Stem Necrosis of Greenhouse Tomato Caused by a Novel *Pseudomonas* sp.

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ABSTRACT

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A fluorescent *Pseudomonas* sp. causes a sporadic stem necrosis in greenhouse tomato (*Lycopersicon esculentum*). The pathogen, like *P. cichorii,* is oxidase-positive and arginine dihydrolase-negative but, unlike *P. cichorii,* is not pathogenic to chrysanthemum or lettuce, utilizes sucrose and D(−) tartrate but not L(+) tartrate, reduces nitrate, grows at 6 C, and produces strain-specific blue, green, or orange water-soluble pigments on King's media A and B.

Stem necrosis and pith rot of greenhouse tomato (*Lycopersicon esculentum* Mill.) have been reported from various parts of the world and have been ascribed to diverse pathogens, including *Pseudomonas cichorii* in New Zealand (10); *P. corrugata* in England (8) and New Zealand (2); *Erwinia carotovora* in Texas (9); *E. carotovora* subsp. *carotovora* in Canada (3); a host of bacterial species, including *P. fluorescens* biotype A, *P. viridiflava,* and *E. carotovora* subspp. *atroseptica* and *carotovora* in Crete (6); and *Fusarium merismoides* in England (5).

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Dark brown discoloration of leaf bases at nodes and adjacent leaf rachises and internodes, followed by cortical and pith necrosis, has been observed on greenhouse tomato cultivars Dombito, Jumbo, and Laura in Ontario for the past few years. Except for a general pith necrosis, these symptoms differ from most of the symptoms reported for the other stem rot diseases of greenhouse tomato. The stem rot caused by *E. carotovora* subsp. *carotovora* is characterized by considerable water-soaking and darkening of the stem exterior, followed by soft rot and wilt of the plant (3,9). In pith necrosis caused by *P. corrugata,* the young leaves become chlorotic, the top half of the plant loses turgidity, and brown to black lesions develop on the stem near the first fruit truss (8). The syndrome for bacterial soft rot of mature tomato plants associated with *E. carotovora* subsp. *carotovora* and *P. viridiflava* in Crete includes pith and xylem browning, leaf yellowing, and disintegration of cortex (6). Tomato stem rot caused by *F. merismoides* is characterized by light brown nodal lesions that darken with age; the lesions girdled stems of young plants (but not those of older, mature plants), causing them to collapse (5). Symptoms of stem bacteriosis of greenhouse tomato caused by *P. cichorii* in New Zealand, however, are similar to those of the disease described here.

Although it occurs only sporadically, the tomato stem necrosis (TSN) disease has caused concern among growers and extension specialists in Ontario. The etiology of the disease is reported here.

MATERIALS AND METHODS

Isolation. Diseased tomato plants were collected from greenhouses in Norfolk and Niagara counties from 1984 to 1986. Stem segments with external brown lesions were washed in running tap water and then rinsed with 0.6% sodium hypochlorite for 1 min. The epidermal and outer layers of cortical tissue were peeled off, and the margins of necrotic tissue were excised and dropped into sterile distilled water in the wells of a spot plate. After 15–20 min, loopfuls of the water were spread on King's medium B (KB) agar. In addition, seeds were aseptically removed from fruit of diseased plants and plated on KB agar. Bacterial colonies that appeared with consistency were selected for pathogenicity tests and diagnostic tests.

Pathogenicity tests. Stems of potted tomato (cv. Bonnybest) plants 3–4 wk old were stabbed with a sterile toothpick that had been coated with 2- to 3-day-old bacterial colonies grown on KB agar at 20 C. After 2–3 wk, the plants were split open to observe the extent of pith discoloration.

Subsequently, mature tomato plants (cvs. CR-6, Dombito, Jumbo, and MR-13) were inoculated as above with selected bacterial strains at the onset of fruit bearing. Each plant was inoculated at a freshly broken leaf base at a basal node and observed for external discoloration for 8 wk.

To determine host range, potted 3- to 4-wk-old plants of chrysanthemum (cv. Florida Marble) and lettuce (cv. Salinas) were inoculated as above, incubated in a moist chamber for 48 hr, and then placed on greenhouse benches. Greenhouse temperatures during the experiment ranged from 20 C (night) to 30 C (midday), and relative humidity ranged from 60% (midday) to 90% (evening to early morning).

Diagnostic tests. The unknown TSN strains and reference *P. cichorii* strains (Table 1) were compared in the following tests: Gram reaction; flagellation; motility; production of diffusible pigments on King's medium A (KA) and KB agar and fluorescence of pigments on KB agar under ultraviolet illumination (366 nm); levan, oxidase, potato rot, arginine dihydrolase, and tobacco hypersensitivity (LOPAT); oxidative-fermentative metabolism of glucose; utilization of selected carbohydrates (glucose, sucrose, erythritol, mannitol, and sorbitol) and organic acids DL(−) lactate, D(−) tartrate, and L(+) tartrate; gelatin and starch hydrolysis; production of catalase, lipase, and tyrosinase; liquefaction of pectate at pH 4.5 and 7.2; nitrate reduction and respiration; and growth in yeast salts (YS) broth at 6, 37, and 41 C. The tests were done according to the methods described by Dhanvantari and Dirks (3) or Dye (4).

Table 1. Bacterial strains used in this study

Strain	Source
Tomato stem necrosis (TSN)	
TSN 1–5 and 10–12	Greenhouse tomato (cv. Dombito), Niagara County
TSN 6–9	Greenhouse tomato (cv. Dombito), Norfolk County
TSN 13 and 14	Greenhouse tomato (cv. Laura), Niagara County
TSN 15 and 16	Greenhouse tomato (cv. Jumbo), Niagara County
Pseudomonas cichorii (PC)	
PC 1–5	Greenhouse lettuce, Norfolk County
PC 6–9	Greenhouse chrysanthemum, Essex County
83–8A	A. R. Chase (from *Schefflera arboricola*)
UCB 464 and 465	D. C. Hildebrand
UCB 467 and 468	D. C. Hildebrand (NCPPB 907 and 1512, respectively)

Table 2. Some key characteristics of strains of the tomato stem necrosis (TSN) pathogen and *Pseudomonas cichorii*

Strain	L	O	P	A	T[a]	Pigments[b]	Nitrate[c] Reduction	Nitrate[c] Respiration	Gelatin hydrolysis[c]	Growth at 37 C[c]
TSN										
1	+	+	−	−	−	Orange	+	+	−	+
2	+	+	−	−	−	Orange	+	+	+	+
3	+	+	−	−	−	Green	+	+	−	+
4	+	+	−	−	−	Green	+	+	−	+
5	+	+	−	−	−	Orange	+	+	−	+
6	+	+	−	−	−	Blue	+	+	+	+
7	+	+	−	−	−	Green	+	+	+	+
8	+	+	−	−	−	Blue	+	+	+	−
9	+	+	−	−	−	Blue	+	+	+	−
10	+	+	−	−	+	Orange	+	+	+	−
11	+	+	−	−	+	Orange	+	+	+	−
12	+	+	−	−	+	Orange	+	+	+	+
13	+	+	−	−	+	Orange	+	+	+	+
14	+	+	−	−	+	Orange	+	+	+	+
15	+	+	−	−	+	Green	+	+	+	−
16	+	+	−	−	+	Green	+	+	+	−
P. cichorii										
PC 1	−	+	−	−	+	Green	−	−	−	ND
PC 9	−	+	−	−	+	Green	ND	−	ND	ND
83–8A	−	+	−	−	+	Green	ND	−	ND	ND

[a] Levan, oxidase, potato rot, arginine dihydrolase, and tobacco hypersensitivity, respectively; + = positive, − = negative.
[b] Diffusing into King's media A and B.
[c] + = positive, − = negative, ND = not done.

RESULTS

The disease usually appeared in April or May in the spring crop. Dark brown blotches on leaf bases extended onto adjacent stem internodes and leaf rachises. Internal browning of pith tissue advanced much beyond the external necrosis. Infrequently, pith tissue broke down, leaving a cavity. Vascular discoloration also occurred, but plants generally did not wilt or collapse. The fruits were free from symptoms. Microscopic examination showed masses of motile bacteria in diseased tissue.

Isolations from 16 of 35 plants collected from six greenhouses yielded off-white colonies of bacteria that produced fluorescent pigment on KB agar. All 16 strains were motile, gram-negative rods (1.52 × 0.57 μm) with one to three polar flagella. The bacterial colonies on KB agar were small (1.5–2.0 mm in diameter), flat, and translucent after 3 days at 25 C. On both KA and KB agar, they produced strain-specific blue, green, or

Table 3. Extent of stem necrosis on tomato (cv. CR-6) 8 wk after inoculation with strains of the tomato stem necrosis (TSN) pathogen or *Pseudomonas cichorii* (PC)[a]

Strain	Nodes affected above inoculation site[b] (no.)
TSN 3	12.50 ± 0.86
TSN 8	10.25 ± 1.03
TSN 9	13.25 ± 0.48
TSN 10	12.25 ± 0.85
TSN 14	11.25 ± 0.85
TSN 16	12.00 ± 1.08
PC 4	13.75 ± 0.48
PC 6	10.66 ± 1.76

[a] A freshly broken leaf base at a basal node of each plant was stabbed with a toothpick coated with 2- to 3-day-old bacterial colonies. Greenhouse temperatures ranged from approximately 20 C (night) to 30 C (midday), and relative humidity ranged from 60% (midday) to 90% (evening to early morning).
[b] Mean plus or minus standard error ($n = 4$).

Table 4. Some physiological and biochemical characteristics of selected strains of the tomato stem necrosis (TSN) pathogen and *Pseudomonas cichorii*[a]

Characteristic[b]	TSN 9, 11, and 13–16	*P. cichorii* 1	9	83-8A	464	465	467	468
Utilization of:								
Glucose	+	+	+	+				
Sucrose	+[c]	−	−	−				
Erythritol	−	−	−	−				
Mannitol	+[c]	+	+	+				
Sorbitol	+	+	(+)	−				
DL(−) lactate	+	+	+	+				
D(−) tartrate	+	−	−	−				
L(+) tartrate	−	+	+	+				
Pectate liquefaction								
pH 4.5	−	−	−	−				
pH 7.2	−	−	−	−				
Starch hydrolysis	+	+	+	+	+	(+)	+	+
Catalase	+	+	+	+	+	+	+	+
Lipase	d		−	(+)	+	+	+	−
Tyrosinase	−		−	−	−	−	−	−
Growth at 6 C[d]	+		(+)	(+)	−	−	−	−

[a] + = positive, (+) = weak reaction, − = negative, d = variable, absence of symbol = not done.
[b] Tested by methods of Dhanvantari and Dirks (3) or Dye (4).
[c] Water-soluble blue pigment produced.
[d] Shake-culture (yeast salts broth) at 28 C for 5 days.

Fig. 1. External symptoms of necrosis on tomato (cv. CR-6) stem and petioles 8 wk after inoculation with bacterial strain TSN 9.

orange diffusible pigments in 4–5 days; pigment production increased with further incubation at the same temperature or in storage at 4 C. All the strains produced pith necrosis in tomato in 2–3 wk. They were positive for levan and oxidase and negative for potato rot and arginine dihydrolase. In contrast, the reference strains of *P. cichorii* were negative for levan production, nitrate reduction or respiration, and gelatin hydrolysis (Table 2), and their colonies on KB agar were larger (3–4 mm in diameter) than those of the TSN strains.

The TSN strains were pathogenic to tomato but not to chrysanthemum or lettuce, whereas the strains of *P. cichorii* were pathogenic to all three hosts. *P. cichorii* produced pith necrosis extending 2–5 cm within 21 days in tomato, stem necrosis and leaf blight in chrysanthemum within 2 wk, and vein discoloration in the petiole and necrotic areas on the leaf blade of lettuce within 2 wk. The TSN strains produced a pith necrosis in tomato similar to that caused by *P. cichorii.*

Six TSN strains and two strains of *P. cichorii* produced similar disease symptoms on mature tomato (cv. CR-6) plants growing under high humidity. Within 2 wk, a blotchy brown discoloration of leaf bases appeared at discontinuous nodes above or below the site of inoculation. Later, brown streaks developed on internodes and adjacent leaf rachises (Fig. 1). The external symptoms spread to 10–13 nodes above the site of inoculation within 8 wk (Table 3). There was considerable pith browning and disintegration of pith in the lower stem. The structural integrity of the tomato plant was, however, maintained, and the fruits did not develop any symptoms. The bacteria were isolated from the pith tissue 8 wk after inoculation but not from the seeds.

All strains of the TSN pathogen and of *P. cichorii* tested hydrolyzed starch (Table 4). The six TSN strains utilized sucrose and D(−) tartrate but not L(+) tartrate and grew within 48 hr at 6 C in YS broth; in these respects they differed from three or more strains of *P. cichorii.*

DISCUSSION

The tomato disease reported here was seldom associated with watery rot of cortex or pith. It appears to be favored by high humidity and nitrogen fertilization, as reported for similar tomato diseases caused by *P. cichorii* (10), *P. corrugata* (8), and *P. viridiflava* and others (6).

The strains of the TSN pathogen and of *P. cichorii* are oxidase-positive and arginine dihydrolase-negative, and they produce similar symptoms in greenhouse tomato plants. But, unlike *P. cichorii,* the TSN strains were not pathogenic to chrysanthemum or lettuce. *P. cichorii* reportedly has a broad host range, naturally infecting diverse plant species and

infecting still more species by artificial inoculation (1). Thus, pathogenically the TSN strains are not typical of *P. cichorii.*

In diagnostic tests, the TSN strains differed from *P. cichorii* in several respects; they utilized sucrose and D(−) tartrate but not L(+) tartrate, reduced nitrate, grew at 6 C, and produced smaller colonies and diversely colored, water-soluble pigments on KB agar. Twelve of the 16 TSN strains hydrolyzed starch; a similar reaction by seven strains of *P. cichorii* tested here is contrary to published information (1). It is worth noting that in the recent *Bergey's Manual,* the starch hydrolysis reaction of *P. cichorii* has been left out (7).

I suggest that the TSN strains be assigned to the group of oxidase-positive, arginine dihydrolase-negative, phytopathogenic, fluorescent pseudomonads, now solely represented by *P. cichorii,* until further taxonomic determination. The taxonomic implications of these findings will be addressed elsewhere.

TSN occurs infrequently and sporadically in Ontario greenhouses. The pathogen appears to be opportunistic, affecting mature tomato plants that have been stressed by such factors as unbalanced nutrition, excessive humidity in the greenhouse, or the onset of fruiting.

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