Ecology and Epidemiology

Bacterial Stem Rot of Greenhouse Tomato: Etiology, Spatial Distribution, and the Effect of High Humidity

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ABSTRACT

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An outbreak of stem rot of the tomato cultivar CR-6 in some Essex County greenhouses in Ontario was caused by *Erwinia carotovora* ssp. *carotovora*. The disease occurred about the time of the first fruit harvest, or later, in the spring crop. Disease incidence was greater in rows under the roof-gutters than elsewhere in the greenhouse. The results of two-directional doublet, runs, and chain analyses of a 9,600-plant stand showed a nonrandom distribution of diseased plants, with marked clumping along the rows. Stem rot and pith disintegration developed more intensively in the affected plants under high humidity.

Stem rot of unknown etiology has occurred sporadically in Ontario greenhouse tomatoes for many years. Tomato stem rots have been attributed to fungi and to several different bacteria. Speights et al (24) described a bacterial stem rot of mature greenhouse tomato in Texas caused by *Erwinia carotovora* ssp. *carotovora* (Jones) Bergey et al. It was characterized by water-soaking of the subepidermal tissue, acropetal breakdown of pith, and, in advanced stages, sloughing of the bark when touched. Butler (2) and Stanghellini (*personal communication*) observed a stem rot of greenhouse tomato in Arizona caused by *E. c.* ssp. *carotovora*, which was characterized by soft rot of lower stems, water-soaking and sloughing of the bark, and eventual wilting and death of the plant. A disease of greenhouse tomatoes with extensive pith necrosis sometimes accompanied by vascular browning and external brown to black lesions was reported to be widespread in England since 1971 and was later found to be caused by a newly named bacterial species, *Pseudomonas corrugata*

Scarlett et al (23). Stem necrosis of mature greenhouse tomato vines in Pennsylvania was ascribed to *Pseudomonas viridiflava* (Burkholder) Dowson (15). *Pseudomonas cichorii* (Swingle) Stapp caused stem necrosis of greenhouse tomatoes in New Zealand, the symptoms being elongated brown streaks on the stem, vascular discoloration, and breakdown of pith tissue leaving a hollow stem (28). A new tomato disease of unknown etiology called "black pith," characterized by browning of stem pith, necrosis of shoot tips, wilting, and death was reported from France by Rieuf and Nourrisseau (22).

An unusually high incidence of stem rot, wilt, and death of tomato plants occurred in the greenhouse tomato production area of Essex County, in Ontario, during the harvest season of 1983. It could not be attributed to any of the diseases commonly seen in local greenhouses. This paper reports the etiology and spatial distribution of, and the effects of high humidity on, that disease and strategies of greenhouse management for disease control.

MATERIALS AND METHODS

Isolation from diseased tissue. The infected tomato stems were surface-sterilized with a 0.6% sodium hypochlorite solution and split open. The margins of discolored pith tissue or vascular strands were excised and suspended in sterile distilled water, and the diffusate was streaked onto King's Medium B (KB) agar plates (13).

Bacterial strains. Eight bacterial strains (TSR) isolated from infected tomato (cultivar CR-6) stems sampled from different greenhouses in the Leamington area of Essex County, Ontario, were designated as TSR strains numbered 01 to 08. The neotype strain of *E. c.* ssp. *carotovora* (PDDCC 5702) was obtained from the Plant Diseases Division, Department of Scientific and Industrial Research, Auckland, New Zealand. They were used in pathogenicity and determinative tests and in greenhouse experiments.

Pathogenicity and determinative tests. The lower portion of the stem of potted 3-wk-old tomato (cultivar Bonny Best) plants was stabbed with sterile toothpicks dipped in 2- or 3-day old bacterial colonies. The stem was cut lengthwise after 1, 2, or 3 wk to examine for pith rot. The following tests were done according to the references in parentheses: nonstaining method for determining gram reaction with 3% KOH (25); flagella staining (17,27); motility under phase contrast microscope; colony morphology on KB agar (13); glucose oxidation/fermentation (10); pectate degradation (6); Kovac's oxidase test and potato rot test (14); sensitivity to erythromycin with 15 μg on paper disks placed on Mueller-Hinton agar plates (7); nitrate reduction (1); Gram staining with Hucker's modification, production of gas from glucose, reducing substances from sucrose, catalase, β-galactosidase with ONPG reagent, phenylalanine deaminase, phosphatase, urease, and indole reaction with Kovac's reagent (9); gelatin liquefaction, H_2S production from peptone and sodium thiosulfate, sodium chloride tolerance, growth at 36 C on nutrient agar and yeast dextrose calcium carbonate-agar in a constant temperature water-bath, acid production from arabinose, ribose, rhamnose, glucose, fructose, galactose, mannose, maltose, lactose, palatinose, trehalose, cellobiose, adonitol, mannitol, sorbitol, α-methyl D-glucoside, aesculin, and salicin, and utilization of L(+) tartaric acid, and sodium malonate in the Ayers, Rupp, and Johnson basal agar medium, and testing aesculin, salicin, and adonitol further in 1% peptone (8). The carbohydrates were filter-sterilized, excepting aesculin and salicin, which were autoclaved.

Greenhouse experiments. Transplants of the tomato cultivar CR-6 were planted in nine double-rows in ground bed of a greenhouse and watered by trickle irrigation. The double-row consisted of 20 plants in two narrowly spaced (75 cm apart) rows of 10 plants each; the walkway between the double-rows was 100 cm wide. Plants in the row were spaced 35 cm apart. They were fertilized weekly with soluble 20:20:20 NPK solution. The plant stand was partitioned into two blocks of four double-rows separated by a buffer row. One block was enclosed by plastic sheets within which a fine water mist maintained a saturated atmosphere and kept the plant surface wet for prolonged periods. The mist was controlled by a solenoid valve responding to drying conditions. Plants were maintained in the normal commercial manner with the lower leaves broken off as they became senescent.

Individual plants in each row within each block were inoculated with one of four selected bacterial strains, TSR 01, 03, or 05 or PDDCC 5702. The inoculation treatments were replicated four times per bacterial strain and randomized within each block. Inoculation was done by stabbing the third or fourth deleafing wound from the base with a sterile toothpick dipped in a 48-hr bacterial growth on KB agar.

The extent of stem rot was measured weekly from the third to the seventh week after inoculation, both directly in centimeters and as the number of nodes traversed by hollowness of stem, which was usually accompanied by the appearance of numerous adventitious root initials on the stem surface. The data were analyzed by obtaining the least significant difference (LSD) for weekly measurements of stem rot to compare the effects of strains within each treatment block. Disease progress curves were plotted to compare the performance of the four bacterial strains under conditions of misting and nonmisting.

Disease survey. Two commercial greenhouses near Leamington, one under plastic and the other under glass, were surveyed for incidence and spatial distribution of stem rot in tomatoes at the peak of the harvest season, in June 1983. In both greenhouses, there were no partitions between adjoining bays, and the roof was constructed in alternating ridges and gutters. Rows of plants ran parallel to these, and the rows below the gutters were designated gutter rows.

Greenhouse No. 1, under plastic, consisted of four sections each covering the area between two gutters or the end wall and a gutter and consisting of 12 rows. The four sections were divided into eight replicates, each spanning the rows from the ridge to the gutter or the end wall; a replicate consisted of one row under the gutter or along the end wall and five other rows. There were 200 plants in each row. Means of disease incidence among all rows, gutter rows vs. other rows, or among other rows were compared by analysis of variance (ANOVA).

Greenhouse No. 2, under glass, joined two houses each with four sections as above. The eight sections were divided into 16 replicates, each consisting of one gutter (or end wall) row and two other rows. There were 200 plants in each row. Means of disease incidence among all rows, gutter rows vs. other rows, or among other rows were compared by ANOVA.

The greenhouse under plastic consisting of four contiguous sections with plant rows established in a rectangular pattern was checked on a plant by plant basis for diseased and healthy plants. Plant and row spacings were as described above. Whether the pattern of disease distribution was random or nonrandom within a crop was determined by analyzing the data as rectangular data matrices and evaluating for the presence of doublets (26), runs, and chains (11,21) of diseased plants. For this purpose, the four sections of the greenhouse were divided into eight contiguous subsections, each measuring 12 rows $\times$ 100 plants. Evaluation of the data was done in two directions, lengthwise along the rows and crosswise across the rows. In either direction, the data from consecutive lengthwise or crosswise counts of equal length were used. A computer program, HARSPAC, was written in BASIC for the spacing analyses on a VAX 750, and it was used to count doublets, runs, and chains. Tests of significance of doublet numbers followed the correction suggested by Converse et al (4).

RESULTS

Symptoms. In the commercial spring crop, naturally infected greenhouse tomato plants (cultivar CR-6) showed a variety of symptoms. Basal leaf scars showed evidence of infection with dark brown lesions. The stem base had become hollow (Fig. 1) and had a water-soaked appearance. Its bark readily sloughed off when handled. Most of the pith had become brown and was in various stages of disintegration. At an advanced state of stem rot, the entire plant wilted (Fig. 1). In commercial greenhouses, the symptoms

appear to have developed about the time of first or second harvest of fruits. With two or more clusters of fruits ripening and the harvest season approaching its peak, it is the time of maximum stress in the spring crop.

Isolation and pathogenicity tests. Isolations made on KB agar from 12 affected tomato vines collected at random from two greenhouses yielded bacteria of several colony types. Washout streaks contained predominantly translucent or dense greenish-white colonies producing green fluorescent pigment diffusing into the agar. Streaks further away from washout contained, among others, flat, off-white, nonfluorescent colonies with a dull surface.

Only the flat, off-white colonies produced brown discoloration and breakdown of pith tissue in 14–20 days after stem inoculation of 3-wk-old potted tomato plants (cultivar Bonny Best). The inoculated plants did not show external evidence of infection, but pith rot was seen on splitting the stem open (Fig. 1). On mature, fruit-bearing plants of cultivar CR-6, pith rot developed in 10–14 days, and water-soaking appeared in the cortex 4 wk after inoculation, wilting occurred shortly afterwards.

After satisfying Koch's postulates, eight pathogenic bacterial strains were selected for characterization and further experiments. The colonies grew to 4–5 mm in diameter with irregular margin on KB agar in 3 days at 28 C.

Characterization of the causal bacterium. The eight tomato stem rot bacterial strains were gram-negative rods with peritrichous flagellation, were oxidase-negative, and fermented glucose rapidly within 24 hr. The phenotypic characters tested were consistent with the TSR strains being *E. c.* ssp. *carotovora*. In the potato rot test, there was less blackening than with *E. c.* ssp. *carotovora*, but the tissue disintegrated in 2 days at 25 C. Pectate medium was pitted in 2 days at 28 C, and then rapidly liquified. Among the characters differentiating soft-rotting erwiniae, the TSR strains were not sensitive to erythromycin, did not produce reducing substances from sucrose or gas from glucose, grew at 36 C, and hydrolyzed gelatin in 2 days at 22 C.

In other biochemical tests, the TSR strains produced H_2S from peptone as well as sodium thiosulfate. They were positive for the production of catalase and β-galactosidase but were negative for phenylalanine deaminase and urease; indole production appeared to be weak as only a faint red coloration developed on addition of Kovac's reagent after 48 hr of growth in shake culture.

The TSR strains produced acid from arabinose, ribose, rhamnose, glucose, fructose, galactose, mannose, lactose, trehalose, cellobiose, mannitol, sorbitol, aesculin, and salicin, but not from maltose, palatinose, adonitol, and α-methyl-D-glucoside. Acid was produced from sorbitol and aesculin when they were steam-sterilized and added to 1% peptone water.

Effect of misting on disease. Under misting, pith rot advanced three to six nodes above the inoculation site in 3 wk, and continued to advance until 6 wk, then became stationary (Table 1). Assessment after 7 wk showed that it had traversed nine to 12 nodes above the inoculation site, and by then the plants were wilting or were dead.

In the block that was not misted, it was not until 4 wk after inoculation that all the strains produced pith rot advancing one to four nodes above the inoculation site. Pith rot advanced from five to nine nodes above inoculation site in 6 wk and did not extend any further. The plants did not wilt. A comparison of disease progress curves under misting and nonmisting conditions in the greenhouse

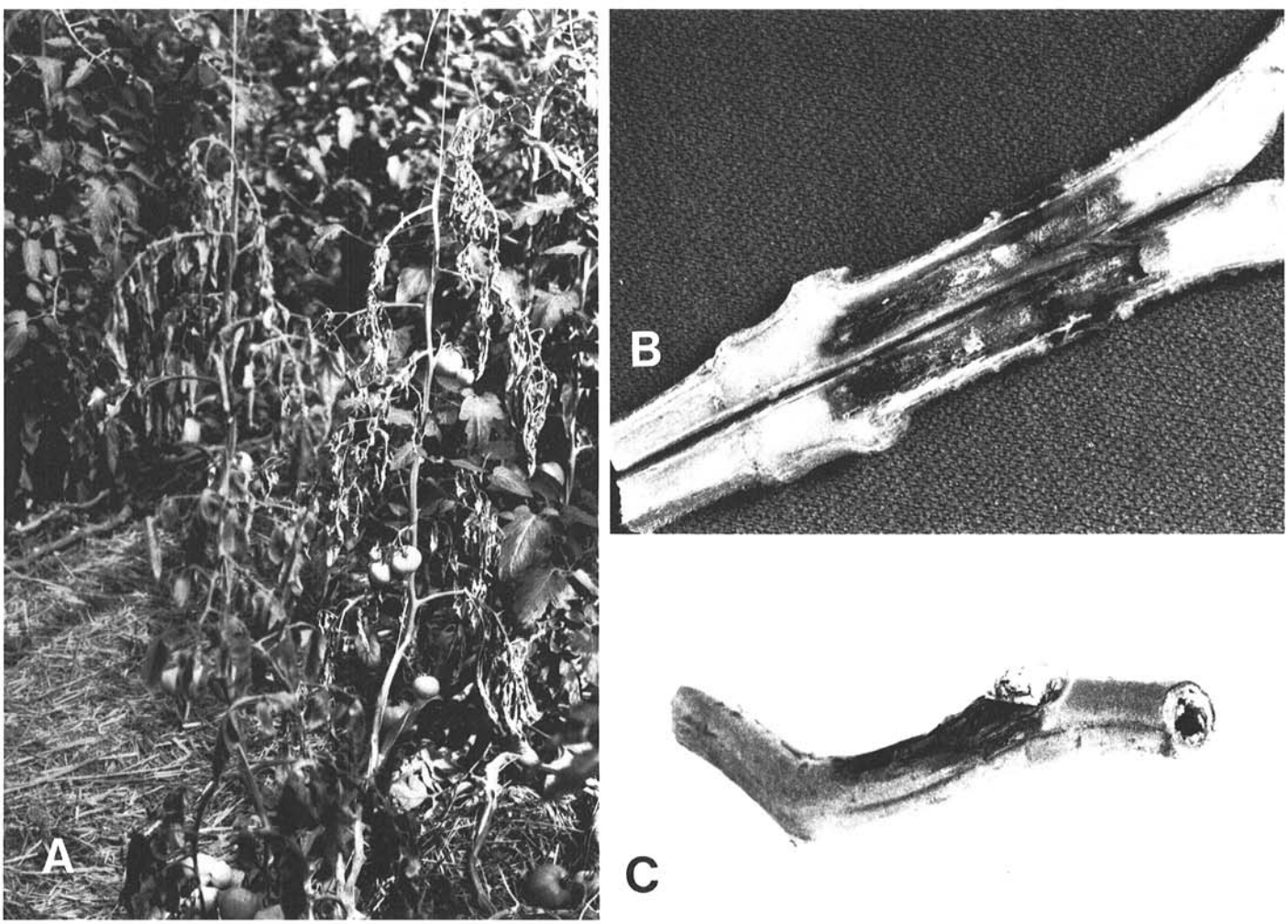

Fig. 1. Tomato stem rot symptoms. **A,** Plant wilting. **B,** Pith necrosis spreading from the point of inoculation. **C,** Hollowed stem after pith disintegration.

TABLE 1. Effect of intermittent misting in greenhouse on extension of stem rot on tomato cultivar CR-6 by three TSR bacterial strains and *Erwinia carotovora* ssp. *carotovora* (PDDCC 5702)

Treatment	Bacterial strain	Nodes affected above inoculation site (no.) (Mean ± S.E.) 3 wk[a]	4 wk	5 wk	6 wk	7 wk
Misting	TSR 01	4.0 ± 1.96	6.8 ± 1.80	10.5 ± 1.71	11.3 ± 1.31	11.5 ± 1.32
	TSR 03	5.0 ± 1.78	6.3 ± 2.10	10.3 ± 0.63	10.8 ± 0.75	10.8 ± 0.75
	TSR 05	6.3 ± 0.85	6.5 ± 0.87	8.8 ± 1.10	9.3 ± 1.03	9.8 ± 1.03
	PDDCC 5702	3.5 ± 1.26	7.0 ± 1.08	10.0 ± 1.68	11.5 ± 1.19	12.3 ± 1.25
	LSD 0.05[b]	2.83	4.69	4.58	3.55	3.53
No Misting	TSR 01	0.0	1.8 ± 1.03	3.0 ± 1.22	5.0 ± 2.20	5.0 ± 2.20
	TSR 03	0.0	2.5 ± 0.87	4.3 ± 1.70	6.8 ± 1.31	6.8 ± 1.31
	TSR 05	2.3 ± 1.65	3.0 ± 1.47	5.8 ± 1.97	6.3 ± 2.13	6.3 ± 2.13
	PDDCC 5702	1.8 ± 1.75	4.0 ± 1.58	6.0 ± 1.47	9.0 ± 0.41	9.0 ± 0.41
	LSD 0.05[b]	3.83	4.07	5.38	5.46	5.46

[a] Weeks after inoculation.
[b] Least significant difference at $P = 0.05$.

TABLE 2. Analysis of variance of incidence of bacterial stem rot on tomato cultivar CR-6 in two commercial greenhouses

Source of variation[a]	Greenhouse 1 df	ms	F	Greenhouse 2 df	ms	F
Among all rows	5	1,800.12	15.49***[b]	2	897.25	8.42**
Gutter rows vs. other rows	1	8,671.52	74.63***	1	1,725.51	16.19***
Among other rows	4	82.27	1	1	69.03	1
Replicates	7	320.71	2.76	15	676.47	6.34**
Error	35	116.20		30	106.56	

[a] See text for experimental details.
[b] ** $P \leq 0.01$; *** $P \leq 0.001$.

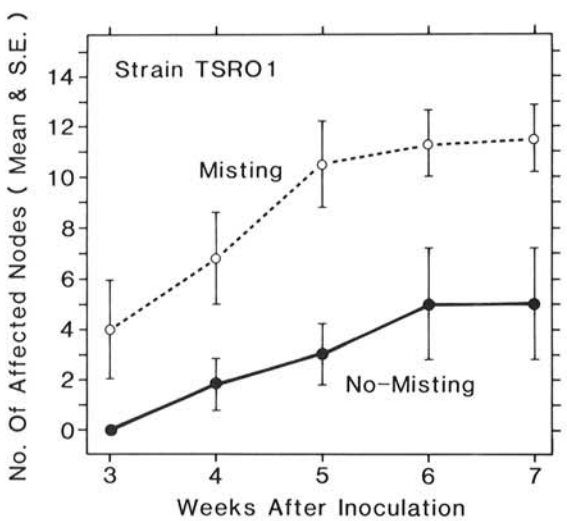

Fig. 2. Disease progress curves of stem rot on tomato cultivar CR-6 inoculated with the bacterial strain TSR 01 under misting and nonmisting conditions in the greenhouse.

for the bacterial strain TSR 01 is shown in Figure 2. Similar curves, not shown here, were obtained for the three other strains.

Disease incidence and spatial distribution. In July 1983, there was an average of 18% dead plants in the two affected commercial greenhouses. By analyzing the disease frequency on a row-by-row basis, a significantly ($P = 0.001$) higher incidence of stem rot and wilt was found in the gutter rows than in the other rows (Table 2). The spatial distribution of the disease is shown in Figure 3. The gutter effect is seen in a comparison of disease incidence by row in Figure 4.

Doublet, runs, and chain statistics on lengthwise and crosswise disease distribution data taken in the plastic greenhouse are contained in Table 3. In lengthwise doublet analyses, there were far more doublets of diseased plants than expected by chance in all the eight subsections, individually as well as in the entire greenhouse ($P < 0.01$). In crosswise doublet analyses, significant increases of doublet numbers over random expectation occurred in four of the eight subsections and in the greenhouse stand overall. There were significantly ($P = 0.01$) more doublets in lengthwise counts than in crosswise counts. Counting diseased plants as linear chains also provided the data for a runs test. Nonrandom distribution of diseased plants would result in a reduction of number of runs and chains compared with random expectation. In addition, the number of short chains would be fewer and the number of longer chains more, if disease distribution were nonrandom. This change in distribution of chain size is illustrated in Figure 5. As with doublet analysis, the lengthwise chain analysis or a simple runs test suggests a significantly ($P < 0.01$) more nonrandom association of diseased plants than does the crosswise analysis.

DISCUSSION

It was established that *E. c.* ssp. *carotovora* was the pathogen causing stem rot, wilt, and death of fruit-bearing tomato plants in the Leamington area greenhouses in the spring of 1983. The greenhouses harboring this disease had been fumigated with methyl bromide. Butler (2) found that methyl bromide reduced but did not eliminate *E. c.* ssp. *carotovora*, whose survival appeared to be greater with increasing depth in the greenhouse soil. Evidence has been accumulating that *E. c.* ssp. *carotovora* persists in the rhizosphere of crop and weed species (5,19,20). Using an anaerobic liquid enrichment and selective medium, McCarter-Zorner et al (18) showed that *E. c.* ssp. *carotovora* contaminated surface and ground water in drains, ditches, streams, reservoirs, and lakes. Butler found that the *E. c.* ssp. *carotovora* strains causing tomato stem rot persisted in tomato and cucumber rhizosphere for over 2 yr; he also suggested that the river-bottom sand used in greenhouse potting mixture might have been contaminated with *E. c.* ssp. *carotovora*. It is conceivable there could be many sources of inoculum of *E. c.* ssp. *carotovora* in Ontario.

Speights et al (24) proposed that the incidence of stem rot of tomato induced by *E. c.* ssp. *carotovora* in Texas greenhouses might be associated with increased humidity under plastic. Stanghellini (*personal communication*) observed increased incidence of pith rot caused by *E. c.* ssp. *carotovora* in Hawaiian tomato greenhouses under the drip lines. In greenhouses in Arizona, Butler (2) associated free moisture and high humidity

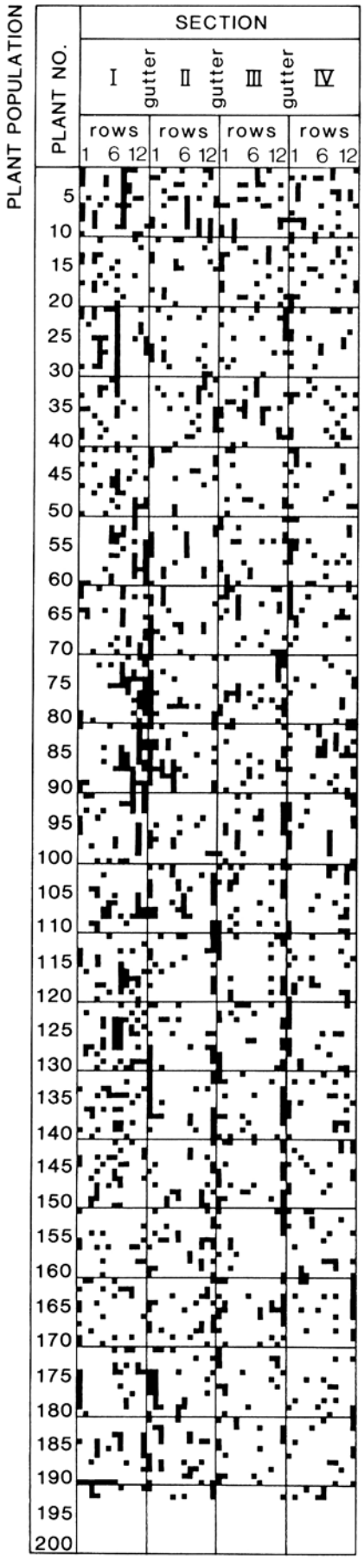

Fig. 3. Spatial distribution of tomato stem rot (cultivar CR-6) in a greenhouse.

with tomato pith rot induced by *E. c.* ssp. *carotovora*. Similar association of high humidity and/or free moisture has been recognized in other diseases induced by *E. carotovora* (12,28). Our study has also shown that extensive stem rot and pith disintegration could occur in greenhouse tomato under conditions of high humidity and free moisture.

Doublet and runs analyses have been compared recently for describing the spatial distribution of diseased plants; the runs test was considered to be more consistent than doublet analysis even when using the error adjustment of Converse et al (4). Chain analysis, taking into account the length of runs of diseased plants, could add a refinement to the runs test. The sum of the number of chains of diseased and healthy plants is the number of runs. By using these methods of evaluating disease distribution it is possible to apply the technique of directional comparisons first suggested by Cochran (3) on a plot basis. Doublets, runs, and chains are methods of describing linear association in a one-directional sequence. In our study, all the three methods resulted in a rejection of the null hypothesis of random distribution of the diseased plants. Evaluation of the stand in both directions indicated a predominantly lengthwise clumping of diseased plants. The presence of chains of considerable numbers of diseased plants could establish further this relationship that might be missed otherwise in a standard runs count. The comparison of gutter rows with the other rows in the greenhouse would support this.

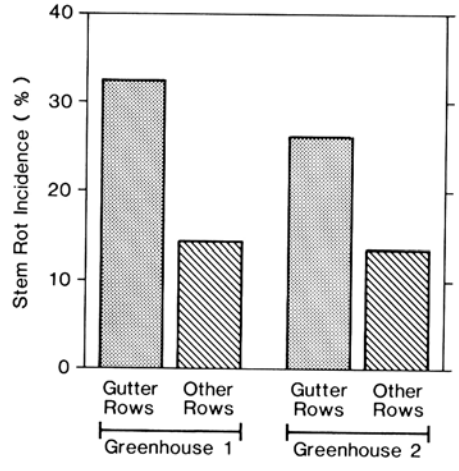

Fig. 4. Percent incidence of tomato stem rot (cultivar CR-6) in two greenhouses showing the difference between rows under the gutter and other rows.

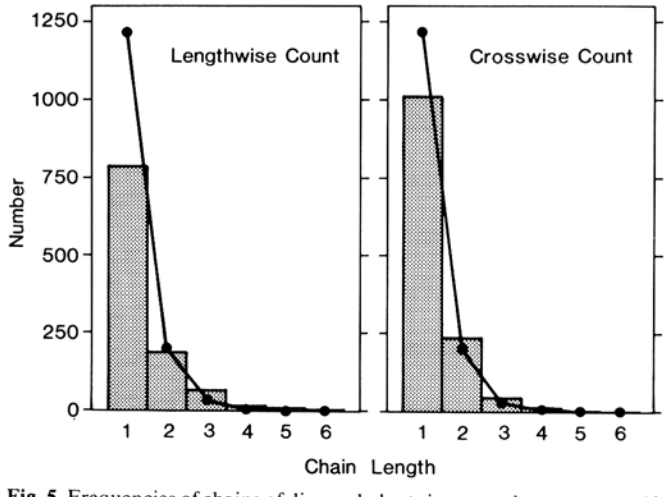

Fig. 5. Frequencies of chains of diseased plants in a greenhouse, counted in two directions (bars) and compared to expectation (—) based on random distribution.

TABLE 3. Doublet, runs, and chain statistics for tomato stem rot in a greenhouse stand of 200 × 48 plants

Greenhouse subsection[a]	Plants (no.)	Diseased plants	Freq.[b] (P)	Doublets			Runs			Chains observed			
				Obs.	Exp.	Z[c]	Obs.	Exp.	Z[c]	1	2	3 or more	X^{2d}
Lengthwise counts													
1	1,200	292	0.243	125	71	6.45	334	443	8.50	113	28	26	34.24
2	1,200	225	0.188	62	42	3.81	326	366	3.81	123	27	13	11.16
3	1,200	202	0.168	79	34	7.77	246	337	9.34	85	20	18	58.78
4	1,200	213	0.178	81	38	7.06	264	351	8.60	87	29	16	43.05
5	1,200	210	0.175	62	37	4.21	296	348	5.10	111	21	16	29.30
6	1,200	177	0.148	61	26	6.88	232	303	8.08	81	23	12	41.68
7	1,200	203	0.169	57	34	3.91	292	338	4.71	107	28	11	16.49
8	1,200	161	0.134	48	21	5.73	226	280	6.63	84	17	12	47.08
All	9,600	1,683	0.175	575	295	16.31	2,216	2,777	28.33	790	195	123	261.33
Crosswise counts													
1	1,200	292	0.243	81	71	1.21	423	443	1.52	157	37	17	2.12
2	1,200	225	0.188	54	42	1.85	343	367	2.29	128	39	4	8.22
3	1,200	202	0.168	46	34	2.09	312	337	2.53	118	31	7	6.81
4	1,200	213	0.178	55	38	2.83	317	351	3.35	115	37	6	11.02
5	1,200	210	0.175	41	37	0.73	338	348	0.90	134	30	5	1.57
6	1,200	177	0.148	42	26	3.15	271	303	3.60	99	33	3	17.23
7	1,200	203	0.169	43	34	1.51	320	338	1.83	125	28	7	3.39
8	1,200	161	0.134	33	21	2.49	257	280	2.77	98	29	1	15.31
All	9,600	1,683	0.175	376	295	4.72	2,615	2,777	5.70	1,014	234	59	26.70

[a] Each subsection is a stand of 12 rows × 100 plants (see text).
[b] Where frequency (*P*) is diseased plants/total.
[c] $Z_{0.05} = 1.96$.
[d] $X^2_{0.05}$ for 2 df = 5.99.

The spatial distribution of bacterial stem rot of tomato in the greenhouse is apparently highly dependent on proximity of healthy plants to infected plants. This could be explained as increased inoculum density in the first-affected plants and its availability for further spread by contact. *E. c.* ssp. *carotovora* is considered opportunistic, with the ability to move rapidly in the tomato tissue (2). Jarvis and Hawthorne (11) tried to use their analyses to make deductions about the origin and dispersal of infective propagules of *Sclerotinia* spp. in lettuce. In our case, the bacteria appear to be splashed on to deleafing wounds on plants in the gutter rows first; it is assumed that they persist in soil from a previous crop as stated by Butler (2). Thence they are spread on tools and fingers along the rows.

Our study has described the distribution of tomato bacterial stem rot in two greenhouses and has provided information of practical value in disease management. It supports the suggestion that the disease first occurs under the roof-gutter, an area subject to water-splash, and it is exacerbated under conditions of high humidity in the greenhouse and free moisture on plant surface. It also supports the conclusion that the disease spreads further along the rows by frequent handling of the stems to lower them on the greenhouse floor, and to remove the bottom leaves by breaking them off as the harvest advances. For this reason, it would be prudent to take hygienic measures to avoid spreading bacteria from plant to plant within the row and from row to row in the greenhouse. Such measures may include doing deleafing and plant tying only when surface moisture has dried, wearing and changing disposable gloves frequently, and directing the water dripping from the gutter bays away from the plants.

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