Annual Report

Fungus and Bacterial Diseases

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1986 Research Summary and Proposal for 1987

Research carried out during 1986 included studies on ringrot, blackleg, and early blight. Results of the studies are summarized below and work proposed for 1987 is outlined. Details of each of the experiments are attached for those who wish to study them more extensively.

Blackleg Research

Irrigation of Erwinia-free plants of 4 cultivars with Erwinia-contaminated water showed that the percentage of stems and daughter tubers contaminated with Erwinia increased as the level of contamination of irrigation water increased. The bacteria were found to be present internally in the stems but only a few plants were found in the plots with blackleg symptoms.

Four liquid seedpiece treatments were applied to commercially cut Norgold Russet seed to determine their effects on Erwinia contamination. Treatments applied were Alcide^{R} (base:activator:water): 1) 0:0:1 (CK); 2) 1:1:10; 3)1:1:23; and 4) 1:1:48. Treatments 2 and 3 significantly reduced surface populations of presumptive Erwinia carotovora on seedpieces. They also significantly ($p=0.05$) increased the number of seedpieces free from Erwinia contamination. Treatments had no significant effect on stand counts, plant vigor or the incidence of blackleg or ringrot symptom expression in the field.

The study reported last year which was funded by the Western Regional IPM Project was carried out again in 1986 both in the San Luis

Valley and Morgan County. The study was designed to determine the relationship between environment and water source (surface vs. well water) and the development of aerial blackleg and populations of Erwinia on potato foliage. Results were nearly identical to those from 1985. Little aerial blackleg was found at either location in Colorado regardless of the water source and very low Erwinia populations were found on potato foliage. A duplicate study in Oregon showed that high Erwinia populations occurred on potato foliage and much more aerial blackleg developed than was found in Colorado.

Ringrot

The final year of the study on the effect of inoculating Russet Burbank and Centennial Russet with varying levels of Corynebacterium sepedonicum showed results similar to those found in previous years. Tubers inoculated with the ringrot bacterium which had not shown symptoms for as much as three years previously produced one plant with probable ringrot symptoms in 1986. The infection was found in the Centennial Russet cultivar in a plant produced from a seed tuber inoculated with C. sepedonicum. Tests to confirm that the symptoms observed were caused by the ringrot organism are pending. The results confirm the fact that in the San Luis Valley tubers exposed to low numbers of Corynebacterium sepedonicum may not produce plants which show visible ringrot symptoms in the field for as long as 3 years after inoculation.

Tuber "melon-ball" scoop seedpieces, inoculated with varying numbers of cells of the ringrot bacterium, were planted in the field near Center, Colorado. "Identical" inoculated seedpieces were planted in similar field plots at other locations in the United States. Two cultivars, Russet Burbank and Norland, were included in this study.

Emergence rates and plant vigor varied greatly within treatment plots in Colorado. Foliar ringrot symptoms only appeared near the end of the growing season after plants had extensive early blight infection and psyllid injury. Only one tuber showed visible ringrot symptoms at the time of harvest. Results should be interpreted with caution because of problems with foliar diseases which made it difficult to read ringrot symptoms late in the season. Russet Burbank plants inoculated with large numbers of Corynebacterium sepedonicum cells showed good ringrot symptoms in other plots planted at the same location and good symptoms were reported when the same experiment was done in other areas in the United States.

Considerable work has been done on the development of improved assay methods to detect the ringrot bacterium. Results have shown that the eggplant assay appears to be affected by plant nutrition, plant age and soil moisture. A vacuum inoculation method which will allow the introduction of precise numbers of bacteria into plants has been developed which will allow us to determine the sensitivity of the eggplant bioassay. Eggplants have also been successfully grown in tissue culture. This will also greatly aid the research.

Preliminary tests using tissue cultured potato microplants have indicated that they may be more sensitive to ringrot bacteria than eggplants. Symptoms appear much faster in these plantlets than in plants grown from tubers in the field or greenhouse.

Assays of a limited number of seed lots which have been exposed to ringrot infected seedlots but which have not shown symptoms in the field have been made to determine if the ringrot bacterium is present in a latent state. Results to date are not conclusive and will be continued.

Early Blight

Cv. Norchip tubers were harvested, graded and treated prior to storage for early-blight control. After storage at 50°F for 4 months, tubers were evaluated to determine the effectiveness of the treatments. Treatments included Captan, Rovral at 5 and 10 ppm and Rovral (5 ppm) combined with BD-11 (10 ppm).

All chemical treatments significantly reduced the average number of early blight lesions per tuber when compared with untreated controls. Chemical treatments did not differ significantly when compared on the basis of the average number of lesions per tuber. However, when data for the percentage of healthy tubers (i.e., tubers with no early blight lesions) were compared, Rovral 5 ppm, Rovral 10 ppm and Rovral 5 ppm + BD-11 10 ppm all performed significantly better than Captan. Rovral 10 ppm was significantly better than Rovral 5 ppm when compared on the basis of the percentage of non-infected tubers. Rovral 5 ppm + BD-11 10 ppm was intermediate between the other two treatments.

1987

Research Proposal

Good progress has been made during the past several years in determining the factors related to recontamination of Erwinia-free potato crops and ways to reduce the problem. We have also made a good start toward better understanding the ringrot disease in the San Luis Valley but much remains to be done and progress will be slow unless additional resources become available. Regardless of these problems, however, these two diseases are serious enough to require that work be continued at a pace as rapid as resources will allow. A graduate student is now working on the ringrot problem. She is, however, unfunded and must not only support herself but also pay tuition out of her own resources. Her contribution has been and will continue to be substantial and we will keep trying to find resources to support her work.

We propose to continue work on already in progress both blackleg and ringrot and to do some work with early dying (Verticillium wilt) if outside funds become available to do so.

Specific areas of research for which funding is requested are as follows.

Blackleg

1. Studies to determine the effects of Erwinia which enter potato stems from irrigation water on growth and productivity of plants produced by Erwinia-free potato seed. This work is important if Erwinia-free seed is to be sold and planted in areas with high Erwinia populations in irrigation water. This work will be combined with studies on the interaction between Erwinia and Verticillium in the early dying problem

in the San Luis Valley but only if additional funds can be obtained from the Western Regional IPM Project to do so.

2. Initial work will be done to determine if Erwinia half-life calculations can be used to predict when the organism will completely disappear from field soil.

Ringrot

1. Work will continue using eggplants and micropropagated potato plantlets to try to develop a more reliable and more sensitive method to detect Corynebacterium sepedonicum. Studies will include work on the numbers of bacterial cells, plant nutrition age and environmental factors required for greatest sensitivity.
2. As detection methods are improved they will be used to study several aspects of ringrot epidemiology in the San Luis Valley including a) the presence of the pathogen in seedlots which have not expressed visible symptoms in the field; and and b) alternate sources of the ringrot bacterium including weeds and other crops, water, insects, etc.
3. We will continue to test means to control ringrot inoculum on seedpiece surfaces chemically as new bactericides become available.

We expect the ringrot work to continue for 3-5 years if significant results are to be achieved.

Budget Request

Plot maintenance	$ 300
Labor	2,700
Travel	1,800
Supplies and Equipment	2,100
TOTAL	$6,900

The Use of Alcide for Seedpiece Treatment in Colorado

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Abstract

Four liquid seedpiece treatments were applied to commercially cut Norgold Russet seed. Treatments (base:activator:water) applied were: 1) 0:0:1; 2) 1:1:10; 3) 1:1:23; and 4) 1:1:48. Treatments 2 and 3 significantly reduced surface populations of presumptive Erwinia carotovora on seedpieces. They also significantly ($p=0.05$) increased the number of seedpieces from which no Erwinia could be recovered. Treatments had no significant effect on stand counts, plant vigor or the incidence of blackleg or ringrot symptom expression in the field.

Materials and Methods

For Erwinia studies, freshly cut cv. Norgold Russet seedpieces (North Dakota seedlot) collected from a commercial seed cutter in Weld County, Colorado were treated by misting with each of four treatments (Table 1). Treatments were applied with a hand-held sprayer and all seedpiece surfaces were thoroughly wetted during treatment.

After treatment, 10 seedpieces from each of five replications per treatment were collected. The surface of each seedpiece was swabbed with a sterile cotton Q-tip^R to determine surface populations of Erwinia. A template (6.5 sq/cm) was used to insure that the same surface area was swabbed on each seedpiece in order for valid statistical comparisons to be made. Swabs were placed in tubes with 1 ml sterile distilled water and allowed to soak for several minutes.

The tubes with the swabs were then vigorously agitated with a vortex mixer to suspend bacteria. Dilutions were then made and spread plated on Stewarts' MacConkey-pectate and crystal violet pectate medium. Plates were incubated at 26°C for 48 hours and typical Erwinia colonies were counted. In cases where Erwinia colonies were too numerous to accurately count, a maximum value of 200 per plate (a very conservative estimate) was assigned. Therefore, the data reported were conservative regarding comparisons between treatments and the water control, i.e., treatment differences were minimized.

The remainder of the treated seedlots were planted in the field at the Plant Pathology/Weed Science Reseach Farm in Fort Collins. Five replications (blocks) per treatment were planted in 4-row plots with 25 seedpieces per row.

For the ringrot study, freshly cut seedpieces were inoculated with Corynebacterium sepedonicum by dipping cut seedpieces into an inoculum slurry prepared by macerating ringrot-infected tubers in water. Inoculated tubers were misted with water plus the three Alcide treatments immediately after inoculation and planted in field plots at the same location as the Erwinia study. Four replications per treatment were planted. Each plot consisted of four rows with 25 treated seedpieces per row.

Data on plant stands, vigor and blackleg or ringrot expression were collected periodically during the growing season. Infected plants were removed from plots or were marked on each reading date to avoid recounting them during subsequent readings. Plant vigor was rated on a scale of 0-10 by comparing plants in treated plots with those in the control (water treated) plot in each replication.

Plants in control plots were arbitrarily given a rating of 5 on each reading date and plants in treated plots were then compared with them on a relative basis. Therfore, plants which appeared less vigorous then the controls received ratings less than 5 and those which appeared more vigorous received ratings greater than 5.

Results

Data on the effect of Alcide on Erwinia surface populations, stand, vigor and blackleg incidence are shown in Table 1. They show that treatments 2 and 3 significantly reduced surface populations of Erwinia and significantly increased the number of seedpieces from which no Erwinia could be recovered ($p=0.05$). Treatments had no significant ($p=0.05$) effect on stand, vigor or blackleg incidence in the field, however, when compared with the water-treated control.

Data on the effect of Alcide treatments on ringrot expression, plant stand and vigor are shown in Table 2. No significant differences were found among treatments.

Discussion

Alcide treatments significantly reduced populations of Erwinia on the cut surface of cv. Norgold Russet seedpieces but had no effect on stand (seedpiece decay) or blackleg incidence. This was probably due to the fact that naturally Erwinia-contaminated seed was used in the study. Erwinia present in lenticels where they usually survive the storage period after invading these sites during the growing season would probably have been protected from the chemical treatments. It is probable, especially in a season such as 1986, which was not highly favorable for seedpiece decay or blackleg expression, that sufficient inoculum was present in the lenticels to

mask the effects of Alcide treatments on disease incidence in the field. If this is true, Alcide may have useful application as a seedpiece treatment to minimize spread of Erwinia during the cutting and handling of Erwinia-free potato seed. The experiment should probably be repeated using tissue culture-derived potato seed cut on a contaminated cutting machine then treated with Alcide. It is, however, also possible that in seasons favorable for seedpiece decay and blackleg expression that treatment of regular (not blackleg free) seedpieces would be beneficial.

The plots received extreme hail injury in early August. This seriously interfered with disease and vigor readings taken in August. This may have resulted in inaccurate field readings of ringrot expression. Perhaps with a longer season infection differences would have been apparent.

Table 1. The effect of Alcide seedpiece treatment on populations of presumptive Erwinia carotovora on seedpiece surfaces, stand, vigor and blackleg incidence in the field. Fort Collins, CO, 1986.

Treatment[1]	Seedpiece Evaluations		Stand Counts (plants/50 feet)		Vigor[5]			Blackleg incidence[6] (plants/plot)			
	average[3] number of EC per seedpiece	% of[4] seedpieces from which EC was recovered	6/9/86	7/2/86	7/2/86	7/22/86	8/1/86	7/2/86	7/22/86	8/2/86	8/25/86
1. 0:0:1	167[2]	80	32.8	35.4	5.0	5.0	5.0	0.0	0.0	0.2	0.0
2. 1:1:10	101*	43*	33.8	34.0	4.6	5.0	5.4	0.0	0.0	0.0	0.0
3. 1:1:23	91*	40*	38.2	38.8	5.6	5.8	5.8	0.8	0.0	0.4	0.0
4. 1:1:48	151	72	32.2	36.0	5.2	5.6	5.4	0.2	0.0	0.4	0.0
LSD value (p=0.05)	54.8	28.2	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD

[1]Base:Activator:Water

[2]Each figure is the average of 5 replications of 10 seedpieces each. When colony numbers were "too numerous to count" a maximum value of 200 was assigned. This occurred only in treatment 1.

[3]Multiply by 10 to calcalate numbers per 6.5 sq cm (1 sq in)

[4]The average percentage of seedpieces from which presumptive Erwinia carotovora was recovered by direct plating.

[5]Scale of 0-10 (0=worst, 10=best) treatment 1 (check) assigned a value of 5.

[6]Average number/plot; blackleg plants were removed from the plots when observed on each date.

Table 2. The effect of Alcide seedpiece treatment on bacterial ringrot symptom expression in the field, Fort Collins, CO, 1986.

Treatment[1]	Stand[4] 7/2/86	Vigor[2] 7/22/86	Vigor[2] 8/1/86	Symptom Expression[3] 7/22/86	Symptom Expression[3] 8/1/86	Symptom Expression[3] 8/25/86
1. 0:0:1	33.0	5.0	5.0	0.0	0.0	3.8
2. 1:1:10	38.5	6.0	5.3	0.5	2.8	4.5
3. 1:1:23	37.8	6.0	5.3	0.3	1.5	4.0
4. 1:1:48	34.8	5.3	5.8	0.3	0.3	2.5
LSD (p=0.05)	NSD[5]	NSD	NSD	NSD	NSD	NSD

[1]Base:Activator:Water

[2]Scale of 0-10 (0=worst, 10=best) treatment 1 (check) assigned a value of 5.

[3]Entries for each date are cumulative from previous dates.

[4]Plants/50 feet of row.

[5]NSD-no significant differences.

The Association of *Erwinia carotovora* in Irrigation Water and Its Influence on Stand, Blackleg, Aerial Blackleg and Daughter Tuber Contamination

G. D. Franc and M. D. Harrison

A study was initiated in 1985 and continued in 1986 to study the effect of *Erwinia* contaminated irrigation water on seedlot performance and daughter tuber contamination. This research was funded primarily by the Western Regional IPM Program. An identical study was done by Dr. Mary L. Powelson and her associates at OSU in Corvallis, Oregon. The generous donation of land and equipment by Bob Kula in NE Colorado and Jim and Rob Jones and Doug Still in the SLV made it possible for this work to be done. This report briefly summarizes the experiments and observations made during the two growing seasons. Final data are being collected and analyzed and a formal report will be prepared when the summaries are completed.

Four identical field plots were established under center pivots in NE Colorado (low elevation) and in the SLV (high elevation) in both years. At each location, one field plot was irrigated with surface water (*Erwinia* contaminated) and one with underground water (*Erwinia*-undetected). *Erwinia* populations in the water were monitored during the growing season. Soil and seed tubers were assayed to determine if *Erwinia* was present. Microloggers, programmed to monitor air temperature and leaf wetness duration, were placed in the plots irrigated with surface water to record environmental parameters. All plots and measurements were duplicated in Oregon (using the same seedlots) to determine factors important to the performance of seedlots and the potential role of contaminated irrigation water on blackleg (stem soft rot) incidence. Russet Burbank and Norgold Russet tubers

were used for the study in 1985 and Russet Burbank only in 1986.

Results showed that the typical blackleg observed in Colorado did not appear to be associated with the use of contaminated irrigation water. Erwinia was present on leaf surfaces but populations were much lower in Colorado than in Oregon. It is not clear if those populations can be attributed to the use of contaminated irrigation water. Leaf surface populations in Colorado were very low compared with those found in Oregon. Little aerial blackleg was found in the field plots in either year. Amounts found were much less in Colorado than in Oregon. Both Ecc and Eca were recovered from plots during the course of the study. Strains are being serologically tested to help determine their sources.

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

G.D. Franc and M.D. Harrison

Abstract

The objective of this study was to determine if whole potato tubers inoculated with serial dilutions of Corynebacterium sepedonicum (ringrot) cells produced plants and tuber progeny that appeared visually healthy but were, nevertheless, infected (latent infection). The occurrence of latent ringrot infections would partially explain the almost cyclical pattern of ringrot symptom appearance in some seed potato production areas. This study was initiated in 1981 and was continued through 1985. The final evaluation will be made in 1986. This report summarizes all the results from the start of the study through 1985.

DEP81 STUDY (initiated 1981, terminated 1984)

Whole Russet Burbank (RB) and Centennial Russet (CR) tubers (mother seed tubers) were inoculated with serial dilutions of C. sepedonicum. Results of studies initiated in 1981 (DEP81) showed that plants of both cultivars had no visible foliar ringrot symptoms (primary symptoms) evident during the first growing season after inoculation (summer 1981) even though individual seed tubers had been inoculated with as many as 6.3×10^8 cells.

Daughter tubers (generation 1 - G1) harvested from DEP81 plots were planted back in the field during 1982. Plants with foliar ringrot symptoms (secondary symptoms) were found in RB but only when the original mother tuber received the maximum inoculum concentration (6.3×10^8 C. sepedonicum cells). All CR plants were symptomless

during 1982. When generation 2 0(G2) tubers were planted in the field in 1983, symptom development occurred only in CR plants when original seed tubers received 10 C. sepedonicum cells in 1981. All other plants for both cultivars appeared healthy. Plant-back of generation 3 (G3) tubers in 1984 produced plants that all appeared healthy.

Tuber bioassays for the same DEP81 seedlots planted in the field during 1982 showed detectable levels of ringrot infection in G1 tubers when mother tubers had received 10^4, 10^6 and 10^8 cells (RB) or 10^6 and 10^8 cells (CR). Assays of G2 tubers in 1983 showed infection only in CR treatments that had received 10^6 cells. All G3 tuber assays in 1984 were negative.

DEP82 STUDY (initiated 1982, terminated 1985)

Primary symptoms for DEP82 (i.e., the first year in the field) only occurred when RB mother tubers received inoculum concentrations of 10^6 and 10^9 cells. Primary symptoms did not occur in CR plants. Secondary foliage symptoms (G1 plants in 1983) occurred in RB only in the treatment receiving 10^4 cells per seed tuber. Both the higher and lower inoculum loads produced healthy appearing plants. Secondary symptoms in CR occurred at the two highest inoculum concentrations (10^9 and 10^6 cells). Symptoms in G2 plants did not develop when Russet Burbank tubers were planted in the field in 1984. Foliar symptoms developed in Centennial at the 10^9 and 10^2 cells per tuber inoculum levels. All G3 plants appeared healthy in the field during 1985. Assays of G1 tubers showed that RB treatments receiving 10^9 cells per tuber were infected and CR treatments receiving 10^6 and 10^9 cells per tuber were also infected. The same results occurred

for G2 tubers assayed in spring 1984. Assays of G3 tubers only showed infection in RB and CR treatments receiving 10^2 cells per tuber.

DEP83 (initiated 1983, terminated 1986)

In DEP83 studies, plants showed primary symptom development for the 10^4, 10^6 and 10^9 inoculum concentrations in RB and 10^1, 10^4, 10^6, and 10^9 in Centennial. In treatments where CR mother tubers received 10^2 cells the plants appeared healthy. Visual inspection of G1 tubers at harvest showed symptoms occurred when mother tubers received inoculum concentrations of 10^6 and 10^9 cells for RB and 10^4, 10^6, and 10^9 for CR. Greenhouse assays of G1 tuber subsamples on eggplant showed that tubers harvested from the treatments with inoculum levels greater than or equal to 10^2 cells per tuber were infected in both cultivars. Foliar symptom development in G1 RB plants occurred when inoculum levels of 10^2, 10^4, 10^6, and 10^9 cells per tuber were used and in CR when 10^4, 10^6 and 10^9 cells per tuber were applied. Assays of G2 tubers showed that treatments receiving 10^6 and 10^9 cells for RB and 10^4 and 10^6 cells for CR were infected. However, G2 plants showed infection for treatments 10^2 and 10^6 for RB and 10^9 for CR.

DEP84 mother tubers were inoculated in 1984 and planted at Fort Collins, Colorado. Foliar symptom development occurred in both cultivars when inoculum levels of 10^6 and 10^9 cells were used. All other inoculum levels produced plants that appeared healthy.

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 established in 1981 (DEP81). Tubers provided by the S.L.V. Research OCenter were used for (almost) identical studies initiated in 1982 (DEP82), 1983 (DEP83), and 1984 (DEP84).

Treatments for the DEP91 study consisted of inoculating tubers with five serial dilutions of Corynebacterium sepedonicum (CS43) and buffer (control). The original CS43 strain was provided by S. Slack, University of Wisconsin-Madison, Madison, WI 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 1/2" needle containing C. sepedonicum (CS43) bacteria suspended in 0.05 M phosphate buffer, pH 7.2. The first foliar ringrot symptoms developed in the eggplants in ca. 12 days after inoculation. On May 25, 1981, the seedlings were uprooted, washed in cold tap water and roots and leaves were removed with a knife previously dipped in ethanol and flamed. The stripped 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 bacterial cells were extracted from the stem segments into the cold buffer by this time due to the osmotic pressure differential. Buffer, containing the extracted cells, was passed through cheesecloth to remove the stem segments and large debris. Cells in the strained buffer were concentrated into a pellet by centrifugation using a Servall refrigerated automatic centrifuge and a SS34 rotor at 11-12 x 103 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 (Whatman 2 was used for the DEP83 study). Serial dilutions of the filtrate were made and lightly stained with 1-2 drops of crystal violet. Aliquots of stained filtrate were placed in a Petroff-Hauser Ocounting chamber and the number of cells per 2.5×10^{-3} mm^2 square were counted using a microscope at 45 X. Fifty squares were counted to determine the average number of cells per 2.5×10^{-3} mm^2. The chamber was then emptied, refilled and the counting procedure repeated (replication I:X = 3.16 cells/2.5×10^{-3} mm^2, S = 1.765. Replication II:X = 318 cells/2.5×10^{-3} mm^2, S = 0.625). An average of 6.34×10^9 cells per ml were determined to be present in the undiluted filtrate. Serial dilutions were made to achieve cell suspensions of 6.34×10^8, 1×10^7, 1×10^5, 1×10^3 and 1×10^2 cells/ml buffer.

Inoculum for the DEP82 study was prepared in a similar manner with minor modifications. The CS43 inoculum for eggplant inoculation was prepared by extracting bacteria from infected tubers harvested the previous fall from infected 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:X = 0.55 cells/5×10^{-8} ml, II: 0.50 cells/5×10^{-8} ml, III: 0.60 cells/5×10^{-8} ml, IV: 0.60 cells/5×10^{-8} ml, S = 0.048).

Eggplants used for inoculum production for the DEP83 and DEP84

studies came from a different seedlot than that used in the DEP81 and DEP82 studies. Both seedlots appeared to react in a similar manner to infection by CS43. 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 prepared 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 Petroff-Hauser squares were counted for each replication (replication I:X = 1.10 cells/5 x 10^{-8} ml, II:X = 0.70 cells/5 x 10^{-8} ml, III: 0.75 cells/5 x 10^{-8} ml, IV:X = 1.00 cells/5 x 10^{-8} ml, V:X = 1.10 cells/5 x 10^{-8} ml, VI:X = 0.95 cells/5 x 10^{-8} ml, S = 0.172). Serial dilutions were made, and tubers were inoculated on the same day.

Inoculum for the DEP84 study was prepared from eggplants inoculated on May 14, 1984. Inoculum came from a DEP82 tuber with ringrot symptoms. Eggplants were uprooted on May 29, 1984 and stems were chopped and placed into buffer (stem wet weight ca. 41.5 grams). Cells were concentrated by centrifugation and resuspended in 15 ml of sterile buffer. Serial 10 fold dilutions were prepared and the cell numbers in the 10^3 dilution were counted (20 squares) (rep. I:X = 11.60, rep. II:X = 10.20; rep. III:X = 11.25, rep. IV:X = 10.50). The original suspension had a concentration of 2.1775 x 10^{11} cells/ml suspension.

Serial dilutions of inoculum for the DEP82, 83 and 84 studies were made to produce inoculum concentrations of 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 (Table 5).

Tubers to be inoculated each year were surface disinfested 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 and replacing the tuber piece. A small piece of sterile wooden toothpick was inserted to hold the tuber piece in place and the entire stolon end (approximately 1/4 - 1/3 of the tuber) was immediately dipped into melted paraffin (Gulfwax) twice. An inoculum 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 only with buffer, were treated in the same manner. All inoculations were done in the order of most dilute to most concentrated inoculum preparations.

Inoculated tubers were planted in a field plot ca 33 ft x 120 ft. Plots were planted by hand on May 28, 1981, May 26, 1982, May 17, 1983, May 17, 1984 (S.L.V.), May 31, 1984 (Ft. Collins) and May 22, 1985. A randomized complete block design consisting of two cultivars, six inoculation treatments and four replications (blocks) each for the DEP81, DEP82, DEP83 and DEP84 field plots was used. 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 all growing seasons 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 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 ODEP82 daughter tubers 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 at the end of the 1983 growing season. Harvest in the San Luis Valley was done on September 18, 1984. The final evaluation of DEP84 plots (Ft. Collins) was done on September 23, 1984. Tubers were not harvested for the DEP84 plots in Ft. Collins. Tuber harvest for DEP82 and DEP83 was done on September 6, 1985.

Tubers harvested from each treatment plot 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 in the laboratory.

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.

A flow-chart diagram of the experimental procedure used is shown in Figure 1.

Results

DEP81 study initially planted May 28, 1981 (San Luis Valley):

Primary foliar ringrot symptoms failed to develop at any time during the first growing season. The plants were water stressed throughout the season and this may be why foliar symptoms did not develop even when mother Otubers were inoculated with as many as 6.34 x 108 C. sepedonicum cells per tuber (Table 1).

Daughter tubers (generation 1 = G1) harvested after the 1981 growing season were determined to be infected with ringrot by the eggplant test (column 2, Table 1) even though mother plants were symptomless. However, G1 tuber infection was only detected in treatments in which the mother tubers were inoculated with at least 10^4 C. sepedonicum cells (Russet Burbank) or 10^6 cells (Centennial).

G1 tubers were replanted in the field on May 26, 1982. Although plants were observed throughout the growing season foliage symptoms were only detected in Russet Burbank plants receiving 6.3×10^8 cells during the previous season. Foliar symptoms were not observed in similarly treated Centennial plants.

Bioassays and field plantings were again repeated in 1983 using

granddaughters (generation 2 - G2) tubers harvested during fall 1982. Bioassays detected ringrot infection only in 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 in Centennial inoculated with 10 cells in 1981. This showed the first evidence that ringrot infection in plants grown from tubers exposed to low levels of inoculum may not express symptoms until the third growing season after the initial inoculation . Thus, ringrot infection may be latent for that period.

Tuber assays and observations of plants in the field failed to show any evidence of ringrot infection during 1984. The DEP81 plots were terminated in 1984.

DEP82 study initially planted May 26, 1982 (San Luis Valley):

Primary foliage symptoms in Russet Burbank developed in 1982 when the mother tuber received at least 10^6 C. sepedonicum cells (Table 2). Foliar symptoms failed to develop in similarly inoculated Centennial plants. G1 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 in tubers harvested from plants inoculated with 10^9 cells in Russet Burbank and 10^9 and 10^6 cells in Centennial. Ten tubers were also planted in the field. Secondary foliage symptoms developed in the field in treatments which received 10^4 cells (Russet Burbank) and 10^9 and 10^6 cells in Centennial. Tubers (G2) harvested from these plants showed that tuber infection had occurred in plants inoculated with 10^9 cells (Russet Burbank) or 10^6 and 10^9 cells (Centennial). Plants produced by G2 tubers planted back in 1984 failed to show ringrot

foliar symptoms in Russet Burbank but Centennial plants derived from treatments inoculated with 10^2 and 10^9 cells per tuber did show symptoms. Assays of G3 tubers in 1985 showed infection for inoculum levels of 10^2 cells for both Russet Burbank and Centennial. No foliar symptoms developed in the field, however. The DEP82 plots were terminated in 1985.

Dep83 study initially planted May 17, 1983 (San Luis Valley):

Primary foliage symptoms developed in Russet Burbank when mother tubers were inoculated with 10^4, 10^6 and 10^9 cells and in Centennial when mother tubers received 10^1, 10^4, 10^6 and 10^9 (Table 3). Centennial tubers receiving 10^2 cells produced plants that were symptomless. Inspection of G1 tubers at harvest showed visible symptoms when mother tubers received 10^6 and 10^9 cells (Russet Burbank) or 10^4, 10^6 and 10^9 cells in Centennial. Assay of G1 tubers on eggplant showed that mother tubers receiving inoculum levels of 10^2 cells or greater produced infected daughter tubers. Secondary foliage symptoms in Russet Burbank occurred in the field in 1984 on plants derived from seed tubers originally inoculated with 10^2 cells or more in 1983. Centennial plants showed symptoms when original seed tubers were inoculated with 10^4 cells or more in 1983. Assays of G2 tubers made in 1985 showed that infection had occurred in treatments receiving 10^9 and 10^6 cells per original seedpiece (R. Burbank) and 10^6 and 10^4 cells per original seedpiece for Centennial. Foliar symptoms for R. Burbank occurred for treatments receiving 10^6 and 10^4, and for Centennial treatments receiving 10^9 cells. The DEP83 plots will be planted for the final time in 1986.

Dep84 study initially planted May 31, 1984 (Ft. Collins):

Primary foliar symptom expression in both Russet Burbank and Centennial only occurred in plants grown from seedpieces receiving 10^6 and 10^9 cells. All other inoculum levels appeared healthy. Tubers were not harvested from these plots.

A comparison of physical cell counts (determined by use of the Petroff-Hauser counting chamber) versus viable cell counts is shown in Table 5. Viable cell counts were lower than expected based upon 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 gave an estimate of 827 viable cells per 1000 cells counted in the Petroff-Hauser chamber or a ratio of 83:100. Therefore, the use of the Petroff-Hauser counting chamber appears to be an accurate method for determining viable cell counts for Corynebacterium sepedonicum produced by the method outlined above. The viable estimate may be 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.

Results from the final phase of the study in 1986 showed that only one plant in stocks inoculated with C. sepedonicum in 1983 showed possible ringrot symptoms in the field. This was a Centennial Russet plant and tests to verify that the symptoms were caused by C. sepedonicum are pending.

Table 1. The effect of Corynebacterium sepedonicum (ringrot) tuber inoculum concentration on symptom expression in progeny plants and daughter tubers - DEP81 study, Center, Colorado, 1981, 1982, 1983, 1984.

Cultivar: Number of cells per seed tuber	The Presence (+) or Absence (-) of Detectable Ringrot in:						
	Mother plants[1]	G1 tubers[2]	G1 plants[3]	G2 tubers[4]	G2 plants[4]	G3 tubers[5]	G3 plants[5]
	summer 1981	spring 1982	summer 1982	spring 1983	summer 1983	spring 1984	summer 1984
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 (G1) tubers harvested in 1981 were replanted in the field in 1982 and symptom expression recorded.

[4] Granddaughter (G2) tubers harvested in 1982 were replanted in the field in 1983 and symptom expression recorded.

[5] Great granddaughter (G3) tubers harvested in 1983 were replanted in the field in 1984 (a total of 20 per inoculum level) and symptom expression recorded. A subsample of 20 tubers per inoculum level (replications were combined) were assayed using eggplants and all appeared healthy.

Table 2. The effect of Corynebacterium sepedonicum (ringrot) tuber inoculum concentration on symptom expression in progeny plants and daughter tubers - DEP82 study, Center, Colorado, 1982, 1983, 1984, 1985.

	The Presence (+) or Absence (-) of Detectable Ringrot in:						
Cultivar: Number of cells per seed tuber	Mother plants[1] summer 1982	G1 tubers[2] spring 1983	G1 plants summer 1983	G2 tubers[2] spring 1984	G2 plants summer 1984	G3 tubers spring 1985	G3 plants summer 1985
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] Tubers were assayed for ringrot infection using the eggplant test.

Table 3. The effect of Corynebacterium sepedonicum (ringrot) tuber inoculum concentration on symptom expression in progeny plants and daughter tubers - DEP83 study, Center, Colorado, 1983, 1984, 1985.

	The Presence (+) or Absence (-) of Detectable Ringrot in:					
Cultivar: Number of cells per seed tuber	Mother plants[1] summer 1983	G1 tubers[2] fall 1983	G1 tubers[3] spring 1984	G1 plants[4] summer 1984	G2 tubers spring 1985	G2 plants summer 1985
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.

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

[4] Daughter (G1) tubers harvested in 1983 were replanted in the field in 1984 and symptom expression recorded. Foliar symptoms were verified by the stem squeeze test on 21 August, 1984.

Table 4. The effect of Corynebacterium sepedonicum (ringrot) tuber inoculum concentration on symptom expression in progeny plants and daughter tubers - DEP84 study, Fort Collins, Colorado, 1984.

Cultivar	Number of cells per mother tuber	Primary foliage symptoms
Russet Burbank	10^9	+
	10^6	+
	10^4	-
	10^2	-
	10^1	-
	Buffer	-
Centennial Russet	10^9	+
	10^6	+
	10^4	-
	10^2	-
	10^1	-
	Buffer	-

Table 5. 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] Aliquots (0.1 ml) of the prepared serial 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, 1983 and Ft. Collins, CO, 1984.

Corynebacterium sepedonicum (isolate CS43) culture slant (DEP81) or CS43 infected tubers (DEP82, DEP83 and DEP84)

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 X G 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.* Repeat for G2 and G3 tubers.

Plant daughter (G1) tubers in the field and observe for foliar symptom development during the second season. Harvest granddaughter (G2) tubers and repeat field and eggplant observations. Repeat with great-granddaughter (G3) tubers.	Inoculate eggplant to detect daughter tuber infection directly

* NOTE: only whole noncut (uninjured) tubers were harvested, assayed and planted.

Comparative Field Test for Bacterial Ring Rot Development - San Luis Valley, CO, 1986

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Colorado State University

Abstract

Tuber "melon-ball scoop" seedpieces, inoculated with varying numbers of the ringrot bacterium, were planted in the field near Center, Colorado. "Identical" inoculated seedpieces were planted in similar field plots at other locations in the United States. Two cultivars, Russet Burbank and Norland, were included in this study.

Emergence rates and plant vigor varied greatly within treatment plots in Colorado. Foliar ringrot symptoms only appeared near the end of the growing season after plants had extensive early blight infection and psyllid injury. Only one tuber showed visible ringrot symptoms at the time of harvest. Results should be interpreted with caution because of problems with foliar diseases which made it difficult to read ringrot symptoms late in the season. Russet Burbank plants inoculated with large numbers of Corynebacterium sepedonicum cells showed good ringrot symptoms in other plots planted at the same location.

Materials and Methods

Potato seedpieces (melon ball scoops) of cv. Russet Burbank and Norland were inoculated with Corynebacterium sepedonicum by Dr. S. Slack (U of W-Madison) and shipped to CSU via Federal Express on 7 May 1986. The seed was received at CSU on 8 May 1986. Inoculated tuber pieces appeared to be in good condition on arrival and were

stored in a coldroom at ca. 40°F until planting time.

Inoculated seedpieces were planted in field plots in the San Luis Valley near Center, Colorado on 17 May 1986. The experimental design used was a randomized block with three replications (blocks) per treatment. Each plot consisted of five rows with five plants per row on a 12 inch spacing between plants and 34 inch (not 36 inch as requested in the protocol) spacing between rows. Seedpieces were placed at least 1.5 inches deep at the time of planting. Two cultivars (Norland and Russet Burbank), each inoculated with three inoculum levels (10^6 cfu/seedpiece, 10^2 cfu/seedpiece and a buffer check), comprised the six treatments included in the study.

Stand counts were taken periodically and weather data (environmental data) were recorded daily at the San Luis Valley Research Center located Ca. 1/4 mile from the plot. In addition, disease expression and plant senenscence data were collected on as near to a weekly schedule as possible.

Results and Discussion

Stand count data are shown in Table 1. Approximately 50% of the plants emerged on or shortly after June 5. Emergence was erratic and varied considerably within single treatment plots.

Data on the estimated percentage of foliage senescence (Barratt-Horsfall scale 0-11) are shown in Table 2. None of the plants in the plots developed visible foliar ringrot symptoms [plants rated on the same dates as senescence readings were made (Table 2)] until 19 August 1986. On this date 1.3% of the Russet Burbank plants inoculated with 10^2 cfu/seedpiece and 2.6% of the Norland plants inoculated with 10^6 cfu/seedpiece showed visible ringrot symptoms.

At the time ringrot symptoms were detected, Ca. 20% to 88% of the foliage was senescent (Table 2) and severe early blight infection and psyllid injury was present.

Tuber evaluations were made on 6 September 1986. Only one tuber was found which showed visible symptoms. This tuber, harvested from a Russet Burbank plot in which seedpieces were inoculated with 10^6 cfu C. sepedonicum, showed both internal and external symptoms of bacterial ringrot and represented 1.7% of the tubers examined. Tubers from all other plots showed no internal or external tuber symptoms.

The large variation observed in the field both within and between treatments makes it difficult to interpret the data. Treatment RB (10^6) produced one tuber with ringrot symptoms but never showed visible foliar symptoms. RB (10^2) on the other hand showed foliar symptoms in a few plants (1.3%) but tubers from this treatment showed no symptoms. The unusually warm, wet year in the San Luis Valley may account for the unusually rapid onset of early blight which made readings difficult. Psyllid injury late in the season also made data collection difficult. Data should be interpreted with these problems in mind. Weather records including air temperature (max and min), solar radiation, soil temperature (max and min) at 2" and 6" depths and wind speed for the period May 1 through September 30 are attached.

Table 1. Plant Emergence in Relation to Corynebacterium sepedonicum inoculation and potato cultivars, San Luis Valley, CO, 1986.

	Treatment	Percentage of Plants[2] Emerged		
		June 5	July 9	July 17
1A.	RB check	61.3	97.3	96.0
2A.	RB 10^2	49.3	94.7	96.0
3A.	RB 10^6	38.7	85.3	86.7
1B.	NORcheck	44.0	93.3	94.7
2B.	NOR 10^2	40.0	90.7	90.7
3B.	NOR 10^6	49.3	93.3	93.3
	RB ave	49.8	92.4	92.9
	NOR ave	44.4	92.4	92.9

[1]Planting date was 17 May 1986.

[2]Data based on the average of 3 replications of 25 plants each.

Table 2. Estimated percentage of foliage senescence in plants inoculated with Corynebacterium sepedonicum, San Luis Valley, CO, 1986.

Treatment	Estimated Percentage of Foliage Senescent[1]				
	July 17	July 30	Aug 7	Aug 13	Aug 19
1A. RB check	0.7	2.0	6.0	12.0	23.5
2A. RB 10^2	0.7	2.0	6.0	7.5	19.5
3A. RB 10^6	0.7	2.0	12.0	12.0	40.5
1B. NOR check	6.0	19.5	46.0	80.5	88.0
2B. NOR 10^2	3.0	15.0	40.5	76.5	80.5
3B. NOR 10^6	3.0	15.0	59.5	80.5	85.0

[1]Each figure is the average of three replications. Ratings are on a scale of 0-11 (0-100%).

Station: Center W | County: Rio Grande | State: Co | Date: May 1981 | Complete Observation: 8 AM MDT | Standard Time in Use | RECORD OF CLIMATOLOGIC OBSERVATIONS

DATE	TEMPERATURE °F: 24 Hours Ending at Observation Max.	Min.	At Observation Dry-bulb	Wet-bulb	Dew Point	Supplemental Readings at Solar Rad Dry-bulb	Wet-bulb	Dew Point	PRECIPITATION: Soil Temperature Time of reading 8 Max	Min	Time of beginning 8 Max	Min	24 Hour Amounts Rain, Melted snow, etc. (in. & hundredths)	Snow, Ice Pellets, Hail (in. & tenths)	At Obsn. Snow, Ice Pellets Hail, Ice on ground (in.)	WIND: Anemometer Dial Reading (Miles)	24 Hour Movement	EVAPORATION (Inches & hundredths): Gage Reading or Amount Added +	Reading When Tank Filled or Amount Removed −	Amount of Evaporation	WATER TEMP. °F: 24 Hours Ending at Observation Max.	Min.	ADDITIONAL DATA/REMARKS
1	74	36	47			717.	80		70	49	62	55					161.1						
2	74	37	50			571.	65		74	52	65	57					142.7						
3	70	36	43			532.	80		70	55	64	58					118.1						
4	71	37	45			625.	30		69	56	65	58					384.9						
5	64	25	40			743.	70		69	53	63	58					222.0						
6	66	26	42			523.	55		70	50	63	57					256.0						
7	63	35	41			669.	70		64	52	62	57					341.8						
8	49	22	34			418.	10		67	49	63	55					155.5						
9	55	27	34			582.	75		60	46	57	52	T	Tsno			221.0						
10	65	22	37			706.	70		61	45	56	51	T				134.8						
11	70	28	28			782.	55		69	43	59	52					230.0						
12	69	34	48			752.	95		72	50	64	55					230.9						
13	70	34	47			741.	85		73	50	65	57					197.3						
14	69	35	46			529.	10		75	53	66	58					157.2						
15	69	33	46			453.	25		68	54	63	58					191.0						
16	58	35	46			418.	10		65	54	62	58					148.2						
17	56	29	33			666.	00		64	48	61	55	.15	1/2"Sno			125.4						
18	63	29	41			715.	95		64	47	59	54	T				106.3						
19	71	36	48			660.	45		70	45	62	53					170.2						
20	74	41	52			584.	60		75	50	66	55					112.7						
21	73	40	50			599.	40		70	54	65	58					266.3						
22	74	43	54			773.	30		72	54	66	59					241.3						
23	75	35	43			847.	30		76	55	69	60					196.3						
24	69	42	61			360.	75		78	55	67	60					84.9						
25	73	34	37			789.	95		69	56	64	60					104.9						
26	72	35	58			717.	80		78	56	69	60					118.8						
27	65	35	48			603.	10		80	58	70	62					92.9						
28	65	36	44			560.	55		77	58	68	62					99.8						
29	49	43	45			157.	25		78	59	67	62	.04				79.5						
30	61	34	40			666.	00		60	50	62	55	.18				127.4						
31	72	39	56			593.	85		65	50	60	54					109.7						
Sum	2068	1053	1384			19066.	10						.37										
Avg.	66.7	34.6	44.6			615.	04					Greatest	.18			5328.9	Adjusted Total						

WS FORM E-22 (4-78)

X=75; 22

U.S. DEPARTMENT OF COMMERCE NOAA NATIONAL WEATHER SERVICE

OBSERVER: S.L.V. Research Center

STATION NUMBER: 05-1458-5

Cente SSW Rio Grande Co June 8 Complete Observation me) 8A Standard Time in Use MDT RECORD OF E AND CLIMATOLOGIC ON VATIONS

DATE	TEMPERATURE °F: 24 Hours Ending at Observation Max.	Min.	At Observation Dry-bulb	Wet-bulb	Dew Point	Supplemental Readings at Solar Radiation Dry Bulb	Wet Bulb	Dew Point	PRECIPITATION: Soil Temperature Time of beginning 2" Max	Time of ending 2" Min	Time of beginning 6" Max	Time of ending 6" Min	24 Hour Amounts Rain, Melted snow, etc. (in. & hundredths)	Snow, Ice Pellets, Hail (in. & tenths)	At Obsn. Snow, Ice Pellets Hail, Ice on ground (in.)	WIND Anemometer Dial Reading (Miles)	24 Hour Movement	EVAPORATION (Inches & hundredths) Gage Reading or Amount Added +	Reading When Tank Filled or Amount Removed −	Amount of Evaporation	WATER TEMP. °F 24 Hours Ending at Observation Max.	Min.	ADDITIONAL DATA/REMARKS
1?	55	41	49				281.	20	75	55	65	56	T				111.7						
2?	58	40	48				468.	05	61	54	60	58					92.6						
3	71	44	51				586.	45	66	52	60	56	.39				153.4						
4	74	44	51				623.	45	75	52	65	57	.07				105.6						
5	73	42	53				508.	75	75	52	67	58	T				128.0						
6	77	44	55				799.	20	75	54	67	58					186.7						
7	80	45	59				690.	05	81	57	72	60					125.7						
8	70	41	44				421.	80	81	59	72	63	.03				101.1						
9	66	42	48				512.	45	74	58	68	62					144.7						
10	64	40	50				490.	25	67	53	63	59	.15				143.1						
11	71	37	52				791.	80	67	52	62	58					97.4						
12	76	42	55				788.	10	80	52	67	57					92.1						
13	79	42	53				632.	70	84	56	72	61					156.3						
14	80	42	60				915.	75	82	59	73	64					78.5						
15	81	37	62				728.	90	84	62	73	65					125.3						
16	84	43	59				695.	60	84	61	76	63					56.9						
17	80	42	57				734.	45	84	62	75	67					113.1						
18	76	42	57				606.	80	86	65	76	69					103.2						
19	76	44	57				599.	40	82	62	73	67	.11				166.7						
20	78	44	59				880.	60	76	59	72	65	T				96.1						
21	81	43	51				629.	00	82	59	73	65					153.1						
22	81	45	53				599.	40	86	62	76	68					83.6						
23	76	48	57				651.	20	87	62	79	67					93.9						
24	67	48	55				325.	60	80	65	74	69					84.3						
25	66	50	54				314.	50	71	64	69	66	T				55.3						
26	76	50	55				675.	25	71	61	66	65	.13				95.1						
27	81	49	57				712.	25	82	62	72	64	.01				51.4						
28	84	47	60				767.	75	87	61	74	66					117.8						
29	75	51	63				405.	15	86	65	77	69	T				94.4						
30	70	50	56				381.	10	78	64	73	67	.07				88.7						
31																							
Sum.	1226	1319	1640				18216.	95					.96				3300.8						
Avg.	74.2	44.0	54.7				607.	23				Greatest	.39				110.0	Adjusted Total					

WS FORM E-22 (4-78)

U.S. DEPARTMENT OF COMMERCE NOAA NATIONAL WEATHER SERVICE

OBSERVER S.L.V. Research Center

STATION NUMBER 05-1458-5

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Center | NW | Rio Grande | CO | July 1986 | 8A | MDT

RECORD OF E... AND CLIMATOLOGIC... OBSERVATIONS

DATE	TEMPERATURE °F: 24 Hours Ending at Observation Max.	Min.	At Observation Dry-bulb	Wet-bulb	Dew Point	Supplemental Readings at Solar Rad: Dry-bulb	Wet-bulb	Dew Point	PRECIPITATION: Soil Temperature Time of beginning 21 Max	Time of ending Min	Time of beginning 6 Max	Type of ending Min	24 Hour Amounts: Rain, Melted snow, etc. (in. & hundredths)	Snow, Ice Pellets, Hail (in. & tenths)	At Obsn. Snow, Ice Pellets, Hail, Ice on ground (in.)	WIND: Anemometer Dial Reading (Miles)	24 Hour Movement	EVAPORATION (Inches & hundredths): Gage Reading or Amount Added +	Reading When Tank Filled or Amount Removed −	Amount of Evaporation	WATER TEMP. °F: 24 Hours Ending at Observation Max.	Min.	ADDITIONAL DATA/REMARKS
1	82	48	58				752.	95	72	60	68	65					52.3						
2	84	45	58				793.	66	85	60	74	63					62.5						
3	81	46	59				518.	00	88	64	77	68					102.9						
4	83	48	59				660.	45	83	77	76	70	T				108.1						
5	76	50	55				536.	50	88	66	78	70					77.5						
6	76	45	62				666.	00	78	60	73	65	.69				79.5						
7	71	44	57				382.	95	82	60	74	64	T				57.4						
8	70	52	57				427.	35	70	59	65	64	T				82.0						
9	70	48	54				466.	20	68	62	72	60	.35				98.2						
10	70	36	51				486.	55	74	57	68	62	.04				85.0						
11	78	42	56				764.	05	70	55	66	59	.22				66.3						
12	82	45	66				401.	45	76	55	67	59					80.2						
13	78	47	49				382.	95	78	58	67	62	.22				83.0						
14	77	44	53				814.	00	75	58	67	62	.07				90.9						
15	79	44	56				715.	95	76	57	68	62					105.0						
16	74	48	61				555.	00	82	59	72	63					94.0						
17	75	43	57				593.	85	75	61	69	65	T				76.5						
18	74	49	61				534.	65	80	62	68	63	T				111.9						
19	78	46	54				514.	30	77	60	68	64	.13				80.8						
20	64	43	53				336.	70	80	57	68	60	.16				34.0						
21	69	48	52				481.	00	70	59	61	61	.08				36.4						
22	72	45	56				505.	05	72	58	64	62	.01				60.9						
23	74	48	54				580.	90	71	59	64	61	T				124.9						
24	74	45	54				714.	10	76	57	65	60	.72				77.3						
25	76	43	57				712.	25	73	54	65	60					86.2						
26	77	42	43				749.	25	74	56	66	61					128.9						
27	78	43	45				784.	40	74	56	66	62					160.4						Solar Rad. hi according to Hi Trans. Change factor to 172.
28	80	36	55				752.	95	77	58	67	62					109.0						
29	83	44	55				741.	85	76	57	67	62					112.8						
30	83	46	59				723.	35	78	62	68	63					96.8						
31	85	45	58				721.	50	80	62	70	64					75.9						
Sum	2373	1398	1724				18770.	11					2.69				2697.5						
Avg.	76.5	45.1	55.6				605.	49				Greatest	.72				87.0	Adjusted Total					

WS FORM E-22 (4-78)

U.S. DEPARTMENT OF COMMERCE NOAA NATIONAL WEATHER SERVICE

OBSERVER S.L.V. Research Center

STATION NUMBER 05-1458-5

X = 85; 36

Station: Center SW | County: Rio Grande | State: CO | Date (Month & yr.): Aug. 1980 | Complete Observation Time: 8A | Standard Time in Use: MDT

RECORD OF E AND CLIMATOLOGIC OBSERVATIONS

DATE	TEMPERATURE °F: 24 Hours Ending at Observation Max.	Min.	At Observation Dry-bulb	Wet-bulb	Dew Point	Supplemental Readings at Solar Dry-bulb	Wet-bulb	Dew Point	PRECIPITATION: Soil Temperature Time of beginning (2") Max	Time of ending (2") Min	Time of beginning (6") Max	Time of ending (6") Min	24 Hour Amounts: Rain, Melted snow, etc. (in. & hundredths)	Snow, Ice Pellets, Hail (in. & tenths)	At Obsn. Snow, Ice Pellets, Hail, Ice on ground (in.)	WIND: Anemometer Dial Reading (Miles)	24 Hour Movement	EVAPORATION (Inches & hundredths): Gage Reading or Amount Added +	Reading When Tank Filled or Amount Removed −	Amount of Evaporation	WATER TEMP. °F: 24 Hours Ending at Observation Max.	Min.	ADDITIONAL DATA/REMARKS
1	76	45	58			Rad. 350.	88		80	64	70	65	T				115.6						
2	77	42	54			529.	76		71	60	66	62					101.3						
3	75	42	48			426.	56		72	60	66	62					100.9						
4	70	42	56			264.	88		70	60	64	62	T				76.4						
5	74	35	49			405.	92		62	56	62	60	.01				107.8						
6	79	42	55			603.	72		67	57	61	59	.03				130.9						
7	79	44	57			560.	72		72	55	64	60					103.2						
8	79	43	58			553.	84		73	57	63	60					78.0						
9	79	42	56			555.	56		74	58	63	60					68.4						
10	74	45	49			529.	76		75	62	65	62					81.8						
11	75	40	54			502.	24		72	57	65	62					74.0						
12	78	45	55			409.	36		72	57	64	61					86.5						
13	79	44	56			577.	92		72	59	61	61	.10				53.2						
14	78	48	57			440.	32		71	56	62	61					106.2						
15	78	44	54			653.	60		73	60	61	61					74.2						
16	83	42	55			619.	20		76	60	61	61					65.9						
17	85	45	59			610.	60		77	59	63	60					76.7						
18	86	47	56			528.	04		79	62	68	62					64.5						
19	85	48	59			546.	96		76	64	67	65	T				84.5						
20	86	45	60			516.	00		84	65	67	65					77.1						
21	82	47	57			553.	84		80	64	68	65	.02				83.9						
22	80	46	58			443.	76		76	63	67	65	T				81.0						
23	75	51	57			380	12		75	62	66	65	.04				77.5						
24	76	50	56			503	96		71	60	65	64	.03				47.6						
25	77	46	55			509.	12		75	60	65	62	T				82.0						
26	75	47	55			412.	80		75	59	65	63					91.8						
27	77	48	53			543.	52		74	60	64	63					71.9						
28	73	47	58			404.	20		75	61	66	63					51.8						
29	72	41	52			361.	20		69	59	65	62					71.0						
30	77	44	52			552	12		69	59	62.	61	.05				110.3						
31	73	42	54			438	60		72	60	65	60					134.8						
Sum	2412	1379	1712			15 289.	08						.28			2	630.7						
Avg.	77.8	44.5	55.2			493.	20					Greatest	.10				84.9	Adjusted Total					

WS FORM E-22 (4-78)

x = 86; 35

U.S. DEPARTMENT OF COMMERCE NOAA NATIONAL WEATHER SERVICE

OBSERVER: S.L.V. Research Center

STATION NUMBER: 05-1458-5

Station: Center | County: Rio Grande | State: CO | Date (Month & yr.): Sept 1981 | Complete Observation Time: 8A | Standard Time in Use: MOT | RECORD OF ... AND CLIMATOLOGI... | ...IONS

DATE	TEMPERATURE °F: 24 Hours Ending at Observation Max.	24 Hours Ending at Observation Min.	At Observation Dry-bulb	At Observation Wet-bulb	At Observation Dew Point	Supplemental Readings at Solar Radiation	Wind Ane-mometer Dial Reading (Miles)	Wind 24 Hour Movement	EVAPORATION (Inches & hundredths): Gage Reading or Amount Added +	Reading When Tank Filled or Amount Removed −	Amount of Evaporation	WATER TEMP. °F: 24 Hours Ending at Observation Max.	Min.	ADDITIONAL DATA/REMARKS
1	69	47	54			428.28		133.7						
2	73	36	50			448.92		89.8						
3	72	35	44			560.72		72.0						
4	75	41	44			533.20		158.3						
5	77	37	49			536.64		115.7						
6	80	40	50			595.12		80.1						
7	74	41	58			345.72		108.6						
8	65	45	50			306.16		84.6						
9	67	43	45			337.12		115.4						
10	55	41	45			405.92		136.8						
11	64	31	38			553.84		92.5						
12	71	33	45			316.48		90.5						
13	70	40	43			299.28		86.2						
14	70	35	42			543.52		157.0						
15	72	34	42			471.28		77.3						
16	69	42	48			417.96		142.1						
17	72	30	38			486.76		157.2						
18	69	36	38			393.88		107.2						
19	73	32	48			435.16		94.6						
20	74	34	38			443.76		159.2						
21	73	26	49			294.12		127.7						
22	70	36	40			318.20		148.0						
23	66	37	45			251.12		123.6						
24	48	39	41			273.48		263.1						
25	58	28	37			431.72		211.0						
26	58	32	39			495.36		248.4						
27	64	23	25			519.44		106.5						
28	66	25	47			464.40		137.4						
29	56	36	43			192.64		75.2						
30	63	22	30			371.52		81.8						
31														
Sum	2033	1059	1311			12,471.72		3781.5						
Avg.	67.8	35.3	43.7			415.72		126.05	Adjusted Total					

PRECIPITATION — Soil Temperature

	Time of beginning 2 Max	Time of ending 11 Min	Time of beginning 6 Max	Time of ending 11 Min	24 Hour Amounts: Rain, Melted snow, etc. (in. & hundredths)	24 Hour Amounts: Snow, Ice Pellets, Hail (in. & tenths)	At Obsn. Snow, Ice Pellets, Hail, Ice on ground (in.)
1	70	56	63	62	T		
2	69	57	63	60	T		
3	69	55	62	59	.02		
4	69	51	62	59			
5	72	55	63	60			
6	72	55	63	60			
7	72	55	63	60			
8	68	55	61	58	.50		
9	63	54	56	55	.09		
10	62	52	58	57	.26		
11	56	45	57	52	T		
12	58	45	55	52			
13	59	48	52	49			
14	59	48	52	49			
15	62	48	53	52			
16	62	47	55	53			
17	62	48	55	54			
18	58	46	53	53			
19	57	49	53	53			
20	61	49	54	53			
21	61	49	57	53			
22	58	43	56	54			
23	56	46	55	54			
24	55	49	54	54			
25	52	44	53	49	.10		
26	45	40	50	50	.02		
27	53	42	49	49			
28	55	42	51	48			
29	57	40	51	47	T		
30	46	42	50	47	.14		
31							
Sum					1.13		
Avg.				Greatest	.50		

WS FORM E-22 (4-78)

X = 80; 22

U.S. DEPARTMENT OF COMMERCE
NOAA
NATIONAL WEATHER SERVICE

OBSERVER: S.L.V. Research Center

STATION NUMBER: 05-1458-5

Ringrot Research

Development of assay methods for Corynebacterium sepedonicum

Beverly Schuld and Monty Harrison

Studies have been underway during the year to test available biological assays for detecting small numbers of Ringrot bacteria (Corynebacterium sepedonicum) in potato tissue.

The eggplant bioassay is the most commonly used biological test for the organism. This method, while known to detect the organism, lacks standardization and critical tests to determine its sensitivity.

Studies during the past year indicate that environmental conditions, such as plant nutrition, plant age, and soil moisture, can affect symptom expression in eggplants. A vacuum inoculation method whereby small numbers of C. sepedonicum cells can be introduced into individual eggplants or potato seedlings has been developed. This will allow studies on the effects of inoculum concentration on symptom expression and thus the determination of test sensitivity. The effects of bacterial strains and inoculation techniques are also being studied.

Eggplants have been successfully propagated in tissue culture. This eliminates plant variability and makes large numbers of plants available for use in bioassays without having to wait for seed germination and seedling growth.

Preliminary tests using tissue cultured potato plantlets for bioassays to detect C. sepedonicum have indicated that this procedure might be superior to the use of eggplants. Studies to date suggest that such plantlets may be more sensitive than eggplants and that they show typical ringrot symptoms much faster than tuber-derived shoots or tomatoes. Symptoms have been visible in potato in about 30 days.

If the optimum conditions for symptom development in potato plantlets can be determined, this method might be useful, especially since it would eliminate the major concern about the susceptibility of eggplants to all strains of C. sepedonicum.

Work has been done on sampling a limited number of seed lots in which visible ringrot symptoms have not been found to determine if ringrot bacteria may be present. Results to date are not conclusive, but work will continue using the most sensitive techniques available and new methods as they are developed.

The Effect of Post-Harvest Treatment on Early-Blight Tuber Infection

Gary D. Franc and Monty D. Harrison

Abstract

Cv. Norchip tubers were harvested, graded and treated prior to storage for early-blight control. After storage at 50°F for ca 4 months, tubers were evaluated to determine the effectiveness of the treatments. Treatments included Captan, Rovral at 5 and 10 ppm and Rovral (5 ppm) combined with BD-11 (10 ppm).

All chemical treatments significantly reduced the average number of early blight lesion per tuber when compared with untreated controls. Chemical treatments did not differ significantly when compared on the basis of the average number of lesions per tuber. However, when data for the percentage of healthy tubers (i.e., tubers with no early blight lesions) were compared, Rovral 5 ppm, Rovral 10 ppm and Rovral 5 ppm + BD-11 10 ppm all performed significantly better than Captan. Rovral 10 ppm was significantly better than Rovral 5 ppm when compared on the basis of the percentage of non-infected tubers. Rovral 5 ppm + BD-11 10 ppm was intermediate between the other two treatments.

Materials and Methods

Potato tubers cv. Norchip were harvested from a commercial field in Morgan County, Colorado, using a mechanical single-row potato digger. Freshly harvested tubers were placed in burlap bags and returned to the CSU Horticultural Farm in Fort Collins for treatment. Tubers were harvested in the morning and treated and stored during the afternoon of the same day (30 September 1986).

Tubers were passed over a mechanical sorter (sizing chains plus a roller table) to remove small tubers and also to approximate the surface (handling) injury typical of many storage operations after harvest. After grading, 100 lbs. of tubers for each treatment were placed in a single layer on burlap bags or newspaper. Spray treatments were applied to the tubers using a small hand-held atomizer and a measured amount of water/chemical suspension. Tubers were rolled and mixed during each individual application to insure uniform coverage. Each 100 lb. sample of treated tubers was split into four 25 lb. samples (replications) and bagged separately. Bagged samples were placed in storage at a regulated temperature of 50°F.

All treatments were applied at the rate of 2.0 gallons of liquid per ton of potatoes. Treatments included: 1) nontreated (dry control); 2) water (wet control); 3) Captan 2.5 lbs 50% wp/100 gallons; 4) Rovral 5 ppm; 5) Rovral 10 ppm, and 6) Rovral 5 ppm + Gustafson-BD11 10 ppm.

After 130 days storage, subsamples of tubers (25 randomly selected tubers per replication) from each treatment were washed in tap water and bleached in 10% chlorox for three minutes. Tubers were rinsed and dried and the number of early blight lesions per tuber was visually counted.

The data for the average number of lesions per tuber and the percentage of tubers with no lesions (i.e., percentage of healthy tubers) were analyzed with a two-way analysis of variance using the MSTAT sofware package and a Zenith personal computer. Tukey's (HSD) test was used for mean separation.

Results and Discussion

Data for the average number of early blight lesions per tuber and the percentage of healthy tubers are shown in Table 1. There were obvious visual differences among treatments, even to the untrained eye.

All chemical treatments significantly reduced the average number of early blight lesions per tuber when compared to the two controls. There were no significant differences among the four chemical treatments when they were compared on the basis of the average number of lesions per tuber. However, when data for the percentage of healthy tubers (i.e., tubers with no lesions) were compared, treatments 4 (Rovral 5 ppm), 5 (Rovral 10 ppm) and 6 (Rovral 5 ppm + BD-11 10 ppm) all performed significantly better than Captan. Treatment 5 (10 ppm Rovral) was significantly better than treatment 4 (5 ppm Rovral) when compared on this basis; treatment 6 (Rovral 5 ppm + BD-11 10 ppm) was intermediate between the two Rovral treatments. The percentage of non-infected tubers in the Captan treatment was not significantly lower than the untreated controls.

The data suggest that rates of Rovral lower than 5 ppm might be effective in controlling early blight infection since the 5 ppm rate was as effective as the 10 ppm rate in reducing lesion numbers. BD-11 does not appear to have any activity against Alternaria solani.

Table 1. The effect of post-harvest treatment on early blight tuber infections - Cv. Norchip. G. D. Franc and M. D. Harrison, 1986.

Treatment[1]	Average No. of Early Blight Lesions per Tuber[2]	Average Percentage of Tubers with no Lesions[2]
1. Nontreated	19.7 a[3]	1% c
2. Water only	19.3 a	0% c
3. Captan[1]	4.0 b	10% c
4. Rovral 5 ppm	1.2 b	46% b
5. Rovral 10 ppm	0.7 b	61% a
6. Rovral 5 ppm + BD-11 10 ppm	1.4 b	57% ab

[1]Treatments were applied at the rate of 2 gal per ton of tubers. Captan was applied at a concentration of 2.5 lbs 50% wp/100 gal.

[2]Each datum entry is the average of four replications. Twenty-five tubers were assayed for each replication.

[3]Treatment means followed by different letters differ significantly (p=0.05).