SURVIVAL OF *ERWINIA CAROTOVORA* IN WISCONSIN SOILS

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Abstract

Erwinia carotovora var. *atroseptica* (van Hall) Dye and *E. carotovora* var. *carotovora* (Jones) Dye were detected in agricultural soils in Wisconsin using baiting and enrichment techniques. These soft rot bacteria could not be detected in soils of potato fields prior to planting of crops in the spring period using standard soil dilution plating techniques on a crystal violet pectate medium, however. A procedure involving incubation of samples in pectate enrichment broth followed by preparation of smears on slides and treatment with a fluorescent antibody stain specific for *E. carotovora* var. *atroseptica* was the most sensitive of the methods tested for detecting the blackleg pathogen.

Erwinia carotovora was isolated more frequently during the spring from fields in which potatoes had been grown the previous year than from fields in which other crops had been grown. It was also isolated from potato tubers and stems that had overwintered in the field. The presence of *E. carotovora* could not be detected in root zone samples of weed plants using the dilution plating method.

Resumen

En suelos agrícolas del estado de Wisconsin, EE. UU. de NA, se detectaron ambos *Erwinia carotovora* var. *atroseptica* (van Hall) Dye y *E. carotovora* var. *carotovora* (Jones) Dye por medio de técnicas de cebo y enriquecimiento. Sin embargo, estas bacterias que causan pudrición blanda, no pudieron ser detectadas en suelos de campos de papa antes de la siembra de cultivos en la época de primavera por medio del método reconocido de serie de dilución con siembra en placas de medio pectato cristal violeta. Un procedimiento que involucra la incubación de muestras en un medio de enriquecimiento con pectato seguido de preparación de un

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frotis sobre portaobjetos y tratamiento con una tinción fluorescente específica para *E. carotovora* var. *atroseptica,* fue el mas sensible de los métodos probados para la detección del patógeno de la pierna negra de la papa. *E. carotovora* fue aislado con mas frecuencia durante la primavera, de campos en los cuales se cultivó papa el año anterior que de campos en los cuales hubieron otros cultivos. Tambien fue aislado de tubérculos y tallos de papa que habían invernado en el campo. No se pudo detectar la presencia de *E. carotovora* en muestras de la zona radicular de malas hierbas mediante la técnica de dilución en placas.

Introduction

During the past 60 years, the question as to whether *Erwinia carotovora* var. *atroseptica* (van Hall) Dye overwinters in the soil in the northern potato growing areas of the United States has been examined by a number of investigators with divergent conclusions. Early investigators concluded that the blackleg pathogen was mainly seed-borne (24, 25, 32, 33). However Patel (28) isolated the pathogen from artificially infested test tubes of soil that had been left buried in the soil throughout the winter under Iowa conditions. In Minnesota, Leach (19, 20) also presented evidence that the blackleg pathogen could in fact overwinter in the soil. When Bonde (2) re-examined the problem in Maine, he was also able to isolate the blackleg pathogen from soil but due to the low level of persistence in the soil he concluded that soil survival was not an important source of inoculum.

In Europe *E. carotovora* has also been found to overwinter in soil and plant debris in Ireland (21) and Germany (11, 36). Graham (12), however, could not isolate it from soil in Scotland and subsequently concluded that soil was not an inoculum source (13). In addition, observations of Perombelon (29, 30, 31) in Scotland and Harrison (23) in Colorado supported the concept that primary inoculum for the blackleg disease did not originate in soil.

Preliminary attempts in Wisconsin to quantify the overwintering population of *E. carotovora* in the soil were not successful (6). Pectolytic *Erwinia* spp. could not be detected in agricultural soils during the spring when a selective crystal violet pectate (CVP) medium was used. Similarly, Meneley and Stanghellini (22) and Burr and Schroth (3) were unable to isolate the pathogen from soil devoid of plant residues in Arizona and California, respectively, with selective media. However, as few as 10 cells/g dry wt in artificially infested soil could be detected using an enrichment broth procedure (3, 22).

The objective of this research was to determine whether *E. carotovora* var. *atroseptica* and *E. carotovora* var. *carotovora* survive the winter in the soil under Wisconsin conditions. A variety of procedures were evaluated

for relative sensitivity including various baiting and enrichment procedures.

In the text, *E. carotovora* var. *carotovora* will be referred to as *Ecc* and *E. carotovora* var. *atroseptica* as *Eca; E. carotovora* will be used when a strain designation is not involved.

Materials and Methods

Soil Sampling — A standard random sampling procedure was used to obtain soil samples. Ten to 20 cores each 2 cm in diameter and 20 cm long taken with an Oakfield soil sampler were combined to form a single sample.

Dilution Plating — A subsample of soil (c. 25 g) was added to 250 ml sterile distilled water (SDW) in a 500 ml bottle. After shaking the suspension for 30-60 min., four serial dilutions (10_2^1 - 10_2^4) were plated on a crystal violet pectate (CVP) medium (6).

Tissue Baiting — Potato tubers were disinfested for 15 min. in 10% Clorox (final concn NaOCl 0.5%), peeled, dipped in 95% ethanol, flamed, and then cut into wedge-shaped segments with a sterile knife. These segments were inserted in soil samples in covered paper cups with perforated bottoms standing in petri plate covers containing water to maintain soil moisture at field capacity. After three days, a small portion of tissue from the edge of decay that often developed on the potato wedge was crushed in 0.2 ml SDW and streaked on CVP medium. A second sample was smeared on a slide and stained with a fluorescent antibody stain (FAS) specific against *Eca* (1).

Enrichment — The enrichment broth of Meneley and Stanghellini (22) was used with the modification that 0.01 M phosphate buffer pH 7.2 was used rather than 0.1% K_2HPO_4. To 10 g of soil, 100 ml of enrichment broth were added, stirred briefly, covered, and incubated for 48 h at room temperature without shaking. Serial dilutions (10_2^1 - 10_2^4) of the broth were plated on CVP and CVP + 0.01% sodium dodecyl sulfate and 0.18% thallium nitrate (Phillips and Kelman, unpublished data); one drop of the enrichment culture was heat fixed to a slide for staining with FAS.

Direct Fluorescent Antibody Staining of Soil Samples — The method described by Allan and Kelman (1) was used. To 10 g of soil 100 ml SDW were added and shaken for 45 min. A flocculating agent was then added, shaken vigorously and the suspension allowed to settle for 60 min. before 40 ml of the supernatant fluid were removed and centrifuged at 12,000 g for 20 min. The pellet was resuspended in 1.0 ml SDW and a drop heat-fixed to a slide for staining with FAS.

Plant and Insect Samples — Tubers that overwintered in the field were sampled by crushing a small portion of a decayed tuber with a glass rod in 0.2 ml SDW on a porcelain depression plate and then streaking the homogenate on CVP medium. Alternatively, small segments of tissue were

placed on tuber slices cut from surface disinfected, peeled tubers and incubated on moist sterile filter paper in petri dishes. If decay occurred in the tuber slices, a small portion of the freshly decayed tissue was tested on CVP medium. Potato stems that had overwintered in fields were sampled by both the direct plating and baiting methods used for overwintered tubers.

Insects present in tubers that overwintered in the field were tested for surface contamination with *E. carotovora* by placing the insects directly on CVP medium. Internal contamination was tested by crushing individual insects with a sterile glass rod in 0.1 ml SDW or in groups of five in 1.0 ml SDW and plating the homogentate on CVP medium.

Weed Root Zones — Weed root zone samples consisted of roots plus any soil that adhered after shaking live uprooted plants (9). Roots were cut into short pieces (1-2 cm) and a sample (15-20 g fresh weight) of soil plus roots was weighed and shaken in a 500 ml bottle with 250 ml SDW for 30-60 min. The resulting suspension was serially diluted and plated in duplicate on CVP medium.

Characterization of E. carotovora — *Erwinia carotovora* was identified by the characteristic colony morphology and the deep depressions it produces on the CVP medium used for isolation (6). *Eca* strains were routinely distinguished from *Ecc* strains by reaction with the fluorescent antibody stain specific for *Eca;* differential biochemical tests were conducted where indicated by the methods described elsewhere (9).

Results

Soil Samples — In the spring of 1973, 1974 and 1975 soil samples were obtained from field plots at the University of Wisconsin Experimental Farm at Hancock, Wisconsin. Each year one plot was sampled in which potatoes infested with *E. carotovora* had been grown the previous year and one plot in which no potatoes had been grown for at least five years. These plots were tested only by the dilution plating method and *E. carotovora* was not detected in any of the samples. The data indicated that inoculum levels were relatively low since previous studies had indicated that it was possible to detect between 10^3 and 10^2 cells of pectolytic *Erwinia*/gm soil by dilution plating on the CVP medium (6).

In May 1975, soil samples were taken from four fields that had been planted with potatoes, four with snap beans and two with field corn the previous season (Table 1). *Erwinia carotovora* was not detected by the dilution plating method, but was detected in two potato-field and two corn-field soil samples by the baiting method. When the tissue used for bait was examined with FAS, stained cells were detected in at least one sample from six of the ten fields sampled. With direct FAS, *E. carotovora* was detected in at least one sample from seven of the ten fields (Table 1).

TABLE 1. — *Comparison of procedures for detection of* Erwinia carotovora, *before planting, in soils from ten commercial potato farms in central Wisconsin in spring 1975.*

Field No.	1974 crop	Method for detection of *E. carotovora*			
		Dilution plating on CVP	Baiting and plating on CVP	Baiting and staining with FAS[a]	Direct staining with FAS[a]
1	potatoes	0/4[b]	1/4	3/4	1/4
2	potatoes	0/4	1/4	2/4	2/4
3	potatoes	0/2	0/2	2/2	1/2
4	potatoes	0/2	0/2	0/2	2/2
5	beans	0/2	0/2	1/2	0/2
6	beans	0/2	0/2	1/2	1/2
7	beans	0/2	0/2	0/2	1/2
8	beans	0/2	0/2	0/2	0/2
9	corn	0/2	1/2	2/2	2/2
10	corn	0/2	1/2	0/2	0/2

[a]Fluorescent antibody stain (FAS) specific for *E. carotovora* var. *atroseptica*.
[b]No. of samples with *E. carotovora*/No. samples tested.

Fields in which potatoes had been grown the previous year were again sampled in May 1976, as well as fields in which other crops had been grown. *Erwinia carotovora* could not be detected by baiting with potato-tuber-tissue segments followed by plating on CVP in any of these fields, but was found with the enrichment method in a third of the fields in which potatoes had been grown (Table 2). It was isolated from only one field in which potatoes had not been grown. Of the 162 *E. carotovora* isolations made from the potato fields, 124 were *Ecc* and 38 were *Eca*. Four *E. carotovora* isolations made from the one non-potato field were all characterized as

TABLE 2. —*Assays for* Erwinia carotovora *in soil in spring 1976 by baiting with tuber tissue segments and by enrichment with a selective pectate broth followed by plating on CVP and staining with FAS*[a].

Crop in 1975	Baiting method			Enrichment method		
	Number of samples	Number with *E. carotovora*		Number of samples	Number with *E. carotovora*	
		on CVP	with FAS		on CVP	with FAS
Potatoes 8	10	0	1		32	11[b]
Other crops 5	7	0	1		25	1[c]

[a]FAS detects only *E. carotovora* var. *atroseptica* and not *E. carotovora* var. *carotovora*.
[b]Of 162 cultures of *E. carotovora* obtained, 124 were var. *carotovora* and 38 var. *atroseptica* on the basis of physiological tests and FAS reaction.
[c]All four *E. carotovora* cultures obtained were var. *carotovora*.

Ecc. Cells reacting with FAS were found in two of 17 slide preparations from tuber tissues used for bait and in 13 of 57 preparations made from the enrichment broth samples (Table 2).

In 1977, 46 fields were assayed for the presence of *E. carotovora* with the pectate enrichment broth followed by plating on CVP (Table 3). *Ecc* was detected in 6 of 21 fields in which potatoes had been grown the previous year and in 2 of 18 fields in which potatoes had last been grown in 1975. *Ecc* was not detected by this procedure in fields in which potatoes had not been grown for 2 years. *Eca* was not detected on the CVP plates but cells which reacted with FAS specific for Eca were detected in smears prepared from the enrichment broth of 8 fields including 2 of 7 fields in which no potatoes had been grown for 2 years (Table 3).

TABLE 3.—*Assays for* Erwinia carotovora *in spring 1977 in field soils using the enrichment method followed by plating on CVP and staining with FAS.*

Rotation sequence	No. of fields	Number with *E. carotovora*	
		Detected on CVP[a]	Detected with FAS[b]
Potatoes in 1976	21	6	5
Other crops in 1976 Potatoes in 1975	18	2	1
Other crops in 1976 & 1975 Potatoes in 1974	7	0	2

[a]All cultures obtained in isolations on CVP were *E. carotovora* var. *carotovora* based on physiological tests. No cultures of *E. carotovora* var. *atroseptica* were obtained.
[b]FAS detects only *E. carotovora* var. *atroseptica* and not *E. carotovora* var. *carotovora*. Positive rating usually based on presence of relatively small numbers of fluorescing cells.

Plant Debris — Potato tubers that had overwintered in the field were collected in May 1974 and 1975. *Erwinia carotovora* was not detected in these tubers by dilution plating, but was found by using tuber tissue bait in six of 15 tubers collected in 1975 (Table 4). Both *Eca* and *Ecc* were isolated. *Ecc* was also isolated from two of 15 overwintered potato stems.

Potato tubers that had overwintered in the soil were extensively decayed and infested with insects. The predominant insect was a sap beetle, *Glischrochilus quadrisignatas* Say. Twenty larvae and three adults were tested for external contamination and 246 larvae and 12 adults for internal contamination but *E. carotovora* was not found in any of the insects.

Weed Root Zones — In the spring of 1974 weed root zones were examined to determine whether they harbored a significant *E. carotovora* population. A total of 76 weeds belonging to nine species was sampled in a field plot in which potatoes infested with *Ecc* and *Eca* had been planted the previous season, but *E. carotovora* was not detected (Table 5).

TABLE 4. — *Assays (by dilution plating and by baiting followed by plating on CVP) for* Erwinia carotovora *in potato tubers and stems that overwintered in the field.*

Year	Tissue Source	Number of samples	Number with *E. carotovora*	
			Dilution plating	Baiting and plating on CVP
1974	Tubers	27	0	-
1975	Tubers	15	0	6[a]
1975	Stems	15	0	2[b]

[a]Both *E. carotovora* var. *atroseptica* and *E. carotovora* var. *carotovora* were isolated from positive samples.

[b]Only *E. carotovora* var. *carotovora* was isolated. In 1977 the enrichment procedure was followed with ten samples and in each case *E. carotovora* var. *carotovora* was isolated.

TABLE 5. — *Weeds tested for the presence of* Erwinia carotovora *in root zones by the dilution plating method using CVP medium.*

Weed species	No. of root samples tested	No. with *E. carotovora*
Agropyron repens (L.) Beauv (quack grass)	15	0
Chenopodium album L. (lamb's quarters)	21	0
Diplotaxis sp. (rocket)	2	0
Erigeron strigosus Muhl. (flea bane)	30	0
Potentilla norvegica L. (rough cinquefoil)	2	0
Taraxacum officinale Weber (dandelion)	1	0
Tragopogon dubius Scop. (western goat's beard)	1	0
Verbascum thapsus Jaq (common mullen)	2	0
Unidentified Caryophyllaceae	2	0

Discussion

Repeated attempts to isolate *E. carotovora* from soil known to contain the organism the previous year were unsuccessful when the dilution plating method with a selective medium (CVP) was used. However, *E. carotovora* could be detected by baiting with tuber tissue segments. Baiting methods have been used by others (15, 16, 19, 27), but the possibility exists that the potato tuber tissue used for bait may be internally contaminated with soft rot bacteria even when reasonable effort to eliminate such contamination is

used. Potato tuber tissue that is ostensibly healthy has been reported to contain bacterial cells (4, 8, 14, 35). Use of an enrichment procedure (3, 22) is therefore preferable to the baiting procedure; furthermore, it is apparently more sensitive than the baiting method (Table 2).

The FAS procedure in combination with the soil enrichment method was the most sensitive of the methods used in detecting *Eca* (Table 2 and 3). Unfortunately, there appear to be many serotypes of *Ecc* (7) and initial attempts to produce an antiserum that will react with most or all isolates have been unsuccessful thus far.

The difficulty in detecting *E. carotovora* in soil in the spring indicates that the population surviving the winter under Wisconsin conditions is very small. The methods used did not allow numerical evaluation of the population, but the failure to detect it on dilution plates indicates it is below 10^3 cells/g dry weight. These observations on relative populations of *Erwinia* spp. are in sharp contrast with data on pectolytic pseudomonads. In these same soils it was not unusual to find pectolytic pseudomonads present at 5 x 10^3 cells/gm dry weight of soil (Cuppels and Kelman, unpublished data). These findings differ from the observations by Sands and Hankin (34) in Connecticut.

During the growing season *E. carotovora* is often present in potato field soils (3, 9) and populations can be relatively high (9). Our results indicate that the population decreases during the winter season. *Erwinia carotovora* was detected in fewer fields in which crops other than potatoes had been grown the previous season than in those in which potatoes were grown (Tables 1, 2 and 3).

The possibility that *E. carotovora* survived preferentially in plant debris was tested; it was detected in both overwintered potato stems and tubers. As in soil, *E. carotovora* population levels in these samples were also below those that normally could be detected by standard dilution plating on selective media.

The presence of *E. carotovora* in rhizospheres of crop and weed plants has been reported (3, 17, 18). Survival of *Eca* was extended up to 20 weeks in artificially inoculated weed rhizospheres (5) but there was no indication that *E. carotovora* was favored in the weed root zones investigated in our field plot (Table 5). Apparently, *E. carotovora* populations were below the level of detection with dilution plating on the selective medium. Much more extensive testing would be necessary to evaluate fully the prospect that survival in weed rhizospheres occurs.

The low level of *E. carotovora* surviving in Wisconsin soils over winter does not represent a significant inoculum source for a crop of table-stock potatoes since at present, seed potatoes may carry a high level of inoculum (10, 26). However, since it can survive in Wisconsin soils, the soil could be a source of primary inoculum for blackleg and soft rot of potatoes when *Erwinia*-free seed tubers are planted. The possibility of

avoiding the pathogen through cultural practices is thereby complicated. Crop rotation may enhance efforts to prevent recontamination of *Erwinia*-free crops from the soil.The data obtained thus far indicate that *Erwinia* are present at detectable levels less frequently when crops other than potatoes are planted.

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