Pectolytic Erwinia spp. in the Root Zone of Potato Plants in Relation to Infestation of Daughter Tubers

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ABSTRACT

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Populations of pectolytic *Erwinia* spp. in the root zones of potato plants (cultivar Sebago) grown from *Erwinia*-infested seed pieces were assayed at periodic intervals during two growing seasons. Populations of *E. carotovora* usually were not detected until seed pieces decayed, at which time high populations were observed; thereafter, populations were variable until tubers were harvested. The maximum number of cells of *E. carotovora* per gram dry wt of roots plus soil detected in root zone samples was 3×10^8. Cells of *E. carotovora* also were detected in soil sampled between plants, but in smaller numbers than in the root zone. Although few plants showed symptoms of blackleg during the growing season, almost all tubers were infested with *E. carotovora* at harvest. Both *E. carotovora* var. *carotovora* and *E. carotovora* var. *atroseptica* were isolated consistently from the root zones of growing plants and from tubers before harvest and in storage. During a 9-mo storage period at 5 C the percentage of tubers from which *E. carotovora* could be isolated decreased from about 90% to 33%. Certain daughter tubers produced on *Erwinia*-free Russet Burbank and Sebago stem cuttings planted in field plots became infested with *E. carotovora* (5-80%) by the time of harvest. With one exception, the strains obtained in isolations from the tubers on a crystal violet pectate medium were characterized as *E. carotovora* var. *carotovora*. However, when a specific fluorescent antibody stain procedure was used, cells of *E. carotovora* var. *atroseptica* were detected in 9/40 of these tubers.

Additional key words: bacterial soft rot.

In the United States the blackleg disease of potatoes *(Solanum tuberosum* L.) which is caused by *Erwinia carotovora* var. *atroseptica* (van Hall) Dye initially was considered to be borne mainly by seed tubers (15, 27, 28, 34, 35). With the development of seed certification agencies in North America, questions arose as to the validity of this assumption. It was difficult to explain why the incidence of blackleg in progeny of seed tubers from ostensibly healthy plants was, in some instances, equivalent to that observed in progeny from plants with symptoms of blackleg (2). Following the publication of Leach's papers on survival of the blackleg pathogen in soil and his classic studies on its transmission by the seed corn maggot (21, 22, 23), a general consensus developed in the United States that the primary inoculum for production of blackleg and soft rot diseases of potato was a resident population in the soil.

In contrast to the situation in the United States, Graham (10) and Perombelon (31, 32) in Scotland and Logan (24) in Ireland concluded that the blackleg pathogen generally does not overwinter in soil. Under some conditions it overwinters in plant debris (9, 10, 24). Cells of *E. carotovora* were shown to survive readily in storage, mainly in tuber lenticels (20, 29, 31, 40, 41). Recent studies in the United States (7, 26, 30) also provide evidence that certified seed potatoes may carry *E. carotovora* in lenticels. Furthermore, preliminary results with *Erwinia*-free stem cuttings in a virus-free seed potato program in Montana indicate that recontamination of tubers from the soil does not occur readily in some localities (37). It seems likely, therefore, that daughter tubers become infested with *E. carotovora* moving from the seed piece into the root zone.

In Scotland, Perombelon (33) reported detection of *E. carotovora* in rhizospheres of ostensibly healthy potato plants; similar findings in the United States were reported by De Boer et al (7) and more recently by Burr and Schroth (4). Pectolytic *Erwinia* spp. also have been isolated from the rhizospheres of several cultivated crop plants and weeds (4, 5, 16, 17, 19, 25, 39).

The objective of this investigation was to examine the population dynamics of pectolytic *Erwinia* in the root zone of potato plants during the growing season in

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Wisconsin. A preliminary report has been presented on this research (7).

The designations *E. carotovora* var. *atroseptica* (van Hall) Dye (*Eca*) as causal agent of blackleg of potato and *E. carotovora* var. *carotovora* (Jones) Dye (*Ecc*) as causal agent of bacterial soft rot of potato, as defined in Bergey's Manual (3) are used in this paper. When the bacterial variety was not identified or when both possibly were involved, reference is made to *E. carotovora* without variety designation.

MATERIALS AND METHODS

Field plots.—In 1972, seed potato tubers (cultivar Sebago) from two different Wisconsin farms were planted in adjacent rows 90 cm apart and with 30 cm between plants in a plot of sandy loam soil at the University of Wisconsin Experimental Farm at Hancock, Wisconsin. The same plot was planted with Sebago tubers from two other Wisconsin farms in 1973. Four rows (7 m in length) were planted with seed pieces from each seed source and an 8-m-wide cultivated area was left between the two seed sources. Another plot in which no potatoes had been grown for at least 27 yr was planted in the same manner.

In 1974, certified Russet Burbank tubers obtained from Montana and certified Sebago tubers from Wisconsin were grown in the greenhouse and used to make stem cuttings. Eighty cuttings of each cultivar were planted in the same plot in which studies were made in 1972 and 1973 and 80 of each in one additional plot in which no potatoes had been grown for 5 yr. In 1975, certified Russet Burbank tubers from Wisconsin were used to make 200 stem cuttings of which 100 were planted in the same plot used in 1972-1974 and 100 in an additional plot in which no potatoes had been grown for 5 yr.

Root zone samples.—Initial potato root zone samples, taken shortly before plants had emerged, consisted of soil that adhered to the seed piece and roots that had formed. Later samples consisted only of roots plus any soil that adhered after shaking the uprooted plant. Although isolations were made only from roots of apparently-healthy plants selected at random, the seed pieces sometimes showed symptoms of decay or had become decayed prior to sampling. Roots were cut into short pieces (1-2 cm) and a sample (15-30 g of soil plus roots) was weighed and shaken in a 500-ml bottle with 250 ml of sterile distilled water (SDW) for 30 to 60 min. The resulting suspension was serially diluted and plated in duplicate on the crystal violet pectate medium (CVP) described by Cuppels and Kelman (5). Plates were incubated for 3 days at about 21 C before *E. carotovora* and total number of colonies were counted. The bacterial populations were calculated on the basis of number of colony-forming units/g dry wt of soil and root material. Dry weights were determined after drying 25-g samples at 105 C for 24 hr. Since the samples included both roots and rhizosphere soil, the inclusive designation "root zone" is used.

Soil samples.—Cores of soil 2 cm in diameter and 20 cm long were taken with an Oakfield soil sampling tube (Soiltest, Inc., Evanston, IL 60202). In the absence of plants, cores were taken at random. When plants were present, cores were taken about 15 cm away from a plant. Four cores were combined to form one sample. A subsample (about 25 g) was added to 250 ml of SDW in a 500-ml bottle. After the suspension had been shaken for 30 to 60 min, serial dilutions were plated on CVP medium and bacteria counts were made as described above.

Tuber samples.—The presence of *E. carotovora* in seed and daughter tubers was determined by the tuber incubation method in which tuber lenticels were punctured and tubers were wrapped in moist paper towels and polyvinylidine film (Saran Wrap) (7). After incubation for 3-5 days at 20 C isolations were made from decayed tissues on CVP medium.

Characterization of Erwinia spp.—Cultures of *E. carotovora* isolated on CVP medium were restreaked and typical colonies were selected for stock cultures and stored on nutrient agar slants at 4 C. All strains were checked for ability to cause soft rot on potato slices.

Four tests were used to distinguish *Eca* from *Ecc*: (i) acid production from α-methyl glucoside (11), (ii) production of reducing substances from sucrose (11), (iii) growth at 36 C, and (iv) reaction with a fluorescent antibody stain. For acid production, cultures were grown for 72 hr in 1% Bacto-peptone broth plus 1% α-methyl glucoside (filter-sterilized). The change in color of the bromthymol blue indicator from green to yellow indicated acid production. For production of reducing substances, cultures were incubated for 48 hr in 1% Bacto-peptone broth plus 4% sucrose (filter-sterilized). One ml of the culture then was added to 5 ml of Benedict Qualitative Solution (Fisher Scientific Co., Chicago, IL 60651) and boiled for 5 min. The formation of a green or orange precipitate indicated that reducing substances had formed. For growth at 36 C, cultures were spotted with a fine needle on CPG medium (1 g casamino acids, 10 g Bacto-peptone, 10 g glucose, 18 g agar, and 1 liter of distilled water) and incubated at 36 C. Cultures able to grow at 36 C produced colonies 1-2 mm in diameter in 48 hr. The reaction of a strain with the fluorescent antibody stain (FAS) specific for *Eca* was determined following the procedure described by Allan and Kelman (1).

For additional confirmation, the pathogenicity of 18 randomly chosen cultures was evaluated by inoculation of potato plants and comparisons were made with data obtained in the above tests. Plants were grown in steamed soil in 12.5 cm-diameter plastic pots in a growth chamber at 90% relative humidity and 19 C with a 12-hr photoperiod and a light intensity of 32,280 lux. When plants were 20 cm high, eight plants per culture were inoculated by puncturing the stem at the lowest leaf axil with toothpicks smeared with bacteria from 48-hr plate cultures. Toothpicks were left in place during the incubation period. Visual assessment of symptoms of blackleg were made at daily intervals for 9 days.

RESULTS

Field plot 1972.—Although the level of seed-tuber infestation with *E. carotovora* was not determined prior to planting, soft rot was observed in a few seed tubers from one farm (source A); only apparently healthy tubers were used for planting, however. Seed tubers from another farm (source B) had no soft rot at time of planting. Fifteen plants from each seed source were

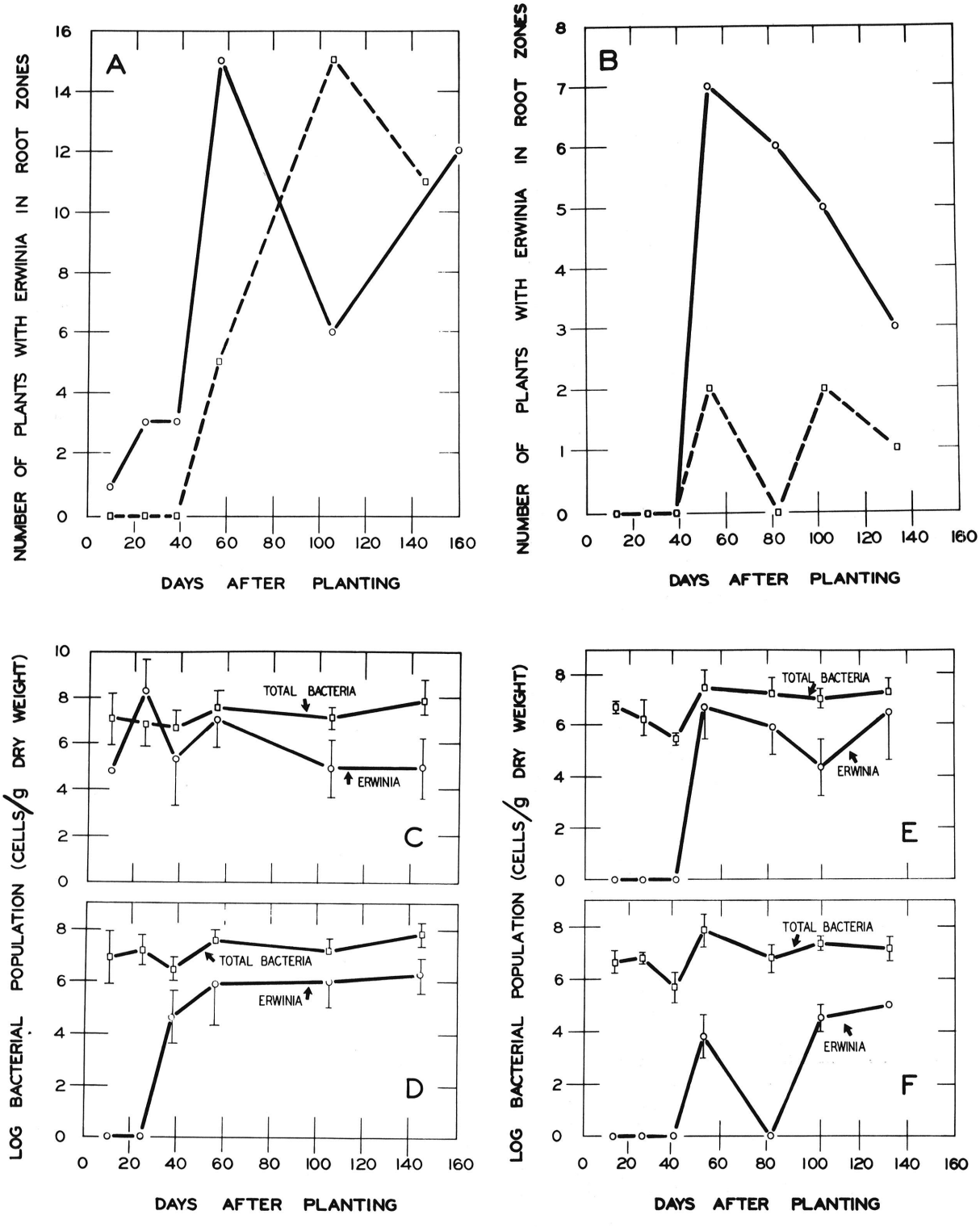

Fig. 1–(A, B). Incidence of Sebago potato plants with *Erwinia carotovora* in their root zones in a 1972 field plot **A)** planted with tubers from one source in which soft rot was present (–) and from another source in which no soft rot was present (- - -) and in a 1973 field plot **B)** planted with tubers from one source with 34% *Erwinia*-infestation (–) and from another source with 1% *Erwinia*-infestion (- - -). **C to F)** Populations of total bacteria and *E. carotovora* (vertical lines indicate standard error) in the root zones of Sebago potato plants in a 1972 field plot planted with tubers in which soft rot was present **C)** and with tubers in which no soft rot was present **D)** and in a 1973 field plot planted with tubers with 34% *Erwinia*-infestation **E)** and with tubers with 1% *Erwinia*-infestation **F)**.

sampled at 10, 24, 38, 56, 105, and 145 days after planting. Ten days after planting, *E. carotovora* was found in 7% of the root zones of sampled plants grown from seed from source A, and in 100% of the plants on the 56th day after planting (Fig. 1-A). *Erwinia carotovora* was not detected in the root zones of plants grown from seed source B until the 38th day after planting. In this plot advanced seed piece decay also was not observed until the 38th day after planting. By the 105th day, 100% of the sampled plants were infested.

The populations of *E. carotovora* in the root zone presented in Fig. 1 were calculated for those plants on which the pathogen was detected and represents a mean root-zone population that may be present under field conditions. The populations on plants from source A were high at all sampling dates, the highest level occurred 24 days after planting (Fig. 1-C). The populations for plants from source B initially were low, but they increased until the 58th day after planting when they reached a level comparable with that for plants from source A (Fig. 1-D). The population of background bacteria in the root zone remained relatively constant at about 10^7 cells/g dry wt throughout the growing season.

Field plot 1973.—Since *E. carotovora* was not detected before planting in the soil of either plot used in 1973, the data of the two plots were combined. Isolations from seed tubers used in 1973 revealed that *E. carotovora* was present on 34% of the seed tubers from one source and on 1% of tubers from a second source. Two and nine plants of 200 grown from the two 1973 seed sources, respectively, developed symptoms of blackleg during the growing season. At 13, 26, 39, 53, 82, 103, and 133 days after planting eight root-zone samples from symptomless plants and eight soil samples were taken from plots of each 1973 seed source. *Erwinia carotovora* was not detected in root-zone samples of plants from either seed source until 53 days after planting at which time a high percentage of the seed tubers had started to decay (Fig. 1-B). The incidence of *E. carotovora* in root zones of plants from the seed with 34% *Erwinia* infestation was almost 90% by this time; but the level of infestation had dropped to 37% by the end of the season. Only 25% of the plants grown from the seed with 1% *Erwinia* infestation had *E. carotovora* in their root zones at any time during the growing season. The mean population of *E. carotovora* in the root zones reflected the level of infestation in the potato seed used for planting (Fig. 1-E, F). The population in individual root-zone samples reached a maximum of 2×10^8 cells/g dry wt for plants grown from seed with 34% *Erwinia* infestation and 1×10^5 cells/g dry wt for plants grown from seed with 1% *Erwinia* infestation. Again, the total background population of soil bacteria remained relatively constant throughout the growing season (Fig. 1-E, F).

The total populations of bacteria and *E. carotovora* in the soils in the field plots were comparable to but lower than the root-zone populations. The total bacterial population varied from 2×10^4 to 3×10^7 cells/g dry wt for individual samples, whereas populations of *E. carotovora* varied from 0 to 2×10^4 cells/g dry wt.

Erwinia carotovora was found on 25-94% of the daughter tubers in samples of 16-30 tubers examined during the growing season (Fig. 2). In August, *E. carotovora* was found on up to 94% of the daughter tubers but the incidence of tuber infestation dropped to about 60% soon after harvest and remained fairly constant for the first 4 mo of storage. *Erwinia carotovora* could be recovered even after 19 mo of storage.

Of 16 *Erwinia* cultures isolated from the soil and root-zone samples, 11 were identified as *Eca*. Of 60 *Erwinia* cultures isolated from the daughter tubers selected at the various sampling times, 28 were *Eca*. The remaining strains were *Ecc*.

Stem cuttings 1974 and 1975.—Stem cuttings were obtained from greenhouse-grown Sebago and Russet Burbank potato plants. Before stem cuttings were rooted, all were tested for the presence of *E. carotovora* by plating 0.1 ml of sap, expressed from the end of the cutting, on CVP medium. No *E. carotovora* was detected on these stem cuttings. During early June, one-half of the cuttings were planted in a field in which the potatoes had been infested with *E. carotovora* during the previous season (old plot). The remainder of the cuttings were planted in a plot in which no potatoes had been grown for 5 yr (new plot). Following standard plating procedures on CVP, *E. carotovora* could not be detected in soil samples taken from either the old or new plot. Furthermore, *E. carotovora* was not detected in eight root-zone samples each of both Sebago and Russet Burbank plants during

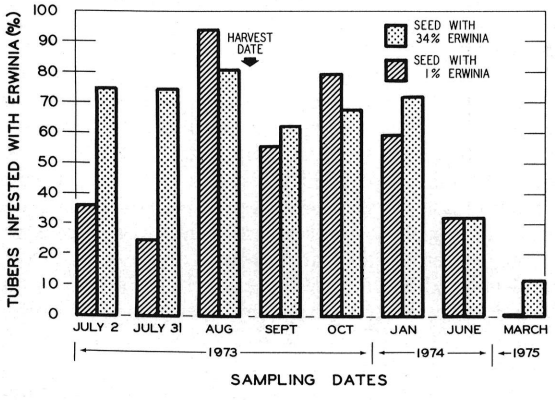

Fig. 2. Percent tubers infested with *Erwinia carotovora* when sampled during the growing season and in storage. Before planting, *E. carotovora* was detected in the lenticels of 34% of the seed tubers from one source and in 1% from another source.

TABLE 1. Percentage of tubers from *Erwinia*-free stem cuttings infested with *E. carotovora* at time of harvest

Year planted	Plot	Time since previous potato crop (yr)	Infested tubers[a] Russet Burbank (%)	Sebago (%)
1974	A	0	10	20
	B	5	5	43
1975	A	0	40	...
	C	5	80	...

[a]Determined by inducing tubers to decay (7) and plating an homogenate of the decayed tissue on crystal violet pectate (CVP) medium. Based on 20 tubers/sample.

July or in four samples from each at harvest in September. Daughter tubers harvested from these plots, however, were infested with *E. carotovora*; bacteria of this species were present on 5 and 10% of the Russet Burbank daughter tubers and on 43 and 20% of the Sebago tubers from the new and old plots, respectively (Table 1). All 30 strains obtained were identified as *Ecc*.

In 1975, Russet Burbank stem cuttings were planted in plots similar to those used in 1974. *Erwinia carotovora* was not detected in the stem cuttings before planting or in soil in the field plots prior to planting. During the growing season, one plant showed symptoms of blackleg and *Eca* was detected in the stem with the FAS procedure. *Eca* was also shown with FAS to be present on the foliage of the plants surrounding the diseased plant. In the new and old plots, 80 and 40% of the daughter tubers, respectively, were infested with *E. carotovora* (Table 1). Twelve strains from tubers of each plot were selected at random and tested with FAS; one strain from the old plot and none from the new plot was *Eca*. However, when FAS-stained smears, prepared from tubers that had been induced to decay by wrapping in polyvinlylidine film were observed, 4/20 and 5/20 tubers of the new and old plots, respectively, were found to contain *Eca* cells.

Characterization of cultures.—Strains identified as *Eca* did not grow at 36 C, but produced acid from α-methyl glucoside and reducing substances from sucrose. All strains with these characteristics reacted positively with FAS prepared against *Eca*. Furthermore, they produced extensive blackening in potato plants near the point of inoculation in 3-4 days, with subsequent collapse and death of the plant. Strains identified as *Ecc* grew at 36 C, did not produce acid from α-methyl glucoside or reducing substances from sucrose, and did not give a positive reaction with the FAS specific for *Eca*. They caused variable symptoms on potato, ranging from local necrotic lesions at the inoculation point to partial collapse and, in one case, death of the plant. The discoloration of affected parts was always brown in contrast to the blackening characteristic of *Eca*.

DISCUSSION

It is difficult to obtain accurate measurements of the population of one bacterial species or strain from a complex environment such as the root zone of a potato plant. Nevertheless, distinctive trends in populations were apparent. The time of rapid increase in the population of *E. carotovora* coincided with the sampling date on which seed pieces first were observed to have lesions (Fig. 1). The counts of *E. carotovora* varied during the growing season. Since total populations were estimated on the basis of populations detected on the selective medium, they were about 1-5% of that expected on a nonselective medium (5). In general, the total populations were within the same range as previously reported for the populations in the rhizospheres of other crop plants (36, 38).

Variation of the population during the growing season may have reflected changes in weather conditions. Soil temperatures and rainfall affect the population of *E. carotovora* in potato fields in Scotland (33). A consistent correlation of *E. carotovora* populations with specific patterns of precipitation or temperature could not be demonstrated in our data, however.

In addition to *E. carotovora*, other pectolytic bacteria commonly were present in the root-zone samples at approximately 1×10^6 cells/g dry wt. Several colony types of pectolytic bacteria were observed on CVP medium. Many of these isolates produced a green fluorescent pigment when plated on King's medium (18) and were probably *Pseudomonas* spp.

The number of plants with *E. carotovora* in their root zones was correlated with percentage of tubers infested with *E. carotovora* at the time of planting. This correlation was most evident in the 1973 plot (Fig. 1-B). The level of seed piece infestation was not determined in 1972 but the seed tubers from the source in which soft rot was present probably had a high level of *Erwinia* infestation. Bacteria may have moved from this highly infested row to the adjacent row which initially had a low level of infestation, resulting in almost equally high percentages of infestation in both rows by the end of the season. Possible movement of *E. carotovora* in the soil was indicated by its presence in soil samples taken in the vicinity of potato plants in plots where it was not detected prior to planting. However, the presence of *E. carotovora* in soil during the growing season does not imply colonization of the soil, but may be due to movement of bacteria from the rhizosphere, as suggested by Harper et al (14).

Although the number of plants with *E. carotovora* in the root zones was relatively low in some instances, almost all daughter tubers became infested (Fig. 2). Therefore, *E. carotovora* may have been present in the root zone of a larger number of plants than the results indicate. The results probably reflect the limitations of the dilution plating technique for root-zone sampling.

Immediately after harvest, a slight decrease in percentage of infested tubers in storage occurred, continuing with time in storage (Fig. 2). The initial decrease may indicate a decline in surface contamination (31) rather than in lenticel infestation.

The infestation of daughter tubers with *Eca* in plots where no plants with symptoms of blackleg were observed, can be explained by the movement of *Eca* from the decaying seed piece to the root zone. If the primary source of inoculum is the seed tuber, tubers grown from stem cuttings should be free of *Eca* if recontamination can be prevented. However, 5 to 80% of the tubers grown from stem cuttings in several plots were infested with *E. carotovora* (Table 1). The recontamination of plants grown from *Erwinia*-free stem cuttings indicated the presence of other sources of inoculum in the experimental field plots where these tests were completed. In Scotland, potato cull piles have been implicated as a source of inoculum which is carried by insects to healthy plants (13). Although no cull piles were present in the immediate vicinity of our plots, other potato plantings were present on the experimental farm where these tests were completed and could have been the source of inoculum which may have been carried by insects to the healthy plants (13, 26). Insects heavily contaminated with *E. carotovora* have been collected from overwintered cull piles and growers' fields in Wisconsin (Kukorowski, Phillips, Chapman, and Kelman, *unpublished*).

Although *E. carotovora* was not detected in the soil from the field plots prior to planting in the spring, the population level may have been below that which can be

detected by direct plating methods on selective media. Soil enrichment techniques have made it possible to enhance detection procedures (25), but Burr and Schroth (4), who used enrichment techniques, could not detect pectolytic *Erwinia* in potato field soils in California after a 4-mo fallow period. Similarly, *E. carotovora* was not detected with enrichment procedures in Wisconsin field soils which had been rotated with other crops for 2 yr after planting in potatoes (Allan and Kelman, *unpublished*). Meneley and Stanghellini (25) suggested that failure to detect pectolytic *Erwinia* in field soils may have been attributable to the lack of sensitivity of standard selective media. However, the application of highly sensitive detection methods confirm prior evidence (5) that pectolytic *Erwinia* populations decrease to very low or nondetectable levels in the absence of potato in Wisconsin. Differences in environmental conditions, in particular soil temperature in Arizona and California in contrast to those in Wisconsin, may provide the basis for observed differences in survival in soil of pectolytic *Erwinia* between different areas.

The main bacterium involved in recontamination was *Ecc* in our experiments in Wisconsin as well as in Scotland (13), British Columbia (8), and Colorado (M. D. Harrison, *personal communication*). Strains obtained from weed rhizospheres and soil in California (4) and British Columbia (8) also were mainly *Ecc* types.

Our results support the observations of Perombelon (32) that the seed piece is the major source of inoculum for blackleg and soft rot of potato. Although preliminary data in Scotland (12) and Montana (37) suggest that recontamination of *Erwinia*-free potatoes can be avoided or minimized, our results indicate that recontamination with *E. carotovora* may be difficult to avoid in Wisconsin. However, daughter tubers, even from infested seed pieces, do not always become contaminated with *E. carotovora* at certain locations in some growing seasons in Wisconsin (Kukorowski, Phillips, Chapman, and Kelman, *unpublished*).

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