ANNUAL REPORT

Fungus and Bacterial Disease Research

Summary of 1983 Results and Research Proposal for 1984

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Introduction

This section summarizes the work done in 1983 and includes new proposals and a budget request for 1984. Detailed reports are attached following the summary for those interested in further detail.

1. Blackleg Research

Objectives

Blackleg research in 1983 continued to emphasize the epidemiology of *Erwinia carotovora* in Colorado. Surveys to determine the presence and approximate populations of *E. carotovora* in surface and underground water samples were continued in 1983. Additionally, research was initiated to determine the role of irrigation water in the recontamination of *Erwinia*-free potatoes and possible means to reduce this threat.

New findings on the long distance transport of *E. carotovora* and its potential relationship to weather systems were made in 1983. Support from a USDA grant enabled initiation of this research.

Objective a. The association of *Erwinia carotovora* with surface and and underground water in Colorado.

Surveys made in Colorado showed that *E. carotovora* was consistently found in water collected from the South Platte and Big Thompson Rivers during a 20-month period from January 1982 through August 1983 (Table 1).

Table 1. The relation of sampling sites to the population of *Erwinia* in "undisturbed" water samples from the South Platte and the Big Thompson Rivers from January 1982 through August 1983.

Site	Mean[1] population (CFU/ml)	Tests for Mean Separation	
		Tukey's HSD	Fisher's (protected) LSD
		South Platte River	
1	0	c[2]	b
2	1.6820	c	b
3	5.0610	bc	b
4	20.5380	ab	a
5	24.3420	a	a
6	18.6460	abc	a
		Big Thompson River	
7	0.0620	b	b
8	0.0510	b	b
9	0.0070	b	b
10	4.4390	ab	a
11	5.5710	a	a

[1] Average for 20 months of samples for each site.

[2] Means with different letters were significantly different (P = 0.05).

Populations of E. carotovora ranged from undetectable to 700 colony forming units (cfu)/ml. The lowest populations were found during the winter months in samples collected at mountainous sites while the greatest populations were found at sites located in the plains during late spring, summer and early autumn.

E. carotovora subsp. carotovora (Ecc) represented 98.0% of the isolates characterized with E. carotovora subsp. atroseptica (Eca) comprising the remaining 2.0%. Eca was isolated primarily during the autumn, winter and early spring months.

Samples of water from rivers, canals, lakes and wells in the San Luis Valley were also tested for the presence of E. carotovora. Almost all of the surface water samples (90%) collected had detectable levels of E. carotovora present. Results showed that Ecc was the predominant isolate found throughout the year. Eca was also isolated from seven Rio Grande Samples (8% of all positive samples) and, usually, early in the year when water temperatures were cool. Data for the Rio Grande and Saguache Creek surveys are shown in Table 2.

The populations of Erwinia in surface waters were quite low, typically below 1 cfu/ml of water and were generally lower than populations estimated in water samples collected in northeastern Colorado. However, populations increased to 1 to 12 cfu/ml of water in late summer and autumn (July-November) in arable regions of the San Luis Valley.

Erwinia was also routinely isolated from water samples collected from the Arkansas River (Table 3). All isolates characterized from the Arkansas River were Ecc.

Table 2. The effect of site location on the presence of *Erwinia carotovora* in water samples - Rio Grande and Saguache Creek surveys, 1982 and 1983.

Site			Number of times sampled	Percentage of samples *Erwinia* (+)	#*Ecc*:#*Eca*[2/]
Designation		Location[1/]			
Rio Grande	1	M	3	67	6:0
	1A	M	17	59	26:0
	1B	M	2	50	0:4
	2	M	23	83	88:0
	3	T	21	81	55:0
	4	A	24	95	135:10
	5	A	23	96	169:10
	6	A	21	86	125:6
Saguache Creek	A	T	20	75	97:0
	B	T	22	100	169:0
	C	A	16	94	92:0

[1/]Site locations are designated as: M = mountainous site location; T = transitional site location; A = site located in an agricultural area.

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

Erwinia was infrequently isolated from well water used for irrigation in Northeastern Colorado and in the San Luis Valley. *Erwinia* was also detected in several well water samples collected near Greeley. However, since well water samples were not consistently positive for *Erwinia* and samplings were not extensive, more rigorous testing is needed before definite conclusions can be made concerning the presence of *Erwinia* in underground water.

Objective b. The role of suppressive soil in the recontamination of potatoes by *Erwinia*.

A study was made in the San Luis Valley to determine if an "*Erwinia*-suppressive" soil could reduce *Erwinia* recontamination of Centennial tubers increased from micropropagation seed stocks.

Potato root samples collected on July 19 had 0% and 2% contaminated with *Erwinia* for "suppressive" and "conducive" (i.e., normal) soil field plots, respectively. Samples collected on August 18 showed an increase in the level of contamination with 6% and 18% of the potato roots contaminated for the "suppressive" and "conducive" field plots, respectively. Fifty potato root samples were assayed for each field plot on each date. Weed-root rhizosphere samples (100 samples) were also collected from the "suppressive" plot. None of the weed root samples had detectable *Erwinia* present.

Objective c. To study the possibility of long distance transport of *Erwinia carotovora*.

Two coastal surveys for *Erwinia* were made in Oregon during 1983. Ocean water, rain water and aerosol samples were collected. One rain sample (out of 1 sample collected) in July and 12 out of 13 samples

Table 3. The presence (+) or absence (0) of Erwinia carotovora in Arkansas River water samples - 1983 and 1984.

Site	May	June	3 Aug	26 Aug	Sept	Oct	13 Dec	29 Dec	Jan
22	0	+	0	0	+	0	NT	NT	NT
2	+	+	+	+	+	+	+	+	+
56	+	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	NT	+
21	+	+	+	+	+	+	+	+	+
#Ecc/#Eca[1/]	23:0	28:0	21:0	25:0	29:0	2:0	34:0	24:0	33:0

[1/]Ecc = Erwinia carotovora subsp. carotovora; Eca = Erwinia carotovora subsp. atroseptica.

collected in December had detectable levels of Erwinia present. Both E. carotovora subsp. carotovora (Ecc) and E.c. subsp. atroseptica (Eca) were found and often both were present in the same rain sample. All sites where ocean water samples were collected were also positive for Erwinia. Data for rain collections made in December, 1983 are shown in Table 4.

Limited aerosol collections in July failed to detect airborne Erwinia cells. Aerosol collections in December resulted in 16 Erwinia isolates collected over a period of 4 consecutive days. Although humidity levels were always very high, positive air samples were collected both when it was raining and when it was not. Characterization of aerosol isolates showed a ratio of seven Ecc isolates to 9 Eca isolates with both [illegible]ies being present in the air simultaneously (i.e., Ecc and Eca both being present on some of the same exposed plates).

Table 4. The detection of Erwinia in rain samples - Newport, OR, December, 1983.

Rain collection site	Date	Presence (+) or absence (-) of Erwinia[1]	Subspeciation results[2] #Ecc:#Eca
Newport	4 Dec	0	NA
	4 Dec	+	12:0
	5 Dec	+	1:0
	6 Dec	+	12:0
	7 Dec	+	11:0
	8 Dec	+	17:0
	9 Dec	+	7:8
	10 Dec	+	11:0
			Total: 71:8
Beverly Beach Park	6 Dec	+	3:15
	7 Dec	+	0:4
	8 Dec	+	10:1
	9 Dec	+	0:15
	10 Dec	+	7:1
			Total: 20:36

[1] Rain samples were collected in sterile buckets ca 3 ft above the ground. Data shown are for both celite and direct enrichments.

[2] Ecc = Erwinia carotovora subsp. carotovora; Eca = E. carotovora subsp. atroseptica.

2. General Potato Pathology

Objective a. To determine the effect of TOPS 2.5, Mertect and Zineb seedpiece treatment on Russet Burbank potatoes in the San Luis Valley.

Treatment of cut Russet Burbank potato seed with fir bark amended by addition of TOPS 2.5, Mertect or Mertect + Zineb significantly increased total tuber yield in the San Luis Valley ($P = 0.05$). Visual beneficial effects of seedpiece treatment, when compared to the fir bark control, were evident in terms of plant vigor, color and degree of defoliation during the latter part of the growing season. There were, however, no measurable effects on plant stand, seedpiece decay, *Rhizoctonia* stem-cankering, *Verticillium* stem infection or early blight foliage infection. Generally, TOPS 2.5 and Mertect treatments were similar and appeared to produce more vigorous plants than treatment with Mertect in combination with Zineb. Data for plant vigor response and tuber yield are shown in Tables 5 and 6.

Objective b. To determine the effect of TOPS 2.5 D seedpiece treatment on stem infection by *Verticillium*.

Micropropagation produced tuber seedpieces (cv Russet Burbank) were treated with TOPS 2.5 D amended with fir bark or fir bark alone. Plants were artificially inoculated with *Verticillium* and later assayed to determine the effect of seedpiece treatment on infection.

Stem inoculations with toothpicks, upon which *Verticillium* had been previously grown, resulted in definite infections. Tissue sections taken from the top, middle, and bottom portions of each stem showed an increasing amount of infection at locations closer to the inoculation site (Table 7).

Table 5. The effect of seedpiece treatment on cv Russet Burbank potato plant vigor - Center, CO, 1983.

Treatment	Relative vigor $(\overline{X})$[1/] as determined on:						
	June 14	June 24	July 19	Aug. 11	Aug. 18	Aug. 25	Aug. 30
1) Fir bark	5.0 a[2/]	5.0 a	5.0 a	5.0 a	5.0 a	5.0 b	5.0 b
2) Fir bark + Tops 2.5	4.5 a	5.5 a	5.9 a	6.1 a*[3/]	5.5 a	6.3 ab*	6.3 ab*
3) Fir bark + Mertect	4.6 a	5.3 a	5.8 a	6.1 a*	5.5 a	6.8 a*	6.5 a*
4) Fir bark + Zineb + Mertect	5.2 a	5.5 a	6.0 a	5.3 a	5.0 a	5.3 b	5.3 b
F Ratio	2.804	0.256	2.513	3.922	1.411	6.895	9.750
	$P>0.1$	$P>0.1$	$P>0.1$	$P<0.05$	$P>0.1$	$P\leq0.025$	$P\leq0.005$

[1/] Rated on a scale of 0-10. Treatment #1 for each replication was arbitrarily assigned a rating of 5. More vigorous treatments were rated greater than 5 and less vigorous less than 5.

[2/] Column means with different letters do not differ significantly (P = 0.05). Mean separation was done using Tukey's HSD test.

[3/] Means designated by "*" are significantly different from the fir bark control (P = 0.05). Mean separation was done using the LSD test.

Table 6. The effect of seedpiece treatment on total yield and grade (cwt/A) of cv Russet Burbank tubers - Center, CO, 1983.

Treatment	Tuber yield (cwt/A)[1/]							
	Total yield	Marketable[2/]	<10 oz	>10 oz	Total US #1	US #2	Grade B	Culls
1) Fir Bark	268.7 b[3/]	191.5 a	161.5 a	11.3 a	172.9 a	18.7 a	50.7 a	19.5 a
2) Fir Bark + TOPS 2.5	312.0 ab*[4/]	239.3 a	212.0 a	7.5 a	219.5 a	19.8 a	50.2 a	15.6 a
3) Fir Bark + Mertect	323.9 a*	249.1 a	212.1 a	13.7 a	225.8 a	23.3 a	45.3 a	20.8 a
4) Fir Bark + Mertect + Zineb	308.7 ab*	228.8 a	184.2 a	23.2 a	207.8 a	21.0 a	53.2 a	28.2 a
F Ratio	3.999 P>0.05	2.972 P>0.1	2.664 P>0.1	1.118 P>0.1	2.099 P>0.1	0.213 P>0.1	0.226 P>0.1	0.580 P>0.1

[1/] Treatment plot size was 100 ft of row (0.0065 Acre). A sample of ca 50 lb was sorted and the proportion of each tuber grade determined. Data were converted to cwt/A for presentation in the table.

[2/] Sum of total US #1 and US #2 yields.

[3/] Column means with different letters differ significantly (P = 0.05). Tukey's HSD test was used for mean separation.

[4/] Column means designated by "*" differ significantly (P = 0.05) from the fir bark control (Treatment 1). An LSD test was used for comparison of means.

Table 7. The effect of TOPS 2.5 D and fir bark on Verticillium infection for artificially inoculated stems - Ft. Collins, CO, 1984.

Treatment	The average number of stem sections and average number of stems with *Verticillium* evident[1/]: stem position[2/] top	middle	bottom	Number stems infected[3/] ($\overline{X}$)
1. TOPS 2.5 + fir bark/uninoculated	0 a	0 b	0 b	0 b
2. Fir bark/ uninoculated	0 a	0 b	0 b	0 b
3. TOPS 2.5 + fir bark/*Verticillium*	1.50 a	2.50 a	2.75 a	3.25 a
4. Fir bark/ *Verticillium*	1.50 a	3.50 a	4.25 a	4.25 a
	NSD[4/]	P=0.01	P=0.01	P=0.01

[1/]Each datum entry point is the average number of stem sections *Verticillium* positive for four replications of five stems each.

[2/]Sections were assayed for *Verticillium* at 12 in., 6 in., and 3 in. above the soil line for top, middle and bottom assays, respectively.

[3/]The average number of stems with at least one section *Verticillium* positive. Each entry is the average of four replications of five plants each.

[4/]NSD = no significant differences found (P=0.05). Mean separation was done using Tukey's (HSD) test.

None of the control plants showed *Verticillium* infection. The only significant differences observed were between *Verticillium* inoculated and non-inoculated treatments ($P = 0.01$). There was a trend for seedpiece treatment with TOPS 2.5 D amended fir bark to have less infection present for the middle and bottom portions of the stem as well as for the average number of stems infected when compared to the similarly inoculated fir bark treatments. However, these differences were not significant ($P = 0.05$).

There was no significant effect of treatment on the average stem weight for potato plants grown in the greenhouse.

3. Ringrot Research

Objectives

Ringrot research continued to emphasize the effect of inoculum concentration on symptom expression. Studies comparing new methods for detection of ringrot were also initiated. Because of problems with the ELISA test for tuber infection, results for this study are not complete.

Objective a. To determine the effect of *Corynebacterium sepedonicum* inoculum concentration on symptom expression in potatoes.

The effect of inoculum concentration on ringrot symptom development in both plants and daughter tubers was studied for the third year in Colorado. Results for 1983 support the previous conclusions that tubers can become infected in the absence of foliar symptom expression in the parent plant. Furthermore, data from 1981 and 1982 show tubers can appear healthy based on visual inspection with latent ringrot infection being detected only by bioassay on eggplant (cv Black Beauty). One treatment (10 cells per tuber) inoculated in May 1981 required three growing seasons before any symptom expression occurred in the field (Table 8).

Table 8. The effect of <u>Corynebacterium sepedonicum</u> (ringrot) tuber inoculum concentration on symptom expression in progeny plants and daughter tubers - DEP 81 study, Center, Colorado.

Cultivar	Number of cells per mother tuber	Primary foliage symptoms 1/ summer 1981	Daughter tuber infection 2/ spring 1982	Secondary foliage symptoms 3/ summer 1982	Grand-daughter tuber infection 2/ spring 1983	Tertiary foliage symptoms 4/ summer 1983
Russet Burbank	6.3×10^8	-	+	+	-	-
	10^6	-	+	-	-	-
	10^4	-	+	-	-	-
	10^2	-	-	-	-	-
	10^1	-	-	-	-	-
	Buffer	-	-	-	-	-
Centennial	6.3×10^8	-	+	-	-	-
	10^6	-	+	-	+	-
	10^4	-	-	-	-	-
	10^2	-	-	-	-	-
	10^1	-	-	-	-	+
	Buffer	-	-	-	-	-

1/ Inoculated tubers were planted in the field in 1981 and symptom expression recorded.

2/ Tubers were assayed for ringrot infection using the eggplant test.

3/ Daughter tubers harvested in 1981 were replanted in the field in 1982 and symptom expression recorded.

4/ Granddaughter tubers harvested in 1982 were replanted in the field in 1983 and symptom expression recorded.

Research Proposal for 1984

Blackleg Research

The following aspects of blackleg epidemiology will be studied:

a. The role of irrigation water in the recontamination of *Erwinia*-free seed stocks.

Emphasis will be placed on determining the significance of *Erwinia* cells in irrigation water in the recontamination of *Erwinia*-free seed. Serological methods will be used to determine if *Erwinia* recovered from tubers produced by *Erwinia*-free stocks are the same as those present in irrigation water. Some monitoring of water sources will continue also.

b. Sources of *Erwinia* in surface waters.

Since we now know that *Erwinia* is continuously present in surface water, including the Rio Grande River, Saguache Creek, the Arkansas River, the South Platte River, and many other streams in Colorado, it is important to determine the source of the contamination. Studies will be made to determine if the bacteria are residents in the water (or stream bed) where they persist and multiply indefinitely or if they are introduced from external sources such as deposition with precipitation from cloud moisture derived from the Pacific Ocean. Intensive studies using funds from the USDA Grant (received in July 1983) supplemented by funds requested in this proposal will be made to determine the

occurrance and extent of long distance dispersal of *Erwinia* cells with weather systems.

c. Biological control of *Erwinia* recontamination of clean seed stocks.

Results from 1983 indicate that a "suppressive factor" exists in a potato field at the Research Center which has been cropped to potatoes for 22 years continuously. Attempts will be made, based upon the availability of funds and time, to determine the nature of the factor. If it is biological in origin, it will be tested as a means of preventing or slowing recontamination of clean potato seed stocks by *Erwinia* introduced with irrigation water or from other external sources.

Ringrot Research

Due to the serious nature of ringrot in the San Luis Valley and because some new techniques for detection and identification of the ringrot organism have been developed, it is now feasible (except for a question of an adequate location to do the work) to begin a detailed study of ringrot in Colorado. As resources will permit it is proposed that we begin in 1984 a study on the following aspects of the ringrot problem:

1) Latency in the tubers in relation to inoculum concentration, variety and the environment.
2) Sources of inoculum and means of spread, including water, insects, machinery and other possible sources.
3) Factors which affect expression of symptoms in the presence of different concentrations of inoculum.

Proposed Budget for 1984

Labor	$2700.00
Travel	1500.00
Supplies/Equipment	1500.00
Total	$5700.00

The Presence of *Erwinia carotovora* in Surface and Underground Water in Colorado

Introduction

Extensive surveys for *Erwinia carotovora* in surface and, to a lesser extent, underground water in Colorado have been made since 1980. Although our results have conclusively shown that *Erwinia* is present in surface waters throughout the year, there is still much that we do not yet know.

Erwinia population studies are being continued on the South Platte, Rio Grande and Arkansas Rivers. These surveys parallel population and nutritional studies on water samples collected in the field and inoculated with selected *Erwinia* strains in the lab.

These studies, coupled with the serological identity of wild-type *Erwinia* isolates from water, may enable us to determine the source of *Erwinia* in the water and its probable ecological role.

Most of the data in this report are taken from Master's thesis research projects done by Mr. Pedro E. Jorge and Mr. Darrell A. Maddox. The theses, when completed, will have much more information than is included in this report.

Materials and Methods

Water samples were collected on a regular basis from specific sites on the Big Thompson River (BTR), the South Platte (SPR), Rio Grande (RG) and the Arkansas Rivers (AR) in Colorado. Collection sites were generally located where roads and bridges crossed the rivers. This increased the likelihood of site access during the winter months. Sites for each river ranged from

samples collected near the head waters in the mountains (non-agricultural) to agricultural areas. Underground water samples in agricultural areas were also collected from wells and ditches used for irrigation purposes.

Although methods varied somewhat, the general methods of sample collection and Erwinia detection were as follows.

Water samples were collected in 10 liter polyethylene jugs previously disinfested with ethanol. The jugs were thoroughly rinsed at the collection site before filling with the sample. Some samples were also collected in pre-sterilized glass jars. "Undisturbed" water samples were collected by immersing the jug in the running water and filling. Samples from the same sites collected after the stream bed was agitated, were also collected in some instances. These latter ("disturbed") samples contained stream bed sediments while the former remained relatively clear.

Temperature and pH were measured for samples collected from each site. Temperature was determined at the collection site while pH was determined for a sample aliquot after being returned to the laboratory in Fort Collins. These data were used to determine the relationship between the Erwinia populations measured, pH and temperature.

Water samples were processed for Erwinia detection using two general methods, i.e., direct enrichment of ca 50 ml water samples and filter concentration of Erwinia in water samples followed by enrichment.

Direct enrichment of water samples was done by adding double strength pectate enrichment medium (2X growth medium) to an equal volume (50 ml) of water sample. Samples were then incubated anaerobically for 96 hr at 26°C.

Samples were filtered by passing the water through a column of finely pulverized diatomaceous earth. The water was drawn through the filter using vacuum pumps. The column retained a percentage of the bacteria present

in the water sample and thus concentrated the cells. The column was then resuspended in single-strength growth medium (ca 200 m) and incubated anaerobically for 96 hr at 26°C.

The incubation period gave sufficient time for any *Erwinia* cells in the enriched sample to increase to numbers great enough to enable detection by streaking onto a semi-selective differential medium (e.g. Stewart's MacConkey pectate). *Erwinia* isolates were transferred and purified using standard bacteriological techniques. *Erwinia* isolates were also characterized by determining identity and pathogenicity (soft-rot) using established methods.

Erwinia populations were estimated in water samples by spread plating aliquots of filter enrichments before the incubation period. These samples were usually plated onto Stewart's MacConkey pectate medium. After 2 or 3 days incubation, the number of *Erwinia* cells in the sample aliquot was determined by counting the number of characteristic *Erwinia* colonies per plate.

Results

Surveys showed that *E. carotovora* was consistently present in water collected from the South Platte (SPR) and the Big Thompson Rivers (BTR) in Colorado during a 20-month period from January 1982 through August 1983. Sites located in the mountains as well as the plains were routinely sampled. The bacterium was readily isolated from "undisturbed" surface water as well as from "disturbed" water containing stream bed sediments.

Erwinia populations in both the "undisturbed" and "disturbed" samples were related to temperature, time of year and sampling site for the BTR and SPR sample series. Populations of the bacterium in the water ranged from

undetectable to 700 colony forming units (CFU)/ml. Lowest populations were found during the winter months at mountainous sites while highest populations were found at sites located in the agricultural (plains) area during late spring, summer and early autumn (Tables 1 and 2). *Erwinia* numbers were consistently higher in "disturbed" than "undisturbed" water samples from sites located in the agricultural areas during the summer months (Table 3). Results suggested that *Erwinia* may multiply in the sediments but concentration of cells by sedimentation of suspended material was also possible.

E. carotovora subsp. *carotovora* (*Ecc*) was the predominant organism isolated from the water, representing 98.0% of the isolates characterized. *E. carotovora* subsp. *atroseptica* (*Eca*) was also isolated occasionally, primarily during the autumn, winter and early spring months.

Erwinia was infrequently isolated from well water used for irrigation in Northeastern Colorado. The relationship of the organism with well water was not conclusively established by this portion of the survey and further investigation is required before final conclusions can be drawn.

Samples of water from rivers, canals, lakes and wells in the San Luis Valley were also analyzed for the presence of *E. carotovora* (Table 4). Almost all of the surface water samples (90%) collected had detectable levels of *E. carotovora* present. Results showed that *Ecc* was readily isolated from rivers and canals throughout the year. *Eca* was also isolated from seven Rio Grande samples (8% of all positive samples) and, usually, early in the year when water temperatures were cool.

Results for individual sites on the Rio Grande River and Saguache Creek are shown in Table 5. The percentage of Rio Grande River water samples which yielded *Erwinia* were less for collections at mountainous sites than for samples collected from the river in agricultural areas. Except for one sample collected at site 1B, all *Eca* isolates were found in water samples collected in the agricultural areas.

Table 1. Effect of time of year (month) on the mean populations of *Erwinia* in undisturbed water from the South Platte River from January 1982 through August 1983.

Month			Mean[1/] populations (CFU/ml)	Tests for Mean Separation	
				Tukey's HSD	Fisher's (protected) LSD
1982	1	(Jan)	1.00	c[2/]	e
	2	(Feb)	0.32	c	e
	3	(Mar)	0.33	c	e
	4	(Apr)	0.67	c	e
	5	(May)	2.17	c	e
	6	(Jun)	10.87	c	cde
	7	(Jul)	0.69	c	e
	8	(Aug)	32.20	abc	bc
	9	(Sep)	27.83	abc	cd
	10	(Oct)	7.25	c	de
	11	(Nov)	8.50	c	cde
	12	(Dec)	5.00	c	de
1983	13	(Jan)	0.60	c	e
	14	(Feb)	0.37	c	e
	15	(Mar)	0.67	c	e
	16	(Apr)	0.35	c	e
	17	(May)	1.40	c	e
	18	(Jun)	60.00	a	a
	19	(Jul)	55.84	ab	ab
	20	(Aug)	18.18	abc	cde

[1/] Average of 6 sites per month.

[2/] Means with different letters were significantly different ($P = 0.05$).

Table 2. The relation of sampling sites to the population of *Erwinia* in "undisturbed" water samples from the South Platte and the Big Thompson Rivers from January 1982 through August 1983.

Site[1/]	Mean[2/] population (CFU/ml)	Tests for Mean Separation: Tukey's HSD	Tests for Mean Separation: Fisher's (protected) LSD
		South Platte River	
1	0	c[3/]	b
2	1.6820	c	b
3	5.0610	bc	b
4	20.5380	ab	a
5	24.3420	a	a
6	18.6460	abc	a
		Big Thompson River	
7	0.0620	b	b
8	0.0510	b	b
9	0.0070	b	b
10	4.4390	ab	a
11	5.5710	a	a

[1/] For location of sites, refer to Figure 1, pg. 8.

[2/] Average for 20 months of samples for each site.

[3/] Means with different letters were significantly different ($P = 0.05$).

Table 3. Comparison of *Erwinia* populations in "disturbed" and "undisturbed" water samples from 11 sites on the South Platte and Big Thompson Rivers from July 1982 through August 1983.

Site[1/]	Mean *Erwinia* population (CFU/ml)			
	14 month period July 1982 - August 1983		6 month period July - September 1982 and June - August 1983	
	Undisturbed	Disturbed	Undisturbed	Disturbed
	South Platte River			
1	0	0	0	0
2	1.47	3.50	3.18	6.33
3	6.52	77.14	13.70	179.00
4	27.34	56.07*[2/]	54.63	121.00*
5	35.26	57.69	80.00	145.40
6	25.71	47.36	56.50	107.33
	Big Thompson River			
7	0	0	0	0
8	0.09	0.58	0.17	0.33
9	0.01	0.30	0.01	0.75
10	3.51	10.15	3.24	23.00
11	7.50	18.07	12.33	39.17

[1/]For location of sites, refer to Figure 1, pg. 8.

[2/]* = Means ("undisturbed" vs "disturbed") significantly different (P = 0.05).

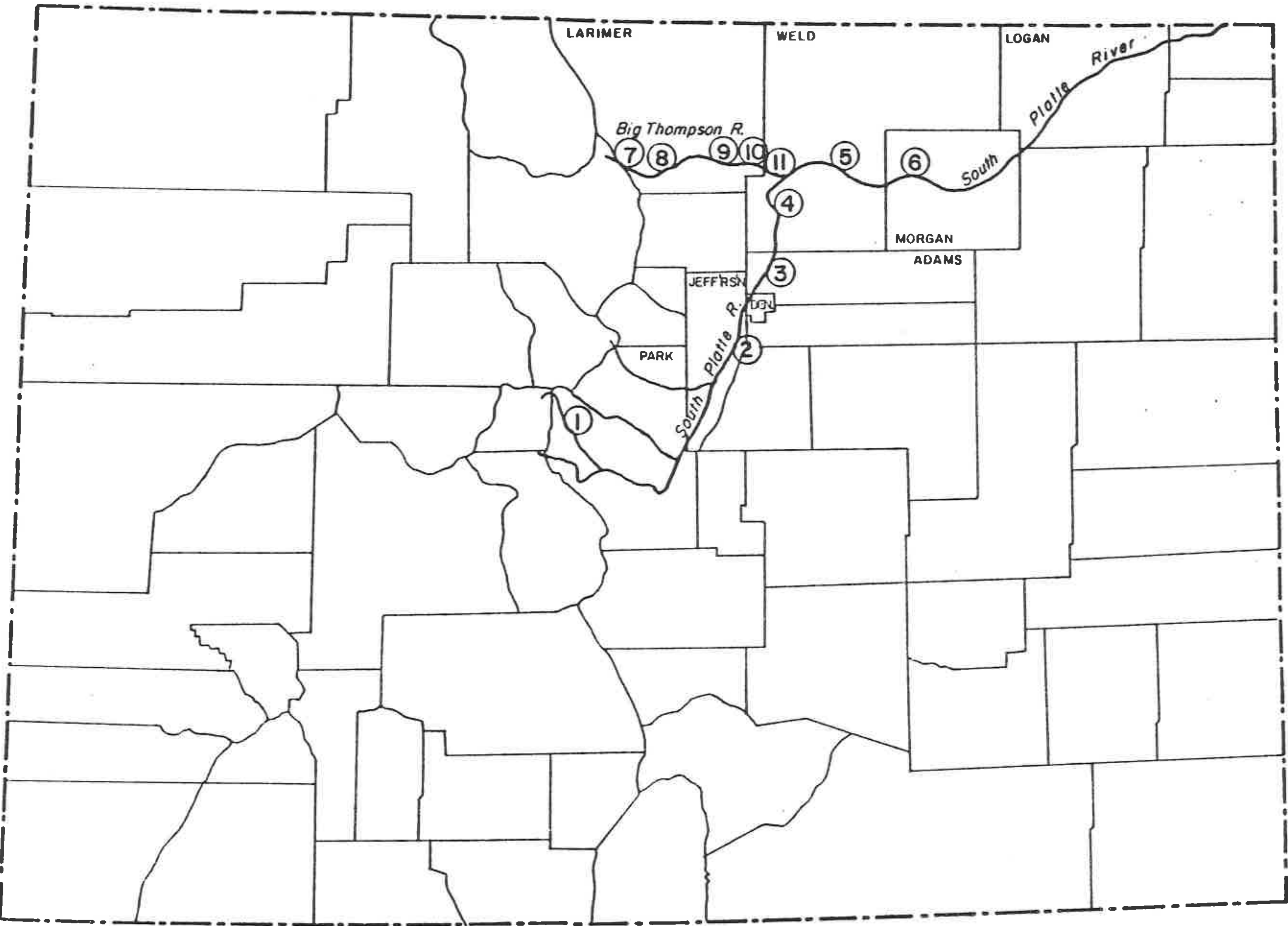

Figure 1. Location of 11 sampling sites on the South Platte and Big Thompson Rivers.

Table 4. Survey results for detection of Erwinia carotovora in surface and ground water sources in Southcentral Colorado, 1983.

Results for:	Source of water sample assayed:[1]	
	surface water	underground water
# samples Erwinia positive	87	9
# samples collected	97	53
% samples Erwinia positive[1]	90%	17%
# isolates characterized[2]	502	30
# isolates Ecc[2]	486	27
# isolates Eca[2]	16	3
% isolates Ecc	97%	90%
% isolates Eca	3%	10%

[1] The smallest volume of samples processed was 50 ml. Most surface water samples were collected from the Rio Grande River and Saguache Creek Underground water samples were collected from wells located in the San Luis Valley.

[2] Isolates from water samples were characterized for subspecies identification after purification on semi-selective differential media and nutrient agar. Ecc = Erwinia carotovora subsp. carotovora; Eca = E. carotovora subsp. atroseptica.

Table 5. The effect of site location on the presence of *Erwinia carotovora* in water samples - Rio Grande and Saguache Creek surveys, 1982 and 1983.

Site			Number of times sampled	Percentage of samples *Erwinia* (+)	#*Ecc*:#*Eca*[2/]
Designation		Location[1/]			
Rio Grande	1	M	3	67	6:0
	1A	M	17	59	26:0
	1B	M	2	50	0:4
	2	M	23	83	88:0
	3	T	21	81	55:0
	4	A	24	95	135:10
	5	A	23	96	169:10
	6	A	21	86	125:6
Saguache Creek	A	T	20	75	97:0
	B	T	22	100	169:0
	C	A	16	94	92:0

[1/]Site locations are designated as: M = mountainous site location; T = transitional site location; A = site located in an agricultural area.

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

Estimations of *Erwinia* populations for the Rio Grande River were made by plating serial dilutions. The populations of *Erwinia* in surface water were quite low, typically below 1 CFU per ml of water and were generally lower than populations estimated in water samples collected in Northeastern Colorado. However, populations increased to 1 to 12 CFU/ml of water in late summer and autumn (July-November) in arable regions of the San Luis Valley. There was a slight tendency for the water samples containing disturbed sediment to have higher *E. carotovora* populations than undisturbed water samples, especially in late summer and autumn. However, these differences were not as pronounced as for samples collected in Northeastern Colorado.

Ecc and *Eca* were also isolated from several underground (well) water samples, but usually only after filter-concentration of 8 to 10 l. Only 17% of the well samples collected were positive for soft rot *Erwinia*. However, one well (sample "S") yielded *Ecc* 57% of the time. This sample was collected after the water contacted the canal surface which may have contributed to the high percentage of positive samples.

Preliminary results for the Arkansas River survey for 1983 and 1984 are shown in Table 6. *Erwinia* was detected in water samples collected every month from May 1983 through January 1984. All isolates were characterized as *Ecc*.

Discussion

These surveys have conclusively demonstrated the widespread presence of *Erwinia* in surface waters commonly used as sources of irrigation water. Although the significance of this finding in relation to potato crops is currently being studied, this evidence suggests that irrigation water, when

Table 6. The presence (+) or absence (0) of Erwinia carotovora in Arkansas River water samples - 1983 and 1984.

Site	May	June	3 Aug	26 Aug	Sept	Oct	13 Dec	29 Dec	Jan
22	0	+	0	0	+	0	NT	NT	NT
2	+	+	+	+	+	+	+	+	+
56	+	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	NT	+
21	+	+	+	+	+	+	+	+	+
#Ecc/#Eca[1/]	23:0	28:0	21:0	25:0	29:0	2:0	34:0	24:0	33:0

[1/] Ecc = Erwinia carotovora subsp. carotovora; Eca = Erwinia carotovora subsp. atroseptica.

contaminated with Erwinia, could perhaps be an important source of inoculum and play a significant role in the recontamination of Erwinia-free potato seedstocks.

Well (underground) water samples often do not have detectable levels of Erwinia present in contrast to surface waters and therefore data interpretation must be conservative. However, since well water samples were occasionally positive and sterile water checks carried through the assay process were consistently negative for Erwinia, the data do suggest that low numbers of Erwinia may be present in some underground water samples. More rigorous testing is needed before definite conclusions can be made about the presence of Erwinia in underground water.

Erwinia surveys on the South Platte, Rio Grande and Arkasas Rivers are continuing with the aid of the USDA grant received in 1983. Serotypical reactions of *Erwinia* isolates may enable us to determine their source and role in the ecology of the water.

The Effect of Suppressive Soil on Recontamination
of Potatoes by *Erwinia*

G.D. Franc and M.D. Harrison

Abstract

A study was made in the San Luis Valley to determine if an apparently "*Erwinia*-suppressive" soil could reduce *Erwinia* recontamination of Centennial tubers increased from micropropagation seed stocks.

Potato root samples collected on July 19 had 0% and 2% contaminated with *Erwinia* for "suppressive" and "conducive" (i.e., normal) soil field plots, respectively. Samples collected on August 18 showed an increase in the level of contamination with 6% and 18% of the potato roots contaminated for the "suppressive" and "conducive" field plots, respectively. Fifty potato root samples were assayed for each field plot on each date. Weed-root rhizosphere samples (100 samples) were also collected from the "suppressive" plot. None of the weed root samples had detectable *Erwinia* present.

Introduction

A field plot in the San Luis Valley has been continuously planted in potatoes since 1962 for the purpose of monitoring soil-borne pathogens. Field studies done in 1978-1979 showed it was difficult to establish *Erwinia* populations on rhizospheres of weeds transplanted into the field. Subsequent studies in weed fallow plots in 1980, 1981, 1982 and 1983 once again showed that there was a very low incidence of weed root contamination by *Erwinia* even though the weed fallow plots were irrigated with water known to contain detectable *Erwinia* populations. A study was initiated in 1983 to

determine if the recontamination of potato roots and tubers was less in the "suppressive" field plot when compared to a nearby "conducive" field plot.

Materials and Methods

Field plots were planted in the San Luis Valley. Seed tubers (cv Centennial) grown in the SLV the previous year from micropropagation tubers were used as the seed source. Two replications of 2 rows each 50 ft long were planted in the "suppressive" field and the "conducive" field. The plots were irrigated with the same *Erwinia*-contaminated water source by center pivot ("suppressive" soil) or furrow irrigation ("conducive" soil).

The estimated percentage of seedpiece decay was determined on 19 July. Five hills per replication were excavated by hand and the mother seedpiece rated for the percentage of decay observed using a Barratt-Horsfall scale (0-11).

Plant height was determined in each plot on 19 July and 11 August. Ten stems per replication were measured from the soil line to the stem apex.

Verticillium infection in stems was determined by plating plant tissue on water agar. Stems used for *Verticillium* assays were collected on 4 August.

Potato roots were assayed for *Erwinia* by direct enrichment. Twenty-five roots were assayed for each replication on 19 July and on 18 August. The presence or absence of *Erwinia* was determined by streaking enrichments onto petri plates of semi-selective differential media (Stewart's MacConkey pectate or crystal-violet pectate). *Erwinia* colonies are characteristic on the media used and were subsequently isolated and characterized.

Tuber samples were harvested from the plots. These tubers will be assayed for the presence of *Erwinia* during April 1984.

Results

The field data are shown in Table 1. Although potato plants appeared more vigorous and stem lengths were greater in the "suppressive" soil this could easily be attributed to different irrigation practices between the two fields.

The "suppressive" soil field plot, continuously cropped to potatoes for 22 years, had two out of 10 stems (20%) while assayed positive for *Verticillium* in contrast to the "conducive" soil in which stem infections were not detected. The "conducive" soil plot had not been cropped to potatoes for many years. Visual inspections of both field plots failed to show any marked differences in the amount of foliar symptoms possibly attributable to *Verticillium*.

Even though both field plots were irrigated with *Erwinia* contaminated water ("suppressive" = center pivot; "conducive" = furrow irrigation) differences in the amount of seedpiece decay and potato root contamination with *Erwinia* were seen. Seedpiece decay (soft-rot) was not found in the "suppressive" soil versus an estimated 1.5% of the seedpieces were decayed in the "conducive" field. Root assays on 19 July showed 0% and 2% of the potato roots *Erwinia* contaminated and on 18 August, 6% and 8%, for the "suppressive" and "conducive" soils, respectively.

Discussion

This study was not extensive enough to demonstrate whether or not the continuously cropped field was indeed suppressive to *Erwinia*. However, these results support the previous observation that it is difficult to establish *Erwinia* on weed-root rhizospheres. This trend appears to be true for potatoes. Tuber assays in April may further support these findings.

Table 1. The comparison of cv Centennial potatoes grown in *Erwinia* "suppressive" and "conducive" soil - Center, CO, 1983.

Assay	Assay of potato plants grown in: *Erwinia* "suppressive" soil[1/]	*Erwinia* "conducive" soil[2/]
Average stem length on:		
19 July	16.0 cm	14.1 cm
11 August	48.6 cm	37.8 cm
Estimated percentage of seedpiece decay	0 %	1.5 %
Percentage of *Verticillium* stem infections	20 %	0 %
Percentage of potato roots *Erwinia* positive:		
19 July	0 %	2 %
18 August	6 %	18 %

A definitive study needs to be done to find out if the soil is "suppressive" to *Erwinia* and the nature of the "suppressive" agent, i.e., suppressiveness may be due to a biological agent, physical characteristics of the soil or a combination of both.

The Presence of *Erwinia carotovora* in Aerosols, Rain and Ocean Water Samples Collected on the Oregon Coast

G.D. Franc and M.D. Harrison

Abstract

Two coastal surveys for *Erwinia* were made in Oregon during 1983. Ocean water, rain water and aerosol samples were collected. One rain sample (out of 1 sample collected) in July and 12 out of 13 samples collected in December had detectable levels of *Erwinia* present. Both *E. carotovora* subsp. *carotovora* (*Ecc*) and *E.c.* subsp. *atroseptica* (*Eca*) were found and often both were present in the same rain sample. All sites where ocean water samples were collected were also positive for *Erwinia*.

Limited aerosol collections in July failed to detect airborn *Erwinia* cells. Aerosol collections in December resulted in 16 *Erwinia* isolates collected over a period of 4 consecutive days. Although humidity levels were always very high, positive air samples were collected both when it was raining and when it was not. Characterization of aerosol isolates showed a ratio of 7 *Ecc* isolates to 9 *Eca* isolates with both subspecies being present in the air simultaneously (i.e., *Ecc* and *Eca* both being present on some of the same exposed plates).

Introduction

Erwinia carotovora causes a decay or soft-rot of potato tubers in the field as well as in storage. It also causes a stem decay of potato plants in the field called potato blackleg.

Until recently, *E. carotovora* was thought to be associated primarily with the growing of susceptible vegetable crops - such as potatoes. However, we have

also found it to be present in natural surface water samples collected in many areas of Colorado and other areas in the United States. These have included ocean water samples as well as samples collected from streams located high in the Rocky Mountains.

Since this discovery, a major thrust of our research has been to demonstrate if E. carotovora can become aerosolized from the ocean and passively carried inland within clouds and deposited with snow and rain. Financial support from the USDA grant received in 1983 has enabled us to pursue this study.

In order to accomplish this task, we must be able to conclusively show that E. carotovora is present on the northwest coast, a major source of Colorado's precipitation, as well as in cloud water (collected as rimed ice) and precipitation samples collected in Colorado. Serological identification of isolates will also be done to determine if the Erwinia strains from different locations are related.

The first step in this study was to conduct surveys along the northwest coast to determine if Erwinia was indeed present and to determine to what extent. If our hypothesis is true, Erwinia could be present in rain and ocean water samples as well as aerosol samples.

A small survey to test this idea was done in July 1983 followed by a more extensive survey in December 1983. Although data are also being collected for snow and cloud water samples, this report deals primarily with some of the observations made in Oregon during December 1983.

Materials and Methods

A survey centered around Newport, OR, was made 4 Dec 1983 through 10 Dec 1983. All samples were collected using pre-sterilized containers and processed at a temporary laboratory at Newport. All media and equipment used in the survey were from the Potato Virus and Weed Research Laboratory in Fort Collins.

Rain samples were collected using 2 4'x 4' sheets of lexan plastic hinged at the apex. The water collected on each sheet during a rain storm drained into a trough which in turn emptied into a plastic bucket attached to the collector. The entire collector was ca 3' above the ground. The entire collector surface was thoroughly surface disinfested with ethanol. A collector was set up at Newport and one collector at Beverly Beach Park.

Sterile water was poured onto the surfaces and assayed for *Erwinia*. Duplicate samples from each trough were treated as replications. Samples were processed by direct enrichment as well as filter concentration of bacterial cells using celite followed by enrichment.

Ocean water samples and several fresh water samples were also collected. These samples were processed for *Erwinia* using the same methods.

All enrichments for *Erwinia* were incubated anaerobically for four days. Aliquots of the enrichment medium were streaked onto Stewart's MacConkey pectate. Characteristic *Erwinia* colonies were purified using standard bacteriological techniques and characterized using standard biochemical tests.

Results

Erwinia was readily detected in Pacific Ocean water samples and several fresh water samples collected from near Tillicum, OR (ca 20 miles south of Newport), to Fort Stevens, OR (near the mouth of the Columbia River). All 22 collection sites had at least one water sample *Erwinia* positive.

Enrichment medium was also poured directly onto wet sand collected along the beaches. Fifteen out of 21 samples collected were *Erwinia* positive.

A total of 173 *Erwinia* isolates from both water and sand were purified and characterized. Of these, 162 (94%) were determined to be *E. carotovora*

subsp. *carotovora* (*Ecc*) and 11 (6%) *E. carotovora* subsp. *atroseptica* (*Eca*).

The data for rain samples are shown in Table 1. Thirteen separate rain samples were collected and all, except the first sample, were *Erwinia* positive over a period of 7 days. The ratio of *Ecc* to *Eca* at the Newport collection site was 71:8; the ratio at the Beverly Beach collection site was 20:36.

The results for aerosol collections are shown in Table 2. Although many samplings failed to show *Erwinia*, collections over a period of four consecutive days gave 16 *E. carotovora* isolates. The ratio of *Ecc* to *Eca* was 7:9.

Discussion

Both *Ecc* and *Eca* were readily detected in ocean and rain water samples. Because of the large volume of water involved it is surprising that *Erwinia* was so easily detected in most samples. Because *Erwinia* was also readily detected in aerosol samples this suggests the *Erwinia* aerosols are readily generated on the coast. The link with precipitation in Colorado has not been proven yet.

Table 1. The detection of *Erwinia* in rain samples - Newport, OR, December, 1983.

Rain collection site	Date	Presence (+) or Absence (-) of *Erwinia*[1/]	Subspeciation results[2/] #*Ecc*:#*Eca*
Newport	4 Dec	0	NA
	4 Dec	+	12:0
	5 Dec	+	1:0
	6 Dec	+	12:0
	7 Dec	+	11:0
	8 Dec	+	17:0
	9 Dec	+	7:8
	10 Dec	+	11:0
		Total:	71:8
Beverly Beach Park	6 Dec	+	3:15
	7 Dec	+	0:4
	8 Dec	+	10:1
	9 Dec	+	0:15
	10 Dec	+	7:1
		Total:	20:36

[1/] Rain samples were collected in sterile buckets ca 3 ft above the ground. Data shown are for both celite and direct enrichments.

[2/] *Ecc* = *Erwinia carotovora* subsp. *carotovora*; *Eca* = *E. carotovora* subsp. *atroseptica*.

Table 2. The results for Erwinia aerosol assays - Newport, OR, December, 1983.

Aerosol sample location and the time collections were initiated		Aerosol sample duration (min)	Number of *Erwinia* colonies on aerosol plates	Subspeciation Results #*Ecc*:#*Eca*[1/]
Newport				
4 Dec	6:20 pm	30	0	NA
5 Dec	6:20 am	30	0	NA
	7:03 am	30	0	NA
	Evening	30	0	NA
6 Dec	6:33 am	30	0	NA
	1:26 pm	30	0	NA
	9:23 pm	30	0	NA
7 Dec	6:34 am	30	0	NA
	2:37 pm	30	0	NA
	9:05 pm	30	0	NA
8 Dec	6:25 am	30	+3	3:0
	9:06 pm	30	0	NA
9 Dec	6:42 am	30	0	NA
	9:57 pm	30	0	NA
10 Dec	8:49 am	30	0	NA
Beverly Beach Shore				
4 Dec	4:38 pm	46	0	NA
5 Dec	8:51 am	60	0	NA
6 Dec	3:48 pm	30	0	NA
7 Dec	11:32 am	30	0	NA
9 Dec	9:30 am	30	0	NA
10 Dec	7:20 am	35	0	NA

continued next page

Table 2 (continued).

Aerosol sample location and the time collections were initiated	Aerosol sample duration (min)	Number of *Erwinia* colonies on aerosol plates	Subspeciation Results #*Ecc*:#*Eca*[1/]
Beverly Beach Park			
6 Dec 2:45 pm	30	0	NA
7 Dec 10:42 am	35	+8	3:5
9 Dec 1:26 pm	25	+2	0:2
10 Dec 9:20 am	30	+1	0:1
Tillicum Beach			
5 Dec 3:41 pm	15	0	NA
9 Dec 4:20 pm	25	0	NA
Ona Beach			
5 Dec 4:21 pm	15	0	NA
9 Dec 3:40 pm	25	+1	0:1
Agate Beach			
6 Dec 9:48 am	33	0	NA
9 Dec 11:06 am	25	0	NA
Moolack Beach			
6 Dec 10:40 am	33	0	NA
7 Dec 12:18 pm	30	0	NA
Fort Stevens			
8 Dec 2:09 pm	25	0	NA
Del-Ray Beach			
8 Dec 3:00 pm	20	0	NA

continued next page

Table 2 (continued).

Aerosol sample location and the time collections were initiated	Aerosol sample duration (min)	Number of *Erwinia* colonies on aerosol plates	Subspeciation Results #*Ecc*:#*Eca*[1]
Tolvana			
8 Dec 3:58 pm	20	0	NA
Nehlem			
8 Dec 4:53 pm	10	0	NA
Yaquina Lighthouse			
9 Dec 10:22 am	25	0	NA
Newport Beach			
9 Dec 2:45 pm	25	+1	1[2]:0

[1] *Ecc* = *Erwinia carotovora* subsp. *carotovora*; *Eca* = *Erwinia carotovora* subsp. *atroseptica*.

[2] Isolates weakly positive on α-methyl glucoside solid medium.

The Effect of TOPS 2.5, Mertect and Zineb Seedpiece Treatment on Russet Burbank Potatoes in the San Luis Valley

G.D. Franc and M.D. Harrison

Abstract

Treatment of cut Russet Burbank potato seed with fir bark amended by addition of TOPS 2.5, Mertect or Mertect + Zineb significantly increased total tuber yield in the San Luis Valley (P = 0.05). Visual beneficial effects of seedpiece treatment, when compared to the fir bark control, were evident in terms of plant vigor, color and degree of defoliation during the latter part of the growing season. There were, however, no measurable effects on plant stand, seedpiece decay, *Rhizoctonia* stem-cankering, *Verticillium* stem infection or early blight foliage infection. Generally, TOPS 2.5 and Mertect treatments were similar and appeared to produce more vigorous plants than treatment with Mertect in combination with Zineb.

Materials and Methods

A field with sandy loam soil cropped continuously with potatoes for 20 years was selected for the study. The field was located near Center, CO in the San Luis Valley with an elevation of ca 7,600 feet. The San Luis Valley is a high desert valley receiving an average of 7 inches of rain per year.

Russet Burbank certified seed potatoes produced in the San Luis Valley were used as the seed source. Each seedpiece treatment plot was planted with a tractor drawn assist-feed 2-row potato planter. Each treatment plot consisted of two planted rows 50 feet long with blank rows on each side and 10 ft buffers at each end. Treatment plots were planted in a randomized complete-block design. of four replications and four treatments (Figure 1). Treatments consisted of a control (fir bark alone), TOPS 2.5 formulated with fir bark, fir bark + Mertect and fir bark + Mertect + Zineb. Soil moisture was good at the time of

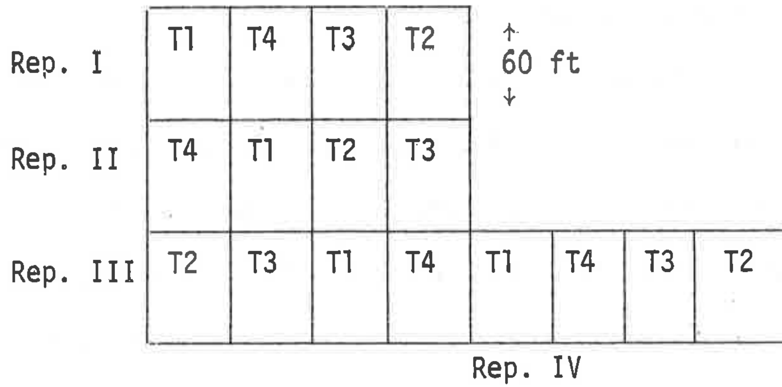

Figure 1. Field plot plan used for Gustafson - TOPS 2.5 field trials - Center, CO, 1983.

Seedpiece treatment designations were:

T1. Fir bark alone
T2. Fir bark + TOPS 2.5
T3. Fir bark + Mertect
T4. Fir bark + Mertect + Zineb

planting on May 17. The plots were irrigated by a center pivot overhead sprinkler throughout the growing season.

Seed tubers were cut by hand into 2 ounce seedpieces using clean knives. Sixteen seedlots of 12.5 lbs each (4 replications x 4 treatments) were placed into sterile paper bags and 56 g of treatment chemical was applied. This produced a net application rate of 1.0 lbs dust per 100 lbs cut seed. The seedpieces were coated by rotating and agitating the bags. Seedpiece coverage was very uniform and caking did not occur. The maximum time between cutting and treatment was 1 hr. The seed was warmed for 10 days at room temperature prior to treatment and planted within 24 hr after treatment. Recommended rates of fertilizer were applied in-row at the time of planting.

Total emergence and emergence rates were determined by counting the number of plants just emerging or well above the soil line in each plot. Emergence counts were made on June 14, June 20 and June 24.

Plant vigor was estimated in each plot throughout the growing season using a scale of 1-10. The control (fir bark) was arbitrarily assigned a value of 5 and more vigorous plots were rated at values greater than 5 and less vigorous plots at less than 5. Vigor ratings were based on general plot appearance and were influenced by plant color, uniformity, fullness of the canopy, height, etc. Vigor ratings were made on June 14, June 24, July 19, August 11, August 18, August 25 and August 30. Ratings for foliar greeness and defoliation were made on August 25 and August 30 using the same scale (10= greenest or least defoliated plots, respectively). Actual stem height (soil line to apex) was measured on July 19 and August 11. Ten stems per treatment plot were measured.

The percentages of mother seedpiece decay and *Rhizoctonia* stem cankering were estimated on July 19. Five hills (plants) in each treatment plot were

uprooted and the amount of decay in the seedpiece was estimated using the Barratt-Horsfall (BH) scale (0-11). The amount of stem cankered below the soil line by Rhizoctonia was estimated for each stem using the same scale.

The severity of early blight (Alternaria solani) infection was estimated using the BH scale on July 19 and August 11. Five disease ratings estimating the percentage of leaflets infected for the top, middle and bottom third of the plant canopy were made in each plot.

Verticillium infection was indirectly determined by estimating the percentage of wilting observed on July 19 (BH scale). Verticillium infection was also measured directly by assaying five stems per treatment plot in the laboratory. Stems were collected and returned to the lab where they were surface disinfested in 10% chlorox. Stems were rinsed in cool tap-water and a cross-section from the top, middle and bottom third of each stem was excised with a sterile scalpel and placed onto water agar. Agar plates were incubated at room temperature for 7-10 days and the presence of Verticillium determined by viewing stem sections with the aid of a microscope for the presence of characteristic Verticillium conidiophores.

Each treatment plot was harvested on September 14 with a two-row potato digger. Tubers were picked by hand into baskets and weighed to determine total yield. A 50 lb sample from each plot was graded to determine the proportion of tubers in each of several grades in each treatment plot. These data were used to calculate the total yield of each grade in cwt per acre.

All data were analyzed in a two-way analysis of variance. Means were compared using an HSD (Tukey's) test. In addition, treatment means were compared to the fir bark control using the LSD test. Barratt-Horsfall readings were converted to percentages using the appropriate conversion table for presentation in this report.

Results

Seedpiece treatment did not significantly influence total stand (Table 1). Plant vigor (Table 2) was significantly affected by seedpiece treatment as determined by ratings made on August 11, August 25, and August 30 ($P \leq 0.05$). Treatment with TOPS 2.5 and Mertect, both formulated with fir bark, were significantly more vigorous than treatment with fir bark alone when compared using the LSD test ($P = 0.05$). Also, treatment with fir bark + Mertect was significantly more vigorous than the fir bark control (Treatment 1) and fir bark amended with Zineb and Mertect in combination on August 25 and 30 when means were compared using a more conservative HSD value ($P = 0.05$). TOPS 2.5 + fir bark was not statistically different from any treatments when means were compared using the HSD test ($P = 0.05$). Vigor ratings were not significantly different on June 14, June 24, July 19, and August 18 ($P \geq 0.05$).

The effect of seedpiece treatment on plant height is shown in Table 3. Significant differences among treatments occurred on July 19 but not on August 11 ($P = 0.05$). TOPS 2.5 + fir bark resulted in significantly taller plants than the fir bark control when means were compared using an HSD or LSD value ($P = 0.05$). The Mertect + fir bark treatment was significantly different from the control only when the LSD value was used for mean comparisons ($P = 0.05$). All fir bark + chemical treatments (treatments 2, 3 & 4) were statistically equivalent on both dates ($P = 0.05$).

The relative amount of defoliation and greenness observed in the plots near the end of the growing season are shown in Table 4. TOPS 2.5 and Mertect + fir bark had significantly less defoliation than Mertect + Zineb + fir bark and fir bark alone on August 25 ($P = 0.05$). Significant defoliation differences were not observed on August 30. Mertect + fir bark treated plots were significantly ($P = 0.05$) greener on August 25 and August 30 than plots treated with Mertect + Zineb or fir bark alone. Plots treated with TOPS 2.5 + fir bark

Table 1. The effect of seedpiece treatment on cv Russet Burbank plant stand - Center, CO, 1983.

Treatment	Average plant stand[1/] determined on:		
	June 14	June 20	June 24
1) Fir bark	47.3 a[2/]	48.4 a	48.3 a
2) Fir bark + TOPS 2.5	48.6 a	49.4 a	49.6 a
3) Fir bark + Mertect	46.0 a	48.6 a	49.0 a
4) Fir bark + Mertect + Zineb	47.3 a	48.3 a	48.3 a
F ratio	1.504	0.830	1.923
	P>0.1	P>0.1	P>0.1

[1/] Average stand for 4 replications of 2 rows for each treatment. Perfect stand is 50 plants (50 ft of row at 1 ft seedpiece spacing).

[2/] Means in columns followed by the same letter do not differ significantly (P = 0.05).

Table 2. The effect of seedpiece treatment on cv Russet Burbank potato plant vigor - Center, CO, 1983.

Treatment	Relative vigor ($\bar{X}$)[1] as determined on:						
	June 14	June 24	July 19	Aug. 11	Aug. 18	Aug. 25	Aug. 30
1) Fir bark	5.0 a[2]	5.0 a	5.0 a	5.0 a	5.0 a	5.0 b	5.0 b
2) Fir bark + TOPS 2.5	4.5 a	5.5 a	5.9 a	6.1 a*[3]	5.5 a	6.3 ab*	6.3 ab*
3) Fir bark + Mertect	4.6 a	5.3 a	5.8 a	6.1 a*	5.5 a	6.8 a*	6.5 a*
4) Fir bark + Zineb + Mertect	5.2 a	5.5 a	6.0 a	5.3 a	5.0 a	5.3 b	5.3 b
F Ratio	2.804	0.256	2.513	3.922	1.411	6.895	9.750
	$P>0.1$	$P>0.1$	$P>0.1$	$P<0.05$	$P>0.1$	$P\leq0.025$	$P\leq0.005$

1/ Rated on a scale of 0-10. Treatment #1 for each replication was arbitrarily assigned a rating of 5. More vigorous treatments were rated greater than 5 and less vigorous less than 5.

2/ Column means with different letters do not differ significantly ($P = 0.05$). Mean separation was done using Tukey's HSD test.

3/ Means designated by "*" are significantly different from the fir bark control ($P = 0.05$). Mean separation was done using the LSD test.

Table 3. The effect of seedpiece treatment on cv Russet Burbank potato plant height - Center, CO, 1983.

Treatment	Average plant height[1/] (cm):	
	July 19	August 11
1) Fir Bark	42.5 b[2/]	52.4 a
2) Fir Bark + TOPS 2.5	47.3 a*[3/]	56.8 a
3) Fir Bark + Mertect	45.8 ab*	55.4 a
4) Fir Bark + Mertect + Zineb	45.5 ab	54.2 a
F Ratio	4.358	1.718
	$P < 0.05$	$P > 0.1$

[1/] 10 stems per treatment per replication were rated by measuring from the soil line to the apex of the stem.

[2/] Means in columns followed by different letters differ significantly ($P < 0.05$). Tukey's Test used for mean separation.

[3/] Means designated by "*" differ significantly ($P = 0.05$) from the fir bark control (LSD test).

Table 4. The effect of seedpiece treatment on the relative defoliation and greenness observed in the field - Center, CO, 1983.

Treatment	Relative defoliation observed[1]		Relative greenness observed[1]	
	August 25	August 30	August 25	August 30
1) Fir Bark	5.0 b[2]	5.0 a	5.0 b	5.0 b
2) Fir Bark + TOPS 2.5	6.3 a*[3]	6.3 a	6.5 ab*	6.0 ab
3) Fir Bark + Mertect	7.0 a*	6.8 a	7.8 a*	7.3 a*
4) Fir Bark + Mertect + Zineb	5.0 b	5.3 a	5.3 b	5.0 b
F Ratio	13.683	2.220	13.588	4.531
	$P \leq 0.005$	$P > 0.1$	$P \leq 0.005$	$P \leq 0.05$

[1] Treatment plots were rated on a scale of 1-10. Treatment #1 for each replication was arbitrarily assigned a rating of 5. Healthier appearing plots (less defoliation or more greenness) were rated greater than 5 and less healthy were rated less than 5.

[2] Column means with different letters differ significantly ($P = 0.05$). Tukey's HSD test was used for mean separation.

[3] Means designated by "*" differ significantly ($P = 0.05$) from the fir bark control.

were not significantly ($P = 0.05$) greener than the plots which received Mertect + fir bark. Treatment with TOPS 2.5 also resulted in significantly greener plants than the fir bark control on August 25 ($P = 0.05$) when means were compared using the LSD value.

Seedpiece treatment did not significantly ($P = 0.05$) affect the percentage of seedpiece decay, Rhizoctonia stem cankering observed in the field or Verticillium stem infection measured by laboratory assays (Table 5). Significant differences did not occur among treatments regarding the estimated percentage of leaflets infected by early blight ($P = 0.05$) (Table 6). Visual ratings for Verticillium, based on foliar wilt, showed very low levels of infection for treatment with Mertect (<4%) and 0% infection for the other three treatments (data not shown).

All seedpiece treatments significantly increased total yield (Table 7) compared to the fir bark control when the LSD was used to compare means ($P = 0.05$). When the HSD value was used for mean comparison Mertect + fir bark was the only treatment with total yields significantly greater than the fir bark control ($P = 0.05$). Although the same trends were observed for total US #1 and marketable yields differences were not statistically significant ($P \geq 0.05$). Yields among the seedpiece treatments were similar for grades US #2, grade B and culls. When the proportion of tubers in each grade (regardless of actual yield) was compared for each treatment the data showed that seedpiece treatment did not significantly affect the proportion of each tuber grade (Table 8) even though total yields were increased (Table 7). Although not significant, treatment of seedpieces with fir bark + TOPS 2.5 had the highest percentage of total US #1 and US #1 less than 10 ounces, the lowest percentage of US #1 greater than 10 ounces, US #2 and culls and the second lowest proportion of grade B tubers.

Table 5. The effect of seedpiece treatment on seedpiece decay, *Rhizoctonia* stem canker, and *Verticillium* wilt infection in the field - Center, CO, 1983.

Treatment	Seedpiece decay[1/]	*Rhizoctonia* stem canker[2/] (% stem cankered)	*Verticillium* wilt[3/] (% infected)
1) Fir Bark	96.0 a[4/]	7.0 a	3.8 a
2) Fir Bark + TOPS 2.5	81.5 a	3.0 a	3.8 a
3) Fir Bark + Mertect	84.0 a	4.0 a	5.0 a
4) Fir Bark + Mertect + Zineb	80.5 a	5.5 a	3.8 a
F Ratio	3.556	1.418	0.070
	P>0.05	P>0.1	P>0.1

[1/] Seedpieces (5 per treatment plot) were rated (0-11) to estimate the percentage of the seedpiece decayed.

[2/] Stems from 5 hills per treatment plot were rated (0-11) to estimate the percentage of stem cankered below the soil line.

[3/] Stems (5 per plant) were plated onto water agar to detect internal stem infection by *Verticillium*.

[4/] Column means with the same letters do not differ significantly (P = 0.05).

Table 6. The effect of seedpiece treatment on infection of cv Russet Burbank potato foliage by *Alternaria solani* (early blight) - Center, CO, 1983.

Treatment	Estimated percentage of leaflets infected:[1]	
	July 19	August 11
1) Fir Bark	0.2 a[2]	14.0 a
2) Fir Bark + TOPS 2.5	0.1 a	13.0 a
3) Fir Bark + Mertect	0.2 a	15.0 a
4) Fir Bark + Mertect + Zineb	0.2 a	16.0 a
F Ratio	0.675	0.253 a
	P>0.1	P>0.1

[1] Foliage infection was estimated by making 5 ratings per treatment plot using the BH scale (0-11) for the top, middle and bottom third of the plant canopy.

[2] Column means with the same letters do not differ significantly (P = 0.05).

Table 7. The effect of seedpiece treatment on total yield and grade (cwt/A) of cv Russet Burbank tubers - Center, CO, 1983.

Treatment	Tuber yield (cwt/A)[1/]							
	Total yield	Marketable[2/]	US #1 <10 oz	US #1 >10 oz	Total US #1	US #2	Grade B	Culls
1) Fir Bark	268.7 b[3/]	191.5 a	161.5 a	11.3 a	172.9 a	18.7 a	50.7 a	19.5 a
2) Fir Bark + TOPS 2.5	312.0 ab*[4/]	239.3 a	212.0 a	7.5 a	219.5 a	19.8 a	50.2 a	15.6 a
3) Fir Bark + Mertect	323.9 a*	249.1 a	212.1 a	13.7 a	225.8 a	23.3 a	45.3 a	20.8 a
4) Fir Bark + Mertect + Zineb	308.7 ab*	228.8 a	184.2 a	23.2 a	207.8 a	21.0 a	53.2 a	28.2 a
F Ratio	3.999 P>0.05	2.972 P>0.1	2.664 P>0.1	1.118 P>0.1	2.099 P>0.1	0.213 P>0.1	0.226 P>0.1	0.580 P>0.1

[1/] Treatment plot size was 100 ft of row (0.0065 Acre). A sample of ca 50 lb was sorted and the proportion of each tuber grade determined. Data were converted to cwt/A for presentation in the table.

[2/] Sum of total US #1 and US #2 yields.

[3/] Column means with different letters differ significantly (P = 0.05). Tukey's HSD test was used for mean separation.

[4/] Column means designated by "*" differ significantly (P = 0.05) from the fir bark control (Treatment 1). An LSD test was used for comparison of means.

Table 8. The effect of seedpiece treatment on the proportion (percentage of total yield) of each tuber grade for cv Russet Burbank - Center, CO, 1983.

Treatment	The proportion of each tuber grade[1/] (% of total yield):						
	Total #1	Marketable[2/]	US #1		US #2	Grade B	Culls
			<10 oz	>10 oz			
1) Fir Bark	64.0 a[3/]	71.0 a	59.8 a	4.1 a	7.0 a	19.0 a	7.4 a
2) Fir Bark + TOPS 2.5	70.1 a	76.6 a	67.7 a	2.4 a	6.5 a	16.0 a	5.2 a
3) Fir Bark + Mertect	69.8 a	77.1 a	65.6 a	4.2 a	7.2 a	14.0 a	6.3 a
4) Fir Bark + Mertect + Zineb	67.3 a	74.1 a	59.7 a	7.5 a	6.8 a	17.3 a	9.1 a
F Ratio	0.489	0.781	1.349	0.785	0.123	0.549	0.681
	P>0.1	P>0.1	P>0.1	P>0.1	P>0.1	P>0.1	P>0.1

[1/] A ca 50 lb tuber sample was sorted for each treatment and the proportion of each grade determined.

[2/] Sum of total US #1 and US #2 yields.

[3/] Column means with different letters differ significantly (P = 0.05).

Discussion

Plots were placed in the San Luis Valley in a field cropped continuously to potatoes for more than 20 years. Under conditions of high disease pressure, treatment of cut Russet Burbank seed with fir bark + TOPS 2.5, Mertect or Mertect + Zineb significantly increased total tuber yield when compared to seedpieces treated with fir bark alone (P = 0.05). However, none of the treatments markedly influenced plant stand, seedpiece decay, *Rhizoctonia* stem-cankering, *Verticillium* stem infection or early blight foliage infection. Visual beneficial effects of seedpiece treatment occurred in terms of plant vigor, greenness and defoliation during the latter part of the growing season. Generally, TOPS 2.5 and Mertect treatments gave very similar results and plants appeared more vigorous than those treated with the combination of Mertect + Zineb.

Reasons for the increased vigor are not clear since none of the disease readings showed affects which could account for the vigor difference. It is possible that treatments reduced the amount of root infection by *Verticillium* or perhaps delayed infection which could have resulted in slower, less severe symptom development even though the stems were positive when isolations were made.

The Effect of TOPS 2.5 D Seedpiece Treatment on Potato Stem Infection by *Verticillium dahliae* (microsclerotial form)

G.D. Franc and M.D. Harrison

Abstract

Tuber seedpieces (cv Russet Burbank) derived from micropropagated stock were treated with TOPS 2.5 D amended fir bark or fir bark alone. Plants were artificially inoculated with *Verticillium* and later assayed to determine the effect of seedpiece treatment on infection.

Stem inoculations with toothpicks, upon which *Verticillium* had been previously grown, resulted in definite infections. Tissue sections taken from the top, middle, and bottom portions of each stem showed an increasing amount of infection at locations closer to the inoculation site. None of the control plants showed *Verticillium* infection. The only significant differences observed were between *Verticillium* inoculated and non-inoculated treatments (P = 0.01). There was a trend for seedpiece treatment with TOPS 2.5 D amended fir bark to have less infection present for the middle and bottom portions of the stem as well as for the average number of stems infected when compared to the similarly inoculated fir bark treatments. However, these differences were not significant (P = 0.05).

There was no significant effect of treatment on the average stem weight for potato plants grown in the greenhouse.

Materials and Methods

A study was done at the Potato Virus and Weed Research Laboratory in Fort Collins, CO, to determine the effect of TOPS 2.5 tuber treatment on *Verticillium dahlia* (microsclerotial form) potato stem infection. The TOPS 2.5 D

preparation used in the study came from lot #386363 (ref. #C-596) and the fir bark was from ref. #C-682.

Russet Burbank tubers from a micropropagation program were used in the study. The average tuber weight was 16.3 grams for 50 tubers weighed (S = 6.348 g). The tubers were produced in the greenhouse and dipped into a giberellic acid preparation on 12 Oct 1983 for the purpose of breaking dormancy. The treated tubers were stored in the greenhouse until treatment and planting.

Tubers for experiment 1 were treated with TOPS 2.5 amended fir bark or fir bark alone and planted on 16 Dec 1983. Fifty randomly selected micropropagation tubers were cut lengthwise using a knife periodically dipped into ethanol and flamed. The resultant 100 seedpieces were divided into two groups for treatment with fir bark containing TOPS 2.5 dust or with fir bark dust alone. The seedpieces for each treatment were coated with the dust by placing them into a separate sterile paper bag containing an excess of the dust treatment and agitating. Seedpiece coverage was uniform and caking did not occur. After 1 hr, tubers were planted into previously steamed, prewatered greenhouse soil mix.

Verticillium inoculum was prepared from a *Verticillium* culture previously isolated from cv Monona potatoes grown in Northeastern Colorado in 1983. This isolate was maintained on potato-dextrose agar (PDA) slants until used.

Wooden toothpicks were autoclaved in PDA medium on 13 Jan 1984 and placed onto the surface of previously prepared PDA petri plates and allowed to cool. The *Verticillium* culture was used to inoculate the toothpicks on the same day. The fungal mycelium was visible within two days and microsclerotial development on the toothpicks was evident at the time of potato stem inoculation on 5 Feb 1984. Similarly treated non-inoculated toothpicks were also included

as a control. Stems were inoculated by inserting toothpicks completely through the stems ca 2.5 cm above the soil line.

Therefore, the treatments for experiment 1 were: (1) TOPS 2.5 amended fir bark stem inoculated with sterile toothpicks; (2) fir bark + stem inoculation with sterile toothpicks; (3) TOPS 2.5 amended fir bark + stem inoculation with *Verticillium*-culture toothpicks; (4) fir bark + stem inoculation with *Verticillium*-culture toothpicks. Four replications of the four treatments (5 pots per treatment) were placed in the greenhouse in a randomized complete block design.

Stems were harvested and the presence of *Verticillium* was determined by first surface disinfesting stems in 10% chlorox and aseptically plating stem cross sections on water agar. Stem cross sections were taken at 3, 6, and 12 inches above the soil line. The presence of *Verticillium* for each section was determined by scanning stem tissue pieces with the aid of a microscope and observing the presence or absence of the characteristic conidiophores after 6 days incubation at approximately 26°C.

Tuber seedpieces for experiment 2 were inoculated directly with the same *Verticillium* isolate (in contrast to stem inoculations using inoculum containing toothpicks).

Seedpieces were cut, as described above, on 17 Dec 1983. Fifty seedpieces were completely immersed in sterile deionized water in a bell-jar. Vacuum was applied (500 mm Hg) until evacuation of air bubbles no longer occurred from the freshly cut surfaces of the seedpieces. The process was repeated.

The remaining 50 seedpieces were placed into sterile distilled water in the bell jar to which 4 PDA *Verticillium*-slant cultures had been added. The cultures were ca one month old at this time and the slant surfaces were very

dark (black) due to extreme microsclerotial formation. The cultures were blenderized (liquified) in an Osterizer blender in a small amount of water for 5 seconds before addition to the bell jar. Seedpieces were vacuum infiltrated with the inoculum as described above for the control treatments. Serial dilutions of the inoculum were done and spread plated onto PDA to estimate the number of *Verticillium* propagules per ml (microsclerotia and hyphae).

Inoculated seedpieces were allowed to air dry for 0.5 hr. The 50 seedpieces for each inoculation treatment were divided into two equal piles for treatment with either TOPS 2.5 amended fir bark or with fir bark alone. All dust treatments were done in sterile paper bags to preclude cross contamination among treatments.

Treated seedpieces for experiment 2 were planted in the greenhouse as described for experiment 1. Stems were harvested on 22 Feb 1983, stored in the cold room overnight, surface disinfested and plated onto water agar the next day as described above.

The harvested stem weight was determined for each treatment before *Verticillium* assays were done.

All data were analyzed in a two-way analysis of variance and means separated using Tukey's HSD test.

Results

None of the plants seedpiece inoculated with *Verticillium* (vacuum infiltration) had detectable *Verticillium* present in any of the stem sections. Serial dilutions of the inoculum used for vacuum infiltration showed ca 3.2×10^6 *Verticillium* propagules present per ml. Even though it is not known what volume of inoculum each seedpiece received, it appears that vacuum infiltration

was not a suitable method for *Verticillium* inoculation in this study.

Stem inoculations with toothpicks, upon which *Verticillium* had been previously grown, resulted in definite infections. Tissue sections taken from the top, middle and bottom portions of each stem showed an increasing amount of infection at locations closer to the inoculation site (Table 1). None of the plants inoculated with sterile toothpicks showed *Verticillium* infection and the only significant differences were between *Verticillium* inoculated and non-inoculated treatments (P = 0.01). However, there was a trend for seedpiece treatment with TOPS 2.5 D amended fir bark to have less infection present for the middle and bottom portions of the stem as well as for the average number of stems infected when compared to the similarly inoculated fir bark treatments.

There was no significant effect of treatment on the average stem weight shown in Table 2 (P = 0.05).

Table 1. The effect of TOPS 2.5 D and fir bark on *Verticillium* infection for artificially inoculated stems - Ft. Collins, CO, 1984.

Treatment	The average number of stem sections and average number of stems with *Verticillium* evident[1/]: stem position[2/] top	middle	bottom	number stems infected[3/] ($\overline{X}$)
1. TOPS 2.5 + fir bark/ uninoculated	0 a	0 b	0 b	0 b
2. Fir bark/ uninoculated	0 a	0 b	0 b	0 b
3. TOPS 2.5 + fir bark/ *Verticillium*	1.50 a	2.50 a	2.75 a	3.25 a
4. Fir bark/ *Verticillium*	1.50 a	3.50 a	4.25 a	4.25 a
	NSD[4/]	P=0.01	P=0.01	P=0.01

[1/]Each datum entry point is the average number of stem sections *Verticillium* positive for four replications of five stems each.

[2/]Sections were assayed for *Verticillium* at 12 in., 6 in., and 3 in. above the soil line for top, middle and bottom assays, respectively.

[3/]The average number of stems with at least one section *Verticillium* positive. Each entry is the average of four replications of five plants each.

[4/]NSD = no significant differences found (P = 0.05). Mean separation was done using Tukey's (HSD) test.

Table 2. The effect of TOPS 2.5 D and fir bark on potato stem weight in the greenhouse - Ft. Collins, CO, 1984.

	The average stem weights (grams) for treatments:[1]	
Treatment	Seedpiece inoculated[3]	Stem inoculated[3]
1. TOPS 2.5 + fir bark/ uninoculated	255.9 a	250.5 a
2. Fir bark/ uninoculated	232.8 a	186.7 a
3. TOPS 2.5 + fir bark/ _Verticillium_	256.9 a	221.4 a
4. Fir bark/ _Verticillium_	238.9 a	223.4 a
	NSD[2]	NSD

[1] Each datum entry is the average weight in grams of four replications of five stems each.

[2] NSD = no significant differences occurred (P = 0.05).

[3] Either seedpiece (vacuum infiltration) or stems (toothpick inoculations) were inoculated with _Verticillium dahliae_ (microsclerotial form).

Dilution End-point Assay of *Corynebacterium sepedonicum* Infectivity on the Potato Cultivars Russet Burbank and Centennial

G.D. Franc and M.D. Harrison

Abstract

The objective of this study is to determine if potato seedpieces inoculated with different numbers of *Corynebacterium sepedonicum* (ringrot) cells can produce plants and tuber progeny that visually appear healthy but are, nevertheless, infected (latent infection). The occurrence of latent ringrot infection may partially explain the almost cyclical pattern of ringrot symptom appearance in some seed potatoes.

Russet Burbank and Centennial tubers were inoculated with serial dilutions of *C. sepedonicum* in order to test this theory. Results for studies initiated in 1981 (DEP81) showed that plants of both cultivars had no foliar ringrot symptom (primary symptoms) expression during the first growing season (summer 1981) even though seedpieces were inoculated with up to 6.3×10^8 cells.

Plant-back of daughter tubers harvested from DEP81 plots in the field during the 1982 growing season produced plants with foliar ringrot symptoms (secondary symptoms) for Russet Burbank plants only when the mother tuber received the maximum inoculation concentration of 6.3×10^8 *C. sepedonicum* cells. All Centennial plants were symptomless during 1982. Plant-back of granddaughter tubers (i.e., with respect to originally inoculated seedpieces during DEP81 study) in the field during 1983 only showed symptom development for Centennial when grandmother seedpieces received 10 *C. sepedonicum* cells. All other plants appeared healthy.

Bioassay of tubers from the same DEP81 seedlots planted in the field during 1982 showed detectable levels of ringrot infection when mother tubers received equal to or greater than 10^4 and 10^6 cells for Russet Burbank and Centennial, respectively.

DEP82 and DEP83 studies, identical to the DEP81 study (except the maximum inoculum concentration was 10^9 cells per tuber), were initiated in 1982 and 1983, respectively.

Primary symptoms for DEP82 only occurred when mother tubers received inoculum concentrations of 10^6 and 10^9 cells for Russet Burbank. Primary symptom development did not occur for Centennial. Secondary foliage symptoms for Russet Burbank only occurred for 10^4 cell inoculum concentrations with both greater and lesser inoculum loads appearing healthy. Secondary symptoms in Centennial occurred for the two highest inoculum concentration (10^9 and 10^6 cells) and corresponded with tuber bioassays done in the greenhouse. Bioassay of Russet Burbank tubers only showed infection when the original mother tubers received 10^9 *C. sepedonicum* cells.

DEP83 studies showed primary symptom development for 10^4, 10^6, and 10^9 inoculum concentrations for Russet Burbank and 10^1, 10^4, 10^6, and 10^9 for Centennial. Treatments where mother tubers received 10^2 cells for Centennial appeared healthy.

Visual inspection of daughter tubers at harvest showed symptoms when mother tubers received inoculum concentrations of 10^6 and 10^9 cells for Russet Burbank and 10^4 , 10^6 and 10^9 for Centennial.

Results indicate that latent ringrot infections can occur in the field. Foliar symptom expression did not occur until the third growing season after the inoculation of one tuber treatment for Centennial in

the DEP81 study. Data also suggests that the environment greatly influences symptom expression.

Materials and Methods

Foundation grade, 1980, Russet Burbank and Red McClure seed potatoes purchased in the San Luis Valley and Centennial seed potatoes provided by the San Luis Valley Research Center were used in the dilution end-point study inoculated in 1981 (DEP81). Tubers provided by the S.L.V. Research Center were used for (almost) identical studies inoculated in 1982 (DEP82) and 1983 (DEP83).

Tuber treatments for the DEP81 study consisted of five serial dilutions of *Corynebacterium sepedonicum* (CS43) and a buffer inoculated control. The original CS43 isolate was provided by S. Slack, University of Wisconsin-Madison, Madison, Wisconsin 53706. Serial dilutions of CS43 cells were prepared for the DEP81 study as follows. On May 6, 1981, eggplant seedlings (*Solanum melongena* "Black Beauty") in the two-leaf stage were inoculated with the aid of a sterile 1 ml tuberculin syringe and 26G ½" needle containing *C. sepedonicum* (CS43) bacteria suspended in 0.05 M phosphate buffer, pH 7.2. The first foliar ringrot symptoms developed in ca 12 days postinoculation. On May 25, 1981, the seedlings were uprooted, washed in cold tap water and roots and leaves were removed with a knife. The remaining stems (approximately 458.5 g wet weight) were cut into ca 1 cm lengths and placed in 4-5 l of cold (40°F) 0.05 M phosphate buffer, pH 7.2 and allowed to stand overnight. The CS43 cells were extracted from the stem segments into the cold buffer at this time due to the osmotic pressure differential. Buffer containing the extracted CS43 cells was passed through cheesecloth to remove the stem segments and large debris. CS43 cells in the strained

buffer were concentrated into a pellet using a Servall refrigerated automatic centrifuge and a SS34 rotor at 11- 12 x 10^3 rpm for 10-12 minutes. Pellets were resuspended in buffer (final volume approximately 350 ml) and filtered under a slight vacuum through Whatman #1 filter paper (DEP83 used Whatman #2). Serial dilutions of the filtrate were made and lightly strained with 1-2 drops of Gram stain crystal violet. Stained filtrate was placed in a Petroff-Hauser counting chamber and the number of cells per 2.5 x 10^{-3} mm^2 square were counted with the aid of a microscope at 45 X. Fifty squares were counted to determine the average number of cells per 2.5 x 10^{-3} mm^2. The chamber was emptied, refilled and the counting procedure repeated (replication I:$\bar{X}$ = 3.16 cells/2.5 x 10^{-3} mm^2, S = 1.765. Replication II:$\bar{X}$ = 3.18 cells/2.5 x 10^{-3} mm^2, S = 0.625) and 6.34 x 10^9 cells per ml were determined to be present in the undiluted filtrate. Serial dilutions were made to result in cell suspensions of 6.34 x 10^9, 1 x 10^7, 1 x 10^5, 1 x 10^3 and 1 x 10^2 cells/ml buffer.

Inoculum for the DEP82 study was prepared in a similar manner with minor modifications. The original CS43 inoculum for eggplant inoculation was prepared from infected tubers harvested the previous fall from plants in the DEP81 study. Eggplant seedlings (cv Black Beauty) in the 4 leaf stage were inoculated as described above on May 6, 1982. Symptoms started to develop on May 20 and stems were harvested on May 24. Bacterial cells from eggplant stems were concentrated by centrifugation on May 25 and counted (replication I:$\bar{X}$ = 0.55 cells/5 x 10^{-8} ml, II: 0.50 cells/5 x 10^{-8} ml, III: 0.60 cells/5 x 10^{-8} ml, IV: 0.60 cells/5 x 10^{-8}, S = 0.048).

Eggplants used for inoculum production for the DEP83 study came from a different seedlot than that used in the DEP81 and DEP82 studies. Both seedlots appeared to react in a similar manner to ringrot infection. Eggplants used in the DEP83 study were planted on March 29, 1983 and transplanted on April 12, 1983. Eggplant seedlings were inoculated on April 26, 1983, as described above, using inoculum from stored DEP81 and DEP82 tubers. Ringrot symptoms were visible in inoculated eggplants on May 6, 1983 and stems were harvested on May 11, 1983. On May 12 cells were concentrated by centrifugation and 20 squares counted for each replication (replication I:$\bar{X}$ = 1.10 cells/5 x 10^{-8} ml, II:$\bar{X}$ = 0.70 cells/5 x 10^{-8} ml, III: 0.75 cells/5 x 10^{-8} ml, IV:$\bar{X}$ = 1.00 cells/5 x 10^{-8} ml, V:$\bar{X}$ = 1.10 cells/5 x 10^{-8} ml, VI:$\bar{X}$ = 0.95 cells/5 x 10^{-8} ml, S = 0.172) serial dilutions made, and tubers were inoculated on the same day.

Serial dilutions of inoculum for the DEP82 and 83 studies were made to produce inoculum concentrations ranging from 10^{10}, 10^{7}, 10^{5}, 10^{3} and 10^{2} *C. sepedonicum* cells per ml. Spread platings (0.1 ml) of serial dilutions onto nutrient-dextrose agar plates were done in 1983 using DEP83 inoculum to determine viable bacterial counts and their relationship to physical counts determined in the Petroff-Hauser counting chamber.

Tubers to be inoculated for each year were surface disinfected with 10% chlorox, rinsed with cool tap water and allowed to dry. Tubers were inoculated by scooping out a section of the stolon end with an "EKCO" fruit baller (2.8 cm diameter), pipetting 0.1 ml inoculum directly into the depression, replacing the tuber piece and inserting a small piece of sterile wooden toothpick to hold the tuber piece in place and immediately dipping the entire stolon end (approximately 1/4 - 1/3 of the tuber) into

melted paraffin (Gulfwax) twice. A volume of 0.1 ml was sufficient to coat the cut surface of the tuber with inoculum when the excised tuber piece was replaced and slight pressure applied. The paraffin sealed the excised tuber piece to the intact tuber and prevented the inoculum from drying out. Control tubers, inoculated with buffer alone, were treated in the same manner. All inoculations were done in the order of most dilute to most concentrated inoculum preparations.

Treatments were planted in a field plot ca 33 ft x 120 ft. Field plots were planted by hand on May 28, 1981, May 26, 1982, and May 17, 1983. Treatment plots were in a randomized complete block design consisting of two cultivars, six inoculation treatments and four replications (blocks) each for the DEP81, DEP82 and DEP83 field plots. Individual treatment plots consisted of 10 treated tubers planted at a 14 inch spacing followed by three Red McClure spacers planted at 12 inch intervals. The plots were cultivated by hand and volunteer potatoes were rogued throughout the growing season. The plots were furrow irrigated during the growing season.

The field plots were observed throughout the growing season during 1981, 1982, and 1983 by Potato Virus Lab research personnel and Potato Certification inspectors and observations on the development of foliar ringrot symptoms were recorded.

On September 21, 1981, the DEP81 plots were harvested. The center three hills in each treatment plot were harvested with a fork and the uninjured tubers were placed in a paper bag. The tubers were placed in cold storage within 12 hours of harvest for assay the following spring. DEP81 daughter tubers were replanted in the field in 1982 at the same time the DEP82 inoculated seedpieces were initially planted. The DEP81

granddaughter and DEP82 daughter tubes were harvested in a similar manner on September 22 and 23, 1982. The DEP81, DEP82, and DEP83 field plots were all harvested on September 15 after the 1983 growing season.

Tubers harvested from each treatment plot for each year were divided into two lots of ca 10 tubers each. The first lot was replanted in the field and observed for symptom development during the year following harvest. The second lot was tested for ringrot infection by bioassay using eggplants.

Bioassays were done by lightly shaving the stolon end of each tuber and excising vascular tissue (potentially infected) with a sterile knife. The tissue was macerated with a sterile mortar and pestle to which a small volume of buffer had been added. Ten tubers per treatment per replication were assayed in this manner. The macerate was injected into eggplants (2 pots of ca 3-4 plants each) and plants were observed for symptom development. This constituted a positive tuber assay.

Tubers for DEP81, 82 and 83 were not tested in 1983 for ringrot by bioassay at the time this report was written. A sample of DEP83 daughter tubers harvested in 1983 was observed in the field and the presence or absence of positive tuber symptoms recorded.

Results

DEP81 study initially planted May 28, 1981:

Primary foliar ringrot symptoms failed to develop at any time during the first growing season. The plants were water stressed throughout the 1981 growing season and, although this was not desirable, did demonstrate that water stress can mask or delay the development of foliar ringrot symptoms when mother tubers were inoculated with as many as 6.34×10^8 *C. sepedonicum* cells per tuber (Table 1).

Table 1. The effect of <u>Corynebacterium sepedonicum</u> (ringrot) tuber inoculum concentration on symptom expression in progeny plants and daughter tubers - DEP81 study, Center, Colorado.

Cultivar	Number of cells per mother tuber	Primary foliage symptoms[1] summer 1981	Daughter tuber infection[2] spring 1982	Secondary foliage symptoms[3] summer 1982	Grand-daughter tuber infection[2] spring 1983	Tertiary foliage symptoms[4] summer 1983
Russet Burbank	6.3×10^8	-	+	+	-	-
	10^6	-	+	-	-	-
	10^4	-	+	-	-	-
	10^2	-	-	-	-	-
	10^1	-	-	-	-	-
	Buffer	-	-	-	-	-
Centennial	6.3×10^8	-	+	-	-	-
	10^6	-	+	-	+	-
	10^4	-	-	-	-	-
	10^2	-	-	-	-	-
	10^1	-	-	-	-	+
	Buffer	-	-	-	-	-

[1] Inoculated tubers were planted in the field in 1981 and symptom expression recorded.

[2] Tubers were assayed for ringrot infection using the eggplant test.

[3] Daughter tubers harvested in 1981 were replanted in the field in 1982 and symptom expression recorded.

[4] Granddaughter tubers harvested in 1982 were replanted in the field in 1983 and symptom expression recorded.

Daughter tubers harvested after the 1981 growing season were determined to be infected with ringrot as determined by the eggplant test (column 2, Table 1) even though mother plants were symptomless. However, daughter tuber infection was only detected in treatments for which the mother tuber was inoculated with at least 10^4 C. sepedonicum cells (Russet Burbank) or 10^6 cells (Centennial).

Daughter tubers were also replanted in the field on May 26, 1982. Although plants were observed throughout the growing season secondary foliage symptoms were only detected in Russet Burbank plants for which the grandmother tuber had received 6.3 x 10^8 cells. Foliar symptoms were not observed for similarly treated Centennial plants.

Bioassays and field plots were once again repeated in 1983 using granddaughter tubers harvested during fall 1982. Bioassay detected ring-rot infection only for Centennial tuber progeny from original seedpieces receiving 10^6 cells two generations prior to the assays. No other ringrot infections were detected. Field plots showed development of symptoms for Centennial inoculated with 10 cells in 1981. This showed the first evidence of ringrot infection for that treatment plot was not expressed until the third growing season after the initial inoculation with ringrot. Thus, ringrot infection was latent for that period.

DEP82 study initially planted May 26, 1982:

Primary foliage symptoms developed in 1982 in Russet Burbank plots when the mother tuber received at least 10^6 C. sepedonicum cells (Table 2). Foliar symptoms failed to develop in similarly inoculated Centennial plants. Daughter tubers harvested in the fall of 1982 were assayed for ringrot infection using the eggplant bioassay during the spring of 1983. Eggplant assays showed detectable ringrot infection for 10^9 cells for

Table 2. The effect of Corynebacterium sepedonicum (ringrot) tuber inoculum concentration on symptom expression in progeny plants and daughter tubers - DEP82 study, Center, Colorado.

Cultivar	Number of cells per mother tuber	Primary foliage symptoms[1] Summer 1982	Daughter tuber infection[2] Spring 1983	Secondary foliage symptoms[3] Summer 1983
Russet Burbank	10^9	+	+	-
	10^6	+	-	-
	10^4	-	-	+
	10^2	-	-	-
	10^1	-	-	-
	Buffer	-	-	-
Centennial	10^9	-	+	+
	10^6	-	+	+
	10^4	-	-	-
	10^2	-	-	-
	10^1	-	-	-
	Buffer	-	-	-

[1] Inoculated tubers were planted in the field in 1982 and symptom expression recorded.

[2] NT = not tested. Daughter tuber infection will be detected by the eggplant test.

[3] Daughter tubers harvested in 1982 were replanted in the field in 1983 and symptom expression recorded.

Russet Burbank and 10^9 and 10^6 cells for Centennial. Ten tubers were also planted in the field. Secondary foliage symptoms developed in the field for treatments receiving 10^4 cells for Russet Burbank and 10^9 and 10^6 cells for Centennial.

DEP82 study initially planted May 17, 1983:

Primary foliage symptoms developed in Russet Burbank when mother tubers were inoculated with 10^4, 10^6 and 10^9 cells and for Centennial when mother tubers received 10^1, 10^4, 10^6 and 10^9 cells (Table 3). Centennial treatments receiving 10^2 cells were symptomless. Inspection of daughter tubers at harvest showed visible symptoms for treatment where mother tubers received 10^6 and 10^9 cells for Russet Burbank and 10^4, 10^6 and 10^9 cells for Centennial.

A comparison of physical cell counts (determined by use of the Petroff -Hauser counting chamber) versus viable cell counts is shown in Table 4. Viable cell counts were lower than expected for physical cell counts (i.e., the cell concentrations in the serial dilutions) for the 10^3 serial dilution and higher than expected for the 10^2 serial dilution. An average of the viable cell counts gives a ratio estimate of 827 viable cells to 1000 cells counted in the Petroff-Hauser chamber or a ratio of 82.7:100. Therefore, the use of the Petroff-Hauser counting chamber is a very accurate method for determining viable cell counts for *Corynebacterium sepedonicum*. The viable estimate is slightly low due to the occurrence of cell pairs often observed with coryneform bacteria. Physical counts can distinguish between closely associated cells while viable counts cannot.

Table 3. The effect of Corynebacterium sepedonicum (ringrot) tuber inoculum concentration on symptom expression in progeny plants and daughter tubers - DEP83 study, Center, Colorado.

Cultivar	Number of cells per mother tuber	Primary foliage symptoms[1] summer, 1983	Daughter tuber infection[2] fall, 1983
Russet Burbank	10^9	+	+
	10^6	+	+
	10^4	+	-
	10^2	-	-
	10^1	-	-
	Buffer	-	-
Centennial	10^9	+	+
	10^6	+	+
	10^4	+	+
	10^2	-	-
	10^1	+	-
	Buffer	-	-

[1] Inoculated tubers were planted in the field in 1983 and symptom expression recorded.

[2] Daughter tubers were visually inspected for symptoms in the field at harvest time. Eggplant tests are not completed at this time.

Table 4. The comparison of *Corynebacterium sepedonicum* cell counts (Petroff-Hauser chamber) versus viable counts (DEP83).

Serial Dilution Plated (# cells/ml)[1]	Number of *C. sepedonicum* cells per ml as determined by viable counts[2]
10^{10}	TNTC[3]
10^{7}	TNTC
10^{5}	TNTC
10^{3}	353 ± 129
10^{2}	130 ± 39
Buffer	0

[1] Cell counts determined in the Petroff-Hauser counting chamber as described in the text.

[2] An aliquot (0.1 ml) of the prepared serial inoculum dilutions were spread plated onto nutrient-dextrose agar. The number of colonies per plate (4 replications) was determined by counting after 6 days incubation at 26° C.

[3] TNTC = Too numerous to count.

Figure 1. Flow chart outline of inoculum preparation for *Corynebacterium sepedonicum* dilution end-point assay - Center, CO, 1981, 1982, and 1983.

Corynebacterium sepedonicum (isolate CS43) culture slant (DEP81) or CS43 infected tubers (DEP82 and DEP83)

Inoculate eggplant seedlings

↓

Wait ca 19 days for infection and symptom development

↓

Uproot, wash in tap water, remove roots and leaves and chop stems into 1 cm segments. Place stems in cold buffer for extraction of CS43 cells.

↓

24 hours with gentle stirring

↓

Remove stems and centrifuge 12200 Xg for 10-12 minutes

↓ ↓

Discard supernatant | Resuspend bacterial pellets in buffer

↓

Filter through Whatman #1 filter paper

↓

Stain and count the number of cells in a known volume, i.e., # cells/ml

↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓

Prepare serial dilutions and inoculate surface-disinfected tubers with 0.1 ml of inoculum

↓

Plant tubers in the field and observe for foliar symptom development. Harvest daughter tubers for further assays.*

← ←

↓ ↓

Plant daughter tubers in the field and observe for foliar symptom development during the second season. Harvest granddaughter tubers and repeat field and eggplant observations.

Inoculate eggplant to detect daughter tuber infection directly

*NOTE: only whole noncut (uninjured) tubers are to be harvested and assayed and planted.

RESEARCH PROPOSAL FOR 1984

David G. Holm

1. The potato breeding and selection program will be continued. Advanced clones will be tested in yield trials. Development of virus-tested seed stocks of the most promising clones will be continued.
2. The Colorado Western Regional Trial will be conducted again in 1984.
3. The study to evaluate the effect of water stress at the time of tuberization on Russet Burbank and Centennial Russet will be continued.
4. The physiological basis for differences in potato clones for nitrogen-use efficiency will be investigated. This information is useful as we try to develop potato clones which utilize nitrogen effectively.
5. Binding studies will be continued to determine if a testing procedure can be developed to evaluate potato clones for susceptibility to blackleg, in cooperation with Rob Davidson.
6. A study will be initiated to compare the performance of 15 line selections of Sangre with our standard seed lot. These selections differ considerably in vine type (in cooperation with Rob Davidson).

BUDGET REQUEST

POTATO BREEDING AND SELECTION PROGRAM	
Labor	$2,390.00
Travel	600.00
Equipment & Supplies	1,300.00
TOTAL	$4,290.00

CULTURAL AND PHYSIOLOGICAL STUDIES	
Labor	$2,500.00
Travel	200.00
Equipment & Supplies	600.00
TOTAL	$3,300.00

GRAND TOTAL $7,590.00

dhm - 3/1/84