Relationship of *Verticillium dahliae* and *Erwinia carotovora* pv. *carotovora* in the Early Dying Disease of Potato

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ABSTRACT

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The effect of simultaneous and sequential inoculations of potato plants with the wilt-causing fungus, *Verticillium dahliae* (Vd), and the soft-rotting bacterium, *Erwinia carotovora* pv. *carotovora* (Ecc), was studied under controlled environmental conditions. All plants were inoculated with Vd by the root dip method. Plants were inoculated with Ecc either through wounded roots, through the cut stem base, or by injection with a micropipette at a leaf axil. Disease severity was assessed by measuring inhibition of stem growth, extent of leaf chlorosis and wilting (disease index), green leaf area remaining at the end of the experiment, and the number of stems in which soft rot developed. Ecc successfully colonized stems inoculated either by the cut stem base or the leaf axil injection method, but not when inoculum was introduced through wounded roots. With the cut stem base inoculation, plants of potato cultivars Norgold Russet and Russet Burbank developed more severe symptoms of early dying in the presence of both pathogens than with either pathogen alone. The effect of concurrent infection with Vd and Ecc on both Norgold Russet and Russet Burbank was synergistic; plant growth was reduced, chlorosis and wilting were increased, and development of stem soft rot was enhanced. Ecc alone did not cause stem soft rot following inoculation by this method, but it resulted in reduced green leaf area in Norgold Russet. When Vd-infected plants were injected with Ecc at a leaf axil, only Norgold Russet developed symptoms that were more severe than with either pathogen alone. Furthermore, soft rot developed in stems of more plants inoculated with both pathogens than when Ecc was present alone.

Additional key words: *Solanum tuberosum*.

Factors associated with the early dying disease complex in potato differ from region to region; in Ohio (13,21), Idaho (4), and New York (22) the two main pathogens in the early dying complex are the vascular wilt fungus, *Verticillium dahliae* Kleb. (Vd) and the lesion nematode, *Pratylenchus penetrans* (Cobb) Filipj. and Schuurm.-Stekh. In Florida, a Verticillium wilt–nematode complex with several other nematode genera has been reported (25). In Indiana, the fungus *Colletotrichum atramentarium* (Berk. & Br.) Taub. may also be an additional component (12,24). In Oregon, *Erwinia carotovora* pv. *atroseptica* and Vd appear to be the most important incitants of early dying (11). In Wisconsin, Vd, *E. carotovora* pv. *carotovora* (Jones) Dye (Ecc), *Fusarium* spp., and *C. atramentarium* have been frequently isolated from infected potato plants showing early dying symptoms (14,20).

The purpose of the present investigation was to determine the extent to which the presence of *E. carotovora* pv. *carotovora* affects the development of the early dying disease in potato in the presence of *V. dahliae* under controlled-environment conditions.

MATERIALS AND METHODS

Plant cultures. Certified seed tubers of cultivars Russet Burbank and Norgold Russet were stored in a cold room at 5 C until used. Seed tubers were removed from the cold room 4–5 days before planting and held at room temperature (24 ± 2 C). If necessary, seed dormancy was broken by treating the tubers with Rindite (a mixture of ethylene chlorhydrin, ethylene dichloride, and carbon tetrachloride [7:3:1, v/v]) 2 wk before planting (10). The tubers were then washed in running water for 10 min, surface sterilized in 0.5% NaOCl for 3 min, and rinsed with tap water. Pieces of tuber tissue containing single eyes were removed with a sterile melon ball scoop, planted in flats containing vermiculite, and allowed to grow in a controlled-environment chamber at 24 C, 75 ± 5% RH, with a 12-hr photoperiod (21,528 lux). Plants were watered every day and were fertilized twice a week with full-strength Hoagland's solution throughout the experiments. Plants were used either for inoculation or as a source of cuttings 3 wk after planting. After inoculation, the plants were transplanted in 12.5-cm-diameter clay pots containing a 3:1 mixture of quartz sand and muck soil, and the pots were arranged randomly on benches in the plant growth chamber.

Cuttings for rooting were made by removing the apical 5–7 cm of the stem with a sterilized knife. The cut ends were dipped in a 4% formulation of 1-naphthaleneacetamide (Rootone No. 10; Amchem Products Inc., Fremont, CA 94536), large leaves were removed, and the stems were inserted in a commercial mixture of peatmoss and vermiculite (1:1, v/v) in flats. Rooted cuttings 3–4 wk old were inoculated with Ecc by stem puncture.

Preparation of inocula. The strain of Vd used in these experiments was initiated from a single conidium of an isolate obtained from a potato stem. This culture was maintained in potato-dextrose agar slants at 5 C and transferred to new slants every 6 mo. The Ecc strain, SR 102, was a highly virulent strain obtained from Arthur Kelman, Dept. of Plant Pathology, University of Wisconsin-Madison, who isolated it from a potato plant. The serogroup of this strain was found to be similar to serogroup XIII (6) by Solke H. De Boer, Research Station, Vancouver, Canada. This strain was maintained in sterile distilled water (SDW) at 24 ± 2 C.

Vd inoculum was prepared by adding a 5-mm-square piece of a 3-wk-old culture growing on potato-dextrose agar to 500-ml flasks containing 100 ml of Czapek-Dox broth (2). The flasks were then incubated on a reciprocal shaker with 80 strokes per minute at room temperature. After 7–9 days, conidia were separated from the mycelium by passing the contents of the flasks through sterilized cheesecloth. The density of the conidial suspension was adjusted to 5×10^4 conidia per milliliter with a hemocytometer.

Bacterial inoculum consisted of 24-hr cultures grown on casamino acids-peptone-glucose medium plates (Bacto-glucose 10 g, Bacto-peptone 10 g, casamino acids 1 g, Bacto-agar 18 g, distilled water 1,000 ml). Bacterial cells were suspended in 10 ml of SDW. This suspension was then adjusted to $\sim 2 \times 10^8$ colony-forming units (cfu) per milliliter using a Spectronic 20 colorimeter (Bausch & Lomb Co.) set at a wavelength of 600 nm. This suspension was diluted to obtain the desired inoculum concentrations. The actual number of colony-forming units present in the original and diluted

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suspensions were determined by plating serial dilutions on crystal violet pectate medium (CVP) (3).

Inoculation procedures. Three methods of inoculation were compared. In the first, the pathogens were introduced into the vascular tissue of stems through wounded roots. Ecc concentrations of 10^6 and 10^8 were used for this purpose. The basal portion of 3-wk-old plants with the seed pieces still attached were washed in SDW and immersed in a suspension of either Vd, Ecc, or Vd plus Ecc. The roots were then clipped at 6 cm from the stem base and left in the suspensions for 30 min. Plants treated similarly in SDW served as controls. The experiment was terminated 28 days after inoculation.

In the second method, the pathogens were introduced into the vascular tissue at the base of the stem where natural inoculum from an infected seed piece would be likely to enter. This method is referred to as the cut stem method. The original seed piece was cut from the stem base prior to inoculation, and the basal portion of the plants including the roots were immersed in a suspension of either Vd, Ecc, or Vd plus Ecc. Ecc concentrations of 10^4, 10^6, and 10^8 were used for Norgold Russet and Ecc concentrations of 10^6 and 10^8 were used for Russet Burbank. The stem base was cut at an angle so that only a small number of roots was removed. Stem base and roots remained in the inoculum suspension for 30 min while plants were exposed to moving air from a fan to increase transpiration and enhance movement of the inoculum suspension into the vascular system. Plants treated similarly in SDW served as controls.

In the third method of inoculation, the stem injury and simultaneous introduction of the pathogen by contaminated insects was simulated. Thirty rooted cuttings were inoculated by clipping the roots immersed in Vd inoculum suspension and another 30 plants were similarly treated except in SDW (day 0). At the desired time intervals, six plants of each set were inoculated with Ecc suspensions of 2×10^3 or 2×10^7 cfu per plant by inserting a 100-μl sterile micropipette (Becton-Dickinson & Co., Parsippany, NJ 07054) at the second leaf axil from the soil line. Prior to inoculation, the bud in the axil was removed and a wound 3–5 mm deep was made by inserting the sharp end of a broken micropipette. The bacterial suspension was taken up by the plants within 4–12 hr. The above experiments were repeated one time.

To determine the populations of Ecc and Vd in plants at the end of the experiment, a 3-cm section of stem immediately above the leaf axil inoculated with Ecc (and the comparable leaf axil in plants that received other treatments) was excised from each stem. The stem sections were surface-sterilized in 0.5% NaOCl solution for 1 min and a 1-cm section was excised from the center and cut into five pieces. This was fragmented for 1 min in 10 ml of SDW in a 50-ml cup of an Omnimixer Homogenizer (Ivan Sorvall, Inc., Norwalk, CT 06856). The resulting suspension was diluted to 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}. One-half milliliter of the diluted suspension was then spread on ethanol streptomycin agar plates (ESA) (17), and 0.1 ml was spread on CVP (3) plates. CVP plates were incubated for 2 days at room temperature (24 ± 2 C), and colonies that formed pits typical of Ecc were counted. ESA plates were incubated at room temperature for 2 wk and colonies that had formed microsclerotia were counted. The two remaining 1-cm pieces were dried for 24 hr at 105 C to determine the dry weight of the tissue used.

Disease measurements. Disease symptoms characteristic of the early dying syndrome were evaluated according to the following criteria in all the experiments: inhibition of stem growth, green leaf area remaining at the end of experiment, disease index (see below), and the number of plants that developed stem soft rot within 7 days after inoculation with Ecc.

Percent change in stem length was determined by using the difference between the stem length at the harvest time and the stem length at the inoculation time.

The area of green leaves remaining at the end of an experiment was measured by means of a leaf area meter model LI-3100 (Lambda Instruments Corporation, Lincoln, NE 68504).

A scale of 0–6 was used for disease indexing according to the estimated percent of foliage that was chlorotic, necrotic, or wilted as follows: 0 = all foliage healthy, 1 = 20%, 2 = 40%, 3 = 60%, 4 = 80%, 5 = 100% of the foliage affected, and 6 = plants completely dead and dried out. The data were analyzed as a completely randomized design, and the treatment means were compared according to Duncan's new multiple range test.

RESULTS

Inoculation via wounded roots. Vd entered wounded roots of cultivar Norgold Russet and caused disease. Vd was reisolated from stems of plants that were inoculated with Vd, but Ecc was not reisolated from stems and apparently did not cause disease. Stem growth, disease index, and green leaf area in Norgold Russet plants were significantly different when Vd was present than when it was absent. There was no significant difference in stem growth, disease index, and green leaf area between plants inoculated with Vd alone and those inoculated with both pathogens. Similar results were obtained when the above experiment was repeated with Russet Burbank, a cultivar more resistant to Ecc (20).

Inoculation via the cut stem base. In Norgold Russet, soft rot was not observed in the cut stems inoculated with Ecc alone. However, soft rot developed at the stem base in a few plants within 7 days after inoculation with Ecc at 10^6 and 10^8 cfu/ml together with Vd

TABLE 1. Effects of *Verticillium dahliae* and *Erwinia carotovora* pv. *carotovora* (Ecc) on plants of potato cultivar Norgold Russet after inoculation via the cut stem base method

Inoculum	Ecc (cfu/ml)	Stems with soft rot[w]	Stem growth after inoculation: Growth (%)	Stem growth after inoculation: Inhibition (%) due to the treatment	Disease index[x]	Green leaf area: Total (cm^2)	Green leaf area: Reduction (%)
V. dahliae[y]		0/6	79 c[z]	51.3	2.1 b[z]	214 d[z]	63.9
E. carotovora pv. *carotovora*	10^4	0/6	151 ab	6.8	0.6 a	508 ab	14.4
	10^6	0/6	147 ab	9.3	0.6 a	488 ab	17.7
	10^8	0/6	124 ab	11.7	0.7 a	393 bc	33.7
V. dahliae[y] + *E. carotovora* pv. *carotovora*	10^4	0/6	117 bc	27.8	2.0 b	325 cd	45.1
	10^6	2/6 (S)	40 d	75.3 (S)	4.2 c	74 e	87.5 (S)
	10^8	1/6 (S)	21 d	87.0 (S)	4.3 c	69 e	88.4 (A)
None		0/6	162 a	0.0	0.0 a	593 a	0.0

[w] Stem soft rot developed within 7 days after inoculation with Ecc: ratio of the number of plants with soft rot to the number of plants inoculated.
[x] A scale of 0–6 was used for disease indexing; 0 = healthy plant, 6 = dead plant.
[y] Roots immersed for 30 min in suspension of 5×10^4 conidia per milliliter.
[z] Mean of six plants; S = synergistic effect; A = additive effect; values followed by the same letter in each column do not differ significantly, $P = 0.05$, according to Duncan's new multiple range test.

(Table 1). This indicated a synergistic effect of Vd and Ecc. The soft rotted tissue was dark brown and discoloration started at the cut stem base and moved progressively upward (Fig. 1).

Stem growth and retention of green leaf area of plants exposed to Vd together with Ecc at concentrations of either 10^6 or 10^8 cfu/ml were significantly less than when Vd was present alone (Table 1). The disease index was also significantly greater when plants were inoculated with Vd together with Ecc at 10^6 and 10^8 cfu/ml than when Vd was present alone.

The concentration of Ecc to which the plants were exposed was important. Stem growth of plants exposed to Ecc alone at inoculum concentrations of 10^4, 10^6, and 10^8 cfu/ml did not differ significantly from that of uninoculated plants (Table 1). However, while the green leaf area of plants exposed to Ecc at concentrations of either 10^4 or 10^6 cfu/ml was comparable to that of uninoculated plants, the green leaf area retained by plants exposed to 10^8 cfu/ml was significantly less than in the uninoculated plants.

In Russet Burbank plants, no symptoms developed in plants inoculated with Ecc alone at any concentrations (Table 2). Stem soft rot was not observed in any of the plants inoculated either with Ecc alone or with both pathogens. However, at the end of the experiment Ecc was reisolated from some of the aboveground stems of plants (16–33%) that had been inoculated with Ecc. At harvest, disease index and green leaf area of plants inoculated with Vd alone or with both pathogens were significantly different from those of uninoculated plants and plants inoculated with Ecc alone. None of the treatments resulted in a significant effect on stem growth.

Inoculation by stem injury. Symptom development was more rapid in the presence of Vd and Ecc than in the presence of Vd alone. The symptoms on day 19 are shown in Fig. 2. Plants inoculated with both pathogens were severely wilted. Plants inoculated with Vd alone were characterized by reduced growth, smaller leaf size, and wilting of the leaves. Control plants and those inoculated with Ecc alone did not show symptoms.

To determine whether the effect of Ecc was altered by the extent of prior colonization by Vd, plants were inoculated with 2×10^3 cfu

Fig. 1. Basal stem soft rot in potato cultivar Norgold Russet 1 wk after inoculation with both *Erwinia carotovora* pv. *carotovora* (Ecc) and *Verticillium dahliae* by using the cut stem base inoculation technique. Similar inoculation with Ecc alone did not cause any basal stem soft rot.

Fig. 2. Interactive effect of *Verticillium dahliae* (Vd) and *Erwinia carotovora* pv. *carotovora* (Ecc) on plants of potato cultivar Norgold Russet in which stems were inoculated by injecting a suspension of Ecc by inserting a micropipette at a leaf axil. Symptoms on day 19 after inoculation, from left to right, plant inoculated with Vd and Ecc (severe wilting and most leaves necrotic), plants inoculated with Vd (plant stunted and some leaves necrotic), and a plant representing either an uninoculated plant or a symptomless plant that had been inoculated with Ecc.

TABLE 2. Effects of *Verticillium dahliae* and *Erwinia carotovora* pv. *carotovora* (Ecc) on plants of potato cultivar Russet Burbank after inoculation via the cut stem base

Inoculum	Ecc (cfu/ml)	Ecc reisolated from stems[w]	Stem growth after inoculation: Growth (%)	Stem growth after inoculation: Inhibition (%) due to the treatment	Disease index[x]	Green leaf area: Total (cm^2)	Green leaf area: Reduction (%)
V. dahliae[y]	...	0/6	217 a[z]	10.3	2.1 b[z]	599 b[z]	30.2
E. carotovora pv. *carotovora*	10^6	2/6	232 a	4.1	0.0 a	834 a	2.8
	10^8	1/6	234 a	3.3	0.0 a	844 a	1.6
V. dahliae[y] + *E. carotovora* pv. *carotovora*	10^6	2/6	219 a	9.5	2.7 c	383 c	55.4 (S)
	10^8	2/6	210 a	13.2	3.0 c	375 c	56.3 (S)
None		0/6	242 a	0.0	0.0 a	858 a	0.0

[w] Symptomless stems from which Ecc was reisolated 28 days after inoculation: ratio of plants from which Ecc was reisolated to the number of plants inoculated.
[x] A scale of 0–6 was used for disease indexing; 0 = healthy plant and 6 = dead plant.
[y] Roots immersed for 30 min in suspension of 5×10^4 conidia per millilter.
[z] Mean of six plants 28 days after inoculation; S = synergistic effect; values followed by the same letter in each column do not differ significantly, $P = 0.05$, according to Duncan's new multiple range test.

of Ecc per plant 2 and 21 days after inoculation with Vd. The results (Table 3) showed that stem growth, disease index, and green leaf area of plants inoculated with Ecc alone on day 2 did not differ significantly from that of uninoculated plants. However, the stem growth was significantly less in plants inoculated with both pathogens than in plants inoculated with either pathogen alone at either date. The disease index on day 19 and 28 was significantly greater in the presence of both pathogens than in the presence of each pathogen alone.

Russet Burbank was inoculated on days 2, 9, and 16 following inoculation with Vd (Table 4). This cultivar was inoculated with 2×10^7 cfu of Ecc per plant, because the ED_{50} (effective dose that causes 50% of the inoculated stems to rot) value (1.36×10^7) for the cultivar Russet Burbank is greater than the ED_{50} value (1.27×10^5) for Norgold Russet (20). One of six Russet Burbank plants inoculated with Ecc on each date developed stem soft rot when both pathogens were present, but no soft rot was observed when Ecc was the sole pathogen present. Disease severity in plants infected by Vd that had been inoculated with Ecc at any time was not significantly different from that of plants inoculated with Vd alone. No significant symptoms were observed in plants inoculated with Ecc alone.

Populations of Ecc on day 28 in Russet Burbank plants inoculated with Ecc alone were not significantly different from those in plants inoculated with both pathogens (Table 4). Likewise, populations of Vd in plants inoculated with Vd alone did not differ significantly from those in plants inoculated with both pathogens. Populations of Ecc and Vd in stems of Norgold Russet plants were not assessed because most of the plants had dried out due to the treatments at the end of the experiment.

Similar results were obtained when the above experiments were repeated (20) except that in Norgold Russet there was more stem soft rot development in plants inoculated with both pathogens than in plants inoculated with Ecc alone, indicating synergism.

DISCUSSION

The two major pathogens colonizing potato stems under Wisconsin conditions are Vd and Ecc (20). Vd is well known as a vascular pathogen causing wilt. Although Ecc in potato is known principally as a soft rotter of stems and tubers (19) and as the causal agent of blackleg (15,23), it also colonizes the vascular tissue (20).

A basic problem encountered in studying the effect of Ecc on early dying disease of potato as caused by Vd was to find a reliable

TABLE 3. Effects of *Verticillium dahliae* (Vd) and *Erwinia carotovora* pv. *carotovora* (Ecc) on potato cultivar Norgold Russet following inoculation of leaf axils with Ecc by micropipette injection 2 and 21 days after root dip inoculation with Vd

Inoculum	Day of inoc. with Ecc after inoc. with Vd	Stems with soft rot[v]	Stem growth after inoculation: Growth (%)	Stem growth after inoculation: Inhibition (%) due to the treatment	Disease index[w]: Day 19	Disease index[w]: Day 28	Green leaf: Total (cm^2)	Green leaf: Reduction (%)
V. dahliae[x]	···	0/6	93 b[y]	46.2	1.8 b[y]	4.5 c[y]	72 c[y]	92.9
E. carotovora pv. *carotovora*[z]	2	0/6	162 a	6.3	0.0 a	0.0 a	1,007 ab	2.1
	21	1/6	222 a	0.0	0.0 a	2.2 b	603 b	41.0
V. dahliae[x] + *E. carotovora* pv. *carotovora*[z]	2	0/6	31 c	82.1 (S)	4.0 c (S)	5.8 d (S)	0 c	100.0
	21	0/6	85 bc	50.9	2.0 b	5.7 d (A)	3 c	99.7
None	···	0/6	173 a	0.0	0.0 a	0.0 a	1,022 a	0.0

[v] Stem soft rot 7 days after inoculation with Ecc. Ratio of plants with soft rot to plants inoculated.
[w] A scale of 0–6 was used for disease indexing 19 and 28 days after inoculation with Vd; 0 = healthy plant, 6 = dead plant.
[x] Inoculum suspension of 5×10^4 conidia per milliliter.
[y] Mean of six plants; S = synergistic effect; A = additive effect. Values followed by the same letter in each column do not differ significantly ($P = 0.05$) according to Duncan's new multiple range test.
[z] Inoculum dosage of 2×10^3 cfu per plant (Strain SR 102).

TABLE 4. Effects of *Verticillium dahliae* (Vd) and *Erwinia carotovora* pv. *carotovora* (Ecc) on potato cultivar Russet Burbank and their populations in stems following stem injection with Ecc by using the micropipette method at 2, 9, and 19 days after root dip inoculation with Vd

Inoculum	Day of Ecc inoc. after Vd inoc.	Stems with soft rot[t]	Increase (%)[u] stem length	Disease index[v]	Green leaf area (cm^2)	Populations at day 28: Ecc	Populations at day 28: Vd
V. dahliae[w]		0/6	74 c[y]	2.1 b[y]	496 b[y]	0.00[z]	6.38 a[z]
E. carotovora pv. *carotovora*[x]	2	0/6	136 a	0.0 a	992 a	5.59 a	0.00
	9	0/6	115 ab	0.3 a	925 a	6.64 a	0.00
	16	0/6	87 bc	0.1 a	904 a	6.74 a	0.00
V. dahliae[w] + *E. carotovora* pv. *carotovora*[x]	2	1/6	83 bc	2.3 b	406 b	6.04 a	6.43 a
	9	1/6	66 c	2.5 b	414 b	6.85 a	6.58 a
	16	1/6	85 bc	2.1 b	577 b	7.38 a	6.20 a
None		0/6	130 ab	0.0 a	956 a	0.00	0.00

[t] Stem soft rot was formed wtihin 7 days after inoculation with Ecc. Ratio of plants with soft rot to plants inoculated.
[u] Percent increase is based on the stem length at the time of inoculation with Ecc.
[v] A scale of 0–6 was used for disease indexing; 0 = healthy plant, 6 = dead plant.
[w] Inoculum suspension of 5×10^4 conidia per milliliter.
[x] Inoculum dosage of 2×10^7 cfu per plant.
[y] Mean of six plants 28 days after inoculation with *V. dahliae*.
[z] Log colony-forming units per gram (cfu/g) dry weight; mean of six plants. Values followed by the same letter in each column do not differ significantly ($P = 0.05$) according to Duncan's new multiple range test.

technique for introduction of Ecc into the vascular tissue of the stem.

Inoculation of plants by clipping roots submerged in an inoculum suspension containing Ecc together with Vd did not result in an increased disease severity over that with Vd alone, and Ecc was not detected in the stems after inoculation. Therefore, it was concluded that Ecc was not introduced into the vascular tissue of stems by this method of inoculation. This is compatible with the results of De Boer et al (7), who found that although the populations of pectolytic *Erwinia* spp. in the root zones of potato plants grown from infested seed pieces increased to as high as 3×10^8 cells per gram dry weight of roots plus soil in the root zone, few plants showed symptoms of blackleg during the growing season. These results suggest that wounded roots are not effective infection sites for pectolytic *Erwinia* spp.

Two other methods were found successful in introducing Ecc into the vascular tissue of the stems. In one method, the potato stems were inoculated by making a wound at a leaf axil by means of a micropipette. This introduced a measured amount of Ecc into the vascular tissue and also simulated natural introduction of the pathogen through stem wounds caused by contaminated insects. In the other method, plants were inoculated with Ecc via the vascular tissue exposed by excising the tip of the stem base while it was immersed in a suspension of inoculum. This method introduced Ecc together with Vd into the vascular tissue at the stem base where plants would be naturally infected via infected seed pieces. The results obtained by these two methods were similar but certain differences were also apparent.

Inoculation of plants with Ecc via the cut stem base technique allowed a longer period of time for the simultaneous development of the two pathogens. When plants were inoculated by this method, the interactive effect of the pathogens was evident on disease index (leaf chlorosis and wilting) and on green leaf area remaining at the end of the experiment both in the susceptible cultivar, Norgold Russet, and in the relatively resistant cultivar, Russet Burbank. On the other hand, when plants were inoculated with Ecc at a leaf axil by means of a micropipette, the interactive effect of the pathogens on stem growth and on disease index was observed only in Norgold Russet, but not in Russet Burbank. In addition, in the cut stem base technique, the absence of stem soft rot in plants inoculated with Ecc alone made it possible to demonstrate the effect of Vd on the development of stem soft rot when both pathogens were present.

The combination of the two pathogens applied both with the micropipette and the cut stem base technique resulted in more stem soft rot development than when Ecc was present alone. From this, it was apparent not only that Ecc enhanced the effect of Vd on stem growth and leaf area, but also that Vd reciprocally enhanced development of stem soft rot. These results are similar to those of Zink and Secor (27) who found that *Fusarium sulphureum, F. oxysporum,* and *V. dahliae* increased the number of blackleg-affected stems per plant under field conditions. They suggested that blackleg and stem soft rot of potato can be the result of a complex etiology involving a bacterial pathogen and a fungal pathogen in certain cases. The existence of these interactions could be a contributing factor in the year-to-year variation in blackleg and stem soft rot which occurs in potato crops.

The overall results obtained here and by Rahimian (20) indicate that plants of cultivars Norgold Russet and Russet Burbank were affected adversely by the presence of both pathogens. The combination of Vd and Ecc inhibited stem growth, decreased in green leaf area remaining at the end of the experiment, increased disease index, and increased the frequency of stem soft rot development.

Natural infection by Ecc is widespread in some commercial potato-growing areas (11,19,20). The data obtained from an experimental field plot and four commercial fields in Wisconsin in 1979, 1980, and 1981 indicated that populations of Ecc as well as Vd increased in the stem tissue during the growing season (20). The results obtained here strongly suggest that Vd and Ecc are interrelated in causing the early dying disease under field conditions.

Although fungus-fungus (1,8), fungus-nematode (9,26), and fungus-virus (18) synergistic relationships have been reported, the synergistic relationship between a fungus and a bacterium had not been reported until recently (5,27). The results obtained here also suggest that a pectolytic bacterium could enhance the development of early dying in potatoes.

Two mechanisms might be involved in the synergistic relationship between Ecc and Vd in early dying disease of potato. First, after inoculation of potato stems, populations of Ecc and Vd in the stems would increase more rapidly when both pathogens are present together than when either one is present alone. According to the results obtained here (Table 4), the populations of Vd and Ecc 28 days after the inoculation of Russet Burbank plants with both pathogens or with either pathogen alone were not significantly different. However, testing of this hypothesis would require a study of the changes of the populations of both organisms in the stem tissue over a period of time following inoculation. Second, the enzymatic activity of Ecc along with that of Vd in the vascular tissue may be synergistic, causing a more rapid breakdown of the host cell wall material and eventually causing a more rapid death of the plants. Recently, Mussell and Stilwell (16) reported that cell walls prepared from tomato, eggplant, and cotton contained enzymes capable of degrading the vegetative mycelium of Vd. Soluble breakdown products obtained in incubating mycelial powders of *Verticillium* with mycelium-degrading enzymes were toxic to tomato cuttings. Toxicity of these preparations was greater if the tomato cuttings had been pretreated with endopoly-galacturonase. They suggested that "pathogenesis in verticillium wilt of tomato results from a sequence of events involving components from both host and pathogen." In light of this suggestion, it is possible that in early dying of potato the pectate enzymes of Ecc would participate in breaking down of the cell wall materials of the host which would be followed by a more rapid breakdown of the mycelium of Vd with the release of toxic products which would eventually produce the symptoms of early dying.

LITERATURE CITED

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