Erwinia carotovora as a Stalk Rot Pathogen of Sunflower in North Dakota

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ABSTRACT

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The etiologic agent of a late-season field stalk rot of sunflower (*Helianthus annuus*) was identified as *Erwinia carotovora*; a pathovar could not be delineated on the basis of biochemical and physiological data. Sunflowers became more susceptible to the disease as they matured, with peak susceptibility occurring at seed set. Resistance to the disease may exist in advanced breeding selections.

Additional key words: serogroup, soft rot

Sunflower (*Helianthus annuus* L.) is an important cash crop in North Dakota that occupies 1–1.2 million ha annually. In 1981 and 1982, diseased sunflowers with external stalk blackening (Fig. 1) similar to that caused by *Phoma macdonaldi* Boerema were collected from the Cargill breeding nursery near Fargo and other areas of North Dakota. The stalk was often hollow, and there was a characteristic inky-black, watery breakdown of the pith (Fig. 2), usually odorless unless the stalk was in an advanced state of decomposition. Affected plants often lodged under the weight of the maturing heads. The head was also affected in some instances (Fig. 3).

Similar disease symptoms in sunflower have been reported elsewhere (1,5,7–9). The causal agent in these cases has been assigned to *Erwinia carotovora* pv. *carotovora* (Jones) Dye (Ecc) (5,8,9) and *E. carotovora* pv. *atroseptica* (Van Hall) Dye (Eca) (5). As we had previous experience with this bacterial pathogen on potato and had isolated *Erwinia* from sunflower (7), we initiated a study to further describe the organism, determine factors affecting it, and elucidate its role in stalk rot of sunflower in North Dakota.

MATERIALS AND METHODS

In 1981, sections of diseased sunflower stalks from the Cargill breeding nursery near Fargo were surface-sterilized in 0.5% sodium hypochlorite for 2 min. The stalks were split aseptically and sap was expressed from the pith tissue at the margins of decay. The expressed sap (0.1 ml) was streaked on crystal violet pectate medium (CVP) (2) and nutrient agar.

Pits characteristic of *E. carotovora* (Ec) appeared on CVP medium after 48 hr. In most instances, no other contaminating strains were observed. Single colonies were restreaked on CVP to ensure purity of the cultures and then tested for acid production from α-methylglucoside, reduction of sucrose, growth at 37 C, and phosphatase activity (6). Additional biochemical characterization of three sunflower strains and two potato strains was done, using MICRO SCAN (115 Patterson St., Hillsdale, NJ 07642) and API (Analytab Products, Plainview, NJ 11803) bacteriologic profile kits. Serogrouping of Ec strains was performed according to the method of DeBoer et al (3). Sunflower stalks and heads from other locations in North Dakota collected in 1982 were treated as described.

Sunflowers were grown in 20-cm pots in the greenhouse under a 16-hr photoperiod. Plants 76 days old were inoculated with 24-hr cultures of Ec by touching a sterile toothpick to a bacterial colony, piercing the stem, and pushing the toothpick into the pith tissue. The toothpick was broken off and the wound was sealed with petroleum jelly. The plants were placed in a moist chamber for 48 hr, then returned to the greenhouse. Pathogenicity was evaluated 12 days after

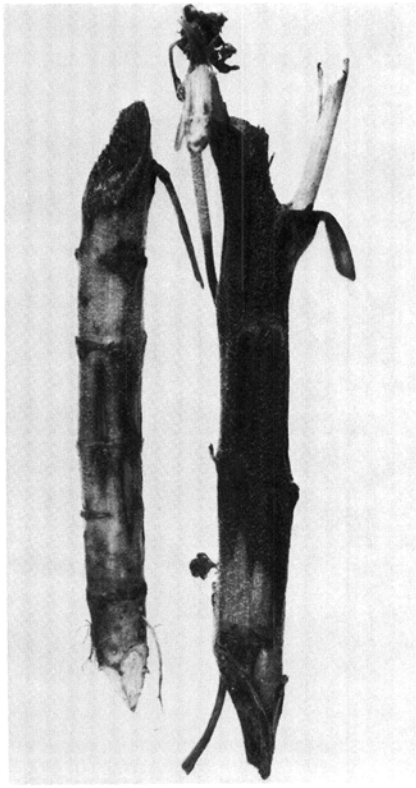

Fig. 1. External blackening of sunflower stalk infected with *Erwinia carotovora*.

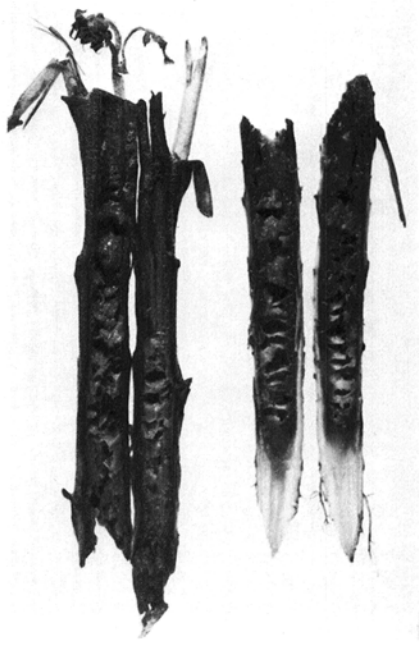

Fig. 2. Internal decay of sunflower stalk infected with *Erwinia carotovora*.

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inoculation by measuring the length of the internal decay and by noting any splitting or blackening of the stalk. Twelve strains of an Ec recovered from sunflowers in 1981 were used in the inoculation plus an Ecc and an Eca from potato and a water control. Three plants were inoculated per strain per treatment. Reisolations from inoculated plants were performed for each strain.

Pathogenicity testing disclosed that plant age had a significant bearing on both inoculation success and severity of stalk rot. Therefore, plants of three inbred sunflower selections, sown at weekly intervals, were inoculated on the same day 61, 68, 74, 82, or 90 days after sowing with three strains of the sunflower Ec, one Eca strain (potato), and one Ecc strain (potato). Inoculations and evaluations were done as previously described.

Fig. 3. Sunflower head with soft rot symptoms caused by *Erwinia carotovora*.

Some "families" of crosses in the Cargill breeding nursery near Fargo appeared to be affected less by the disease than others late in the growing season. To determine if resistance to stalk rot is present in breeding material, the three inbreds previously inoculated plus nine inbred and hybrid selections of varying genetic background (Table 1) were inoculated with four strains of the sunflower Ec 68 days after sowing, as previously described.

RESULTS AND DISCUSSION

Twelve strains of Ec were isolated in pure culture from sunflower stalks collected from the breeding nursery. Tables 2 and 3 show the results of biochemical, physiological, and cultural tests used to characterize the sunflower Ec strains and the relationship between the sunflower Ec strains and known strains of *Erwinia* from potato. All strains from both hosts reacted similarly in the detailed API and MICRO SCAN profiles. Minor variances were noted in the Voges-Proskauer and gelatinase tests.

Table 1. Evaluation for resistance[a] of 12 hybrid sunflower selections

Sunflower selection[b]	Days to maturity	Percent black[c]	Percent split[d]	Extent of internal decay (mm) at 88 days	Rating[e]
Hybrid 1	116	46	18	357	S
Hybrid 2	115	27	9	245	S
Hybrid 5	110	27	18	225	S
Hybrid 7	113	70	60	207	S
Hybrid 4	121	27	46	200	S
Inbred 5	109	...[f]	...	197	S
Inbred 2	106	57	100	181	S
Hybrid 3	117	14	29	130	MR
Inbred 1	107	29	29	103	MR
Inbred 3	114	...	...	89	MR
Hybrid 6	109	0	30	59	R
Inbred 4	112	...	...	14	R

[a] Resistance evaluated with four different strains of the sunflower *Erwinia carotovora*. All data were grouped across strains for each sunflower selection.
[b] Cargill, Inc., breeding selections.
[c] Expressed as the proportion of plants with blackening of external stem tissue. A minimum of eight plants was evaluated per sunflower selection.
[d] Expressed as the proportion of plant stalks that split open near the point of inoculation. A minimum of eight plants was evaluated per sunflower selection.
[e] S = susceptible, MR = moderately resistant, R = resistant.
[f] Data not recorded.

Table 2. Biochemical, physiological, and cultural characteristics of sunflower and potato strains of *Erwinia carotovora*

Diagnostic test	Ecc (2 strains from potato)	Eca (2 strains from potato)	Ec (12 strains from sunflower 1981)	Ec (7 strains from sunflower 1982)
Growth at 37 C	+[a]	−	+	+
Acid from α-methylglucoside	−	+	+	+
Sucrose reduction	−	+	+	I
Phosphatase activity	−	−	−	−
Rot on potato slices	+	+	+	+
Pits on CVP medium	+	+	+	+
Yellow pigment	−	−	−	−

[a] + = 80% or more of strains positive, I = 21–79% of strains positive, − = 20% or less of strains positive.

All strains were identified as *Enterobacter agglomerans*, with an estimated probability of correct identification ranging from 95.6 to 99.9%. In recent bacterial taxonomic schemes (10), the *Enterobacter agglomerans–Erwinia herbicola* complex appears as *Enterobacter agglomerans* in much of the medical literature and as species of *Erwinia* in the botanical literature. Since microbiological profile systems are designed primarily for animal pathogens, *Erwinia* appears as *Enterobacter agglomerans*.

According to the diagnostic tests of Graham (6) used for species identification, all our sunflower strains of *Erwinia* are *carotovora*. The 19 strains reacted identically when subjected to the common diagnostic tests used to differentiate pathovars of Ec (Table 2), with the exception of sucrose reduction, for which 15 of 19 were positive. Using these tests, however, we were unable to assign the strains of Ec we recovered as a known pathovar (Table 2). Mazzucchi and Mazzi (8) and Richeson (9) identified the Ec affecting sunflower in their area as pv. *carotovora*. Fucikovsky et al (5) demonstrated that pvs. *carotovora* and *atroseptica* are both involved in a soft rot of sunflowers in Mexico. Since Richeson (9) did not perform many of the biochemical tests we feel necessary for Ec pathovar differentiation, we cannot ascertain the complete identity of the Ec he recovered. Some researchers may be tempted to label our strains "high temperature atrosepticas" because they react as an *atroseptica* pathovar in the common diagnostic tests but are able to grow at 37 C, unlike true *atroseptica* pathovars. We feel it is inappropriate at this time to make such a designation, however.

Most of the Ec recovered from sunflower could not be placed into any known serogroup (M. L. Powelson, *personal communication*), although strain SFIB belongs to serogroup XXVII and strains SF Stalk 1, SF3, SF9, and SF10 belong to XL (3,7). The antiserum with which the latter four sunflower strains reacted was prepared by M. L. Powelson to an Ecc isolated from Norgold Russett potato seed originating from North Dakota. This only serves to further confuse the problems we have with assigning the Ec strains we have isolated from sunflower to a known pathovar. It also raises questions concerning the possible pathogenic adaptation of an Ec from one host to another.

Koch's postulates were completed when sunflowers inoculated with 12 strains of Ec developed symptoms similar to those described from the original diseased sunflower stalks. Each Ec strain was recovered from the inoculated sunflowers. When inoculated into sunflower, Ecc and Eca from potato caused similar but less severe stalk decay

(Table 4), and they could be reisolated. Attempts to cause stem soft rot of Norgold Russet potato with the sunflower strains were unsuccessful, however. Two of 21 plants inoculated with three different strains produced only a trace of decay. In the same greenhouse experiment, potato strains of Ec did cause a rapid stem soft rot (blackleg) of potatoes. This is somewhat surprising, since the sunflower strains we tested caused soft rot of potato tuber slices (7).

Table 3. Results of API and MICRO SCAN biochemical characterization of three sunflower strains (SF6, SF8, SF12) and two potato strains (Ecc, Eca) of *Erwinia carotovora*

Test		Ecc	Eca	SF6	SF8	SF12
ONPG (β-galactosidase)		+/+[a]	+/+	+/+	+/+	+/+
Arginine dihydrolase		−/−	−/−	−/−	−/−	−/−
Lysine decarboxylase		−/−	−/−	−/−	−/−	−/−
Ornithine decarboxylase		−/−	−/−	−/−	−/−	−/−
Citrate		+/+	+/+	+/+	+/+	+/+
H_2S		−/−	−/−	−/−	−/−	−/−
Urease		−/−	−/−	−/−	−/−	−/−
Tryptophan deaminase		−/−	−/−	−/−	−/−	−/−
Indole		−/−	−/−	−/−	−/−	−/−
Voges-Proskauer		+/+	−/−	+/+	−/+	−/+
Gelatinase		+/N[b]	−/N	−/N	−/N	−/N
Oxidase		−/N	−/N	−/N	−/N	−/N
Glucose		+/+	+/+	+/+	+/+	+/+
Mannitol		+/N	+/N	+/N	+/N	+/N
Inositol		−/−	−/−	−/−	−/−	−/−
Sorbitol		−/−	−/−	−/−	−/−	−/−
Rhamnose		+/+	+/+	+/+	+/+	+/+
Saccharose		+/N	+/N	+/N	+/N	+/N
Melibiose		+/+	+/+	+/+	+/+	+/+
Amygdalin		+/N	+/N	+/N	+/N	+/N
Arabinose		+/+	+/+	+/+	+/+	+/+
Sucrose		N/+	N/+	N/+	N/+	N/+
Adonitol		N/−	N/−	N/−	N/−	N/−
Esculin		N/+	N/+	N/+	N/+	N/+
Malonate		N/−	N/−	N/−	N/−	N/−
Estimated percent probability of correct identification	API	99.9	95.6	95.6	95.6	95.6
	MICRO SCAN	97.5	97.5	97.5	97.5	97.5

[a] Results of API/results of MICRO SCAN.
[b] N = test not available in profile.

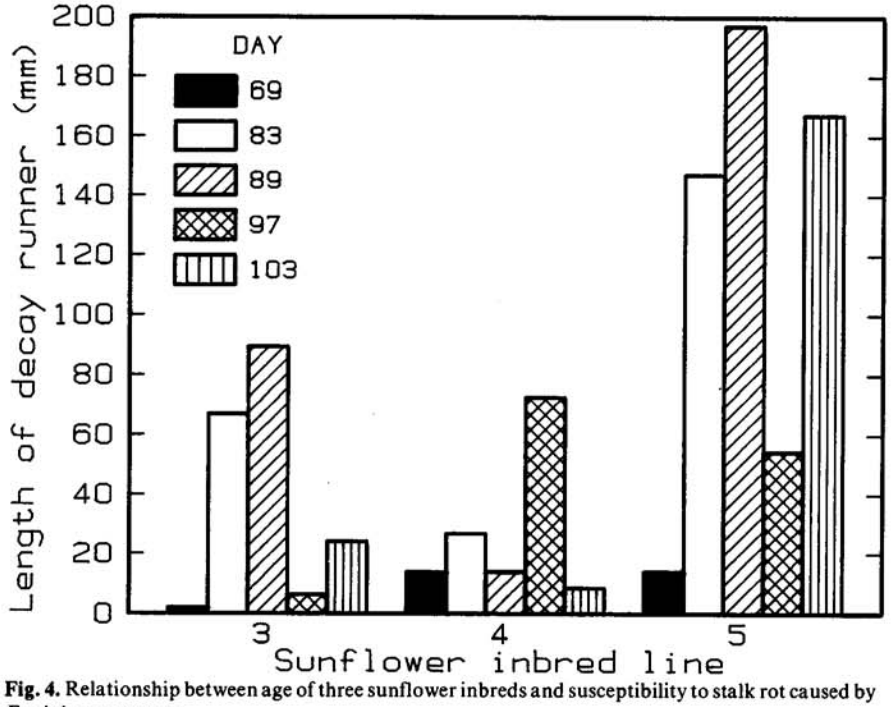

Fig. 4. Relationship between age of three sunflower inbreds and susceptibility to stalk rot caused by *Erwinia carotovora*.

Sunflowers became more susceptible to Ec infection as plants matured (Fig. 4). Maximum symptoms occurred at about 90 days or later, at heading time. Relatively young plants were more resistant to stalk rot decay than plants nearing senescence, a finding previously reported by Richeson (9).

Five of the 12 sunflower selections were either moderately resistant or resistant to infection by Ec in the greenhouse (Table 1). Resistance was not related to the date of maturity for the various inbreds tested ($r = 0.29$ NS). The mean length of the internal decay appeared to be the most useful criterion for evaluating resistance and could easily be used as a breeding and screening tool. The response of the hybrid sunflower selections to Ec infection in the greenhouse did not always correlate with that observed in the field, possibly because of the differing environments. Resistance and susceptibility studies should be confirmed by field tests. More research is needed in this area to elucidate the mechanisms of resistance in sunflower to this stalk rot pathogen.

Differences in virulence occurred among the four Ec sunflower strains tested, with SF12 being the most virulent (Table 4). The mean length of the internal decay was the most useful criterion for evaluating the virulence of these bacteria.

The stem blackening associated with this disease is often centered around a petiole axil, suggesting that Ec enters the plant at this site. Ec probably enters through wounds caused by insects, hail, or mechanical damage. The axil of the petiole often collects water and may serve as a favorable site for both Ec residence and insect activity. The sunflower budworm (*Suleima helianthana* Riley) preferentially oviposits in this axil, and the larvae feed at and exit from the same location (4).

Table 4. Assessment of virulence[a] of four *Erwinia carotovora* strains (SF6, SF8, SF11, SF12) recovered from sunflower and two strains (Eca, Ecc) isolated from potato

Treatment or strain	Percent black[b]	Percent split[c]	Extent of internal decay (mm)
SF6	50	64	197
SF8	43	33	197
SF11	5	23	151
SF12	33	19	480
H_2O	0	0	0
Eca	...[d]	...	72
Ecc	...	...	42

[a] Virulence evaluated with nine genetically different sunflower hybrids.
[b] Expressed as the proportion of plants with blackening of external stem tissue. A minimum of 12 plants was evaluated per treatment.
[c] Expressed as the proportion of plant stalks that split open near the point of inoculation. A minimum of 12 plants was evaluated per treatment.
[d] Data not recorded.

Our evidence indicates an undefined pathovar of Ec is involved in a stalk rot of sunflower in North Dakota. The organism occurs sporadically throughout the state, and diseased sunflower stalks and heads are usually observed only after an extended wet period in the latter part of the growing season. This suggests that Ec may be able to infect only stressed and/or senescing plants when conditions are optimum. Because of the ubiquity of Ec (7), the role that sunflowers may play in recontamination of stem-cut seed stock of potato warrants further consideration. A sunflower-potato rotation is not recommended because of Ec and other diseases, including Verticillium wilt. The role of *Erwinia* in so-called accelerated senescence (premature death, early dying) of sunflowers is unknown, but *Erwinia* has the potential to be a component of this complex.

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