PtR5) was selected from a wild-type strain by plating 10^9 cells onto a complete agar medium (18) containing the following ingredients per liter: 10 g of casamino acids, 5 g of yeast extract, 3 g of K_2HPO_4, 1 g of KH_2PO_4, and 15 g of Bacto agar (Difco). Rifampicin at 100 μg/ml was added after the medium was autoclaved. Rifampicin-resistant strains were subcultured and characterized for fluorescence on King's medium B (KMB) (14), oxidase (16) and arginine dihydrolase negativity (24), hypersensitivity on tobacco (15), and pathogenicity and virulence on tomato to assure their relationship to the wild type. Inoculum was prepared from 24-hr shake cultures of PtR5 in 50 ml of minimal broth (18) at 25 C. The suspensions were diluted with sterile distilled water (SDW) to approximately 10^7 colony-forming units (cfu/ml), as determined by standard turbidimetric and dilution plate techniques. A pneumatic sprayer held at a distance of 25–35 cm was used to apply inoculum until runoff to leaves of susceptible field-grown tomato plants. Lesions were

observed 7 days after inoculation, and the pathogen was isolated from these lesions. Noninoculated leaves on susceptible greenhouse-grown tomato plants were used as controls.

In October of year 1, approximately 225 g of diseased or nondiseased tomato leaves was placed in 18-cm^2 nylon mesh bags (3-mm mesh). One bag of diseased leaves and one bag of nondiseased leaves were positioned at the soil surface (0 depth) and at depths of 8 and 18 cm. The experiment was conducted at two locations: the Sodus Horticultural Experiment Station, Sodus, MI, (Kalamazoo loamy sand) and the Michigan State University (MSU) Botany and Plant Pathology Research Center in East Lansing (Colwood clay loam). The Sodus and East Lansing sites were overseeded to rye and winter wheat, respectively.

Bags at the Sodus site were sampled after 1 and 2 mo. A portion of the leaves were removed, and the bags were returned to their original positions. The East Lansing site was left undisturbed until April of the following year, when leaf bags were removed from both sites and the remaining leaf tissue evaluated for the presence of the pathogen.

For isolation of the pathogen, leaf tissue was macerated in SDW and the SDW-tissue suspension was allowed to rest undisturbed for 3 hr. From these suspensions, 0.1-ml samples were removed and spread onto the surface of the same complete agar medium as used previously with the exception that 25 μg/ml of cycloheximide was added along with the 100 μg/ml of rifampicin after autoclaving (CRC agar). Five plates were prepared from each suspension (bag) and incubated at room temperature for 5 days. Colonies with phenotypes characteristic for strain PtR5 were selected for further characterization. Isolates that fluoresced on KMB and were oxidase- and arginine dihydrolase-negative and hypersensitive-positive were tested for pathogenicity to tomato.

In year 2, the overwintering experiment was repeated only at the East Lansing site. Susceptible greenhouse-grown tomato plants were used as sources of both diseased and nondiseased leaf tissue. Leaf bags were buried in November and sampled in May.

Field survey. A survey for the presence of the bacterial speck organism was conducted prior to tomato planting in April 1984 at the MSU Botany and Plant Pathology Research Farm in East Lansing. The field had been planted to tomatoes inoculated with rifampicin-resistant mutant strains of *P. s.* pv. *tomato* in four of the preceding 5 yr (PtR5 in 1979 and 1980 and PtFr in 1982 and 1983). Overwintered surface debris and weeds from several taxonomic families growing in or near the field were sampled. Collections included fruit, leaves, stems, roots, and rhizosphere soil from tomato and roots and leaves of shepherd's purse (*Capsella bursa-pastoris* (L.) Medic.), dandelion (*Taraxacum officinale* Weber), narrow-leaved plantain (*Plantago lanceolata* L.), white clover (*Trifolium repens* L.), quackgrass (*Agropyron repens* (L.) Beauv.), and Kentucky bluegrass (*Poa pratensis* L.). Nonrhizosphere soil was also collected. Two 2-g samples were collected from each plant part, rhizosphere soil, and nonrhizosphere soil and placed in flasks containing 100 ml of SDW; the flasks were then shaken on a wrist-action shaker at 180 rpm for 30 min. The wash water was serially diluted with SDW, and 0.1-ml samples were spread on the surface of CRC agar. After 72 hr, plates were examined under near-ultraviolet light (254 nm). Fluorescent colonies were identified and subcultured by streaking onto CRC agar. Bacteria from single colonies were increased overnight in 50 ml of complete broth (18) with wrist-action shaker incubation. The broth was then placed into a 1,000-ml beaker and diluted with SDW to give a final concentration of approximately 5×10^7 cfu/ml as determined by absorbance readings made with a spectrophotometer (0.26 OD at 580 nm). Plants were inoculated with suspect strains by immersing the aboveground portions of three plants of the susceptible tomato cultivar Pik Red in the suspension for 1 min. The plants were removed and incubated in a mist chamber for 96 hr. Twenty-three strains were tested, including a known *P. s.* pv. *tomato* strain that served as a positive control. Three plants immersed in SDW alone served as negative controls. After incubating, plants were returned to a greenhouse bench for 7 days to allow symptoms to develop.

In April 1985, a second survey was conducted in the tomato production area of southwestern Michigan. Sample sites included fields where recent outbreaks of bacterial speck had occurred. The plants were sampled over a 5-wk period.

Thirty-four foliage samples of 20 plant species (emphasis on members of the Solanaceae) were collected, placed over ice, and transported back to the laboratory. The collection included red root pigweed (*Amaranthus retroflexus* L.), yarrow (*Achillea millefolium* L.), quackgrass, rye (*Secale cereale* L.), common chickweed (*Stellaria media* (L.) Cyrillo), vetch (*Vicia* sp.), *Agropyron* sp., dandelion, milkweed (*Asclepias syriaca* L.), ragweed (*Ambrosia artemisiifolia* L.), pineapple weed (*Matricaria matricarioides* (Less.) Porter), yellow nutsedge (*Cyperus esculentus* L.), prostrate knotweed (*Polygonum aviculare* L.), mare's-tail (*Erigeron canadensis* (L.) Cronq.), curled dock (*Rumex crispus* L.), purslane (*Portulaca oleracea* L.), black nightshade (*Solanum nigrum* L.), volunteer tomato, jimsonweed (*Datura stramonium* L.), and horsenettle (*Solanum carolinense* L.). To increase detection of low *P. s.* pv. *tomato* numbers, a vacuum infiltration assay procedure was used (10). Tomato plants (susceptible cv. Pik Red) were grown in the greenhouse for 5–6 wk (15–20 cm tall) in 10-cm-diameter pots filled with a synthetic soil medium. Four or five plants for each sample were removed from the soil with minimum injury to the root system, vacuum-infiltrated, and replanted into the pots after treatment. Control plants were infiltrated with SDW. The treated plants were placed on a greenhouse bench and examined periodically for disease development. Isolations were made from plants with suspect lesions. Typical colonies were identified and checked for fluorescence.

Effect of tillage on survival. In October of year 1, a plot at the MSU Botany and Plant Pathology Research Farm containing plants infested with strain PtR5 was subdivided into three sections. One section was plowed, one was plowed and planted with rye, and the third was left with undisturbed standing tomato plants. The following May, each section was plowed and prepared for tomato culture according to the standard commercial procedures for the area. Care was taken to prevent movement of soil from one section to another during cultural operations. In each section, three rows were direct-seeded and three rows were hand-planted with greenhouse-grown susceptible tomato transplants (cv. Pik Red) that were visually free from bacterial speck symptoms.

The experiment was repeated in year 2 with several modifications. On 15 May, 6-wk-old transplants were hand-planted into the field. The field had been planted the previous year with tomato plants infested with a rifampicin-resistant mutant strain (PtFr) of *P. s.* pv. *tomato*. Plantings were made in ground that had been fall-plowed, spring-plowed, or left untilled. Tilled plots were prepared by plowing with a moldboard plow to a depth of 20 cm. Final plot preparation was done just before planting using a disk harrow and cultimulcher on both the fall- and spring-plowed plots. Plants were placed in rows 1.5 m apart with an in-row spacing of 0.6 m. There were five plants for each of three replications per treatment arranged in a randomized complete block design.

Plants were examined for disease at approximately 10-day intervals for 1 mo. Leaves with lesions were sampled, taken to the laboratory, and tested for the presence of strain PtFr by standard isolation procedures using CRC media. Colonies that were rifampicin-resistant and fluorescent were further tested for pathogenicity as described previously.

RESULTS

Pathogen survival. Rifampicin-resistant *P. s.* pv. *tomato* was recovered from

diseased tomato leaf tissue overwintered 26 wk on the soil surface or buried 8 or 18 cm in either loamy sand or clay loam soil (Table 1). No attempt was made to quantify the results, since the degree of leaf decomposition varied among soil depths and types. All suspect PtR5 colonies tested were fluorescent on KMB, oxidase- and arginine dihydrolase-negative, hypersensitive-positive, and pathogenic to tomato. No rifampicin-resistant bacteria were recovered from noninfested (control) leaf tissue.

Field survey. The bacterial speck organism could not be detected in association with the roots or leaves on any of the various nonhost plants tested in 1984 using CRC agar. Also, *P. s.* pv. *tomato* was never isolated from roots or from the rhizospheres of overwintered tomato plants or from residue-free soil. However, fluorescent, rifampicin-resistant colonies were isolated from tissue samples taken from dried tomato fruits, stems, and leaves that had overwintered as surface debris. Only colonies from the tomato leaf and stem segments were pathogenic. None of the colonies from the fruit caused typical speck symptoms after inoculation.

In 1985, a vacuum infiltration assay reported (11) to detect *P. s.* pv. *tomato* at levels approaching 10 cfu/ml of leaf washings was used to increase the level of detection. In preliminary studies, the method was successful in detecting the rifampicin-resistant mutant at levels similar to those previously reported for a wild-type strain. Cells of *P. s.* pv. *tomato* were not detected in any of the 20 species of weeds representing 11 families collected from various locations in the tomato production areas of southwestern Michigan in 1985. However, *P. s.* pv. *tomato* was recovered from volunteer tomato plants in June 1985 from a field planted to tomatoes the preceding season. Also, bacterial speck was observed on numerous volunteer tomato plants in surveys conducted in 1986 and 1987 (C. T. Stephens, *unpublished*).

Effect of tillage on survival. In our studies, the ability of overwintered *P. s.* pv. *tomato* to cause disease in greenhouse-grown, spring-planted tomatoes depended on the environment and tillage system. In the first experiment, no speck-infested plants, regardless of tillage type, were detected throughout the season. However, the environmental conditions after planting were unfavorable (warm and dry) for natural development of the disease.

In the second experiment, there was a significant difference ($P = 0.01$) between tillage types in both incidence and severity of bacterial speck. When a bacterial speck-infested field was fall-plowed, no disease occurred on plants planted the following spring. In contrast, the incidence of bacterial speck was 89% in both spring-plowed and untilled treatments (Fig. 1A). The number of lesions per plant, used to indicate severity of infection, was 3.4 and 12.8, respectively, for spring-plowed and untilled treatments (Fig. 1B).

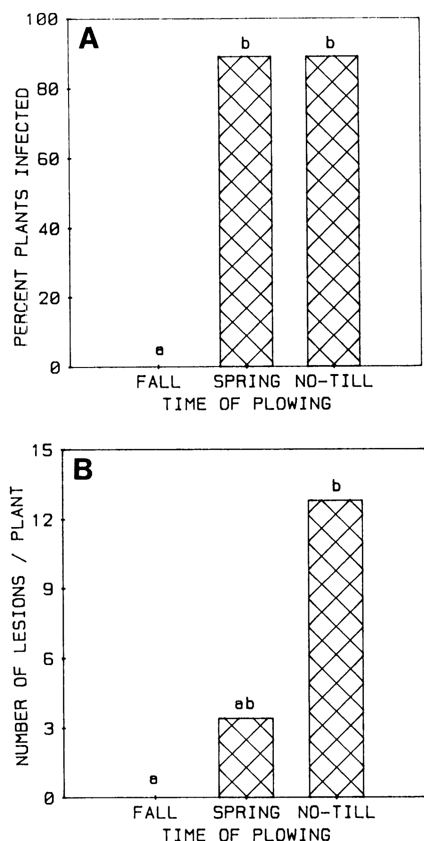

Fig. 1. Effect of tillage system on the **(A)** incidence and **(B)** severity of bacterial speck on the susceptible tomato cultivar Pik Red resulting from overwintered cells of a rifampicin-resistant strain of *Pseudomonas syringae* pv. *tomato* grown at East Lansing, MI, in 1985. Bars with the same letter are not significantly different ($P = 0.01$) according to Duncan's multiple range test.

DISCUSSION

The causal agent of bacterial speck, *P. s.* pv. *tomato*, has been reported to survive for various lengths of time in both field and laboratory studies (1,5,8,19,20), but whether it is capable of serving as an initial inoculum source for a succeeding crop has not been demonstrated. The organism reportedly survived for up to 28 wk in pasteurized soil in the laboratory (8,20) and was capable of surviving for 30 wk in an unsterilized field soil held in the laboratory at constant room temperature and moisture levels (5). In Georgia, McCarter et al (19) detected low populations of *P. s.* pv. *tomato* in buried host debris after 81 days at 18 C in a Tifton loamy sand, but not after 27 days at 33 and 38 C. In Michigan, we found that the organism survived through the winter in tomato debris and perhaps seeds, in both loamy sand and clay loam soils. This study differs from the laboratory experiments in the natural variations in soil type, temperature, moisture, and microbial activity normally occurring during the overwintering phase and known to influence plant decomposition rates (4,6).

In the colder northern climate of Michigan, as diseased host material senesces and desiccates and as air temperatures decrease, the bacteria may shift into a hypobiotic metabolic state (17). Bacteria in a hypobiotic state are more likely to survive than are active cells. During Michigan winters, the ground soil frost typically extends to a

Table 1. Overwintering of *Pseudomonas syringae* pv. *tomato* (strain PtR5) in tomato leaves in Michigan field soils

			Recovery[a]							
		Depth of burial	November		December		April		May	
Year	Soil type, location	(cm)	Infected	Control	Infected	Control	Infected	Control	Infected	Control
1979–1980	Loamy sand, Sodus[b]	0[c]	+	−	+	−	+	−	...	...
		8	+	−	+	−	+	−	...	...
		18	+	−	+	−	+	−	...	...
	Clay loam, East Lansing	0	...	...	...	...	+	−	...	...
		8	...	...	...	...	+	−	...	...
		18	...	...	...	...	+	−	...	...
1980–1981	Clay loam, East Lansing	0	...	...	...	...	...	...	+	−
		8	...	...	...	...	...	...	+	−
		18	...	...	...	...	...	...	+	−

[a] + = Rifampicin-resistant, fluorescent bacterium pathogenic on tomato was recovered from tomato leaf tissue, − = no rifampicin-resistant bacteria were recovered.
[b] Results were identical for both replications.
[c] Soil surface.

depth of 15–20 cm. In addition to reducing rate of plant decomposition, the low temperatures may directly favor the survival of *P. s.* pv. *tomato* (3). If *P. s.* pv. *tomato* shows the same pattern as for several other phytopathogenic pseudomonads (7,21), greatest reductions in viable *P. s.* pv. *tomato* occur during the warm spring months when soil temperatures increase and microbial activity resumes, resulting in a faster degradation of debris.

Schneider and Grogan (22) detected the bacterial speck organism in rhizospheres of several nonhost plants as well as in numerous California soil types. McCarter et al (19) reported finding *P. s.* pv. *tomato* on weed hosts at one location in Georgia but not at a second location. In our study, the organism did not appear to be a native resident on weed hosts. However, the organism was consistently isolated from volunteer tomatoes, making these a potentially important source of inoculum for early planted tomatoes. This suggestion is supported by the recent report of Jones et al (12) that volunteer tomatoes were important in the epidemiology of *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye in Florida.

The overwintered strains of *P. s.* pv. *tomato* can serve as initial inoculum in the spring, and this ability may be related to cultural and environmental conditions in the field. We used three different tillage treatments in order to manipulate the levels of surface debris present at planting. These treatments represented high (untilled), medium (spring-plowed), and low (fall-plowed) residual levels of infested soil debris. When environmental conditions were unfavorable for disease development (i.e., warm and dry), as in 1984, the organism was not detected in any of the treatments. However, when environmental conditions were highly favorable for disease development, as in 1985 when heavy rains followed planting, bacterial speck developed in both the spring-plowed and untilled treatments but not in the fall-plowed treatment. In the fall-plowed treatment, the plant debris apparently decomposed before planting. In contrast, in the spring-plowed and untilled treatments, a large amount of undecomposed debris was present either beneath or on the soil surface at planting time. Since *P. s.* pv. *tomato* survives in association with plant debris, it is likely that spring-plowed and untilled fields are more likely to favor overwintering of the bacterial speck organism. Bacteria isolated from suspect lesions were identified as being rifampicin-resistant, which indicates that the source of infection was overwintered *P. s.* pv. *tomato*, not the seed or transplants.

On the basis of our results, we believe that crop debris management and elimination of volunteer tomatoes are important strategies in controlling bacterial speck. Ideally, a good crop rotation program should be followed in order to allow adequate time for the decomposition of infested debris and the elimination of volunteers. However, because the possibility exists that inoculum could be moved from infested debris or volunteers in adjacent fields to current crops by high winds and blowing rain, fall plowing should be done to bury debris and seeds. Growers who are unable to do fall plowing because of erosion problems should consider fall disk harrowing to incorporate debris into the soil; this encourages greater soil-debris contact and speeds the decomposition process. These fields could then be seeded to rye or other suitable winter-cover crops to reduce winter erosion losses before normal spring tillage.

ACKNOWLEDGMENTS

We thank Susan Getz for her contribution to the initial overwintering study and Thomas Stebbins for his assistance in conducting the field survey and field-baiting experiments.

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