RECENT DEVELOPMENTS IN UNDERSTANDING EARLY DYING OF POTATO VINES -- THE ROLE OF ERWINIA CAROTOVORA

by
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During the past 3 years one of our major research priorities has been to determine the pathogenic organisms associated with the early dying syndrome of potato vines in Oregon. Verticillium dahliae is the most important fungal pathogen associated with decreased potato yields due to early dying, and this disease becomes particularly severe in fields after several years in potato production. Other fungal pathogens, e.g. Fusarium spp. and Colletotrichum atramentarium may be associated with early dying in some areas, but in the irrigated potato circles in Oregon's Columbia Basin, we were unable to demonstrate that these pathogens cause significant disease.

Nevertheless early dying of potato vines may be severe when either the bacterial pathogen, Erwinia carotovora is involved or when a complex of pathogenic organisms is involved. Studies were initiated this past year to determine if the bacterial pathogen, E. carotovora was associated with early dying symptoms.

A. SEASONAL INCIDENCE AND CAUSE OF BLACKLEG AND A STEM SOFT ROT OF POTATOES IN OREGON

Blackleg of potatoes occurs wherever potatoes are grown, and is responsible for weakened plants, premature death, and loss of stand. Erwinia carotovora var. atroseptica (Van Hall) Dye (Eca) and E. carotovora var. carotovora (Jones) Dye (Ecc) were involved in blackleg infections in Colorado (6), but Eca was the predominant pathogen. Ecc was isolated with few exceptions only from plants collected in the warmer areas of the state. Atypical blackleg symptoms, i.e. diseased plants with brown to black discoloration of the vascular tissue and/or pith, were found in Arizona, and 30.6% of the bacterial isolates from these plants were Ecc(9). Blackleg symptoms occur frequently in the center pivot irrigated potato circles of Oregon's Columbia Basin, but a widespread stem soft rot symptom, atypical of blackleg and different from that described by Stanghelleni and Meneley (9) also occurs. Stems appear translucent, watery and are soft and mushy to the touch. The seasonal distribution of Eca and Ecc and their association with blackleg and stem soft rot symptoms were determined to aid in understanding this disease syndrome.

MATERIALS AND METHODS

Stems from potato plants (Solanum tuberosum L. "Kennebec") with typical blackleg and stem soft rot symptoms were collected throughout the growing season (June through August) from one commercial potato field (48.7 ha) in the Columbia Basin of Oregon. This field was selected because it was representative of many other fields with plants showing similar symptoms. At each sampling time, the percentage of plants with blackleg and stem soft rot symptoms was visually estimated by making a random survey of a different area 55 meters x 21 meters.

Plants with blackleg and/or stem soft rot symptoms were brought back to the laboratory for isolation and identification of the bacteria associated with the symptoms. Pieces of stem tissue, dissected from the advancing margin of the rot, were suspended in 1.0 ml sterile water and aliquots of the suspension were streaked onto crystal violet pectate agar (CVP) (2). After 48 hours of incubation at 22°C, typical E. carotovora-like colonies were subcultured on fresh CVP medium. Single colonies were then transferred to sugarless-nutrient agar on which

they were maintained. All isolates were tested for ability to cause soft rot of potato tuber slices.

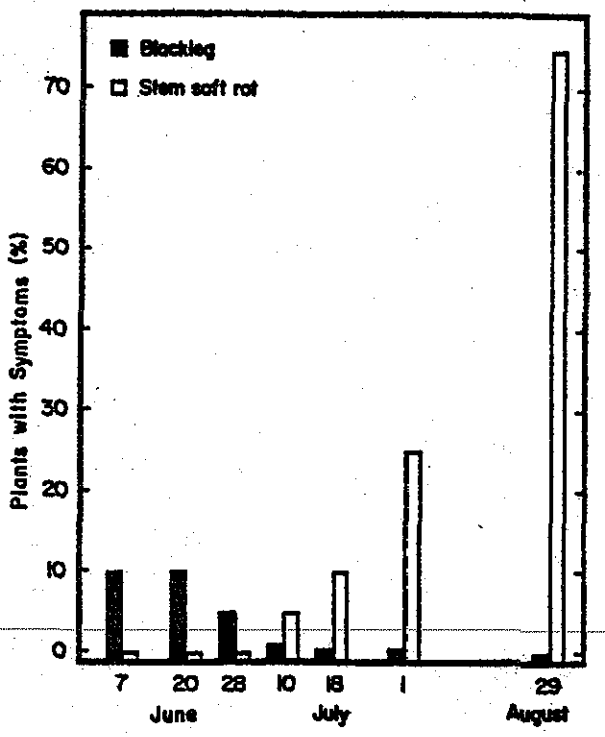
Eca was distinguished from Ecc by acid production from ∝ -methyl glucoside (5), and growth at 37°C. For additional confirmation, the pathogenicity of 30 randomly chosen isolates was evaluated by inoculation of potato plants grown in a greenhouse at 18°C. Stems of 6-week-old potato plants (cultivar Russet Burbank) were punctured with moist toothpicks smeared with bacteria cells from 24-hour-old cultures grown on casamino-peptone-glucose agar. The toothpicks were left in place, and the plants were examined for symptoms 6 days later.

A total of 141 Erwinia isolates from different plants (68 from plants with blackleg symptoms and 73 from plants with stem soft rot symptoms), were isolated and identified as either Eca or Ecc.

RESULTS AND DISCUSSION

The incidence of plants with typical blackleg symptoms was generally low throughout the season (Fig. 1). In early June about 10% of the plants had blackleg symptoms, but as the season progressed it became difficult to find plants with typical symptoms. Plants with blackleg symptoms in the early season probably died or were overgrown by the vines of surrounding healthy plants. During July the vines of previously healthy looking plants began to show a stem soft rot symptom. Initially, stems at the soil surface appeared watery and translucent rather than "inky black" which is characteristic of blackleg.

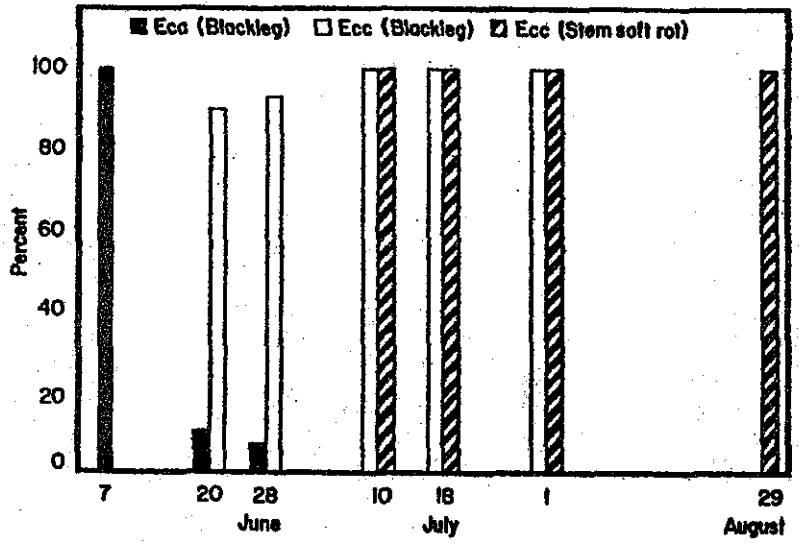
Figure 1. The seasonal incidence of plants with blackleg and stem soft rot symptoms in a center pivot irrigated circle of "Kennebec" potatoes, 1979.



The onset of stem soft rot occurred in early July, and at that time only vines near the soil surface showed symptoms. As the season progressed, the incidence of this symptom increased dramatically and the disease was no longer solely associated with vines near the soil surface, but also occurred in the upper canopy. By the end of August, 75% of the plants had stem soft rot and the vines were dying.

The frequency of isolation of Eca and Ecc from diseased plants varied greatly as the season progressed. Eca was recovered early in the season (Fig. 2) when blackleg symptoms were predominant (Fig. 1). Later in the season, the frequency of Ecc isolations increased. Ecc was recovered from some stems with blackleg symptoms, but always from plants with watery stem soft rot symptoms. Eca was never recovered from plants with only stem soft rot symptoms. Although Eca is primarily tuber-borne (8) Ecc can be tuber-borne and also soil-borne. The origin of the effective current season inoculum for inciting stem soft rot symptoms in the irrigated circles of Oregon's Columbia Basin needs to be determined.

Figure 2. Seasonal incidence of Erwinia carotovora var. atroseptica (Eca) and E. carotovora var. carotovora (Ecc) and their recovery from plants with blackleg or stem soft rot symptoms.



B. ERWINIA CAROTOVORA: A BACTERIUM ASSOCIATED WITH EARLY DYING OF POTATO VINES

MATERIALS AND METHODS

Plots were established in two potato circles in the Columbia Basin of Oregon. One circle (Boardman) was in its second year of potato production following 3 years in alfalfa; the second circle (Hermiston) was in its fourth year of potato production with winter wheat the only rotation crop. Norgold Russet seed pieces from the same seed lot were used throughout the study. Plots were planted on April 5, 1979 in the Columbia Basin.

The occurrence of E. carotovora varieties on the seed pieces was determined. Selected tubers were wounded with sterile toothpicks. The tubers were wrapped individually with moist paper towelling, sealed in plastic bags, and incubated at 20 C for 96 hours. Small

sections of tuber tissue, dissected from the advancing margin of rot pockets, were suspended in sterile water and aliquots of the suspension were streaked onto crystal violet pectate agar (CVP) (2). After 48 hours of incubation, typical E. carotovora-like colonies were selected and transferred to fresh CVP medium, and subsequently to sugarless nutrient agar on which they were maintained throughout the study. All isolates were tested for ability to cause soft-rot of potato tuber slices.

Two tests were used to distinguish E. carotovora var. atroseptica (Eca) from E. carotovora var. carotovora (Ecc): acid production from α-methyl glucoside (5) and growth at 37 C.

Representative Ecc and Eca isolates were sent to Solke DeBoer, Canadian Department of Agriculture for serogroup typing.

Antisera were produced in rabbits against whole, glutaraldehyde-fixed cells of serogroup I (Eca) and serogroup V (Ecc) because these bacteria were present on the seed pieces used in this trial. Bacterial cells were grown on casamino-peptone-glucose agar at 24 c for 48 hr, suspended in a phosphate buffered saline solution (PBS), pH 7.2, 0.01 M buffer, and washed three times by centrifugation and then resuspended in PBS. The final concentration was adjusted to 10^9 cells/ml and the cells were fixed in glutaraldehyde (1). Rabbits were given five 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 by heart puncture 1 week after the final injection. Antisera titers were determined by the drop agglutination methods and the sera were purified further to separate out globulins. These globulins were suspended in PBS and stored frozen.

During the growing season at the onset of symptom expression and then 3 weeks later, the number of potato plants with early dying symptoms was determined by counting the number of diseased and symptomless plants in 800 ft. of row. Two hundred stems from plants with early dying symptoms were collected at the onset of symptom expression and again 3 weeks later in both plots in the Columbia Basin. The plants were brought back to the laboratory for isolation and identification of associated pathogens.

For isolation of fungi, a stem section about 5 cm long from near the soil-line was surface sterilized in a 5% commercial bleach solution for 3 min. The epidermis was removed and five 0.5 cm long segments were cut and placed onto water agar containing 100 ug/ml streptomycin. The culture plates were incubated at 22 C for 1 week after which they were examined for the presence of V. dahliae, Fusarium spp. and C. atramentarium.

Isolations for bacteria were made from below-ground stem sections. Pieces of stem tissue were suspended in 1 ml of sterile water and aliquots of the suspension were streaked onto CVP medium to determine the occurrence of E. carotovora. The same procedure as outlined previously was followed for maintaining the cultures and for Erwinia varietal determination.

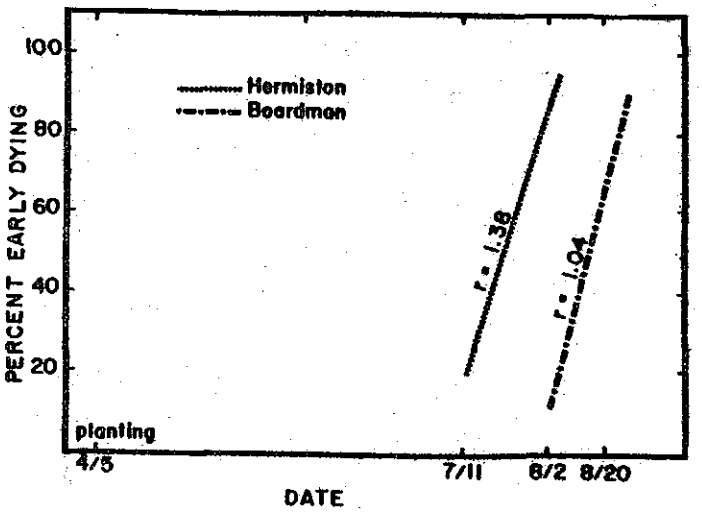
Agar plates for double diffusion assay tests were prepared according to the procedure of DeBoer, et al. (4). Wells, 3 mm in diameter and 4 mm apart, were cut in sets of six peripheral wells surrounding a center well. E. carotovora isolates were grown at 24 C for 48 hr on sugarless nutrient agar slants, the cells were harvested in 0.5 ml of sterile distilled water, and a drop of liquified phenol was added to the cell suspension. The center well was filled with undiluted globulins; two wells on opposite sides of the center well were filled with the homologous cell suspension and the remaining four wells were filled with suspensions of unknown bacteria. The plates were incubated at 24 C for 24 hr before examination for precipitation bands.

RESULTS

Disease Progress. Early dying of the potato vines occurred in all plots but the onset of symptom expression differed among locations (Fig. 1). In the Columbia Basin, early dying symptoms were not evident until 97 (Hermiston) and 118 (Boardman) days after planting. Almost all the plants had symptoms of early dying within 3 weeks after the onset of symptoms in each of the test plots.

The apparent infection rate (r) (9) was calculated from the percentage of plants with early dying symptoms during the growing season. Calculated r values were 1.38 and 1.04 per unit per day for Hermiston and Boardman, respectively (Fig. 3). The epidemic progressed at about the same rate in all plots with the incidence of early dying more than doubling each day after the onset of symptoms.

Figure 3. Disease progress curves (apparent infection rate, r) for early dying of Norgold Russet potatoes at two locations in Oregon, 1979.



The r values for the two plots were similar, therefore we can assume that the influence of environment was similar at the two sites. The only difference among the plots was the date of symptom initiation.

Because symptom initiation differed with plot location but the apparent infection rates were similar, we can hypothesize that there were different kinds and/or amounts of effective inoculum to start the disease in each plot. Since the onset of symptoms occurred sooner after planting in the Hermiston plot, we can conclude that there was more effective inoculum in this plot than in the Boardman plot.

V. dahliae was probably the primary pathogen responsible for early dying symptoms in the Hermiston plot (Fig. 4), where it was isolated 82% and 64% of the time from diseased plants collected at the start and end of the epidemic, respectively. Ecc was the only variety of E. carotovora isolated and it occurred with V. dahliae 18% and 20% of the time in isolations made in July and August respectively. Rarely was Ecc isolated alone from diseased plants in this field.

Figure 4. Percent of Norgold Russet plants with early dying symptoms at Hermiston from which Verticillium dahliae (V) alone, Erwinia carotovora (E) alone and V. dahliae and E. carotovora (V & E) together were isolated.

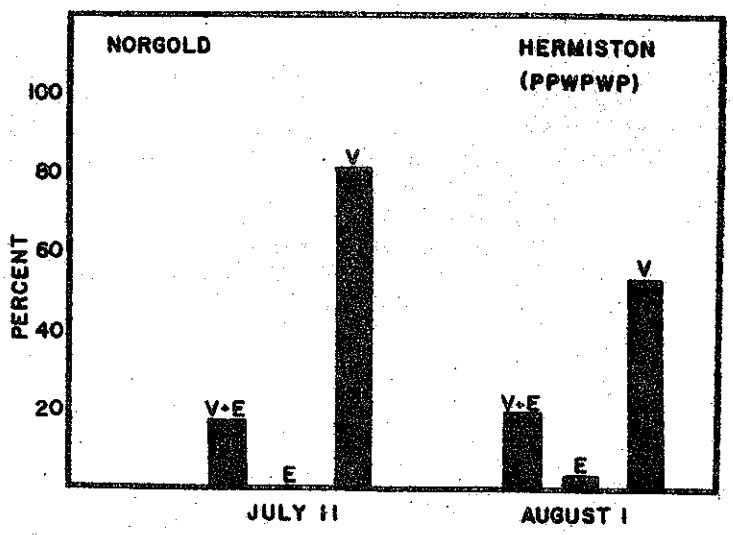
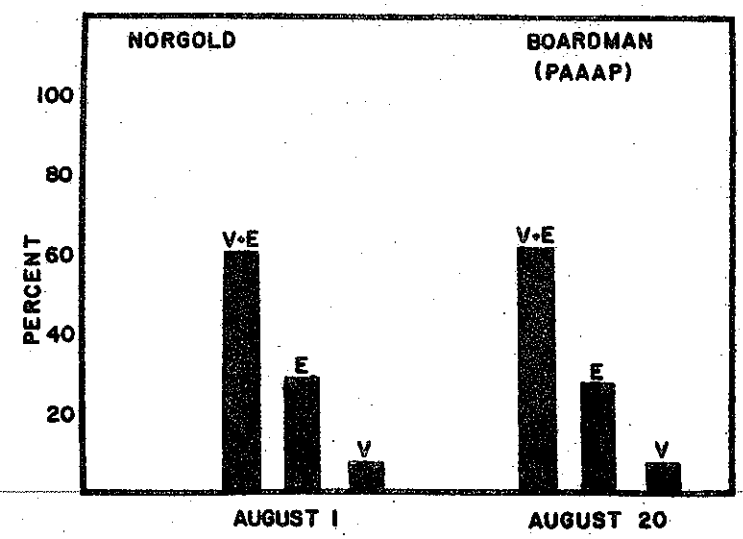


Figure 5. Percent of Norgold Russet plants with early dying symptoms at Boardman, OR from which Verticillium dahliae (V) alone, Erwinia carotovora (E) alone and V. dahliae and E. carotovora (V & E) together were isolated.



Early dying of the vines occurred in the Boardman plot, but the onset was late in the season (Fig. 3). Based on isolations from 400 plants that died prematurely, the frequency of V. dahliae (8%) and E. carotovora (39%) alone probably did not account for the disease in this field (Fig. 5), but the pathogens in combination may have been a significant factor in early dying. The E. carotovora variety recovered from the diseased plants was exclusively Ecc.

Sources of Erwinia carotovora inoculum. Recent studies on potato blackleg and soft rot caused by Eca and Ecc suggest that the seed piece is an important inoculum source (3). Nevertheless, in some experiments where plants were grown from stem cuttings to avoid tuber-borne pathogens, a small percentage of the plants became infected by Ecc and occasionally by Eca. This suggests that the seed piece may not be the only source of inoculum (3, 4). Generally blackleg symptoms occur on stems in a single hill and are limited in occurrence within a given field. Early dying symptoms on the other hand, are generally widespread within a field.

Epidemiological and ecological studies involving E. carotovora have been hampered by the difficulty in identifying strains of these bacteria, but a recent serotyping scheme for E. carotovora by DeBoer (4) has allowed rapid classification into serogroups. Eca and Ecc differ serologically and, by immunodiffusion, Ecc can be classified into 18 different serogroups and Eca into four (4).

In the Boardman plot, 39% of the plants with early dying symptoms were infected by E. carotovora alone (Fig. 3) and 69% were infected by both V. dahliae and E. carotovora. All of the E. carotovora isolates were Ecc. In fact, Eca was never recovered from plants with early dying symptoms (Table 1) and consequently is not considered an important factor in the early dying syndrome in the Columbia Basin production area.

Table 1. The incidence of Erwinia carotovora var. carotovora (Ecc) and E. carotovora var. atroseptica (Eca) in Norgold Russet plants with early dying symptoms grown at two locations in Oregon.

Location	Eca (%)	Ecc (%)
Hermiston	0	15
Boardman	0	91

If we accept the hypothesis that Ecc and Eca are primarily seed-borne pathogens, then heavily infected seed lots are necessary to produce wide spread early dying of potato vines, and the bacteria recovered from diseased plant tissue should be the same serologically as those associated with the seed pieces.

The predominant E. carotovora variety associated with the seed lot used in this study was Eca (Table 2). In fact, of the 50 tubers sampled, Eca was recovered from 99% of the tubers whereas Ecc was recovered from only 11%. All Eca isolates belonged to serogroup I while all Ecc isolates were serogroup V.

Antiserum to serogroup V (Ecc) was used in immunodiffusion tests to determine if the Ecc isolates recovered from plants with early dying symptoms from Boardman and Hermiston were the same serologically as those isolated from seed pieces. Serogroup V isolates were never recovered from plants with early dying symptoms in the two plots in the Columbia Basin although it had been present on 11% of the tubers planted. Identification of these serogroups from diseased plants is currently being determined by our laboratory. Failure to recover serogroup V isolates suggests that seed-borne Ecc inoculum of serogroup V is not involved in the early dying syndrome in the Columbia Basin, and that inoculum sources of Ecc other than the seed piece may be involved in early dying of potato vines.

Table 2. Percent tubers from which Erwinia carotovora var. atroseptica (Eca) and E. carotovora var. carotovora (Ecc) were isolated and the percent of each variety isolated.

Erwinia carotovora variety	Tubers (%)	Isolates (%)
Eca	99	90
Ecc	11	10

Traditionally Ecc and Eca have been regarded as pathogens that cause blackleg and tuber rots. Our research provides new information that suggests that E. carotovora plays a role in the early dying syndrome of potato vines.

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