

Soil and Seed Tubers as Sources of Inoculum of *Erwinia carotovora* pv. *carotovora* for Stem Soft Rot of Potatoes

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ABSTRACT

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To determine the relative importance of soil strains and seed tuber strains of *Erwinia carotovora* pv. *carotovora* (Ecc) as primary inoculum for potato plant infection in the field, a soil enrichment technique and a modification of the tuber incubation method were used to isolate Ecc strains from soil samples and seed tubers, respectively. The strains were characterized by the double diffusion method. Tubers from three Russet Burbank seed lots were naturally contaminated with Ecc, and all strains recovered from the seed tubers were serologically different from those isolated from soil. Ecc strains recovered from potato stems during the growing season were identified serologically with antisera produced against seed tuber strains and soil

strains. In 1980, seed tuber serogroups were obtained most frequently in isolations from symptomless plants in two of three experiments. In two experiments during 1981, 11 and 15% of the strains isolated from symptomatic stems were serogroups characteristic of soil strains, whereas 14 and 4%, respectively, were serogroups characteristic of seed tuber strains. A majority of the strains isolated from stems in three of five experiments were different from the known soil or seed tuber serogroups. Other sources of inoculum, such as irrigation water, may be involved in the stem soft rot disease.

Additional key word: blackleg.

Potato plants with blackleg symptoms are found wherever potatoes are grown and frequently occur in the center-pivot-irrigated fields in the Pacific Northwest. In this area, however, stem soft rot is a more common symptom than typical blackleg (17). Both *Erwinia carotovora* pv. *carotovora* (Jones) Bergey, Harrison, Breed, Hammer and Huntoon (Ecc) and *E. carotovora* pv. *atroseptica* (Van Hall) Dye (Eca) can cause blackleg under field conditions (14, 17, 18). The symptom produced by Eca is typically a black, wet rot of the stem. Occasionally, Ecc causes blackleg symptoms that are indistinguishable from those caused by Eca. Ecc is the primary causal agent of stem soft rot in Oregon (17), and symptoms vary. Stems are light tan to dark brown and appear translucent and watery.

Both the soil (2, 17) and seed tubers (7, 16) have been implicated as sources of primary inoculum of Ecc. However, epidemiological studies aimed at identifying the primary inoculum responsible for plant infection have been hampered by difficulty in identifying strains of Ecc and Eca. A recent serotyping technique developed by De Boer et al (5) has allowed rapid classification of bacterial strains into serogroups. By means of the Ouchterlony double diffusion method (15), *E. carotovora* strains were originally classified into 18 serogroups (5). Additional strains have been identified, and according to De Boer (*personal communication*), there are now 40 different serogroups of *E. carotovora*.

We used the serotyping scheme developed by De Boer et al (5) to identify naturally occurring soil and seed tuber strains of Ecc. Ecc strains recovered from potato stems were also serotyped to determine if they were of soil or seed tuber origin.

MATERIALS AND METHODS

Soil isolations. In 1980, soil samples were collected 2 mo before planting from three agricultural fields; in 1981, samples were collected from two fields 1 wk before planting and 10 wk after planting. The samples collected from a depth of 3–20 cm were

placed in plastic bags and stored at 5 C until processed. About 4 g of soil was placed in a 40-ml vial, then soil enrichment pectate medium (13) was added to fill the vial. The vials were capped with Parafilm, incubated at 22 C for 2 days, then serially diluted in sterile water and plated on crystal violet pectate medium (CVP) (3) to determine the presence of *E. carotovora*. Biochemical and serological tests were performed on all strains.

Seed tuber isolations. For the 1980 and 1981 studies, symptomless potato seed tubers (cultivar Russet Burbank) weighing 100–200 g each were selected from three seed lots (80A, 81A, and 81B) that had been grown and certified in Montana the previous year. A modification of the tuber incubation method proposed by De Boer and Kelman (7) was used to detect the presence of *E. carotovora* on the seed tubers. The tubers were rinsed to remove soil, punctured 10 times with sterile toothpicks, wrapped individually in moist paper toweling, sealed in two plastic bags, and incubated at 24 C for 4 days. The tubers were then examined for soft rot pockets. Small sections of tissue dissected from the advancing margin of the rot were suspended in 1 ml of sterile water, and aliquots of the suspension were streaked onto CVP. The isolated *E. carotovora* strains were characterized biochemically and serologically.

Field plots. In 1980, plots were established in two center-pivot-irrigated fields in the Columbia Basin (plots EF3 and RF) and in a solid-set-sprinkler-irrigated field in the Klamath Basin of Oregon (plot KF). Seed tubers from seed lot 80A were cut into seed pieces weighing 42–57 g, and the seed pieces were suberized at 13 C for 2 wk before planting. On 6 April, seed tubers were planted in an area 35 × 5 m at the two locations in the Columbia Basin. Rows were 86 cm apart and between-plant spacing was 23 cm. On 4 June, a plot of similar size was seeded at the same rate at the Klamath Basin location. Plot RF was located in a field previously cropped three times to potatoes, and plot EF3 was located in a field that had never been cropped to potatoes. Plot KF had previously been cropped eight times to potatoes. Potatoes in the KF and RF plots had been grown in rotation with winter wheat, whereas alfalfa had been grown for 6 yr in the EF3 plot on previously native desert land. None of the fields was irrigated before planting.

In 1981, two center-pivot-irrigated commercial fields (48.5 ha each) in the Columbia Basin were chosen for test plots. One (EF 51)

was planted with seed lot 81A on 25 March and the other (EF 10) was planted on 6 April with seed lot 81B. Both fields had a row spacing of 86 cm and a plant density of about five hills per meter of row. Both fields had been cropped to potatoes in 1977 and 1979, with winter wheat planted in alternate years. Neither field was irrigated before planting, and both were irrigated the first time during the first week of May.

Plant isolations. In 1980, potato stems were collected triweekly beginning in early June at the RF plot, in late June at the EF3 plot, and in late July at the KF plot. In the laboratory, isolations were made from ground-line stem sections 10 cm long. Each segment was washed in running tap water and surface-sterilized by a 3-min immersion in 0.5% sodium hypochlorite. A section 1 cm long was removed from the middle of the segment and coarsely dissected into pieces that were suspended in 1 ml of sterile distilled water. After incubation at 22 C for 30 min, the suspension was agitated and aliquots were streaked onto CVP to determine the presence of *E. carotovora*. Strains isolated were biochemically and serologically characterized.

In 1981, 20–40 plants with stem or petiole soft rot symptoms were collected biweekly from a different area (about 55 × 21 m) within each field beginning in mid-June. Plant samples were assayed for *E. carotovora* and subsequent isolated strains were characterized biochemically and serologically.

Antisera production. Antisera to strains from seed tubers and soil were produced in rabbits against whole, glutaraldehyde-fixed bacterial cells. The bacterial cells were grown on casamino acid-peptone-glucose agar (6) at 24 C for 48 hr, then the cells were suspended in a 0.013 M phosphate buffer saline solution at pH 7.2. The cells were centrifuged and resuspended in fresh buffer three times, then fixed in glutaraldehyde at a final concentration of about

10⁹ cells per milliliter (1). Rabbits were given seven intramuscular injections of a 0.5-ml cell suspension emulsified with an equal volume of Freund's incomplete adjuvant at weekly intervals. The rabbits were bled from the marginal ear vein weekly after the fourth injection. Antisera titers were determined by the immunodiffusion method. Additional intramuscular injections were given when necessary to maintain high antibody titers. The sera were stored frozen; when in use, sera were kept at 5 C with thimerosal (1 µg/ml) added as a preservative.

Biochemical and serological characterization. All strains of *E. carotovora* were maintained on sugarless nutrient agar slants throughout the study. Eca strains were distinguished from Ecc strains by the production of acid from α-methylglucoside (8) and absence of growth at 36 C. All strains were tested for ability to cause soft rot of potato tuber slices.

Agar plates for double diffusion assay tests were prepared with wells (3 mm in diameter and 4 mm apart) cut in sets of six surrounding a center one (5). Unknown and homologous Ecc strains were grown at 24 C for 24 hr on sugarless nutrient agar slants. The cells were harvested in 0.5 ml of sterile distilled water, and 10 µl of liquefied phenol was added to the cell suspension. The suspensions were then vigorously mixed on a vortex mixer to solubilize cell wall antigens. The center well was filled with an antiserum produced against either a seed or a soil Ecc strain, and two wells on opposite sides of the center well were filled with the homologous cell suspension. The remaining four wells were filled with unknown cell suspensions of Ecc strains. The plates were incubated at 24 C for 48 hr, then examined for precipitin bands.

Ecc strains recovered from the seed tubers and the soil to which antiserum was produced were serologically identified by S. H. De Boer, Agriculture Canada, Vancouver Research Station.

RESULTS

TABLE 1. Serogroups of *Erwinia carotovora* pv. *carotovora* isolated from the soil in 1980 and 1981 experimental plots

Year	Location	Number of soil samples tested	Serogroups
1980 ^a	EF3	12	0
	RF	12	XXIX
	RF	12	0
1981	EF 10 preplant ^b	12	XXXV
	EF 51 preplant ^b	20	XXXV
	EF 10 postplant ^c	10	XXXVII
	EF 51 postplant ^c	10	IV, VIII, XXVIII

^a Sampled 2 mo before planting.

^b Sampled 1 wk before planting.

^c Sampled 10 wk after planting.

TABLE 2. Number of tubers contaminated by *Erwinia carotovora* pv. *carotovora* (Ecc) serogroups among Ecc strains isolated from symptomless seed tubers from three seed lots

Serogroup	Seed lot 80A ^a		Seed lot 81A ^b		Seed lot 81B ^c	
	No. of tubers	No. of strains	No. of tubers	No. of strains	No. of tubers	No. of strains
III	1	1	7	13	0	0
V	32	68	3	5	1	2
VII	0	0	1	2	0	0
XI	0	0	2	2	0	0
XV	29	44	4	7	0	0
XVIII ^d	0	0	3	3	0	0
XXXIII	0	0	3	3	0	0
XXXVI	0	0	16	31	1	1
Untyped	0	0	6	10	0	0
Total	62	113	45	76	2	3

^a 68 tubers tested.

^b 87 tubers tested.

^c 78 tubers tested.

^d An *E. carotovora* pv. *atroseptica* serogroup, but initially some Ecc strains typed into it (5).

Soil strains. In 1980, Ecc was recovered from five of 12 soil samples taken before planting at one plot (RF), and one of the strains was identified as serogroup XXIX (Table 1). In 1981, Ecc was detected in at least 60% of the preplant soil samples taken at both locations. All seven strains from EF 10 and 13 of the 14 strains from EF 51 were characterized as serogroup XXXV. Ecc was recovered in 40% of the postplant soil samples at both locations. At EF 10, one strain of four was identified as serogroup XXXVII. In contrast, three of the four soil strains at EF 51 were identified as IV, VIII, or XXVIII. Serogroup XXXV was not detected in the postplant soil samples. Five of the soil strains remained unclassified serologically.

Seed tuber strains. Seed tubers from two of the three seed lots were frequently contaminated with Ecc (Table 2). Of the 68 seed tubers sampled in the 1980 study, 91% yielded Ecc strains. In 1981, 59 and 3% of the tubers from seed lots 81A and 81B, respectively, were contaminated with Ecc. In all seed lots, the predominant *E. carotovora* pathovar isolated was Ecc. Eca was recovered from only two tubers in seed lot 80A and was not detected in the 1981 seed lots.

All the Ecc strains recovered from seed tubers in 1980 were classified in three serogroups (Table 2). Most were contaminated with only one serogroup but 10% were contaminated with both V and XV. In 1981, 87% of the Ecc strains from seed lot 81A were typed serologically; serogroups III, XV, and XXXVI occurred most frequently, with XXXVI representing 41% of the strains. Although serogroup XXXVI was isolated eight times from tubers contaminated with other known serogroups, combinations of other known serogroups on the same seed tuber rarely occurred. Two strains from one tuber in seed lot 81B were both serogroup V; a second tuber was contaminated with bacteria of serogroup XXXVI.

Plant strains. Although typical blackleg or stem soft rot symptoms were not observed on any of the basal stems in 1980, Ecc was isolated from 14% of the stems sampled. The soilborne serogroup XXIX was not isolated from stems in the RF plot (Table 3) but was recovered from 13% of the stems in the EF3 and KF plots, where Ecc was not detected in the soil before planting. The

seed tuber serogroup V was recovered from plants from all three plots, whereas XV was obtained from only two, RF and KF (Table 4). At the RF and KF plots, 88 and 73%, respectively, of the stem strains were the same serogroups as the seed tuber strains, whereas at EF3, only 9% of the stem strains were the same serogroups as the seed tuber strains (Table 5).

In 1981, Ecc was the only pathovar of *E. carotovora* recovered from symptomatic stems. At the site planted with seed lot 81A (EF 51), 11% of the stem strains were serologically identical to the soil serogroup (Tables 3 and 5). A similar trend (15%) was observed at the second site (EF 10). Although the soilborne serogroup XXXVII was not detected in soil samples at site EF 51, 11% of the strains recovered from diseased plants were this serogroup. Likewise, 1 and 6%, respectively, of the strains recovered from stem isolations at site EF 10 were serogroups IV and VIII, neither of which was detected in soil samples. Seed tubers from seed lot 81A were contaminated with several serogroups, but serogroup VII was involved more frequently in plant infection than the other known seed tuber serogroups (Table 4). At the site planted with this seed lot (EF 51), 14% of the strains recovered from diseased tissues were characteristic of serogroups isolated from seed tubers (Table 5). Seed tubers of seed lot 81B were rarely contaminated with Ecc, and of the two serogroups recovered from the seed tubers, only serogroup V was associated with plant infection (Table 4). Only 4% of the strains recovered from symptomatic plants at this site (EF 10) were the same serologically as this seed tuber serogroup (Table 5).

DISCUSSION

Some strains of Ecc recovered from soil samples were serologically the same as the strains obtained in potato stem isolations. The association of soil serogroups with stem soft rot symptoms suggests that the soil can be an important source of inoculum. Maher et al (9) reported that serogroup XXIX, detectable in the soil before potatoes were planted, was obtained frequently in stem isolations during the season and was the predominant serogroup isolated from daughter tubers at harvest. In our study, seed tuber serogroups of Ecc were the predominant serogroups recovered from stem isolations in two of the three plots in 1980. In these two plots (RF and KF), Verticillium wilt was widespread. In contrast, plants at the EF plot did not show symptoms. Plants in fields with Verticillium wilt die prematurely, whereas the canopy in noninfested fields remains lush throughout the growing season. Consequently, the microclimate in the Verticillium wilt fields may have been more conducive to infection by seed tuber strains than by soil strains. In 1981, seed tuber serogroups were isolated less often than soil serogroups from symptomatic stems and petioles. Nevertheless, the findings suggest that seed tubers contaminated with Ecc can also serve as an inoculum source. De Boer (4) reported supportive evidence that contaminated planting stock can serve as an Ecc inoculum source. Tubers were artificially contaminated with cells of an Ecc serogroup not previously found in British Columbia; 10 wk after planting, the same serogroup was recovered from all sampled foliage, roots, and daughter tubers. Thus, the source of primary inoculum of Ecc for potato plant infection may be the soil or the seed tuber or both.

Not all the known soil serogroups and seed tuber serogroups were involved in plant infection. For example, in the 1981 study, two soil strains, serogroups XXXV and XXVIII, were never recovered from stem isolations. Similarly, serogroups III and XXXVI, two of the most prevalent seed tuber serogroups in seed lot 81A, were not recovered from symptomatic stems. In another study conducted the following year in which seed tubers contaminated with serogroup III were used, this serogroup was frequently recovered from diseased stems and petioles (M. L. Powelson and J. D. Apple, unpublished). Similar results were reported by De Boer et al (5). Certain serogroups, eg, III, IX, XI, and XVI, were isolated from potatoes in British Columbia, whereas others were isolated rarely or not at all. The environmental factors governing the amount of inoculum required for a specific Ecc serogroup to cause disease under field conditions are unknown. Many Ecc serogroups

are capable of causing disease in potatoes, however, and these may originate from different sources.

Of the Ecc strains obtained from stem or petiole isolations made during the course of our study, 57% were serologically different from the seed tuber and soil strains to which antiserum was produced. Although Ecc was not detected on all the seed tubers evaluated or in all the soil samples assayed, the methods used may favor the detection of a restricted number of serogroups because only the most virulent ones or the most numerous ones are likely to predominate. In addition, different serogroups may predominate under different soil environments. The temperature of tuber incubation influences the recovery of *E. carotovora* pathovars (16).

TABLE 3. Soil serogroups represented among *Erwinia carotovora* pv. *carotovora* strains isolated from symptomless plants at three locations in 1980 and from symptomatic plants at two locations in 1981

Serogroup	Number of strains				
	1980 fields ^a			1981 fields	
	EF3	RF	KF	EF 51 ^b	EF 10 ^c
IV	0	...
VIII	13	...
XXVIII	0	...
XXIX	...	0
XXXV	0	0
XXXVII	15
No. of strains tested	103	40	30	121	97
No. of stems sampled	595	260	340	121	97

^aPlanted with seed lot 80A.

^bPlanted with seed lot 81A.

^cPlanted with seed lot 81B.

^dNot applicable, as serogroups not detected in soil samples.

TABLE 4. Seed tuber serogroups represented among *Erwinia carotovora* pv. *carotovora* strains isolated from symptomless plants at three locations in 1980 and from symptomatic plants at two locations in 1981

Serogroup	Number of strains				
	1980 fields ^a			1981 fields	
	EF3	RF	KF	EF 51 ^b	EF 10 ^c
III	0	0	0	0	...
V	9	27	19	1	4
VII	12	...
XI	3	...
XV	0	8	3	0	...
XVIII	0	...
XXXIII	1	...
XXXVI	0	0
No. of strains tested	103	40	30	121	97
No. of stems sampled	595	260	340	121	97

^aPlanted with seed lot 80A.

^bPlanted with seed lot 81A.

^cPlanted with seed lot 81B.

^dNot applicable, as serogroups not detected on seed tubers.

TABLE 5. Percentage of *Erwinia carotovora* pv. *carotovora* strains isolated from potato stems that were characterized as soil serogroups, seed tuber serogroups, or untyped strains

Year	Location	Percent		
		Soil serogroups	Seed tuber serogroups	Untyped strains ^a
1980	EF3	...	9	91
	RF	0	88	12
	KF	...	73	27
1981	EF 51	11	14	71
	EF 10	15	4	85

^aStem strains serologically different from soil and seed tuber serogroups.

^b*E. carotovora* pv. *carotovora* not detected in soil samples.

At 25 C, Ecc was recovered more frequently than Eca from tubers artificially infested with equal numbers of both organisms. Similarly, at 24 C, Ecc was the predominant pathovar isolated from the rhizosphere after enrichment (10,13).

Ecc has also been detected in the rhizosphere of many cultivated (10,13) and noncultivated plants (2,12). McCarter-Zorner et al (12) reported that Ecc was the predominant organism recovered from weed species in both Scotland and Colorado and hypothesized that these rhizosphere inhabitants may be a source of inoculum for recontamination of *Erwinia*-free seed stocks. Serological techniques should help establish the significance of rhizosphere populations of Ecc with respect to current-season stem soft rot and blackleg of potatoes.

Inoculum of Ecc may have been introduced into the crop during the growing season in irrigation water. Ecc has been isolated frequently from lakes, reservoirs, rivers, canals, and creeks in the United States and Great Britain (11). In 1981 (M. D. Harrison, *personal communication*) and 1982 (M. D. Harrison, *personal communication*, and M. L. Powelson and J. D. Apple, *unpublished*), Ecc was recovered from the Columbia River, the major source of irrigation water for the Columbia Basin production area. A contaminated water supply may explain, in part, the presence of different serogroups of Ecc in soil samples after irrigation. The effectiveness of this inoculum in inciting disease under field conditions is currently being investigated.

A knowledge of the importance of different sources of inoculum of Ecc and their effectiveness in inciting disease under different environments and management practices is essential if disease tolerances for blackleg and stem soft rot are to be included in seed certification standards. This paper presents evidence that both soilborne strains and seed tuber strains of Ecc can cause disease symptoms under field conditions.

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