Erwinia carotovora var. *carotovora* on Bell Peppers in Ohio

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ABSTRACT

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Sources of *Erwinia carotovora* var. *carotovora* (*Ecc*) inocula in bell pepper fields were investigated in relation to postharvest soft rot. *E. carotovora* was found on the roots of field-grown transplants and on the roots and foliage of mature plants. The bacterium overwintered in soil in fields where peppers were rotated with cabbage and potatoes but not with soybeans. In a study on previously untilled soil, greenhouse-grown transplants remained free of *Ecc*, whereas artificially infested transplants retained the bacterium on their roots throughout the summer. High populations of *Ecc* on peduncles of fruits collected at a commercial packing house were associated with high incidence of soft rot. Washing peppers before packing greatly increased the amount of decay. This was reduced by chlorinating the wash water.

Bacterial soft rot of bell peppers (*Capsicum annum* L.), caused by *Erwinia carotovora* var. *carotovora* (*Ecc*), is the most destructive market disease of this crop (1,4,5,14). Soft rot bacteria invade pepper fruits through wounds, especially through peduncles broken during harvesting. Rot starts in the peduncle and calyx tissues, and the entire fruit is reduced to a watery mass within 2–6 days. High moisture content increases susceptibility to soft rot; therefore, the disease is normally reduced by harvesting during dry weather and by keeping the fruit cool and dry during packing and storage (10). In Ohio, however, it often is necessary to harvest during rainy weather, and traditionally peppers are washed before packing. These practices allow bacteria to enter the broken peduncle and predispose it to soft rot by causing watersoaking.

E. carotovora is common on the aerial surfaces of plants (18,19), in the root zone (3,6,8,11,12,15,17), and in soil (7,15). In our initial studies, peppers from certain fields rotted very quickly, even without washing. This suggested that high inoculum levels of *E. carotovora* in these fields might have been an important factor in determining the incidence of postharvest decay. The purpose of this study was to identify sources of primary inoculum in the field before harvest and during postharvest handling.

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MATERIALS AND METHODS

Media and characterization of strains. Bacterial cultures were maintained on L agar (13) and stored in 40% (v/v) glycerol at −20 C. Pectolytic bacteria were isolated from soil, and plant material on crystal violet-pectate agar (CVP) with 0.1% sodium dodecyl sulfate was added (6,16).

Tests for identifying soft rot *Erwinia* spp. were performed as described by Graham (9). The medium used for lactose, maltose, and trehalose utilization tests consisted of 1.0% Bacto peptone, 1.0% test-sugar, 0.005% bromthymol blue, and 1.5% agar in distilled water. Utilization of α-methylglucoside was tested on a medium developed by Phillips (16). Reference strains *E. carotovora* var. *atroseptica* SR8, *Ecc* SR206, and *E. chrysanthemi* SR120A were obtained from A. Kelman, University of Wisconsin, Madison.

Isolation from soil and plant tissue. Overwintering of *E. carotovora* in commercial pepper fields was investigated with the soil enrichment technique of Meneley and Stanghellini (15). A composite sample of 10–15 cores was taken from an area of about 1,350 m^2. Sodium polypectate (No. 6024, Orange Products Div., SunKist Growers, Ontario, CA 91761) was used without further purification. Enrichment cultures were incubated in a BBL GasPak anaerobic system (Becton, Dickinson, and Co., Cockeysville, MD 21030) for 48 hr at 25 C. Serial dilutions were then spread on CVP plates and incubated aerobically.

E. carotovora was isolated from peduncle sections taken adjacent to the fruit. The epidermis and cortex from each of three sections were removed and ground together in 3 ml of 0.15 M NaCl. Serial dilutions of the homogenate were spread on CVP agar. Sections were 1.5 cm long and had mean surface areas of about 5 cm^2. To determine root zone populations, 2 g (wet weight) of small roots and adhering soil were shaken in 10 ml of sterile distilled water for 1 hr. Roots from three plants were combined for each sample. The washings were dilution-plated on CVP agar and incubated anaerobically at 25 C for 2 days. Colonies were examined with a dissecting scope using oblique substage lighting. Several typical, rough, pit-forming colonies were selected from each isolation, purified on L agar, and characterized as described above.

Determination of soft rot potential. Peppers collected from fields and a commercial packing line were held under high humidity at 26 C for 5–8 days to determine the potential incidence of soft rot. Each sample consisted of four replicates of 10 peppers each. Fruits were sprayed to runoff with sterile water, then sealed in polyethylene bags. Peppers were examined daily and removed from the bags as soon as they started to rot. Fruits with visible insect damage were not included in the samples.

RESULTS

Isolation and identification of *Ecc*. Pectolytic bacteria were isolated from rotted peppers from 24 commercial lots; 61 isolates were obtained for further characterization. Isolations were made only from fruits that developed rot within 2–5 days after harvest. All isolates were Gram-negative, nonpigmented, facultative-anaerobic rods; all formed pits on CVP agar, rotted potato slices and pepper fruits, grew at 37 C, and utilized lactose and trehalose but not maltose or α-methylglucoside. Reducing sugars were formed by 29% of the isolates. On the basis of these properties, the bacteria were identified as *Ecc* (2,9). All *Erwinia* strains obtained from soil, roots, and stems were also identified as *Ecc*.

Frequently, peppers not affected by *Ecc* developed a slight peduncle rot that took 10 days or more to progress into the fruit. The rotted tissue fluoresced strongly under ultraviolet light (375 nm), and pectolytic, green-fluorescent *Pseudomonas* spp. were isolated from the tissue. These isolates were not further characterized, since pathogenicity tests on detached fruits indicated that they were not

aggressive enough to be responsible for major storage losses.

Occurrence of *Ecc* on roots and foliage and overwintering in field soil. *Ecc* was detected on stems, leaves, fruits, and roots of 10 of 10 mature pepper plants during September 1977 in plots at the Ohio Agricultural Research and Development Center, Wooster. The plants were from a field that had been rotated with potatoes and *Ecc* was detected in a composite sample of the soil at the time of planting by the enrichment method. Estimation of bacterial populations on stems and roots was difficult because individual plants harbored either very high or very low numbers of *Ecc*. Stem, peduncle, leaf, and fruit prints made on CVP agar indicated that *Ecc* was prevalent on stems and peduncles but was only occasionally found on the waxy surfaces of leaves and fruits. Epidermal and cortical tissues from the peduncle contained 40–300 viable cells per section. Populations in the root zone ranged from undetectable (< 100 cells per gram of soil and roots) to 10^4 cells per gram; the average of seven plants was 5×10^3 cells per gram.

In a study during 1978 on two commercial farms near Sandusky and Huron, OH, field soil and transplants were examined as primary sources of soft rot inocula and *Ecc* populations were monitored during harvest in late August and early October. *Ecc* was detected by a soil enrichment technique in field soil at planting (15 June) in four of five sites previously planted to cabbage and another site rotated with potatoes. *Ecc* was not found in soils from former soybean (nine sites) or pepper (one site) fields (Table 1). At the same time, roots of grassy weeds taken from these fields were plated on CVP agar; *Ecc* was found only on grasses from one former cabbage field. Populations of *Ecc* were high on the roots of one of two lots of field-grown transplants from Georgia, but none could be found on greenhouse-grown transplants from Ohio (three lots) and Florida (one lot). Each lot of transplants was sampled twice. During dry weather in late August of 1978, *Ecc* was not detected in most root and stem samples, even though it had been present in the soil and on transplants at planting (Table 1). After several days of rain in early October, *Ecc* could still not be demonstrated on stems, but root zone populations had increased at all sites on both farms and ranged from 50 to 3×10^5 cells/g of soil and roots. Fruits collected at the Sandusky farm did

Table 1. Presence of *Erwinia carotovora* var. *carotovora* in field soil, on roots of pepper transplants, and on peduncles and roots of mature plants and occurrence of soft rot during 1978

		16 June		30 August			11 October		
Site	Prior crop	Soil[a]	Transplants[a]	Roots[b]	Peduncle[c]	Rot[d]	Roots[b]	Peduncle[c]	Rot[d]
Huron, OH									
1	Cabbage	+	−	+	−	+++	++	++	+++
2	Cabbage	+	−	−	−	−	++	−	−
3	Cabbage	−	−	−	−	++	++	−	++
4	Cabbage	+	−	−	−	−	++	−	++
5	Cabbage	+	−	−	−	−	+++	−	+++
6	Pepper	−							
Sandusky, OH									
1	Soybeans	−	−	−	−	−	++	+++	−
2	Soybeans	−	−	−	−	−	+	−	−
3	Soybeans	−	−	−	−	−	+	−	−
4	Soybeans	−	−	−	−	−	++	−	−
5	Soybeans	−	−	−	−	−	+	−	−
6	Soybeans	−	+	−	−	−	++	−	−
7	Soybeans	−	+	+	−	+	+	−	−
8	Soybeans	−	+	−	−	+	++	−	−
9	Soybeans	−	+	−	−	−	++	+	−
Wooster, OH									
1	Potatoes	+	−				+++	−	−
2	Untilled	−	−				−	−	−
2	Untilled	−	+[e]				+++	−	+++
3	Untilled	−	−				−	−	−
3	Untilled	−	+[e]				++	−	−
4	Untilled	−	−				−	−	−

[a] Data not quantified.
[b] − indicates <50 cells/g of roots and adhering soil; +, 50–100 cells/g; ++, 5×10^2 to 5×10^3 cells/g; and +++, $>10^5$ cells/g.
[c] + indicates 10–100 cells/peduncle section; ++, 10^2–10^4 cells per peduncle section; and +++, $>10^4$ cells per peduncle section.
[d] + indicates soft rot incidence ≤10%; ++, 11–29%; and +++, 30–45%.
[e] Transplants artificially infested.

Table 2. Populations of *Erwinia carotovora* var. *carotovora* on commercial lots of unwashed pepper fruits and incidence of soft rot before and after washing in 1976

	19 August			2 September		
		Rot (%)[b]			Rot (%)	
Commercial grower	Bacteria[a] per peduncle	Unwashed	Washed	Bacteria per peduncle	Unwashed	Washed
A	800	7	95*[c]	60,000	100	100
B	40	0	54*	600	5	60*
C	500	5	95*	10,000	70	95*

[a] Number of bacteria in epidermal and cortical tissues removed from a 1.5-cm peduncle section with an average surface area of 5 cm^2 taken adjacent to the fruit. Mean of two samples of three stems each.
[b] Mean of four samples of 10 fruit each.
[c] *Significantly different from unwashed peppers from same source on same date ($P = 0.05$).

not rot when held under high humidity, but the incidence of fruit rot was slight to moderate at the Huron farm (Table 1).

In a similar study, greenhouse-grown seedlings, transplanted into noninfested soil, remained free of *Ecc*, whereas artificially infested transplants at the same sites maintained populations of *Ecc* over the summer. The land used for this experiment (Table 1, Wooster sites 2–4) had been in sod the previous year and *Ecc* could not be detected in the soil by the enrichment technique. At sites 2 and 3, half of the transplants were infested with *Ecc* by drenching them with a water suspension of bacteria (10^8 cells/ml) before planting. Infested and control plants at the two sites were separated by 50 and 300 ft, respectively, by sweet corn plantings. On 11 October, the mean respective root zone populations of infested plants were 3×10^5 and 4×10^3 cells/g of soil and roots. In contrast, *Ecc* could not be found on control plants at sites 2, 3, and 4.

***E. carotovora* on peppers after harvest.** Peppers from a commercial packer were examined for the presence of *Ecc* on stems and evaluated for soft rot potential before and after washing (Table 2). Samples were obtained from three growers on two dates. In each case, four samples of 20 fruit each were taken from hoppers as they came in from the field, and a corresponding set of samples was taken after washing. The incidence of soft rot in unwashed peppers varied greatly with source and date of harvest and was positively related to the number of soft rot bacteria associated with the peduncles. Washing in water containing 25 ppm chlorine greatly increased the incidence of soft rot on both dates. Although this concentration of chlorine eliminated bacteria from the wash water, it did not completely disinfest the peduncles. Because it was not feasible to increase the concentration of chlorine used on the commercial packing line, we simulated the washing process in the laboratory, using peppers picked from a field with high epiphytic populations of *Ecc*. Fruits were washed and brushed by hand in water containing either 0 or 250 ppm free chlorine. The incidence of soft rot was 93 $\pm$ 6% and 21 $\pm$ 10% for peppers washed in unchlorinated and chlorinated water, respectively.

DISCUSSION

Susceptibility of peppers to bacterial soft rot is increased by high moisture content, high nitrogen fertilization, jagged stem breaks, and hydrocooling (10). Results of this study demonstrate that *Ecc* is frequently found on the stems and roots of pepper plants in Ohio and suggests that high resident populations of *Ecc* in the field can also increase the amount of postharvest soft rot.

Field-grown transplants and overwintering populations in soil were identified as primary sources of *Ecc* inocula. The bacterium overwintered in fields previously planted with cabbage and potatoes but not soybeans. Similar results have been reported by De Boer et al (7), who found that *E. carotovora* overwintered more frequently in Wisconsin fields rotated with potatoes than with beans or corn. In our study on previously untilled soil not containing *Ecc*, greenhouse-grown transplants remained free of the pathogen, whereas infested transplants maintained the bacterium on their roots throughout the summer. These findings suggest that, in connection with other control measures, rotation of peppers with agronomic crops such as corn and soybeans and the use of *Ecc*-free transplants may be beneficial in controlling soft rot. The appearance of *Ecc* on roots of pepper plants at all sites on the Sandusky farm in 1978 may reflect either our inability to detect very low and possibly nonuniform overwintering populations of the pathogen in soil or secondary spread by insects, machinery, and workers.

In a study of seasonal variation in populations of *Ecc* on leaf surfaces of tobacco, Tsuyama (19) reported that soft rot bacteria spread rapidly from roots to aerial parts of the plant during periods of high humidity. In our studies, the highest epiphytic populations were noted on sampling dates (Table 1, 11 Oct., and Table 2, 2 Sept.) that were preceded by several days of rain. The 1978 growing season was exceptionally dry. As a result, stem populations were low and postharvest decay problems were minimal. On the basis of our observations and those of Tsuyama (19), it is likely that *Ecc* survives in the root zone of pepper plants during the summer and then moves to the foliage at the onset of rainy weather in late September. This period, when soft rot is most severe, also coincides with the decay of unmarketable fruits that have been left on the ground during earlier havestings. These fruits accumulate and by the end of September may be an important source of secondary inoculum. We are therefore uncertain whether the peduncle populations obtained in late September are the result of actively multiplying epiphytic *Ecc* populations or simply contamination of the foliage by bacteria from decaying fruits.

After harvest, peppers are stored in large hoppers, then washed and packed. The environment in these containers is frequently warm, humid, and ideal for multiplication of *Ecc*. Fruits may also become contaminated by contact with other partially rotten peppers. These factors may have contributed to the high populations of *Ecc* on peduncles of peppers in the packing house (Table 2).

In an initial visit to the packing house, we found that wash water on the packing line was not chlorinated and contained 10^6 cells of *Ecc* per milliliter. Addition of 25 ppm free chlorine eliminated bacteria from the wash water but did not decrease soft rot incidence. This may have been because watersoaking of broken peduncle tissues during washing made the fruit more susceptible to soft rot (10) and because this concentration of chlorine did not disinfest the peduncle. In laboratory tests, increasing the chlorine concentration to 250 ppm decreased the incidence of rot by disinfesting the surface of the stem, but enough bacteria were present internally that the treatment did not completely offset the adverse effect of washing.

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