Interaction of Fungal Wilt Pathogens and Potato Blackleg

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ABSTRACT

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A field study was done to determine the effects of combined inoculations with *Erwinia carotovora* subsp. *atroseptica* (Eca) or *E. carotovora* subsp. *carotovora* (Ecc) and *Fusarium sulphureum*, *F. oxysporum*, *F. solani*, or *Verticillium dahliae* on the incidence of seed tuber decay and blackleg. Treatments inoculated with Eca resulted in significantly greater seed tuber decay, blackleg, and reductions in stand and yield compared with treatments inoculated with Ecc. No significant differences were observed between the uninoculated controls and treatments inoculated with Ecc for the variables measured. A significant positive correlation existed between the percentage of seed tuber decay and of blackleg stems per plant early in the season. This correlation diminished following postplant inoculations with fungal wilt pathogens. Postplant inoculations with *F. sulphureum*, *F. oxysporum*, *F. solani*, and *V. dahliae* significantly increased the incidence of blackleg, with no significant effect on seed tuber decay. Of the different pathogen combinations tested, preplant inoculation with Eca caused the most early-season blackleg, and Eca plus *V. dahliae* caused the most midseason blackleg. The greatest reduction in yield occurred in plots containing seed tubers that were inoculated before planting with Eca plus *F. sulphureum*. The results of this study indicate that interactions may exist between the blackleg bacterium and the common fungal wilt pathogens of potato. This interaction of combined infection may cause an increased incidence of blackleg over that caused by these pathogens alone.

Potato blackleg, caused by *Erwinia carotovora* subsp. *carotovora* (Jones) Dye (Ecc) and *E. carotovora* subsp. *atroseptica* (van Hall) Dye (Eca), is a widespread and persistent disease found in every country in which potatoes are grown. Temperature is the most important factor that determines which subspecies causes infection. In North Dakota, losses of up to 50% have been observed due to blackleg, but overall losses average 2–5%.

Blackleg is a complicated disease influenced by many environmental and nonenvironmental factors, including pathogens often found accompanying *Erwinia* bacteria in blackleg-affected plants (17). Extensive surveying of the potato-producing areas of North Dakota revealed that three species of *Fusarium*, two species of *Verticillium*, *Colletotrichum atramentarium* (Berk. & Br.) Taub., and *Rhizoctonia solani* Kühn are often associated with blackleg-infected potato plants (7). Common soilborne pathogens that have been reported to increase the incidence of blackleg include *Pythium butleri* Subramanium (1), *Phoma exigua* Desm. var. *foveata* (Forster) Boereman (11), *F. caeruleum* (Lib.) Sacc. (19), and *F. sulphureum* Schlecht. (8).

Considering the recent economic importance of "early dying" disease of potatoes, thought to be caused by combined effects of *Verticillium* and *Erwinia* (9,18), and recent evidence of the soilborne nature of *E. carotovora* (2–4, 12,16), it is important to understand the influence that bacterial-fungal interactions have on the blackleg disease complex. This is particularly important because *Fusarium* spp. and *Verticillium* spp. are present in many soils for long periods.

This study was conducted to examine the combined effects of either *F. sulphureum*, *F. solani* (Mart.) Sacc., *F. oxysporum* Schlecht., or *V. dahliae* Klebahn with either Ecc or Eca on seed decay and the expression of blackleg.

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MATERIALS AND METHODS

The cultures of fungi and bacteria used in this study were selected from a group of isolates obtained from a potato disease survey of North Dakota in 1977 (7).

Fusarium spp. were increased on potato-dextrose agar for 14 days under 12-hr fluorescent lights (5,000 lux) at room temperature. *V. dahliae* was increased on potato-dextrose agar in the dark at 21 C for 35 days. Propagule suspensions of each fungal pathogen were made by homogenizing the contents of each petri plate with distilled water in a Waring Blendor for 15 sec. Final inoculum suspensions contained approximately 10^5 propagules per milliliter of liquid.

Ecc and Eca were increased on tryptic soy agar (Difco, Detroit, MI) in the dark at 27 C for 48 hr. Cell suspensions were prepared by washing the growth from the surface of the medium with a stream of distilled water. The final inoculum concentration was approximately 10^6 colony-forming units per milliliter of liquid. All inoculum preparations were refrigerated and used within 3 hr.

The experiment was located on a Bearden silt loam plot at the Red River Valley Potato Research Farm near Grand Forks, ND. A randomized complete block design, replicated 12 times, was used to evaluate the treatments shown in Table 1. Each replicate consisted of 56 B size seed tubers of the cultivar Norgold Russet, spaced 30 cm within the row with 102 cm between rows. Each block was separated by a 6-m alley and bordered by two rows of uninoculated potatoes.

Inoculations with the tuber-rotting pathogens Eca, Ecc, and *F. sulphureum* were made before planting. Preplant inoculation was done by forcing the stem end of each seed tuber onto two steel pins mounted in the bottom of a plastic container containing the inoculum. The resulting wound approximated the wound made during planting with a pick-type planter.

Wilt-inducing pathogens *F. solani*, *F. sulphureum*, *F. oxysporum*, and *V. dahliae* were inoculated to newly emerged plants 26 days after planting. The root system was damaged by inserting a steel blade 10 × 20 cm into the soil approximately 5 cm from base of the plant to a depth of 20 cm. Upon removal of the blade, an opening remained in the soil into which 50 ml of inoculum suspension was dispensed.

Seed piece decay (percentage of the seed tuber decayed) and preemergence blackleg readings were taken 25, 36, and 44 days after planting by hand digging five plants per treatment per replicate on each date. The numbers of wilted plants and of plants with one or more stems with blackleg were recorded 30, 40, 54, 63, 82, and 93 days after planting. Isolations (16) were periodically made throughout the season from 10% of the diseased stems and seed tubers to confirm the causal agent and to correlate the disease reading with treatment. Yields were measured 109 days after planting. Treatment means were tested by the least significant difference (LSD) method.

RESULTS

No significant reduction in stand was observed in plots where Ecc was inoculated alone or in combination with *F. sulphureum* compared with the uninoculated controls. However, inoculation of seed tubers with Eca before planting resulted in a 23.5% reduction in stand compared with the uninoculated water (H_2O) control (Table 1), but no further reduction in stand over Eca alone was observed by combining *F. sulphureum* with Eca as a preplant inoculation.

A significant positive correlation existed between the percentage of seed tuber decay and of blackleg stems (preemergence and postemergence) per plant at 25, 36, and 44 days after planting (r = 0.78, 0.67, and 0.45, respectively). This correlation decreased with time as seed tuber decay approached 100%. The highest levels of seed tuber decay were always associated with seed that had been inoculated with Eca before planting. At 25 days after planting, inoculation with Eca before planting (Eca-preplant) had caused an average of 80.8% seed tuber decay compared with 35.7% decay caused by Ecc-preplant, whereas only 8.1% decay was present in the H_2O-preplant control. The addition of *F. sulphureum* to Eca-preplant did not significantly increase seed tuber decay.

Inoculations with Ecc alone and in combination with *F. sulphureum*-preplant initially caused 35.7 and 27.3% seed tuber decay, respectively, at 25 days after planting. This was significantly more decay than was present in the H_2O-preplant control (8.1%). However, at 36 and 44 days after planting, there was no significant difference in seed tuber decay between the H_2O-preplant control and either Ecc alone or in combination with *F. sulphureum*-preplant.

No significant increase in seed tuber decay could be detected in any treatment 10 days after postplant root inoculations (36 days after planting) with *V. dahliae, F. solani, F. oxysporum*, and *F. sulphureum* (Table 1). However, the postplant addition of these fungal pathogens significantly increased the percentage of blackleg stems per plant in treatments preplant inoculated with Eca (33.8–38.5%), compared with preplant inoculation with Eca but not postplant root inoculation (19.0%). Postplant root inoculations with fungal pathogens did not cause an increase in percentage of blackleg stems per plant compared with a combination of Eca and *F. sulphureum* preplant inoculation (24.8%) (Table 2). Seed tuber decay and percentage blackleg stems per plant readings were discontinued after 44 days because of the complete decay of most seed tubers and the rapid breakdown of small blackleg stems.

Significantly greater numbers of blackleg plants were found up to 54 days after planting in plots combining root inoculation with *F. sulphureum, F. solani, F. oxysporum*, or *V. dahliae* following preplant inoculation with Eca (Table 2), compared with plots having preplant inoculations with either Eca plus *F. sulphureum* or Eca alone. The number of visible blackleg plants was almost zero by 63 days after planting and later in all treatments because of the rapid and complete breakdown of earlier existing blackleg stems.

At no time after planting was a significant number of blackleg plants observed in any plot where Ecc-inoculated tubers had been planted. The highest incidences of blackleg were observed between 30 and 40 days after planting.

In general, wilt symptoms were most evident 82 days after planting (Table 2). The incidence of wilted plants was most severe after inoculation with *V. dahliae*. All three *Fusarium* spp. were about equal in causing wilt.

Yield was reduced an average of 35% in all plots where preplant inoculation with Eca occurred alone or in combination. No other preplant or postplant inoculation reduced yield as compared with the uninoculated control (Table 1).

A total of 230 samples of diseased plant tissue were randomly collected throughout the season from the field experiment. About 2,000 individual isolations were made from seed tubers and plant stems (14). In all treatments, the inoculated pathogen was consistently recovered from diseased samples. Of the pectolytic (6) bacteria isolated from diseased stem and tuber tissue, biochemical tests identified 63% as Eca and 28% as Ecc. Of the four wilt pathogens used in postplant inoculation treatments, *V. dahliae* was recovered most frequently from stem tissue.

DISCUSSION

The data on blackleg presented in this study agree with research conducted by us in previous years (8). In this study, a 23.5% reduction in stand occurred when seed tubers were inoculated with Eca

Table 1. Effect of *Fusarium* spp. and *Verticillium dahliae* on potato stand, seed tuber decay, blackleg stems per plant, and yield

Treatment[a]	Stand (%)[b]	Yield (kg)[c]	Seed tuber decay (%)[d] Days after planting 25	36	44	Blackleg stems (%)[e] Days after planting 25	36	44
H_2O-preplant	97.9	23.2	8.1	35.7	72.0	0.0	0.0	0.0
H_2O-postplant	98.1	25.1	2.3	20.0	54.0	0.0	0.0	0.0
Eca-preplant	74.9	15.8	80.8	81.1	93.4	14.5	19.0	36.5
Ecc-preplant	96.4	26.8	35.7	49.4	61.1	0.0	0.7	0.0
F. sulphureum-postplant	98.8	25.9	4.4	21.6	81.8	0.0	0.0	0.0
F. solani-postplant	96.9	25.1	0.8	15.0	46.0	0.0	0.0	0.0
F. oxysporum-postplant	96.7	24.1	5.3	17.3	44.3	0.0	0.0	0.0
V. dahliae-postplant	96.9	25.7	5.3	19.0	64.5	0.0	0.0	1.8
Eca-preplant + *V. dahliae*-postplant	71.4	16.7	86.4	84.4	97.1	23.0	34.0	30.0
Ecc-preplant + *V. dahliae*-postplant	97.3	26.0	26.4	32.3	74.0	0.0	0.0	0.0
Eca-preplant + *F. oxysporum*-postplant	74.6	16.4	81.0	88.8	95.7	24.7	38.5	35.3
Ecc-preplant + *F. oxysporum*-postplant	97.5	28.2	24.8	46.4	78.8	0.0	0.0	3.8
Eca-preplant + *F. solani*-postplant	82.6	17.5	78.3	96.8	100.0	22.2	34.0	36.3
Ecc-preplant + *F. solani*-postplant	97.5	23.8	28.0	40.8	82.3	7.2	0.8	2.0
Ecc-preplant + *F. sulphureum*-preplant	99.4	23.9	27.3	47.7	64.4	0.0	0.0	0.0
Eca-preplant + *F. sulphureum*-preplant	76.0	14.9	81.1	96.8	100.0	19.0	24.8	32.7
Ecc-preplant + *F. sulphureum*-postplant	93.2	23.8	20.0	33.0	83.6	0.8	0.0	0.0
Eca-preplant + *F. sulphureum*-postplant	75.8	16.8	82.7	89.7	95.4	19.7	33.8	32.7
LSD, $P = 0.05$	3.4	3.9	13.0	18.5	18.1	10.8	13.5	13.4

[a] Eca = *Erwinia carotovora* subsp. *atroseptica;* Ecc = *E. carotovora* subsp. *carotovora.*
[b] Percentage of plants emerged 26 days after planting; mean of 12 replicates.
[c] Total weight of all tubers harvested from a 10-m plot; mean of six replicates.
[d] Percentage of the seed tuber decayed; mean of five seed tubers per treatment from six replicates.
[e] Percentage blackleg stems per plant; mean of a five-plant sample per treatment from six replicates.

Table 2. Effect of *Fusarium* spp. and *Verticillium dahliae* on the incidence of potato blackleg and wilt

	Blackleg plants (%)[b]						Wilted plants (%)[c]			
	Days after planting						Days after planting			
Treatment[a]	30	40	54	63	82	93	54	63	82	93
H_2O-preplant	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0	4.1	2.1
H_2O-postplant	0.2	0.2	0.0	0.0	0.7	0.4	0.3	0.0	1.4	2.1
Eca-preplant	13.6	8.4	4.4	0.3	0.0	0.5	0.4	0.0	1.1	0.9
Ecc-preplant	0.2	0.0	0.0	0.0	0.0	0.4	0.0	0.0	4.9	4.1
F. sulphureum-postplant	0.0	0.0	0.0	0.0	1.4	0.7	0.2	0.0	2.1	2.7
F. solani-postplant	0.0	0.2	0.0	0.0	0.7	0.9	0.0	0.0	4.2	1.8
F. oxysporum-postplant	0.0	0.0	0.0	0.0	0.0	1.4	0.2	0.0	3.2	1.7
V. dahliae-postplant	0.0	0.0	0.0	0.0	0.2	0.9	0.7	0.0	13.2	8.3
Eca-preplant + *V. dahliae*-postplant	15.1	18.7	14.6	0.0	0.3	0.4	0.9	0.0	3.1	2.6
Ecc-preplant + *V. dahliae*-postplant	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	8.6	7.5
Eca-preplant + *F. oxysporum*-postplant	17.1	18.8	13.5	0.0	0.0	0.5	1.0	0.0	1.9	0.5
Ecc-preplant + *F. oxysporum*-postplant	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.3	2.1	2.4
Eca-preplant + *F. solani*-postplant	17.5	14.5	11.5	0.0	0.0	0.0	0.2	0.0	1.5	1.2
Ecc-preplant + *F. solani*-postplant	0.5	0.0	0.0	0.0	0.3	0.5	0.3	0.0	5.8	1.4
Ecc-preplant + *F. sulphureum*-preplant	0.0	0.0	0.0	0.0	0.0	1.4	0.7	0.0	5.2	2.8
Eca-preplant + *F. sulphureum*-preplant	16.3	9.0	2.9	0.0	0.0	0.7	0.4	0.0	2.6	1.1
Ecc-preplant + *F. sulphureum*-postplant	0.0	0.2	0.0	0.0	0.9	0.4	0.4	0.0	3.0	2.4
Eca-preplant + *F. sulphureum*-postplant	9.8	11.5	10.9	0.3	0.0	1.0	0.6	0.2	0.9	1.3
LSD, $P = 0.05$	2.7	2.4	2.7	NS	0.7	0.8	0.7	NS	2.1	1.8

[a] Eca = *Erwinia carotovora* subsp. *atroseptica*; Ecc = *E. carotovora* subsp. *carotovora*.
[b] Plants with one or more blackleg stems, mean percentage of 12 replicates.
[c] Plants with one or more wilted stems, mean percentage of 12 replicates.

before planting, whereas seed inoculated with Ecc produced stands equal to the uninoculated control. Examination of seed tubers for decay showed that Eca had caused extensive seed tuber decay early in the season and continued to do so throughout the first 8 wk. Ecc, however, caused less seed tuber decay initially and ceased within 4 wk after planting.

In a separate greenhouse study (R. T. Zink, *unpublished*), Eca was a more aggressive seed tuber rotter than Ecc at 12 C, whereas Ecc tended to cause more seed tuber decay at 30 C. The relationship between these two bacteria and temperatures appears to be the same under field conditions (15). This is in agreement with Molina and Harrison (13), who reported that Ecc causes more severe disease at high soil temperatures than does Eca under the same soil conditions. Isolations made from rotted seed tubers during the course of the field study showed that Eca was more pathogenic early in the season than Ecc, and Ecc was seldom isolated at any time during the study. Based on this information and the cool soil conditions in North Dakota at planting time (average daily range for May at 10-cm depth is 9.3–12.6 C), Eca should be considered the more important pathogen in early-season seed tuber decay.

The incidence of blackleg, both in percentage of stems infected per plant and number of plants infected, was directly related to seed tuber decay. In plots where seed tuber decay was high, emergence was reduced, the percentage of blackleg stems per plant was high, and the total incidence of blackleg plants was greatest.

Expressing the level of infection as a percentage of the stems per plant showing blackleg symptoms provided accurate measurement of the increase in blackleg infection over a short period. Other workers have reported only the number of plants infected, with no regard for the extent of the infection (13,19). The latter method overlooks preemergence blackleg and the relationship between seed tuber decay and the number of stems infected per plant. In this study, by measuring seed tuber decay and percentage of stems infected, it was possible to determine the immediate effect of wilt-causing fungi on the extent of the blackleg infection.

The increase in percentage of blackleg stems was not significantly different with any of the four fungi, which indicates that a general detrimental effect existed rather than a specific synergistic relationship between the wilt fungi and Eca.

Two hypotheses may explain the role that fungal pathogens have in blackleg development. Based on the previous work of Huguelet et al (8) and von Munzert et al (19), in which the fungi associated with Eca were both dry-rotting *Fusarium* spp., the increase in blackleg could be considered a function of the increased rate of seed piece decay wherein the infection literally destroys the seed pieces and the newly developing stems (5). The alternative hypothesis would be active or passive transport of the bacteria from the rotting seed piece into the stem by systemic activity of the fungal pathogens present in the system.

These hypotheses seem valid when considering a dry-rotting fungus such as *F. sulphureum*, but not valid with wilt pathogens. In the case of *V. dahliae, F. solani*, and *F. oxysporum* as postplant inoculations, it seems more likely that colonization of the roots and vascular system would generally weaken the plant, making it more susceptible to the blackleg bacteria already established in the seed piece. However, Kotcon et al (10) have pointed out that in the case of potato early dying disease, which often involves *Verticillium, Fusarium*, and *Erwinia*, there is no decrease in total root length.

Preemergence and early postemergence blackleg are usually accompanied by extensive seed piece decay, whereas midseason blackleg is usually associated with plant stress. Fungal pathogens, as well as injury (eg, hail or root pruning at cultivation), cause additional plant stress and may act as triggers to the development of increased blackleg. Midseason blackleg is more noticeable because early-season blackleg accompanied by seed piece decay tends to result in missing plants. However, both early-season and midseason blackleg can have a significant effect on yield.

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