Identification and Host Range of an *Erwinia* Pathogen Causing Stem Rots on Hydroponically Grown Plants

ANDREW C. SCHUERGER, Senior Plant Pathologist, and JEAN CARLSON BATZER, Technical Specialist, The Land, EPCOT Center, P.O. Box 10000, Lake Buena Vista, FL 32830

ABSTRACT

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A bacterium pathogen causing stem rot and vascular wilt symptoms on hydroponically grown vegetables was identified by biochemical tests and cellular fatty acid compositions as *Erwinia carotovora* subsp. *carotovora*. Eighteen different plant hosts were screened for resistance to the pathogen. Stems of hydroponically grown plants were inoculated (with a micropipette technique) with 100-μl bacterium suspensions at several dosage levels between 3.4×10^4 and 3.4×10^8 cfu/ml. Pole bean (*Phaseolus vulgaris* 'Glastada'), Chinese bitter melon (*Momordica charantia* 'Park 5188'), and luffa gourd (*Luffa aegyptiaca*) were the most resistant; plants failed to develop stem rot or wilt symptoms at any of the dosage levels tested. Potato (*Solanum tuberosum* 'Russet Burbank') and tomato (*Lycopersicon esculentum* 'N-65') were classified as moderately resistant because inoculated tissues developed rot symptoms at dosage levels of 10^6 and 10^8 cfu/ml, but not at 10^4 cfu/ml. Winter squash (*Cucurbita maxima* 'Banana Pink Jumbo'), pepper (*Capsicum annuum* 'Early Thickset'), buffalo gourd (*Cucurbita foetidissima* 'Az. Synthesis 1'), tobacco (*Nicotiana tabacum* 'Coker 48'), and cucumber (*Cucumis sativus* 'Vetomil') were rated as moderately susceptible because stem rot symptoms occurred on 10–50% of the plants inoculated at the 10^4-cfu/ml dosage level. Plants within the Cruciferae and cantaloupe (*Cucumis melo* 'Chilton') were the most susceptible; stem rot symptoms were observed within 3 days after inoculation on at least 50% of the plants inoculated at the lowest dosage level of 3.4×10^4 cfu/ml. Horticultural activities that increased wounding of plants generally increased the incidence of disease.

Additional keywords: aeroponic, API 20E

In the spring of 1983, a stem rot and vascular wilt caused by an *Erwinia* species first appeared on hydroponically grown plants in greenhouses at The Land, EPCOT Center, Florida. Outbreaks of bacterial stem rot occurred primarily on crucifer and cucurbit species grown in aeroponic systems or drip-irrigated sand beds. Outbreaks were often severe, causing rapid and extensive damage on Chinese cabbage and winter squash varieties between 1983 and 1985. Horticultural activities that increased wounding of plants generally increased the incidence of disease. Preliminary tests established the pathogen as an *Erwinia* species (19).

The origin of the stem rot pathogen and the means by which it was introduced into the plant-growing areas at The Land have not been determined. Samples from the greenhouse environment in 1983 and 1984 indicated the pathogen was endemic. It was recovered from greenhouse aerosols, dipteran insects, separate batches of hydroponic solutions, and the rhizospheres and phyllospheres of several plant species. The pathogen was not recovered from seeds of cucurbit and crucifer cultivars grown at the time of the initial disease outbreaks, nor was it recovered from well water used for the hydroponic solutions. Butler (2) suggested that river-bottom sand used in a hydroponic system in Arizona might have been the primary source of inoculum of an *Erwinia* pathogen, but we were not able to confirm whether the sand used at The Land was contaminated before the construction of the facility.

Jenkins and Averre (11), and Speights et al (20) isolated several *Erwinia* species from the stems of infected tomato plants grown in commercial hydroponic greenhouses, but they did not characterize the taxonomic affinity of the recovered pathogens. In other studies, both *Erwinia carotovora* subsp. *carotovora* (Jones) Bergey et al (2,6) and *E. c. atroseptica* (van Hall) Dye (1) were identified as the causal agents of bacterial stem rots of hydroponically grown cucumbers and tomatoes in commercial greenhouses. Symptoms were water-soaking of subepidermal parenchyma tissues, breakdown of the stem pith acropetal to the points of infection, and sloughing of the outer stem when mechanically disturbed (6,20).

The objectives of this study were to identify the etiology of the disease and to determine the pathogenicity, virulence, and host range of the pathogen. Portions of this research have been presented previously (19).

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MATERIALS AND METHODS

Strains of the *Erwinia* stem rot pathogen were compared with two reference strains of each of the following: *E. c. carotovora; E. c. atroseptica; E. c. betavasculorum* Thomson, Hildebrand, and Schroth; and *E. chrysanthemi* McFadden, Burkholder, and Dimmock (Table 1). Twelve strains of the pathogen were isolated from macerated stem tissues of diseased plants growing in the vegetable-production greenhouses at The Land (Table 1). One additional strain (K12) was obtained from ornamental kale (*Brassica oleracea* L. 'Emperor Red') exhibiting severe stem rot symptoms in a landscape bed within 50 m of the greenhouses. Nutrient agar (NA) was the primary isolation medium for all stem-tissue samples. Koch's postulates were completed on winter squash (*Cucurbita maxima* Duchesne 'Pink Banana Jumbo') and pak choi (*B. rapa* L. 'Lei Choi') for all 13 strains of the pathogen isolated from hydroponically grown plants.

Strains of the pathogen and of *E. c. carotovora* were grown for 24 hr at 30 C on NA plates. Strains of *E. c. atroseptica* were grown on NA for 24 hr at 24 C. Strains of *E. c. betavasculorum* were grown for 24 hr at 30 C on Luria media, consisting of 10 g of tryptone, 5 g of yeast extract, 8 g of NaCl, 0.25 g of $MgSO_4 \cdot 7H_2O$, and 17.5 g of Bacto Agar (Difco Laboratories, Detroit, MI 48232) in 1 L of deionized water. The pH of the solidified Luria medium was 7. Tests were conducted at room temperature (approximately 24 C) with four repetitions, three for *E. chrysanthemi*. Biochemical tests were conducted as described previously (4,7–9, 13,18,21).

The effects of different temperatures on the growth of all bacterium strains were determined by dispensing 1 μl of a 1×10^4 cfu/ml suspension of each strain onto each of ten NA plates, then incubating the petri dishes in a dark growth chamber for 48 hr at 15, 30, 37, and 39 C. Temperatures were selected based on previous reports on the use of temperature to differentiate taxons of *Erwinia* species (7,8,17,21). Bacteria that were capable of growth at specified temperatures produced colonies at least 1–2 mm in diameter. The experiment was conducted three times.

Strains were also characterized with the API 20E Research System. Strains were grown on NA at 30 C for 24 hr, then suspended in 9 ml of sterile deionized water (SDIW). The test galleries were handled as instructed by the manu-

facturer, except that they were incubated at 30 C instead of 37 C.

Cellular fatty acid compositions of all bacteria were determined with gas–liquid chromotography by Microbial ID, Inc. (115 Barksdale Professional Ct., Newark, DE 19711), according to the procedures of De Boer and Sasser (5).

Eighteen plant species and cultivars (Table 2) were screened for their relative resistance to one strain of the *Erwinia* pathogen (PBJB) isolated from infected winter squash plants. PBJB was selected for these tests based on the results of the pathogenicity tests used to confirm Koch's postulates. It produced consistently severe stem rot symptoms in winter squash and pak choi.

Seeds for the host-range tests were sown in a commercial potting soil. When seedlings were between 14 and 21 days old, they were transplanted into three 0.3-m-deep sand beds in a 3.7 m × 7.3 m greenhouse. Plants were watered twice daily with a hydroponic nutrient solution composed of the following inorganic salts (all except Sequestrene 330 obtained from Sigma Chemical Co., St. Louis, MO 63178): 4.99 mM $Ca(NO_3)_2 \cdot 4H_2O$, 4.75 mM KNO_3, 1.23 mM KH_2PO_4, 2.06 mM $MgSO_4 \cdot 7H_2O$, 0.046 mM H_3BO_4, 9.10 μM $MnSO_4 \cdot H_2O$, 6.10 μM $ZnSO_4 \cdot 7H_2O$, 3.80 μM $CuCl_2 \cdot 2H_2O$, 2.60 μM $(NH_4)_6 Mo_7O_{24} \cdot 4H_2O$, and 89.5 μM Sequestrene 330 (Ciba-Geigy Corp., Greensboro, NC 27419) providing ferric-diethylenetriaminepentaacetic acid equivalent to 5 $mg \cdot L^{-1}$ of ferric iron. Diurnal fluctuations for the host-range tests were between 25 and 32 C, and 70 and 95% relative humidity.

Greenhouse space limitations required that each repetition of the experiment be split into three separate trials. Each trial was composed of six of the 18 cultivars. Four plants per cultivar were assigned to each of three replicated plots per trial. Plants of pole bean (*Phaseolus vulgaris* L. 'Glastada') and pak choi were included in each trial as controls to ensure that disease development was consistent throughout the experiment. In preliminary tests, pole bean was found to be highly resistant and pak choi highly susceptible to the pathogen.

Inoculum was prepared from 24-hr cultures grown on NA at 30 C. Bacteria were transferred to glass tubes containing 9 ml of SDIW, vortexed, and optical densities (OD) measured at 560 nm with a spectrophotometer. Bacterial suspensions were adjusted to an OD of 0.30. The numbers of bacteria in stock suspensions for the host-range experiments were estimated by serial dilutions and ranged from 1.4×10^8 to 8.2×10^8 cfu/ml, with a mean of 3.4×10^8 cfu/ml. Stock suspensions were diluted to obtain desired inoculum levels.

Inoculations were made with calibrated micropipettes, sensu Lum and Kelman (14). Individual plants within each plot were wounded at two locations per plant by inserting sterile micropipettes 4–6 mm into stem or petiole tissues. Wounded tissues were inoculated by injecting 100 μl of either SDIW or bacterial suspensions containing approximately 3.4×10^4, 3.4×10^6, or 3.4×10^8 cfu/ml. Each plant within the Leguminosae, Solanaceae, and Cucurbitaceae was inoculated at the axil of the first fully expanded leaf basipetal to the primary shoot meristem, and at the axil of the first true leaf. Each plant in the Cruciferae was inoculated at the bases of two leaf petioles on opposite sides of the plant.

Eighteen inoculations at each dosage level were evaluated for each cultivar. Plants were observed daily for 14 days, harvested, and then dissected to inspect for internal symptoms. Symptoms were rated as follows: 1 = no external or internal symptoms at the site of inoculation; 2 = no tissue maceration or foliar wilt, but with bacterial ooze or stem discoloration at the site of inoculation; 3 = maceration of stem tissue at the site of inoculation between 4 and 14 days, but no foliar wilt during the experiment; 4 = maceration of stem tissue at the site of inoculation within 3 days, but no foliar wilt during the experiment; and 5 = maceration of stem tissues and leaf wilt acropetal to the inoculation site during the experiment. Disease-severity values were compared by an analysis of variance followed by a Bonferroni (Dunn) mean separation test ($P = 0.05$). The analysis utilized mean ratings of three observations partitioned for each combination of crop, inoculation site, and experiment repetition; thus, the data were considered approximately continuous. For each crop, the percentage of inoculations developing stem maceration was recorded for each dosage level. The experiment was conducted three times.

RESULTS AND DISCUSSION

Biochemical characterization of the reference strains of *E. c. carotovora, E. c. atroseptica, E. c. betavasculorum,* and *E. chrysanthemi* (Table 3) was consistent with previously described reports (7,8,13,21). Based on the results of the biochemical tests, two phenotypic groups were identified within the 13 bacterium strains isolated from hydroponically grown plants (Table 3). Bacteria in group I (10 strains) tested positive for reducing substances from sucrose, and acid from palatinose and α-methylglucoside, phenotypic characteristics generally associated with *E. c. atroseptica* (5, 12,21). Bacteria in Group II (three

Table 1. Reference strains, *Erwinia* strains from plants within or near hydroponic greenhouses, and plant hosts from which bacteria were originally obtained

Bacteria	Strain	Plant host
	Reference	
Erwinia carotovora subsp. *carotovora*	Ecc26[v]	*Solanum tuberosum* L.
E. c. carotovora	Ecc63[v]	*S. tuberosum*
E. c. atroseptica	Eca31[v]	*S. tuberosum*
E. c. atroseptica	Eca6[v]	*S. tuberosum*
E. c. betavasculorum	Ecb173[w]	*Beta vulgargis* L.
E. c. betavasculorum	Ecb184[w]	*B. vulargis*
E. chrysanthemi	SR80[x]	*Syngonium* sp. Schott
E. chrysanthemi	SR81[x]	*Philodendron selloum* K. Koch
	From greenhouse and landscape plants	
	PBJB[y]	*Cucurbita maxima* Duchesne cv. Banana Pink Jumbo
	S2[y]	*C. pepo* L. cv. Sundance
	C1[y]	*Cucumis sativus* L. cv. Asunta
	C2[y]	*C. sativus* cv. Vetomil
	CC2[y]	*Brassica rapa* L. Pekinensis Group cv. WR-60
	PU1[y]	*C. pepo* cv. Spookie
	BS[y]	*C. pepo* cv. Butterstick
	HS[y]	*C. maxima* cv. Golden Hubbard
	K12[z]	*Brassica oleracea* L. Acephala Group cv. Emperor Red
	PC3[y]	*B. rapa* L. Chinensis Group cv. Lei Choi
	TD[y]	*B. rapa* Pekinensis Group cv. Tropical Delight
	CCT[y]	*B. rapa* Pekinensis Group cv. China Pride
	MG[y]	*Tagetes patula* L. cv. Harmony Boy

[v] From S. H. De Boer, Vancouver, British Columbia.
[w] From M. N. Schroth, University of California, Berkeley, CA.
[x] From J. A. Bartz, University of Florida, Gainesville, FL.
[y] From within The Land greenhouses, EPCOT Center, FL.
[z] From landscape beds 50 m from The Land greenhouses, EPCOT Center, FL.

strains) were negative for these tests, suggesting a closer affinity to *E. c. carotovora.* Although Cho (3) found reducing substances from sucrose unreliable for differentiating subspecies of *E. carotovora,* other workers (7,8,21) have found this test to be reliable. Group II strains showed some similarities to *E. chrysanthemi* by producing indole and not producing acid from trehalose (7,8). However, Group II strains did not produce phosphatase, were not sensitive to erythromycin, and could not utilize malonate, indicating greater affinity to *E. carotovora* subspecies than to *E. chrysanthemi* (7,8). Both Group I and Group II strains were not able to utilize galacturonate, cellobiose, or melibiose, indicating a difference from *E. c. betavasculorum* (21).

Strains in Group I grew at 37 C, but not at 39 C (Table 3), a characteristic of *E. c. carotovora* (7,8). Strains in Group II grew at 37 and 39 C, which suggested a greater affinity towards *E. chrysanthemi* (7). Bacterial growth at different temperatures has been described as a variable taxonomic characteristic for species of *Erwinia* (10). However, Perombelon (17) used temperature as a primary selective factor to isolate and identify soft rot erwinias directly from plant material, and other workers (7,8,21) appear to have utilized temperature effectively to help separate species and subspecies of *Erwinia.* Growth at various temperatures (Table 3) was a consistent test in our study for differentiating species and subspecies of

Table 2. Plants tested for resistance to *Erwinia carotovora* subsp. *carotovora* (strain PBJB)

Plant species and cultivar	Common name	Seed source
Cruciferae		
Brassica rapa L. Pekinensis Group cv. WR-60	Chinese cabbage	Takii Seed, Kyoto, Japan
Chinensis Group cv. Lei choi	Pak choi	W. Atlee Burpee Seed, Warminster, PA
B. oleracea L. Acephala Group cv. Dwarf Siberian	Kale	W. Atlee Burpee Seed, Warminster, PA
Botrytis Group cv. Green Comet	Broccoli	Harris Moran Seed, Rochester, NY
Botrytis Group cv. White Contessa	Cauliflower	Sakata Seed, Yokohama, Japan
Gonglylodes Group cv. Purple Danube	Kohlrabi	Sakata Seed, Yokohama, Japan
Raphanus sativus L. cv. Cherry Belle	Radish	W. Atlee Burpee Seed, Warminster, PA
Cucurbitaceae		
Cucumis melo L. Reticulatus Group cv. Chilton	Cantaloupe	Auburn University
C. sativus L. cv. Vetomil	Cucumber	Bruinsma Seed, Holland
Cucurbita maxima Duchesne cv. Banana Pink Jumbo	Winter squash	Hollar Seed, Rockyford, CO
C. foetidissima H.B.K. cv. Az. Synthesis 1	Buffalo gourd	University of Arizona
Momordica charantia L. cv. Park 5188	Chinese bitter melon	Park Seed, Greenwood, SC
Luffa aegyptiaca Mill.	Luffa gourd	Park Seed, Greenwood, SC
Solanaceae		
Capsicum annuum L. var. *annuum* cv. Early Thickset	Bell pepper	Park Seed, Greenwood, SC
Solanum tuberosum L. cv. Russet Burbank	White potato	University of Wisconsin
Nicotiana tabacum L. cv. Coker 48	Tobacco	Coker Seed, Hartsville, SC
Lycopersicon esculentum Mill. cv. N-65	Tomato	University of Hawaii
Leguminosae		
Phaseolus vulgaris L. cv. Glastada	Pole bean	Nickerson-Zwaan-dp, Holland

Table 3. Biochemical reactions of *Erwinia* strains

	Strains from greenhouse and landscape plants[y]		Reference strains			
Biochemical tests	**Group I**	**Group II**	***Erwinia chrysanthemi***	***Erwinia carotovora* subsp. *carotovora***	***Erwinia carotovora* subsp. *atroseptica***	***Erwinia carotovora* subsp. *betavasculorum***
Pectolytic on CVP	+[z]	+	+	+	+	+
Oxidation fermentation	+	+	+	+	+	+
Reducing substances from sucrose	+	−	−	−	+	+
Erythromycin sensitive	−	−	+	−	−	−
Utilize malonate	−	−	+	−	−	−
Produce indole	−	+	+	−	−	−
Produce phosphatase	−	−	+	−	−	−
Acid from						
lactose	+	+	−	+	+	+
trehalose	+	−	−	+	+	+
palatinose	+	−	−	−	+	+
maltose	−	−	−	−	+	+
α-methylglucoside	+	−	−	−	+	+
galacturonate	+	+	+	+	+	−
cellobiose	+	+	+	+	+	−
melibiose	+	+	+	+	+	−
Growth at						
15 C	+	+	+	+	+	+
30 C	+	+	+	+	−	+
37 C	+	+	+	+	−	+
39 C	−	+	+	−	−	+
API 20E codes	1205173	1245173	1247173	1205173	1205173	1005133

[y] Land isolates = 13 isolates obtained from plants with stem rot at, or near, The Land, EPCOT Center. Group I: PBJB, S2, K12, C1, C2, PU1, BS, CC2, PC3, HS. Group II: MG, TD, CCT.

[z] Symbols: + = all strains positive; − = all strains negative.

Erwinia.

Bacteria tested with the API 20E system were classified by a seven-digit code (Table 3). A comparison between these results and the published results of Mergaert et al (16) indicates strong similarities between the *E. chrysanthemi, E. c. carotovora,* and *E. c. atroseptica* reference strains in each study. Mergaert et al (16) could not differentiate between strains of *E. c. carotovora* and *E. c. atroseptica* based solely on the API 20E seven-digit codes. Similarly in our study, bacteria in Group I, *E. c. carotovora* reference strains, and *E. c. atroseptica* reference strains (Table 3) had identical API 20E seven-digit codes (1205173). Strains UCPPB 1931, UCPPB 188, and NCPPB 2792 in the Mergaert et al study (16) were listed as *E. c. atroseptica* and produced an API 20E seven-digit code of 1005133. In contrast, the reference strains of *E. c. betavasculorum* used in our study produced the same API 20E code (Table 3). Additional work on identification of *Erwinia* subspecies with the API 20E system might clarify the discrepancy between the observed results of the two studies.

The fatty acid ratios of 12 of the 13 bacterium strains isolated from hydroponically grown plants were similar to the *E. c. carotovora* reference strains used in the present study (Table 4), and the ratios were consistent with published results for *E. c. carotovora* (5). The following fatty acids were determined: dodecanoic acid (12:0), tetradecanoic acid (14:0), hexadecanoic acid (16:0), 9-hexadecanoic acid (16:1), and 9-octadecanoic acid (18:1). *E. c. carotovora* strains have benchmark ratios of >3.71, <4.87, and <2.70 for 12:0/14:0, 16:0/12:0, and 16:1/18:1 fatty acids, respectively, and *E. c. atroseptica* strains have benchmark ratios of <3.71, >4.87, and

Table 4. Fatty acid ratios of the reference strains of *Erwinia* spp. and the *Erwinia* strains isolated from plants within or near hydroponic greenhouses

Bacteria	Strains	Fatty acid ratios[z] 12:0/14:0	16:0/12:0	16:1/18:1
	Reference			
Erwinia carotovora subsp. *carotovora*	Ecc63	4.35	4.14	1.68
E. c. carotovora	Ecc26	4.34	4.15	1.75
E. c. atroseptica	Eca31	2.24	4.99	3.22
E. c. atroseptica	Eca6	1.92	5.44	4.48
E. c. betavasculorum	Ecb173	<0.10	>10.00	1.82
E. c. betavasculorum	Ecb184	<0.10	>10.00	1.87
E. chrysanthemi	SR80	2.99	4.35	2.82
E. chrysanthemi	SR81	4.21	4.33	2.82
	From greenhouse and landscape plants			
	PBJB	4.43	4.18	1.73
	S2	3.91	4.22	2.09
	C1	4.61	4.17	1.69
	C2	4.58	4.19	1.68
	CC2	4.69	4.26	1.69
	PU1	4.61	4.19	1.72
	BS	4.60	4.26	1.76
	HS	4.43	4.15	1.63
	K12	4.74	4.17	1.72
	PC3	4.72	4.33	1.66
	TD	4.10	4.31	1.88
	CCT	1.66	4.25	1.51
	MG	4.52	4.31	1.69

[z] The fatty acid ratios are for dodecanoic acid (12:0), tetradecanoic acid (14:0), hexadecanoic acid (16:0), 9-hexadecanoic acid (16:1), and 9-octadecanoic acid (18:1).

Table 5. Plant resistance to *Erwinia carotovora* subsp. *carotovora* (strain PBJB)

Plant host	Dosage level (cfu/ml)[x] 3.4×10^4 Tissue maceration[y] (%)	3.4×10^4 Disease severity rating[z]	3.4×10^6 Tissue maceration (%)	3.4×10^6 Disease severity rating	3.4×10^8 Tissue maceration (%)	3.4×10^8 Disease severity rating
Highly susceptible						
Pak choi	100	5.0 a	100	5.0 a	100	5.0 a
Broccoli	100	5.0 a	100	5.0 a	100	5.0 a
Cauliflower	94	4.7 a	100	5.0 a	100	5.0 a
Kale	94	4.7 a	100	5.0 a	100	5.0 a
Chinese cabbage	89	4.3 ab	100	5.0 a	100	5.0 a
Kohlrabi	55	3.0 b	83	4.4 abc	100	5.0 a
Radish	50	2.8 b	88	4.2 abc	100	4.9 a
Cantaloupe	55	2.6 bc	100	4.0 abc	100	4.8 a
Moderately susceptible						
Winter squash	31	2.0 bcd	94	3.9 bc	100	4.4 ab
Pepper	25	1.6 bcd	63	3.6 cde	100	4.4 abc
Tobacco	19	1.7 bcd	63	3.4 bc	69	3.4 c
Buffalo gourd	13	1.4 cd	25	2.1 def	94	4.4 abc
Cucumber	25	1.6 cd	44	2.3 def	88	3.8 bc
Moderately resistant						
White potato	0	1.1 cd	14	2.3 def	88	3.4 c
Tomato	0	1.1 cd	31	1.9 def	88	3.2 c
Highly resistant						
Chinese bitter melon	0	1.2 cd	0	1.6 def	0	1.6 d
Luffa gourd	0	1.0 d	0	1.5 ef	0	1.6 d
Pole bean	0	1.0 d	0	1.0 f	0	1.0 e

[x] Mean inoculum dosages delivered to individual inoculation sites at 100 µl per inoculation.

[y] Inoculation points in which tissue maceration was observed after 14 days.

[z] Values represent means for 18 inoculations. Rating scale: 1 = no external or internal symptoms at inoculation site; 2 = no tissue maceration or foliar wilt, but with bacterial ooze or stem discoloration at inoculation site; 3 = maceration of stem tisue at inoculation site between 4 and 14 days, but no foliar wilt during the experiment; 4 = maceration of stem tissue at inoculation site within 3 days, but no foliar wilt during the experiment; and 5 = maceration of stem tissues and leaf wilt acropetal to the inoculation site during the experiment. Values in columns followed by the same letters are not significantly different based on analysis of variance and a Bonferroni (Dunn) mean separation test ($P = 0.05$).

>2.70, respectively (5). Strain CCT had an unusually low 12:0/14:0 ratio, but normal 16:0/12:0 and 16:1/18:1 ratios compared to *E. c. carotovora* reference strains. Consistent with the report of De Boer and Sasser (5), the percentages of dodecanoic acid were low for *E. chrysanthemi* reference strains SR80 and SR81, which produced low 12:0/14:0 and high 16:0/12:0 ratios (Table 4). Standard fatty acid ratios for strains of *E. c. betavasculorum* are not available (Sasser, *personal communications*), but our data are provided for future comparisons. Based on the results of biochemical tests, the tolerance to high temperatures, and cellular fatty acid compositions, we conclude that the stem rot pathogen encountered at The Land was *E. c. carotovora.*

Stem rot symptoms developed most rapidly on the Cruciferae. Stem or petiole tissue at the point of inoculation became water-soaked and collapsed within 3 days following plant inoculation. Foliar wilt appeared to be the result of the maceration of parenchyma tissues within the stem or petiole cortex, interfascicular zones, and nonconducting vascular regions, rather than the result of the occlusion of vascular bundles.

Symptomatic tissues on several plant species within the Cucurbitaceae developed large quantities of bacterial ooze. Plant foliage often wilted before stem maceration was observed externally. When stems were longitudinally dissected at 14 days, vascular browning was observed up to 90 cm acropetal to inoculated leaf axils, but it did not extend more than a few centimeters basipetal to inoculation sites. In advanced stages of the disease, tissues within the entire cross-section of the stems decayed and collapsed. However, symptoms on Chinese bitter melon (*Momordica charantia* L. 'Park 5188') and luffa gourd (*Luffa aegyptiaca* Mill.) were limited to slight discoloration of internal stem tissues at the sites of inoculation. Pole bean was the only legume used in the host-range experiment, and symptoms were not observed in any of the inoculated stems.

Diseased tissues of species within the Solanaceae did not produce bacterial ooze. The main stems of peppers (*Capsicum annuum* L. 'Early Thickset') developed black, dry necrotic lesions at the points of inoculation, usually resulting in the breakage of lateral branches at the inoculated node. Stem necrosis in peppers usually did not extend into pith tissues. In contrast, the inoculation sites on tobacco plants (*Nicotiana tabacum* L. 'Coker 48') developed black, water-soaked necrotic lesions. Black vascular bundles extending several centimeters above the inoculation sites were observed in dissected stems of tobacco. Some of the inoculated tobacco plants developed chlorosis and wilt. In advanced stages of the disease, pith tissues degenerated resulting in hollow stalks. Symptoms were similar to those described for tobacco cultivars infected with *E. c. carotovora* (15). Symptoms on tomato (*Lycopersicon esculentum* Mill. 'N-65') were similar to those observed on tobacco, except that pith necrosis and the development of a hollow stalk were not observed.

Disease-severity values derived from the host range (Table 5) did not always correlate with the natural occurrence of disease in the greenhouses. For example, the crucifers broccoli, cauliflower, Chinese cabbage, kale, kohlrabi, and pak choi received the highest disease-severity values in the host range, with most of the inoculated plants exhibiting wilt symptoms during the experiments. However, broccoli and kohlrabi typically failed to develop stem rot in greenhouses under standard horticultural practices. Both broccoli and kohlrabi grow with leaves erect; thus, the leaves do not contact the drip-irrigated sand beds. Therefore, plant wounding was reduced, and the petioles were less likely to be in contact with the sand. Togashi and Sakamoto (22), working with soft rot pathogens of Chinese cabbage, concluded that soil contact of the petiole at the wrapping stage increased the multiplication of *Erwinia* species and caused an increase of disease.

In another example, winter squash and radish (*Raphanus sativus* L. 'Cherry Belle') had similar disease-severity ratings at all inoculum densities tested (Table 5). However, devastating losses of winter squash frequently were observed during 1983 and 1984; and no outbreaks of stem rot were observed on radish, even though radishes were grown as ground covers below trellised winter squash plants. Observations of the horticultural practices used in the squash and radish intercropping suggested that the key difference between the two crops was the degree of wounding of the plants. Winter squash plants were hand-twisted up plastic strings, fastened with clips, their lateral stems removed, and the vines pruned heavily for aesthetic reasons; whereas radish plants were never pruned during their cropping cycle. Jenkins and Averre (11) demonstrated that wounds were required for infection by *Erwinia* species in hydroponic systems. Cultural practices in greenhouse operations were found to be as critical to reducing disease incidence and severity of *E. c. carotovora* as the relative resistance of host plants.

A primary objective of the host-range study was to determine whether combinations of plant species could be used in The Land that would reduce the occurrence of severe epidemics. Thus, a seasonal crop rotation program was developed that has proven effective in suppressing outbreaks of stem rot caused by *E. c. carotovora.* In particular, the growing season for crucifer species was limited to the coolest winter months between November and March, and intercropped plots containing cucurbit and crucifer species were eliminated. In addition, the use of crop species that were highly susceptible to the pathogen and to mechanical damage, for example pak choi, was discontinued. Although the pathogen can still be recovered from the drip-irrigated sand beds at The Land, the crop rotation program and the cultural practices described above have limited disease outbreaks since 1985 to a few isolated cases.

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