INSECT TRANSMISSION OF *ERWINIA CAROTOVORA* VAR. *CAROTOVORA* AND *ERWINIA CAROTOVORA* VAR. *ATROSEPTICA* TO POTATO PLANTS IN THE FIELD

J.W. Kloepper[1], J.W. Brewer[2], and M.D. Harrison

Abstract

Adult fruit flies (*Drosophila melanogaster* Meig.) artificially contaminated with *Erwinia carotovora* var. *carotovora* (Jones) Dye (*Ecc*) and/or *E. carotovora* var. *atroseptica* (van Hall) Dye (*Eca*) readily transmitted the bacteria to plants in the field that had been injured by crushing the stem. Injured, inoculated plants developed disease symptoms when maintained at high relative humidities. *Erwinia* was transmitted to ten-hour-old injuries and they became infected as frequently as freshly made wounds. Insect transmission of *Eca*, *Ecc*, and mixtures was greatest during the afternoon, which was the warmest part of the day. *Ecc* was transmitted significantly less frequently during the cold morning than during afternoon or evening hours. A potato cull pile placed in a commercial potato field attracted a natural insect population which increased during the season. Both *Ecc* and *Eca* were isolated from uninoculated rotting tubers in the cull pile and from insects associated with the pile from May through September. These naturally infested insects transmitted *Ecc* and/or *Eca* from the cull pile to artificially injured field plants during July and August at distances as great as 183 m from the cull pile. No *Erwinia* was isolated from injured plants in a neighboring control field, which lacked a cull pile, further than 6 m upwind from the cull pile. We suggest that insects are important agents in the epidemiology of potato blackleg and soft rot even in areas with low relative humidities.

Resumen

Moscas adultas de la fruta (*Drosophila melanogaster Meig*) contaminadas artificialmente con *Erwinia carotovora* var. *carotovora* (Jones) Dye (*ECC*) y/o *E. carotovora* var. *atroseptica* (van Hall) Dye (*ECA*) transmitieron fácilmente la bacteria a plantas en el campo que habían sido dañadas por rompimiento del tallo. Las plantas dañadas e inoculadas desarrollaron síntomas de la enfermedad cuando estuvieron mantenidas

---

[1]Formerly Research Assistant Colorado State University; currently Assistant Professor University of California, Berkeley; [2]Professor of Entomology and Professor of Plant Pathology, respectively, Colorado State University, Fort Collins, CO 80523. Published with approval of the Colorado State University Experiment Station as Scientific Series Paper No. 2536.
Received for publication June 20, 1980.
KEY WORDS: *Drosophila*, potato cull piles, potato blackleg, soft rot, bacteria.

a alta humedad relativa. *Erwinia* fue transmitida a heridas efectuadas diez horas antes y ellas se infectaron tan frecuentemente como heridas efectuadas en el momento. La transmisión por insectos de *ECA*, *ECC* y mezclas fue mayor durante la tarde, la parte más calurosa del día. *ECC* fue transmitida significativamente menos durante las mañanas frías que durante las tardes en horas del atardecer. Una pila de papa de descarte colocada en un campo de papa comercial atrajo una población natural de insectos, la cual aumentó durante la estación. Ambas *Erwinia* fueron aisladas de tubérculos podridos desde la pila de descarte, y de insectos asociados con la pila un desde Mayo hasta Setiembre. Estos insectos naturalmente infectados transmitieron *ECC* y/o *ECA* desde la pila de desearte a plantas dañadas artificialmente en el campo durante Julio y Agosto a distancias tan grandes en un campo vecino que carecía de pila de descarte (control) más allá de 6 m desde la pila, en dirección contraria al viento.

Sugerimos que los insectos son agentes importantes en la epidemiología de la pierna negra y la pudrición blanda en areas con baja humedad relativa.

Introduction

The use of stem cut stock as an approach to the control of potato blackleg caused by *Erwinia carotovora* var. *atroseptica* (van Hall) Dye (*Eca*) and *Erwinia carotovora* var. *carotovora* (Jones) Dye (*Ecc*) is gaining acceptance due to the superior performance of such seed. However, low levels of *Erwinia* are re-introduced quite rapidly into fields planted with stem cutting stocks (2, 9, Harrison unpublished data). *Erwinia*-contaminated insects and bacterial aerosols are suggested as possible sources of these bacteria. Graham *et al.* (4) isolated identical serotypes of *Ecc* from infected potato plants in the field and from insects collected from nearby vegetable cull piles and suggested that the insects were responsible for the transmission of *Erwinia* from the cull piles to the potato plants.

Molina *et al.* (8) caged *Drosophila melanogaster* Meig, which had acquired *Eca* from laboratory cultures, on healthy, injured plants in the greenhouse and found that they readily transmitted the bacterium. Harrison *et al.* (5) showed that insects from potato cull piles in Scotland transmitted *Erwinia* to injured plants in the greenhouse. The relevance of such insect transmission to *Erwinia*-free potato fields is unknown, especially in arid environments of the western U.S.

The purpose of this study was to determine the importance of insect transmission of *Ecc* and/or *Eca* under field conditions in the semi-arid climate of Colorado. The work was conducted during 1976 in the San Luis Valley in southern Colorado. Studies were designed to determine if artificially contaminated insects can transmit *Ecc* or *Eca* to wounded plants under field conditions and if transmission in the field results in infection. The

most favorable time of day for transmission and subsequent symptom development and the acquisition of *Erwinia* from a field source by naturally occurring insects and its transmission to healthy injured plants in the field were also studied.

Materials and Methods

Transmission of Erwinia *spp. by Artificially Contaminated Insects—D. melanogaster* used in the study were maintained aseptically on a medium consisting of instant mashed potato buds, baker's yeast, white vinegar, and pineapple juice in a 2:1:1:1 ratio. During the studies adult insects were first immobilized in a freezer for 5 minutes to facilitate handling. Insects were artificially contaminated with the bacterium to be tested by placing them (in groups of about 50) into bottles containing 24 hr old cultures of *Ecc*, *Eca*, or both organisms growing on nutrient agar for 1 hour. Previous experiments (Harrison unpublished) showed that a high percentage of insects held under such conditions become contaminated with bacteria. Control insects were exposed for the same period to sterile nutrient agar.

The study was conducted in a commercial potato field where 21 plants were randomly selected and thinned to 2 stems per plant 2 days prior to use. Both stems on each plant were artificially injured at 10 points per stem by crushing the tissue with surface-disinfested pliers. Injured plants were then covered with nylon mesh cages to prevent visitation and/or contamination by "wild" insects since previous field observations (5) showed that plant sap oozing from such wounds is commonly visited by numerous insects. To determine the period that wounds remained susceptible to insect transmission of *Erwinia*, contaminated flies were released into 3 replicate cages with injured plants 0, 1, 2, 4, 6, 8, and 10 hours after injury on 3 different days. Plants exposed to non-contaminated flies served as controls. Contaminated and non-contaminated flies were removed from the cages after 2 hours and the plants were covered with plastic bags to maintain high humidity around the injured sites. The plastic bags were also covered with paper bags to prevent sunburn. Forty-eight hours after exposure to the flies 1 of the 2 stems from each replicate plant was removed and the 10 injured sections of tissue per stem were individually macerated in sterile water. The resulting suspension was plated on Stewart's medium (10) and incubated for 48 hr at 26°C. Isolation of *Erwinia* from wounded potato tissue was considered evidence of successful insect transmission.

Influence of Time of Day on Insect Transmission of Erwinia *and Disease Development*—The effect of environmental conditions on insect transmission of *Erwinia* was studied in experiments similar to those described above at 3 different time periods during the day (6:00 a.m. to 4:00 p.m., 12:00 noon to 10:00 p.m., and 6:00 p.m. to 4:00 a.m.). For

each time period potato plants were injured and artificially contaminated flies were allowed to visit the wounds as described above. Transmission of bacteria was detected by isolation from the injured sites 2 hours after exposure. Successful infection (the development of disease) was determined by observing the stems remaining on the plants which were covered with plastic bags to maintain high relative humidity for 2 days, and noting the appearance of soft rot symptoms. Thus, the level of insect transmission was measured by sampling the wounded sites on 1 stem 2 hours after exposure to contaminated flies and also by following the development of symptoms on the second stem on each plant.

Acquisition and Transmission of Erwinia *by Naturally Occurring Insects*—An inoculum source was created in a commercial potato field by dumping approximately 1.8 metric tons of culled potatoes in a field planted with cv. Russet Burbank potatoes. An 8 acre field approximately 238 m long and 76 m wide was divided approximately in half (Fig. 1). The cull pile was placed in the southwest corner of the lower half of this field (designated as field 2). Potatoes in field 1, which was upwind (in terms of the prevailing wind) from field 2, served as the control. Three line transects were established across each field as illustrated in Figure 1. Sample sites were established at 3, 6, 12, 24, 48, and 76 m from the cull pile along each of the transects in each field. Additional sampling sites also were established at 98, 120, and 134 m from the zero point in field 1 (Fig. 1) and at 98, 158, and 183 m from the pile in field 2 (Fig. 1).

Wounds were provided for the potential insect vectors by periodically injuring plants at the sampling sites along each transect throughout the season as shown in Table 3, using surface-disinfested pliers. Nylon mesh cages were placed around half the injured plants at the 3 m sampling sites to exclude visitation by insects. The amount of injured tissue per plant was increased by crushing the total length of plant stems as the season progressed to improve chances of visitation and bacterial transmission. Plants were also injured at some locations on August 3 using a "hail simulator" which consisted of a flail-like device made of metal chains and bolts attached to a metal handle (11). This device caused severe plant injury. Eight to 27 hours after injury, sections of injured tissue were removed and macerated in sterile water. The resulting suspension was streaked on Stewart's medium and incubated for 48 hr at 26°C. Samples of tissue from plants at duplicate locations in the control field were always taken and processed in the same way for comparative purposes. Insects were collected from field 2 at each designated sample point at the beginning of each experiment to determine if contaminated insects were present in the field. The insects were macerated in groups in sterile water and streaked on Stewart's medium as described above.

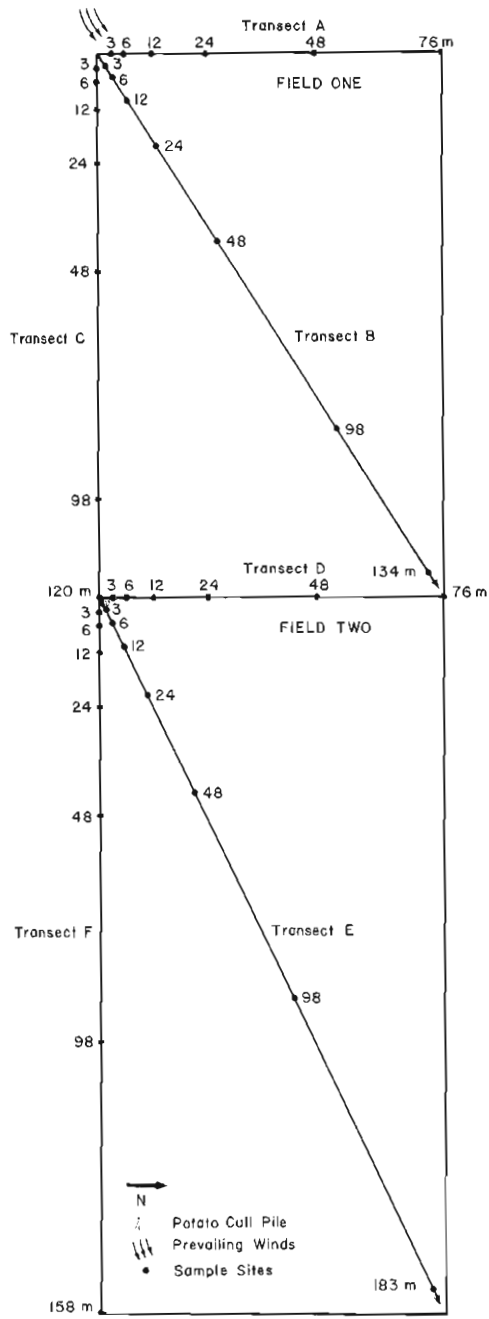

FIG. 1. Schematic diagram of the experimental field showing transects and sample locations.

All data were analyzed using a 2 way analysis of variance. If a significant F resulted, treatment means were separated using Tukey's HSD Test.

Results

Transmission of Erwinia *spp. by Artificially Contaminated Insects*—Fruit flies readily transmitted *Ecc*, *Eca*, and mixtures to injured potato plants in field tests (Table 1). The percent transmission ranged from 15 to 52%. Injured potato plants remained susceptible to transmission for at least 10 hr (Table 1). There were no significant differences in the level of transmission regardless of whether insects were allowed access to fresh injuries or to those created up to 10 hours earlier. The results were similar regardless of whether *Eca*, *Ecc*, or a mixture were used. During the course of the experiment air temperatures ranged from 5.0 to 27.2°C with an average of 19.5°C. Relative humidity ranged from 18-74% with an average of 45%.

TABLE 1.—*Transmission of* Erwinia *by artificially infested* Drosophila melanogaster *to potato plants and the infection of injured plants.*[1]

Hours after injury	Mean Percent Transmission[2]			Mean Percent Infection[2,3]		
	Ecc[4]	Eca	Ecc + Eca	Ecc	Eca	Ecc + Eca
0	15	31	23	2	0	12
1	18	28	37	2	3	12
2	31	29	20	6	7	6
4	25	52	45	8	6	11
6	34	33	30	2	1	7
8	21	12	30	0	1	2
10	20	32	20	1	7	4

[1] Mean of 3 replicates.
[2] Means were not significantly different (P = 0.05) on any of the tests.
[3] Infection was indicated by symptom development.
[4] *Eca* = *Erwinia carotovora* var. *atroseptica*; *Ecc* = *Erwinia carotovora* var. *carotovora*.

The results of observations on disease development were similar to the transmission data; however, the percent infection was less than the percent transmission and ranged from 0 to 12%. There were no significant differences in the mean percentage of infection of injuries ranging from 0 hours to 10 hours old. Again, differences were not significant regardless of the organism used. Injuries from control plants did not yield *Erwinia* and no soft rot symptoms were ever observed on these plants.

Influence of Time of Day on Insect Transmission of Erwinia *and on Disease Development*—The mean percentage of insect transmission of

Ecc was significantly lower during the 6:00 a.m. to 4:00 p.m. time period than for the other 2 test periods (Table 2). There were no significant differences in insect transmission during the various time periods when *Eca* was used as the inoculum, nor when a combination of *Ecc* and *Eca* was used. Infection levels were consistently highest for all 3 inocula during the 12:00 noon to 10:00 p.m. period, which is the warmest portion of the day in San Luis Valley (max. 27.2°C; min. 15.0°C; mean 21.1°C on the day of the experiment). With *Ecc* the difference was significant when compared to the other time periods. The mean percentage of infection was lowest for *Ecc* during the 6:00 p.m. to 4:00 a.m. (max. temperature 26.1°C; min. 10.6°C; mean 17.0°C) period and was significantly different (P = 0.05) from the 12.00 noon to 10.00 p.m. period but not from the 6:00 a.m. to 4:00 p.m. period (max. temperature 25.0°C; min. 5.0°C; mean 19.0°C). Differences in the level of *Ecc* infection during the 3 time periods were not significant (P = 0.05). When the 2 varieties of *Erwinia* were combined in the inoculum, however, the mean percentage infection was significantly higher (p = 0.05) during the 12:00 noon to 10:00 p.m. period than the other test periods.

TABLE 2.—*The effect of time of day on transmission of* Erwinia *by artificially infested* Drosophila melanogaster *to potato plants and on plant infection.*

Time period of injury	Mean Percent Transmission[1]			Mean Percent Infection		
	Ecc[2]	*Eca*	*Ecc* + *Eca*	*Ecc*	*Eca*	*Ecc* + *Eca*
6 a.m. - 4 p.m.	11B[3]	19A	18A	2AB	1A	2B
12 Noon - 10 p.m.	32A	43A	40A	7A	8A	21A
6 p.m. - 4 a.m.	21A	31A	31A	0B	2A	0B

[1] Mean of 3 replicates.

[2] *Eca* = *Erwinia carotovora* var. *atroseptica*; *Ecc* = *Erwinia carotovora* var. *carotovora*.

[3] Tukey's test used for mean separation. Different letters within a column indicate means which differ significantly (P = 0.05).

Acquisition and Transmission of Erwinia *by Naturally Occurring Insects*—Insects naturally acquired *Ecc* and *Eca* presumably from the potato cull pile. Both organisms were isolated from insects collected up to 3 m from the cull pile (Table 3). The insects apparently transmitted the bacteria to artificially injured potato plants. *Eca* was first recovered on July 26 (Table 3) when the organism was isolated from injured plants located 3 m from the potato cull pile. Tests for the presence of *Erwinia* on injured tissues on caged plants at the same distance from the cull pile were negative. Both *Ecc* and *Eca* were isolated from flies and potato tubers collected from the cull pile from June through September. *Ecc* was later

TABLE 3.—*Transmission of* Erwinia *from a potato cull pile to healthy injured potato plants by insects in the field.*

	Locations of injured plants (m from source of inoculation or from zero point in control)	Method of Injury	Contaminated Insects		Injured Plants Yielding *Erwinia*	
			Location[1] inoculum (m from source)	Bacterium from recovered specimens	Location[1] (m from inoculum source)	Bacterium recovered from specimens
June 28	3, 6, 12, 24, 49, 76, 98, 158, 183	Stems crushed[6] (10 places per plant)	--	--	--	--
July 5	3, 6	Stems crushed (20 places per plant)	--	--	--	--
July 12	3, 6	Stems crushed (20 places per plant)	--	--	--	--
July 21	3	Stems crushed (20 places per plant)	3	*Eca*[2]	--	--
July 26	3	All stems and petioles on each plant completely crushed	--	--	3[3]	*Eca*
August 3	3, 6, 12, 24, 49, 76, 98, 158, 183	3 plants at each site injured with "hail simulator"	3	*Ecc*[4]	6	*Ecc*
August 10	24	3 plants at each site completely crushed	--	--	24	*Eca* & *Ecc*
August 17	6, 76, 158, 183	5 plants at each site completely crushed	--	--	183, 6[5]	*Ecc* & *Eca* & *Ecc*

[1] Distance (m) from cull pile – 3 replicate plants (1 along each transect).
[2] *Eca* = *E. carotovora* var. *atroseptica*.
[3] Caged injured plants at same location yielded no *Erwinia*.
[4] *Ecc* = *E. carotovora* var. *carotovora*.
[5] Plant was 6 m upwind from the cull pile in the neighboring control plot (120 m downwind from origin).
[6] Plants were crushed with disinfested pliers.

isolated from injured plants at distances of 6 m on August 3 and August 17, 24 m on August 10 and 183 m on August 17. Wounded tissue removed from potato plants in the control plot (field 1) failed to yield *Erwinia* in all cases except on August 17 when isolations from a plant located 120 m from the zero point along transect C were positive; however, the plant was only 6 m upwind from the potato cull pile of the test field. *Eca* was isolated in field 2 from insects 3 m from the cull pile on July 21, and *Ecc* was isolated from insects collected at the same distance from the cull pile on August 3.

Discussion

The repeated detection of *Erwinia* in injured plant sites up to 183 m from the cull pile, which was the bacterial and insect source, but not from plants in a neighboring control field strongly suggests that the insects transmitted *Erwinia* from the cull pile to the injuries. These results also indicate that insects probably disseminate *Erwinia* in the field over a considerable distance from their sources. To detect insect transmission of *Erwinia* from the cull pile to field plants, it was necessary to provide a large number of injuries to be sampled. This is not surprising considering the nearly infinite number of potential receptor sites in a potato field. While we cannot conclusively state that the *Erwinia* isolated from injured plants in the field actually originated from the potato cull pile, nor that it was transmitted from there to the plants by insects, this explanation of the results is justified since none was ever isolated from plants in the control field. The assertion that insects transmitted the *Erwinia* isolated from wounded plants is supported by the fact that *Erwinia* was never isolated from injuries on the caged plants in the field with the cull pile. It is possible, of course, that the bacteria were transmitted to the injured plants as aerosols as suggested by Graham and Harrison (3). This possibility cannot be totally ruled out but, since *Erwinia* spp. was not isolated from injured plants caged to prevent insect visitations, the probability is that insects were responsible for the transmission that was observed. The possibility that airborne *Erwinia* cells could have been prevented from reaching injured plants by impaction on the screen surrounding them should be investigated. Experiments using marked *Erwinia* isolates should be conducted to determine that the bacteria were actually transmitted by insects from the cull pile.

In addition to naturally transmitting *Erwinia*, insects artificially contaminated with *Ecc*, *Eca*, or mixtures also readily transmitted the bacteria to injured plants. It was surprising that the injured potato tissue remained susceptible to bacterial infection for 10 hr under the low relative humidities

(as low as 18% at mid-day during the study period) that exist in the San Luis Valley. However, relative humidity has been shown to be higher under a plant canopy than normally indicated by measurements in the macro climate. Differences in relative humidity in the macro and micro climate in arid regions may explain the longevity of plant wounds as infection courts for bacteria. However, it is also possible that bacteria, at least *Erwinia* spp., are more capable of infecting field grown plants through partially desiccated wounds than is commonly recognized. Regardless, these results show that *Erwinia* contaminated insects can transmit the bacterium to injured plants for a considerable time after the injury occurred, suggesting that insect transmission may be much more important in the epidemiology of blackleg and bacterial soft rot than previously considered.

Data on transmission and infection at various periods of the day suggest that levels of both transmission and successful infection are highest from 12:00 noon to 10.00 p.m. when temperature and insect activity are generally at their peak. If increased insect activity is, in fact, associated with increased transmission of bacteria, as our data suggest, control of insect populations may be a valuable approach to disease control.

Insect transmission of *Erwinia* may be an important source for the reintroduction of the pathogen into *Erwinia*-free potato lots even if infection does not occur. Survival of transmitted *Erwinia* on potato leaves or stems could potentially result in introduction of bacteria into the rhizosphere during rains and/or sprinkler irrigation. *Erwinia* spp. are well adapted to rhizosphere survival and subsequent infection of developing tubers (1).

Our work indicates that insects can readily transmit *Erwinia* to injured plants and that the bacteria thus transmitted can cause infection even though the injuries may be at least 10 hours or more old. Consequently, it becomes extremely important to reduce potential sources of bacterial inoculum and also to maintain a reasonable insect control program. Culled potatoes dumped in open areas are probably the major source of *Erwinia* for insect vectors. Many of the insects collected in the field in Colorado and shown to be contaminated with *Erwinia* (6,7) also reproduce and/or overwinter in such potato refuse piles. Consequently, elimination of potato dumps would reduce the problem in 2 ways. First, by lowering the level of bacterial inoculum available for acquisition by naturally occuring insects and second reducing the overwintering and reproduction sites would also reduce the populations of the vectors. A combined approach of the use of high quality, stem-cutting seed stock, the elimination of the practice of dumping culled potatoes in municipal dumps or other locations near potato fields and the implementation of a practical insect control program to reduce populations of files around the existing inoculum sources could possibly eliminate potato blackleg or reduce it to very low levels in the Colorado potato industry in the San Luis Valley.

Literature Cited

1. Deboer, S.H., D.A. Cuppels, and A. Kelman. 1978. Pectolytic *Erwinia* spp. in the root zone of potato plants in relation to infestation of daughter tubers. Phytopathology 68:1784-1790.
2. Graham, D.C. and J.L. Hardie. 1971. Prospects for the control of potato blackleg disease by use of stem cuttings. Proc 6th Br Insectic Fungic Conf 1:219-224.
3. Graham, D.C. and M.D. Harrison. 1975. Potential spread of *Erwinia* spp. in aerosols. Phytopathology 65:739-741.
4. Graham, D.C., C.E. Quinn, and M.D. Harrison. 1976. Recurrence of soft rot coliform bacterial infections in potato stem cuttings: an epidemiological study on the central nuclear stock production farm in Scotland 1967-74. Potato Res 19:3-20.
5. Harrison, M.D., C.E. Quinn, I. Ann Sells, and D.C. Graham. 1977. Waste potato dumps as sources of insects contaminated with soft rot coliform bacteria in relation to recontamination of pathogen-free potato stocks. Potato Res 20:37-52.
6. Kloepper, J.W. 1976. The role of insects in the epidemiology of potato blackleg. M. Sc. Thesis. Colorado State University. 90 pp.
7. Kloepper, J.W., M.D. Harrison, and J.W. Brewer. 1979. The association of *Erwinia carotovora* var. *atroseptica* and *Erwinia carotovora* var. *carotovora* with insects in Colorado. Am Potato J 56:351-361.
8. Molina, J.J., M.D. Harrison, and J.W. Brewer. 1974. Transmission of *Erwinia carotovora* var. *atroseptica* by *Drosophila melanogaster*. I. Acquisition and Transmission of the Bacteria. Am Potato J 51:245-250.
9. Perombelon, M.C.M., R. Lowe, and E.M. Ballantine. 1976. Contamination by *Erwinia carotovora* of seed potato stocks of stem cutting origin in the process of multiplication. Potato Res 19:335-347.
10. Stewart, D.J. 1962. A selective-diagnostic medium for the isolation of pectinolytic organisms in the Enterobacteriaceae. Nature 195:1023.
11. Workman, M., C. Denhard, and J. Gough. 1979. Hail Adjustor's Handbook. Colorado State University Experiment Station Special Ser. Bull. 14.
12. Yates, W.W. and S.W. Linquist. 1952. Further studies on dispersion of flies tagged with radioactive phosphoric acid. J Econ Entomol 45:547-548.