THE ASSOCIATION OF *ERWINIA CAROTOVORA* VAR. *ATROSEPTICA* AND *ERWINIA CAROTOVORA* VAR. *CAROTOVORA* WITH INSECTS IN COLORADO.[1-4]

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Abstract

Ten genera from 9 families of dipterous insects collected in the field in the San Luis Valley of Colorado were contaminated with *Erwinia carotovora* var. *atroseptica (Eca)* and *Erwinia carotovora* var. *carotovora (Ecc)*. *Erwinia*-contaminated insects were collected from settling ponds near potato warehouses, potato cull piles on growers' farms, municipal dumps, lettuce and potato fields. The percentage of contaminated insects reached 14.5% in early spring but decreased as the season progressed. Insects were more commonly contaminated with *Eca* than *Ecc* early in the season. Later the proportion of contamination by *Ecc* increased while that of *Eca* decreased. We suggest that insects may play a potentially important role in the epidemiology of potato blackleg especially in the reintroduction of *Erwinia* into *Erwinia*-free potato fields.

Resumen

Insectos dípteros de 10 géneros agrupados en 9 familias colectados en el campo en el Valle San Luis de Colorado se encontraban contaminados con *Erwinia carotovora* var. *atroseptica (Eca)* y *E. carotovora* var. *carotovora (Ecc)*. Se colectó insectos contaminados con *Erwinia* de lagunas de sedimentación cerca de almacenes de papa, pilas de papa desechada en chacras, depósitos municipales para basura y de campos de lechuga y papa. El porcentaje de insectos contaminados alcanzó el 14.5% durante el inicio de primavera pero fue decayendo con el progreso de la temporada. La contaminación de insectos fue mas frecuente con *Eca* que con *Ecc* durante los inicios de la temporada. Mas tarde la proporción de contaminación con *Ecc* aumentó mientras la de *Eca* se redujo. Sugerimos que los insectos pueden jugar un papel potencialmente importante en la epidemiología de la pierna negra de la papa especialmente en lo referente a la reintroducción de *Erwinia* a campos de papa libres de *Erwinia*.

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Introduction

Potato blackleg caused by *Erwinia carotovora* var. *atroseptica (Eca)* and perhaps by *Erwinia carotovora* var. *carotovora (Ecc)* has been controlled through the use of *Erwinia*-free stocks derived from *Erwinia*-tested stem cuttings (2). Low levels of *Erwinia* recontamination have been detected in fields planted from *Erwinia*-free stocks after as little as 2 years' cultivation (2). Sources of the recontamination are not fully known but insects are implicated as a possible means of dissemination (2, 3, 4, 5, 6, 8).

In Scotland, insects were observed visiting injured plants in *Erwinia*-free fields and insects coming from a vegetable dump near an *Erwinia*-free potato field carried serotypes of *Ecc* identical to those found in the dump (3). Many of the same serotypes were subsequently isolated from infected plants in the field. Harrison et al. (4) found that 3 to 5% of the insects collected from potato cull piles in Scotland carried either *Ecc* or *Eca*. They found that the percentage of contaminated insects increased during the summer growing season.

Harrison et al. (4) also reported that *Eca* was isolated from insects only early in the season. Later in the season isolations from contaminated insects showed that *Ecc* became more abundant.

Molina et al. (6) found that fruit flies (*Drosophila melanogaster*) readily acquired *Eca* from laboratory cultures and transmitted it to healthy but injured potato plants in the greenhouse, and Harrison et al. (4) reported that insects collected from potato cull piles readily transmitted *Ecc* to injured potato plants in the greenhouse. Based upon these studies it has been suggested (3, 4, 6) that *Erwinia*-contaminated insects may contribute to the reintroduction of the bacteria into *Erwinia*-free potato fields.

The objectives of this project were to determine 1) the extent of insect contamination by *Erwinia* in the dry Colorado environment, 2) sources from which insects acquire *Erwinia*, 3) the insect vectors and 4) the seasonal abundance of *Ecc* and *Eca*.

Materials and Methods

The study was conducted in the San Luis Valley area in south central Colorado during 1975 and 1976. The San Luis Valley is a high mountain valley with an area of ca 2.43 million hectares (6.0 million acres) and an elevation of about 2,316 m (7,600 ft). It has a growing season of 90-110 days and an average annual precipitation of 17.6 cm (6.9 in) (1961-1977). General insect collections were made from several sources in the San Luis Valley during the 1975 and 1976 growing seasons. Insects were collected from potato fields, lettuce fields, on-farm potato cull piles, potato cull piles at municipal dumps and settling ponds associated with potato warehouses in Rio Grande County during 1975. In 1976 collections were made from these same sources as in 1975 plus potato cull piles in Saguache, Conejos, Costilla and Alamosa Counties; from spinach and carrot fields in Costilla County; and from barley fields, and a soil pile removed from potato fields in Rio Grande County.

In addition to the general collections, 3 cull piles in 1975 and 6 in 1976 were sampled repeatedly during both growing seasons. Cull pile temperatures were continuously recorded 30 cm below the surface with a recording thermograph[1]. To determine how long bacteria survived in the cull piles, core samples were removed from the 6 cull piles in 1976 at monthly intervals. The tuber tissue was suspended in water and plated on Stewart's medium (10).

Insects were collected with sweep nets sterilized between collections by dipping in 95% ethyl alcohol and transferred either individually or collectively to clean vials. Each time an unidentified insect was collected, a specimen was preserved in 70% ethyl alcohol. The specimens of insects found to be contaminated with *Erwinia* were sent to the Insect Identification and Beneficial Insect Introduction Institue, USDA, Agricultural Research Center, Beltsville, Maryland, 20705, for identification.

Vials containing the insects were placed in a freezer for ca 5 minutes to inactivate the insects. Individual insects were removed and bacterial isolations were made from them. At the beginning of the study, individual specimens were crushed in a drop of sterilized water between surface sterilized glass slides and a loopful of the resulting suspension was streaked on Stewart's McConkey-pectate medium. After mid season in 1975, insects were plated directly by crushing them with a sterile cotton swab on the sides of petri dishes and streaking across the plates containing Stewart's medium. After 48 hours incubation at 26°C the plates were examined and 1-5 suspected *Erwinia* colonies were transferred to nutrient agar from each plate. Cultures were purified by plating on nutrient agar in petri dishes and selecting single colonies for identification.

Colonies were identified using the following diagnostic tests described by Graham (1): 1) ability to rot potato slices, 2) oxidase production, 3) oxidation/fermentation test (glucose), 4) production of acid from lactose, maltose, trehalose, and α-methyl glucoside, 5) production of reducing compounds from sucrose, and 6) production of indole from tryptophan.

Results

A total of 3,376 insects was collected in 1975 and 10,317 in 1976. Isolation data (Table 1) showed that 1.3 percent of the total insects collected in 1975 and 2.1 percent in 1976 were contaminated with *Erwinia*. Ten genera and at least 11 species of dipterous insects were found to carry *Erwinia*. Insects from other orders were never contaminated. Only 5 gen-

[1]Dial Thermometer Division, Marshalltown Manufacturing, Inc., Marshalltown, Iowa.

TABLE 1. — *Insects collected in the field and levels of contamination with* Erwinia carotovora *var.* carotovora *and/or* Erwinia carotovora *var.* atroseptica, *1975 and 1976.*

Identification	Number Collected		Percent contaminated with *Erwinia*	
	1975	1976	1975	1976
Diptera				
Family Sphaeroceridae				
Leptocera spp.	268	778	1.1	4.5
Family Drosophilidae				
Drosophila busckii	-	1,484	-	2.7
Drosophila melanogaster	-	1,933	-	4.1
Drosophila spp.	1,591	-	1.5	-
Family Anthomyiidae				
Hylemya platura	217	267	2.8	5.2
Family Ephyridae				
Allotrichoma spp.	408	-	2.5	-
Unidentified spp.	-	14	-	0
Family Scatopsidae				
Scatopse fuscipes	22	2,056	4.5	1.1
Family Sciaridae				
Bradysia sp.	-	97	-	1.0
Family Calliphoridae				
Protophormia terraenovae	-	441	-	2.7
Phormia spp.	-	115	-	6.1
Unidentified spp.	-	130	-	0
Family Chironomidae				
Unidentified genus	-	233	-	1.3
Family Tethimidae				
Pelomyia cruciata	-	60	-	1.7
Family Muscidae				
Unidentified spp.	-	73	-	0
Family Milichiidae				
Unidentified spp.	-	63	-	0
Family Ceratopogoniade				
Unidentified spp.	-	82	-	0
Unidentified Diptera	870	2,146	0	0
Hymenoptera				
Family Braconidae				
Pentapleura	-	53	-	0
Coleoptera				
Family Staphylinidae				
Unidentified spp.	-	246	-	0
Homoptera				
Family Cicadellidae				
Macrosteles fascifrons	-	46	-	0

era of dipterous insects were found to be contaminated with *Erwinia* in 1975 while 9 genera were found to carry the bacteria in 1976. The percentages of insects in individual genera which were contaminated with *Erwinia* ranged from 1.0% for *Bradysia* sp. to 6.1% for *Phormia* sp. (Table 1).

The sources from which insects were collected are presented in Table 2. Insects contaminated with *Erwinia* in 1975 and 1976 were collected from settling ponds associated with potato warehouses, cull potatoes located at municipal dumps, on-farm potato cull piles and lettuce fields. The highest percentage of *Erwinia*-contaminated insects was found in lettuce fields in 1975, with specimens taken from cull piles located on growers farms or at municipa

TABLE 2. — *Sources from which insects were collected in 1975 and 1976 and the percentage of* Erwinia-*contaminated insects from each source.*

Source of collection	Number collected	Percent contaminated with *Erwinia*
	1975	
Settling ponds[1]	365	0.3
Municipal dumps[2]	872	1.3
On-farm potato cull piles	1,261	1.4
Potato fields	477	0
Lettuce fields	401	3.5
	1976	
Settling ponds[1]	781	0.9
Municipal dumps[2]	254	4.3
On-farm potato cull piles	8,556	2.3
Potato fields	91	1.1
Lettuce fields	257	0.4
Soil piles[3]	80	0
Barley fields	52	0
Spinach fields	113	0
Carrot fields	133	0

[1]Drainage pits associated with potato warehouses.
[2]Culled potatoes at Monte Vista and Center city dumps.
[3]Piles of rocks and soil removed from nearby fields.

dumps ranking second. In 1976 insects collected from potato cull piles were most often contaminated. Relatively few contaminated insects were collected from other sources in either year. Only 1 *Erwinia*-contaminated insect was collected from a potato field in 1976 and none was found in 1975. None of the insects collected from spinach or carrot fields in 1976 carried *Erwinia* although both crops are susceptible to *Ecc*.

The percentage of contaminated insects in 1976 was highest in May reaching 4.9% (Table 3) then declining steadily during the season. The same trend occurred in the general collection in 1975 (Table 3). The data from insects collected from 6 cull piles at weekly intervals during the season (Table 4) show that the percentage of contaminated insects declined as the season progressed at 4 of the 6 locations. Percentage contamination was significantly correlated with time at those 4 locations (P=0.05). At the other 2 locations regression analysis did not show a significant correlation between the percentage of contaminated insects and time of collection. Mean contamination percentages for all locations also showed a significant (P=0.01) negative linear correlation with time.

TABLE 3. — *Percentage of insects contaminated with* Erwinia car1979) tovora.

Month	Number collected[1]	Insects carrying *Erwinia* number	percent	Bacterium isolated *Ecc*[2]	*Eca*[3]
				%	%
		1975			
June	327	7	2.1	43	71[4]
July	2,008	19	0.9	47	58
August	1,041	18	1.8	100	0
		1976			
May	1,619	79	4.9	37	63
June	3,856	103	2.7	53	47
July	1,889	20	1.1	37	67
August	2,014	10	0.5	36	67
September	488	3	0.6	100	67
October	451	0	0	-	-

[1]Totals of all collections for a given month.
[2]*Ecc*=*Erwinia carotovora* var. *carotovora*
[3]*Eca*=*Erwinia carotovora* var. *atroseptica*
[4]Percentages may exceed 100% since insects sometimes carried both *Ecc* and *Eca*.

Core samples from the 6 cull piles studied throughout 1976 (Table 4) showed that the presence of *Erwinia* in the piles followed a trend very similar to the insect contamination. No *Erwinia* was detected in piles 4 and 5 after June while piles 1, 2 and 3 contained detectable *Erwinia* through July and the organism was found in pile 6 through September.

No consistent relations ip was found between cull pile temperature and insect contamination or presence of *Erwinia* in the piles. Therefore, the data are not presented here.

Data on the relative proportions of *Ecc* and *Eca* isolated from insects collected from cull piles during the course of the season (Tables 3 and 5) show some interesting trends. Table 3 shows that the percentage of insects contaminated with *Ecc* in the general collection increased from 37% in June to 100% in August while *Eca* contamination declined from 71% in June to

TABLE 4. — *Percentage of insect specimens contaminated with* Erwinia carotovora *collected from 6 cull piles, sampled weekly in 1976.*

Date	Cull Pile[1]						Mean for all locations
	1	2	3	4	5	6	
May[2]	4.6	10.8	1.9	3.2	11.4	5.0	6.4
June	6.0	6.7	1.1	1.0	5.9	14.5	4.4
July	3.1	2.0	2.2	0	0	5.7	2.6
August	0	0	0.5	-	-	1.2	0.6
September	-[3]	-	0	-	-	1.7	0.9
October	-	-	-	-	-	0	0
r=[4]	−1.0**	−.986*	−.57	−.95*	−1.0**	−.776	−1.0**

[1]Cull pile locations:

1, 2, 3 — Near potato warehouses in Rio Grande County (9, 8 and 6 M north of Monte Vista, Colo., respectively).

4, 5 — On-farm location in Alamosa County

6 — On-farm location in Rio Grande County

[2]Monthly values are totals for 4 weekly samples taken at each location.

[3]No insects were collected.

[4]Regression analysis was used to correlate decline in % contamination with time.

*significant at P=.05

**significant at P=.01

0% in August. The same general relationship was found with *Ecc* in 1976, however, the percentage of *Eca* did not decline as in 1975. When the data from the 3 cull piles in 1975 and the 6 piles in 1976 which were sampled repeatedly during the season are examined (Table 5) it is apparent that in 2 of the 3 cull piles in 1975 and 4 of the 6 in 1976 there was a distinct tendency for the proportion of *Ecc* to increase and for *Eca* to decrease as the season progressed. In 1975 *Eca* completely disappeared from the samples by August in 2 of the cull piles sampled. In the other cases (pile number 2 in 1975 and numbers 3 and 6 in 1976) *Eca* isolation frequencies remained essentially as high in July, August and September as they were in May. The most rapid shifts from *Eca* toward *Ecc* appeared to be in cull piles where *Erwinia* contamination totally disappeared early in the season, especially in 1976.

Discussion

The data show that insect contamination with *Erwinia* commonly occurs in an arid region such as Colorado and is not a phenomenon restricted to more moist regions. The level of *Erwinia* contamination was unevenly distributed among several fly species independent of the abundance of a specific insect species suggesting that contamination was purely accidental (Table 1). For example, *Drosophila* spp. constituted almost 50%

TABLE 5.—*Percentages of* Erwinia-*carrying insects with either* Erwinia carotovora *var.* carotovora *or* Erwinia carotovora *var.* atroseptica *in relation to time at six locations, 1976.*

Date	1 *Ecc*[1]	1 *Eca*[2]	2 *Ecc*	2 *Eca*	3 *Ecc*	3 *Eca*	4 *Ecc*	4 *Eca*	5 *Ecc*	5 *Eca*	6 *Ecc*	6 *Eca*
						1975[3]						
June	33	100[4]	0	100	0	100	-	-	-	-	-	-
July	60	60	0	100	86	14	-	-	-	-	-	-
			0	100	-		-	-	-	-	-	-
August	100	0	-	100	0		-	-	-	-	-	-
						1976						
May[5]	33	67	58	42	50	50	67	33	17	83	0	100
June	50	53	42	54	10	100	100	0	50	50	79	21
July	60	40	67	33	22	78	0	0	0	0	78	22
August	0	0	0	0	33	67	0	0	0	0	40	60
September	0	0	0	0	0	0	0	0	0	0	100	67

Column numbers 1–6 are the Cull pile number.

[1]*Ecc*=*E. carotovora* var. *carotovora*
[2]*Eca*=*E. carotovora* var. *atroseptica*
[3]Only 3 cull piles sampled intermittently in 1975.
[4]Some insects carried both *Ecc* and *Eca;* consequently, values can add to over 100%.
[5]Totals of 4 weekly samples/month.

of the insects collected in 1975, but only 1.1% carried *Erwinia* while *Scatopse fuscipes* accounted for only 0.7% of the insects collected but 4.5% of the individuals carried *Erwinia*. Differences in insect behavior or life cycles may be important in determining the role of insects as *Erwinia* vectors. Detailed studies of the insect groups commonly contaminated with *Erwinia* should be conducted to determine the factors affecting contamination.

The large number of different insect groups carrying *Erwinia*, however, suggests that any dipterous insect associated with cull piles or other *Erwinia* sources could acquire the bacteria and perhaps act as a vector of the bacterium to healthy plants. Although the percentages of insects carrying *Erwinia* seem small (1.1 - 6.1%, Table 1), they represent thousands of potential vectors present in the total insect population.

The predominant source of *Erwinia*-contaminated insects found in this study was potato cull piles. These are numerous and widely scattered in the San Luis Valley, and are often located beside potato fields. Insects were collected at cull piles early in the spring before they appeared at other sources. It seems quite probable that the cull piles are overwintering and/or breeding sites for some insect groups and thus may represent an important initial source of contaminated insects. Since culled potatoes are often diseased, the possibility of contamination of any insects visiting or residing in the pile is high.

Erwinia-contaminated insects were only collected from lettuce fields late in the season and only *Ecc* was isolated from the insects. Potato cull piles could serve as the source of insects which introduces *Ecc* into the lettuce. Studies comparing *Erwinia* serotypes present in cull piles with those found on insects and lettuce plants such as reported by Graham et al. (3) could be used to determine if insects do, in fact, transmit *Ecc* to lettuce. The absence of *Erwinia*-contaminated insects in spinach and carrot fields may be related to the absence of an *Erwinia* source, since these fields were located approximately 80 km (49.7 mi) southeast of the potato-growing area. Cull piles were not found near any of the spinach and carr1979) t fields sampled.

In Scotland the percentage of *Erwinia*-contaminated insects increased as the season progressed (3) while in our studs the percentages decreased. Therefore, insect vectoring of *Erwinia* to healthy potato plants may be more easily controlled in Colorado where the highes levels of contaminated insects apparently occur before potato plants have emerged (June 1-15). The elimination of cull piles, settling ponds, and other sources of *Erwinia* early in the spring may decrease the total *Erwinia* population in the environment as well as prevent bacterial spread by insects.

Declining percentages of *Erwinia*-contaminated insects at the 6 cull piles sampled weekly in 1976 (Table 4) appear to be related to the decreasing survival of *Erwinia* in the desiccating cull piles. *Erwinia* was isolated at high levels from cull piles early in the season but declined about the same time that *Erwinia* contamination of insects fell off.

Temperatures monitored in the 6 cull piles during the season were not correlated with decreased levels of *Erwinia*-contaminated insects. Apparently the decline in numbers of viable bacteria in cull piles was not due to high temperatures but rather to some other factor, possibly moisture.

The predominance of *Ecc* in the population of *Erwinia* reintroduced into fields planted from stem-cutting (7, 9 Harrison unpublished) material may be related to the trend toward increased proportions of *Ecc* compared with *Eca* carried by insects as the season progresses. This phenomenon was evident in 1975 (Table 3) and at several locations monitored in 1976 (Table 5). It is possible that insects range away from cull piles and other food sources to potato or other vegetable fields only late in the season when *Ecc* is often the predominant organism carried by potential vectors.

We do not have an adequate explanation for the tendency for the proportion of *Ecc* carried by insects to increase at most locations as the season progresses; however, there are at least two possibilities. In Scotland, the levels of *Ecc* and *Eca* carried late in the season by insects seem to be related to the time that cull potatoes are dumped (4). Piles dumped at harvest time the previous year yielded insects carrying more *Ecc* than Eca late in the season, while those dumped closer to planting time yielded insects with high levels of *Eca* late in the season. The exact time that cull piles sampled in our experiment were dumped was not always determined but the tendency for the *Eca* to *Ecc* shift to be more pronounced in cull piles which ceased to yield contaminated insects early in the season in 1976 suggests that this may be a factor in Colorado (those were probably early dumped piles which dried up early in the season). This may explain why *Eca* was more persistent in some of the cull piles sampled in 1976 (Table 5). A second possibility is related to the moisture levels in cull piles. *Ecc* seems to be able to survive over a wider range of environmental conditions than *Eca* (5, 8). All of the cull piles sampled weekly in 1976 (Table 5) gradually dried out as the season progressed except number 2 in 1975 and 6 in 1976. Cull pile 2 was flooded from June 1 to 15 when a nearby irrigation ditch broke. During that period the levels of *Eca* carried by insects increased rather than declined as it did at the other locations. Cull pile 6 was sprinkled during the season to create favorable conditions for bacteria for an extended time. Insects collected from this cull pile carried variable proportions of *Ec* and *Eca* during the season. It is possible that *Ecc* survives (or multiplies) better than *Eca* in dry cull piles and that *Eca* populations increase when the piles are moist to make the populations more equal.

Further studies on the association of *Erwinia* with insects are needed to confirm the phenomenon of the shift from *Eca* toward *Ecc* as the season progresses. Experiments designed to measure the effect of single factors on this trend, such as cull pile age and moisture, should be undertaken. If this shift toward *Ecc* is verified and the factors responsible for it determined, the data could be important in explaining the predominance of *Ecc* in potato stocks derived from stem cuttings when planted in the field in potato growing areas. Modifications of the practice of disposing of cull potatoes such as dumping earlier in the winter or spring could be beneficial in terms of reducing total insect contamination and hence the danger of recontamination of clean stocks.

Our work confirms the previous report from Scotland (4) of the trend toward *Ecc* predominance on insect vectors, however, we are unable to explain the factors responsible for this phenomenon at this time.

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