# ANNUAL REPORT

## Fungus and Bacterial Disease Research

## Summary of 1984 Results and Research Proposal for 1985

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### Introduction

This section summarizes work done in 1984 and includes new proposals and a budget request for 1985. Detailed reports are attached following the summary for those interested in further detail.

### 1. Blackleg Research

Two aspects on the epidemiology of *Erwinia carotovora* were emphasized during 1984.

Studies on the potential for long distance transport of *E. carotovora* in the atmosphere were continued to determine the source of viable cells routinely found in remote surface waters. Studies were also made to determine if a replication of *Erwinia* could occur in surface waters. Studies were also made to determine the significance of irrigation water as a source of contamination by *E. carotovora* in relation to the production of *Erwinia*-free seed potatoes and also to determine if an *Erwinia*-"suppressive" soil would reduce the level of recontamination.

Support from a USDA grant and Advanced Genetic Sciences along with support from the potato industry enabled this research to be done.

Objective a. The long distance transport of Erwinia and replication of Erwinia in surface waters.

A study was initiated in 1983 to determine if the long distance dispersal of E. carotovora in atmospheric aerosols could occur. If cells are being transported, an aerosol source and means of cell deposition, i.e., a "sink", must be identified by the presence of Erwinia.

Preliminary results have shown that an Erwinia serogroup common to ocean water, rain water and aerosol samples collected on the Oregon Coast was also recovered from snow and surface water samples collected at remote sites in the Colorado Rocky Mountains. Overall, the results of this study have shown considerable evidence supporting the hypothesis that long distance transport of E. carotovora occurs in the atmosphere. Considerably more research will be required, however, to obtain the final proof that this does indeed occur.

Laboratory studies have not demonstrated that replication of Erwinia occurs in surface water. These results were based on data generated from batch cultures that were a crude approximation of actual surface water environments. Field studies of Erwinia populations generally showed that the concentration of Erwinia is greatest during the summer months and lowest during the winter months.

Objective b. The role of irrigation water in the recontamination of Erwinia-free seed stocks and the potential for biological control.

A study was made to determine if a specific field soil found in the San Luis Valley would suppress or slow Erwinia-recontamination of Erwinia-free Sangre, Russet Burbank and Centennial Russet potato roots and tubers.

Results showed that Erwinia recontamination of potato roots occurred in the field (Table 1). However, Erwinia was not recovered from daughter tubers. There was no strong evidence for an Erwinia-suppressive factor present in the field soil. Greenhouse studies using the field soil are being made to test the hypothesis further.

Table 1. The effect of different soil sources on *Erwinia* contamination of potato roots, Center, CO, 1984.

| Cultivar: Soil source[1] | Number of colony forming units per gram root ($\bar{X}$)[2] | | | Percentage of root samples with at least one *Erwinia*[3] | | |
|---|---|---|---|---|---|---|
| | 17 Jul | 7 Aug | 27 Aug | 17 Aug | 7 Aug | 27 Aug |
| Sangre: | | | | | | |
| 1) "suppressive" | 354 | 0 | 0 | 25% | 50% | 0% |
| 2) suppressive + 1% conducive | 111 | 0 | 0 | 25% | 50% | 0% |
| 3) "conducive" | 0 | 0 | 0 | 25% | 75% | 0% |
| 4) conducive + 1% suppressive | 0 | 0 | 0 | 25% | 50% | 0% |
| Russet Burbank: | | | | | | |
| 1) "suppressive" | 0 | 0 | 0 | 25% | 50% | 0% |
| 2) suppressive + 1% conducive | 0 | 0 | 0 | 0% | 0% | 0% |
| 3) "conducive" | 0 | 0 | 0 | 0% | 50% | 0% |
| 4) conducive + 1% suppressive | 0 | 0 | 0 | 25% | 25% | 25% |
| Centennial Russet: | | | | | | |
| 1) "suppressive" | 0 | 0 | 0 | 25% | 0% | 0% |
| 2) suppressive + 1% conducive | 0 | 0 | 0 | 25% | 0% | 0% |
| 3) "conducive" | 0 | 0 | 219 | 0% | 75% | 25% |
| 4) conducive + 1% suppressive | 0 | 0 | 0 | 0% | 50% | 0% |

[1] See text for soil source descriptions.

[2] Assay sensitivity was ca 100 cells per gram of root.

[3] Assay sensitivity was ca 1 cell per gram or root.

2. General Potato Pathology

Objective a. To determine the effect of TOPS 2.5 seedpiece treatment on Solanum tuberosum "Centennial Russet" performance.

Cut "Centennial Russet" potato seedpieces were treated with fir bark alone and fir bark + TOPS 2.5 Dust. Seedpieces were planted in the field in the San Luis Valley, CO, and plots were evaluated throughout the growing season.

Although seedpiece treatment with fir bark + TOPS 2.5 Dust significantly increased the total stand ($P = 0.05$) when compared to the fir bark control there were no significant effects on the number of stems per hill, average stem length (plant height), vigor, the estimated percentage of defoliation observed near the end of the growing season, the amount of seedpiece decay, Rhizoctonia stem cankering and the incidence of stem infection by Verticillium ($P = 0.05$). Lastly, seedpiece treatment did not significantly affect total tuber yield, marketable yield, yield of US #1 or US #2 ($P = 0.05$).

Objective b. To determine the effect of fir bark + Zineb and fir bark + Mertect seedpiece treatment on Solanum tuberosum cv. "Centennial Russet" performance.

Cut "Centennial Russet" potato seedpieces were treated with fir bark, fir bark + Zineb and fir bark + Mertect. Treated seedpieces were planted in the field in the San Luis Valley, CO, and plots were evaluated throughout the growing season.

Although both seedpiece treatments significantly increased total stand ($P = 0.05$) when compared to the fir bark control there were no significant effects on the average number of stems per hill, plant height (stem length), plant vigor, defoliation observed near the end of the growing

season, seedpiece decay, Rhizoctonia stem cankering, the incidence of Verticillium stem infection and, lastly, no effect on tuber yield and grade (P = 0.05).

Objective c. To determine the effect of Talc + TBZ seedpiece treatment on Solanum tuberosum cv. "Centennial Russet" performance.

Cut "Centennial Russet" potato seedpieces were treated with fir bark alone and talc + TBZ. Seedpieces were planted in the field in the San Luis Valley, CO, and plots were evaluated throughout the growing season.

Seedpiece treatment with talc + TBZ significantly increased the final stand counts and the average number of stems per hill when compared to the fir bark control (P = 0.05). Although tuber yields for marketable grades were unaffected (P = 0.05), talc + TBZ seedpiece treatment resulted in significantly more grade "B" tubers (P = 0.05). This effect is probably due to the increased number of stems per hill.

There was no effect of seedpiece treatment on the average plant height (stem length), plant vigor, estimated percentage of defoliation observed in the plots near the end of the growing season, the amount of mother seedpiece decay and Rhizoctonia stem cankering observed in the field and, lastly, no effect on the incidence of stem infection by Verticillium (P = 0.05).

3. Ringrot Research

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

The objective of this study is to determine if whole potato tubers

inoculated with different numbers of Corynebacterium sepedonicum (ringrot) cells can produce plants and tuber progeny that visually appear healthy in the field 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 potato production areas. This study was initiated in 1981 and was continued through 1984.

Russet Burbank and Centennial whole tubers (mother tubers) were inoculated with serial dilutions of C. sepedonicum. Results for studies initiated in 1981 (DEP81) showed that plants of both cultivars had no visible foliar ringrot symptom (primary symptom) expression evident during the first growing season (summer 1981) even though individual seed tubers had been inoculated with as many as $6.3 \times 10^8$ cells.

Daughter tubers harvested from DEP81 plots were planted back in the field during 1982. Plants with foliar ringrot symptoms (secondary symptoms) resulted for Russet Burbank but only when the original mother tuber received the maximum inoculation concentration of $6.3 \times 10^8$ 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 for both cultivars appeared healthy. Plant-back of great granddaughter tubers in 1984 resulted in plants that appeared healthy. No symptoms were detected.

Tuber bioassays for 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. Tuber assays in 1983 showed infection for Centennial treatments that had received $10^6$ cells. All tuber assays in 1984 were negative.

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 during the spring of 1983 and 1984. Bioassay of Russet Burbank tubers only showed infection when the original mother tubers received $10^9$ C. sepedonicum cells. Tertiary symptoms did not develop for Russet Burbank tubers planted in the field during 1984. Foliar symptoms developed for Centennial at the $10^9$ and $10^2$ cells per tuber inoculum level.

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. Greenhouse assays of tuber subsamples on eggplant showed that tubers harvested from the inoculum levels greater than or equal to $10^2$

cells per tuber were infected for both cultivars. Secondary foliar symptom development for Russet Burbank occurred for inoculum levels of $10^2$, $10^4$, $10^6$ and $10^9$ cells per tuber and $10^4$, $10^6$ and $10^9$ cells per tuber for Centennial.

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

## Research Proposal for 1985

a) Information from an additional growing season is needed to determine the role of irrigation water in the recontamination of Erwinia-free potatoes.

The recontamination (rates and levels) of Erwinia-free seedlots versus inoculum levels present in irrigation water needs to be determined. Studies must be done in the field since the resident soil microflora may influence recontamination rates.

These studies will be similar to those done in 1984 with one major exception; different inoculum concentrations will be prepared and watered into the treatment plots to determine the relationship of levels of water contamination to recontamination of seed potatoes.

b) Long-distance transport of Erwinia.

This is a continuation of an ongoing research project funded primarily by a USDA grant. Our results have conclusively shown that Erwinia can be routinely recovered from surface waters in Colorado even when collected from remote mountain streams during the winter months. We also have

preliminary evidence that these cells may be transported to Colorado in specific weather systems. Continued research is needed to determine the source of cells found in the snowpack and remote mountain streams. Studies may also be made to determine if cells can replicate in surface waters as well as their ability to survive in various aerosolized states (i.e., environmental conditions approximating those encountered by bacterial aerosols present in cold clouds).

c) Ringrot Research

Two additional growing seasons (1985 and 1986) will be required to finalize the data on the effect of inoculum concentration on ringrot symptom expression in the San Luis Valley. The data currently show that latent infections do result in both Russet Burbank and Centennial. The effects of inoculum concentration, however, vary from year to year.

If time and resources permit, a study will be initiated to determine if the ringrot bacterium can be perpetuated in a symptomless state in micropropagation-derived material.

Proposed Budget for 1985

| | |
|---|---|
| Plot Maintenance | $ 250.00 |
| Labor | 2450.00 |
| Travel | 1500.00 |
| Supplies and Equipment | 1500.00 |
| Total | $5700.00 |

# The Epidemiology of *Erwinia carotovora*

Gary D. Franc and Monty D. Harrison

## Introduction

Two theses on the association of *E. carotovora* with irrigation water in Colorado have been completed at C.S.U. Thesis abstracts from both theses are included in this report.

A preliminary report on the long distance transport of *E. carotovora* is also included. This research is still being continued.

## Association of *Erwinia carotovora* with Irrigation Water in Northeastern Colorado (Pedro E. Jorge):

*Erwinia carotovora* (*Ec*) was found consistently 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. The bacterium was isolated from "undisturbed" surface water as well as from "disturbed" water containing stream bed sediments.

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

*Erwinia* populations in both the "undisturbed" and "disturbed" samples were related to temperature, time of the year and sampling location. Populations of the bacterium in the water ranged from 0 to 700 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) areas during late spring, summer and early autumn.

During the summer months, Ec numbers were consistently higher in "disturbed" than "undisturbed" water samples from sites located in the agricultural areas. This suggests that Erwinia may either multiply in the sediments or be concentrated by the sedimentation of suspended material.

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

Association of Erwinia carotovora with Irrigation Water in Southcentral Colorado (Darrell A. Maddox):

The presence of Erwinia carotovora (Ec) in surface and underground (well) water was studied, using filter concentration and anaerobic enrichment techniques. Ec was commonly isolated from surface water in Southcentral Colorado. Ec was isolated from water samples collected at sites in mountainous (over 80 km from potato producing areas), transitional and arable regions during every month of the year in 1982 and 1983. Erwinia carotovora subsp. carotovora (Ecc) was the predominant subspecies isolated. Of 1,029 strains, 999 (97.1%) were identified as Ecc and 30 (2.9%) as Erwinia carotovora subsp. atroseptica (Eca). Eca was found primarily in water samples collected in the spring months and from arable regions. Erwinia chrysanthemi was never isolated from water in Southcentral Colorado.

Ec populations were generally low in the Rio Grande river and Saguache Creek. However, populations reached over 6,000 colony forming units (cfu)/l of water in samples collected in late summer and autumn months from arable regions. Month and sample site had the most significant effects on Ec

populations. Water temperature, pH, water turbidity and stream flow rate had no significant effects on Ec populations.

Ec was isolated infrequently from well water samples collected in the San Luis Valley. Only 15.8% of the samples yielded Ec and it was only detected after filter concentration of 3.4 to 10 l ($\bar{X}$ = 6.9 l) of water.

## Long Distance Dispersal of Erwinia in Atmospheric Aerosols (Gary D. Franc):

A study was initiated in 1983 to determine if the long distance dispersal of E. carotovora in atmospheric aerosols could occur. A preliminary report of the results was made (Franc et al., 1984a, b).

Ocean water samples were collected from sites along ca 285 km of Oregon coastline during extensive surveys made in 1983 and 1984 (Franc et al., 1984b). Of 202 E. carotovora strains recovered, 182 (90%) were identified as Ecc and 20 (10%) as Eca. Results clearly demonstrated that both Ecc and Eca were present in the Pacific Ocean surf as well as water collected several kilometers away from shore.

Rain water samples were also collected at several locations near Newport, Oregon during the same period of time that ocean water surveys were made. Seventeen separate rain water samples were collected. Of these, 16 (94%) yielded E. carotovora. Although relatively large volumes of rain water were routinely processed for each sample ($\bar{X}$ = 3.8 l), in 41% of them E. carotovora could be detected by enriching 50 ml subsamples, showing that at least one viable cell was present. In ocean water 50% of the 50 ml subsamples had at least one viable cell present. A total of 177 strains of E. carotovora isolated from rain water were characterized; 75% were Ecc and 25% Eca.

Aerosol collections on the Oregon coast in December 1983 yielded 16 stains of E. carotovora (7 Ecc and 9 Eca) collected during the same time that ocean water and rain samples were collected. The relative humidity was always near 99% during the period when successful collections were made. Airborne cells of E. carotovora were collected whether or not rain was falling. Extensive aerosol sampling in March failed to detect E. carotovora probably due to unusually warm dry weather and extensive sunshine which prevailed during that period.

If cells are transported through the atmosphere they should appear inland in the precipitation which would serve as the proposed "sink" or means of cell deposition. Viable E. carotovora cells were recovered from fresh snow samples collected in Colorado, Oregon and Utah (Franc et al., 1984a).

One of eight samples (13%) collected approximately 424 km inland from the Oregon coast yielded E. carotovora. All strains (6) were characterized as Ecc. Two of six (33%) samples collected in Utah were also Erwinia positive. Twenty-one strains from this source were identified; all were Ecc.

The most extensive series of snow samples was collected over a two year period in Colorado. All samples were collected during the winter months at remote sites in the Rocky Mountains. Snow cover ranged from ca 1.5 to 3.0 m during the period collections were made. Of 105 snow samples processed ($\overline{X}$ = 6.7 l water), 12 (11%) yielded E. carotovora. Ecc was recovered from eight of the positive samples and Eca from four. Fifty-nine Ecc and 36 Eca strains were identified. Erwinia-positive snow samples were collected from Rabbit Ears Pass (elevation 2873 m), Cameron Pass (elevation 3135 m) and Gore Pass (elevation 2917 m).

Surface water samples were also collected monthly from the Elk and Yampa rivers (Franc et al., 1984a) in areas near the sites where snow samples were collected in Colorado. Results showed the E. carotovora was recovered

from water throughout the year. All strains (153) were characterized as Ecc.

Numerous strains recovered from all of the sources i.e., ocean water, coastal rain, coastal aerosols, inland snow and mountain stream water, were serotyped using the scheme developed by De Boer et al. (1979). The distribution of Ecc serogroups recovered from the different sources is shown in Table 1. The recovery of serogroups common to all sources was considered additional evidence for long distance transport of viable cells of E. carotovora.

Preliminary results have shown that a serogroup common to ocean water, rain water and aerosol samples collected on the Oregon Coast was also recovered from snow and surface water samples collected at remote sites within the Colorado Rocky Mountains (Table 1). Overall, the results of this study, to date, have shown considerable evidence supporting the hypothesis that long distance transport of E. carotovora occurs in the atmosphere. Considerably more research will be required, however, to obtain the final proof that this does indeed occur.

## References Cited

1. De Boer, S.H., R.J. Copeman, and H. Vruggink. 1979. Serogroups of Erwinia carotovora potato strains determined with diffusible somatic antigens. Phytopathology 69:316-319.

2. Franc, G.D., M.D. Harrison, and D. Maddox. 1984a. The presence of Erwinia carotovora in snow and surface water in the United States. Proc. Int. Conf. on Potato Blackleg Disease. Edinburgh, Scotland. 26-29 June, 1984. (in press).

3. Franc, G.D., M.D. Harrison, and M.L. Powelson. 1984b. The presence of Erwinia carotovora in ocean water, rain water and aerosols. Proc. Int. Conf. on Potato Blackleg Disease. Edinburgh, Scotland. 26-29 June, 1984. (in press).

Table 1. The distribution of Erwinia carotovora subsp. carotovora (Ecc) serogroups recovered from ocean water, rain water and aerosols collected on the Oregon coast and from snow and surface water collected in the Rocky Mountains of Colorado.

| Serogroup Designation[1] | Percentage[2] of Ecc strains in each serogroup recovered from: | | | | |
|---|---|---|---|---|---|
| | Ocean water | Rain | Aerosols | Snow | Surface water |
| III | 1.5 | 6.1 | -- | -- | -- |
| IV | 4.5 | -- | -- | -- | 4.5 |
| V | 0.8 | -- | -- | -- | 3.6 |
| VII | 0.8 | -- | -- | -- | -- |
| IX | --[3] | 1.0 | -- | -- | -- |
| XI | 0.8 | -- | -- | -- | -- |
| XII | 2.3 | -- | -- | -- | 1.8 |
| XIII | -- | 1.0 | -- | -- | -- |
| XIV | 0.8 | -- | -- | -- | -- |
| XV | -- | -- | -- | -- | 19.6 |
| XVI | -- | -- | -- | 10.3 | 14.3 |
| XVIII | -- | -- | -- | -- | 2.7 |
| XIX | 1.5 | 1.0 | -- | -- | -- |
| XXVIII | -- | 1.0 | -- | -- | -- |
| XXIX | 11.3 | 7.1 | 42.9 | 2.6 | 4.5 |
| XXXIII | 4.5 | 3.0 | -- | -- | -- |
| XXXIV | -- | 2.0 | -- | 5.1 | 2.7 |
| XXXV | 0.8 | -- | -- | -- | -- |
| XXXVIII | 1.5 | -- | -- | -- | -- |
| XXXIX | -- | -- | -- | 5.1 | -- |
| CC601 | -- | -- | -- | -- | 2.7 |
| CC602 | 10.5 | 13.1 | -- | -- | 11.6 |
| CC651 | 0.8 | 6.1 | -- | 10.3 | 4.5 |
| CC652 | 6.8 | 1.0 | -- | -- | -- |

[1]Serogroup designations after De Boer et al. (1979). Most strains were tested against a range of 30 different sera. Only reactions of identity are presented in the table.

[2]The percentage totals for strains recovered from a given source do not equal 100% because it was not possible to assign all strains to a specific serogroup. The total number of strains tested were: ocean water, 133; rain, 99; aerosols, 7; snow, 39; surface water, 112.

[3]Serogroup not detected.

Preliminary Report: Testing of different soils for suppression of *Erwinia* recontamination of *Erwinia*-free potatoes in the San Luis Valley.

Gary D. Franc and Monty D. Harrison

## Abstract

A study was made to determine if a specific field soil found in the San Luis Valley would suppress or slow *Erwinia*-recontamination of *Erwinia*-free Sangre, Russet Burbank and Centennial Russet potato roots and tubers.

Results showed that *Erwinia* recontamination of potato roots occurred in the field. However, *Erwinia* was not recovered from daughter tubers. There was no strong evidence for an *Erwinia*-suppressive factor present in the field soil. Greenhouse studies using the field soil are being made to test the hypothesis further.

## Introduction

A field cropped continuously to potatoes for more than 20 years consistently had fewer weed roots and potato roots with detectable levels of *Erwinia* present when compared to other fields in the San Luis Valley. Reports are in the literature concerning reduction of *Erwinia* contamination of potatoes. This effect is reported to be due to PGPR bacteria that are commonly found in the soil. Therefore, a study was made to determine if the soil in question was 1) suppressive and 2) if suppressive, whether or not the suppressive agent was biological and could, therefore, be transferred to a conducive soil.

Field plots using *Erwinia*-free seed potatoes were planted in the San Luis Valley to test this hypothesis. A greenhouse experiment using potted

soil and Kochia scoparia as a bioassay was also made. This is a preliminary report because not all experiments have been completed.

## Materials and Methods

Erwinia-free tubers derived from a micropropagation program were used in the study. The tubers were planted by hand into rows at one foot intervals. Blank rows were left between planted rows. Cultivars Sangre, Russet Burbank and Centennial Russet were planted for the study.

Two separate field plots were planted with two soil sources (treatments) within each field plot. Four replications were included for each treatment. The first field plot was the apparently "suppressive" soil and the two soil sources were: 1) the original suppressive soil and 2) the original suppressive soil + 1% (v/v) conducive soil roto-tilled into the row prior to planting. The second field plot was "conducive" soil and the two soil sources were: 3) the original "conducive" soil and 4) conducive soil + 1% (v/v) suppressive soil roto-tilled into the row prior to planting. The first and second field plots were irrigated by center pivot and furrow irrigation, respectively. The water was periodically assayed and was found to contain detectable levels of Erwinia during the growing season.

Potato plant roots were assayed three times during the growing season to determine the level of re-contamination versus soil type. Plant roots (one plant per treatment per replication) were collected on 17 July, 7 August and 27 August. Roots were individually bagged, placed on ice and returned to Ft. Collins for assay. Roots were snipped into piles and weighed and placed into 10 ml of sterile deionized water. The suspensions were vigorously mixed and an 0.1 ml aliquot plated onto crystal violet

pectate medium for detection of Erwinia (direct plating). After plating, 10 ml of twice strength enrichment growth medium was added to the root suspensions for detection of Erwinia following a four-day anaerobic incubation (direct enrichment). Theoretically, direct enrichment will detect one living Erwinia cell on the root sample and was therefore ca 100 times more sensitive than direct plating in the experiment.

Tubers were harvested from the plots on 18 September and stored until assay in December and January. Five tubers per treatment plot (240 tubers total) were wrapped in wet paper towels and incubated anaerobically for four days at 22°C to trigger soft-rot pocket formation. Tissue removed from rot pockets was suspended in sterile distilled water, mixed vigorously and streaked onto growth medium for detection of Erwinia. Aseptic technique was practiced throughout all assays.

## Results

There is no evidence to suggest that soil included in the study is Erwinia suppressive (Table 1). Most direct platings failed to detect Erwinia. Assay sensitivity was ca 100 cells per gram of root. However, direct enrichment of roots (assay sensitivity ca one cell per gram of root) showed that many roots were contaminated.

The data show that potato plants grown from Erwinia-free tubers can become contaminated during the first growing season. However, tuber assays failed to detect Erwinia after ca 3 months storage at 40°F (data not shown). The negative results may be due to the sample size assayed.

## Discussion

Previous assays in the same field plots (1980, 1981, 1982 and 1983) showed that very few weed and potato roots were contaminated with *Erwinia*. This was surprising since the plots were routinely irrigated with *Erwinia*-contaminated water and also since weed-root surveys at other locations in the San Luis Valley showed that contaminated roots could be routinely found. The results of this experiment do not support the previous observation because contaminated potato roots could be readily found in the same plots previously believed to be suppressive. It is possible that the suppressive factor is no longer present due to changes in the cropping of the field plot during the last two years.

Root populations were very low for the plants in the field plots. Higher populations, i.e., by watering in *Erwinia* inoculum, may allow differentiation between soil types included in the study. "Baiting" of, or selecting for, suppressive biological agents found in soil has been done using plant pathogens in other disease situations. If the *Erwinia* populations were too low in the field in 1984, triggering of a biological *Erwinia*-suppressive agent may not have occurred. Greenhouse studies using higher *Erwinia* inoculum levels are now being made.

Table 1. The effect of different soil sources on *Erwinia* contamination of potato roots, Center, CO, 1984.

| Cultivar: Soil source[1] | Number of colony forming units per gram root ($\bar{X}$)[2] | | | Percentage of root samples with at least one *Erwinia*[3] | | |
|---|---|---|---|---|---|---|
| | 17 Jul | 7 Aug | 27 Aug | 17 Aug | 7 Aug | 27 Aug |
| *Sangre*: | | | | | | |
| 1) "suppressive" | 354 | 0 | 0 | 25% | 50% | 0% |
| 2) suppressive + 1% conducive | 111 | 0 | 0 | 25% | 50% | 0% |
| 3) "conducive" | 0 | 0 | 0 | 25% | 75% | 0% |
| 4) conducive + 1% suppressive | 0 | 0 | 0 | 25% | 50% | 0% |
| *Russet Burbank*: | | | | | | |
| 1) "suppressive" | 0 | 0 | 0 | 25% | 50% | 0% |
| 2) suppressive + 1% conducive | 0 | 0 | 0 | 0% | 0% | 0% |
| 3) "conducive" | 0 | 0 | 0 | 0% | 50% | 0% |
| 4) conducive + 1% suppressive | 0 | 0 | 0 | 25% | 25% | 25% |
| *Centennial Russet*: | | | | | | |
| 1) "suppressive" | 0 | 0 | 0 | 25% | 0% | 0% |
| 2) suppressive + 1% conducive | 0 | 0 | 0 | 25% | 0% | 0% |
| 3) "conducive" | 0 | 0 | 219 | 0% | 75% | 25% |
| 4) conducive + 1% suppressive | 0 | 0 | 0 | 0% | 50% | 0% |

[1] See text for soil source descriptions.

[2] Assay sensitivity was ca 100 cells per gram of root.

[3] Assay sensitivity was ca 1 cell per gram or root.

Appendix I. Number of *Erwinia* CFU per gram of potato root.

| Field | Cultivar | Soil[1] | Replication | | | | | Replication | | | | | Replication | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | I | II | III | IV | $\bar{X}$ | I | II | III | IV | $\bar{X}$ | I | II | III | IV | $\bar{X}$ |
| "Suppressive"[5] | Sangre: | 1) | 0 | 0 | 0 | 1414 | 354 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 2) | 444 | 0 | 0 | 0 | 111 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | R. Burbank: | 1) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 2) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Centennial: | 1) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 2) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| "Conducive"[6] | Sangre: | 3) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 4) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | R. Burbank: | 3) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 4) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Centennial: | 3) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8763 | 0 | 0 | 0 | 2191 |
| | | 4) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

[1] 1) = original "suppressive" soil; 2) = original suppressive + 1% (v/v) conducive; 3) = original "conducive" soil; and 4) = original conducive soil + 1% (v/v) suppressive.

[2] Assays on 17 July, 1984.

[3] Assays on 7 August, 1984.

[4] Assays on 27 August, 1984.

[5] Field soil cropped continuously to potatoes for 20+ years.

[6] Field soil cropped to potatoes for two years.

Appendix II. Number of potato roots *Erwinia* positive after direct enrichment (one root assayed per treatment replication).

| Field | Cultivar | Soil[1] | Replication[2] | | | | | Replication[3] | | | | | Replication[4] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | I | II | III | IV | % | I | II | III | IV | % | I | II | III | IV | % |
| "Suppressive"[5] | Sangre: | 1) | 0 | 0 | 0 | 1 | 25 | 0 | 0 | 1 | 1 | 50 | 0 | 0 | 0 | 0 | 0 |
| | | 2) | 1 | 0 | 0 | 0 | 25 | 1 | 1 | 0 | 0 | 50 | 0 | 0 | 0 | 0 | 0 |
| | R. Burbank: | 1) | 1 | 0 | 0 | 0 | 25 | 1 | 0 | 0 | 1 | 50 | 0 | 0 | 0 | 0 | 0 |
| | | 2) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Centennial: | 1) | 0 | 1 | 0 | 0 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | | 2) | 0 | 0 | 1 | 0 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| "Conducive"[6] | Sangre: | 3) | 0 | 0 | 1 | 0 | 25 | 0 | 1 | 1 | 1 | 75 | 0 | 0 | 0 | 0 | 0 |
| | | 4) | 1 | 0 | 0 | 0 | 25 | 1 | 1 | 0 | 0 | 50 | 0 | 0 | 0 | 0 | 0 |
| | R. Burbank: | 3) | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 50 | 0 | 0 | 0 | 0 | 0 |
| | | 4) | 0 | 0 | 1 | 0 | 25 | 0 | 1 | 0 | 0 | 25 | 0 | 0 | 0 | 1 | 25 |
| | Centennial: | 3) | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 75 | 1 | 0 | 0 | 0 | 25 |
| | | 4) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 50 | 0 | 0 | 0 | 0 | 0 |

[1] 1) = original "suppressive" soil; 2) = original suppressive + 1% (v/v) conducive; 3) = original "conducive" soil; and 4) = original conducive soil + 1% (v/v) suppressive.

[2] Assays on 17 July, 1984.

[3] Assays on 7 August, 1984.

[4] Assays on 27 August, 1984.

[5] Field soil cropped continuously to potatoes for 20+ years.

[6] Field soil cropped to potatoes for two years.

Final Report: Gustafson

# The Effect of TOPS 2.5 Seedpiece Treatment on *Solanum tuberosum* "Centennial Russet" Performance

Gary D. Franc, Monty D. Harrison and Darrell A. Maddox

## Abstract

Cut "Centennial Russet" potato seedpieces were treated with fir bark alone and fir bark + TOPS 2.5 Dust. Seedpieces were planted in the field in the San Luis Valley, CO and plots were evaluated throughout the growing season.

Although seedpiece treatment with fir bark + TOPS 2.5 Dust significantly increased the total stand ($P = 0.05$) when compared to the fir bark control there were no significant effects on the number of stems per hill, average stem length (plant height), vigor, the estimated percentage of defoliation observed near the end of the growing season, the amount of seedpiece decay, *Rhizoctonia* stem cankering and the incidence of stem infection by *Verticillium* ($P = 0.05$). Lastly, seedpiece treatment did not significantly affect total tuber yield, marketable yield, yield of US #1 or US #2 ($P = 0.05$).

## Materials and Methods

A field with sandy loam soil cropped continuously to potatoes for ca. 23 years was selected for the study. The field was located near Center, CO in the San Luis Valley (SLV). The SLV is a high desert valley (2316 m) that receives ca. 18 cm rain each year. Therefore, successful crop production requires irrigation throughout the growing season.

Centennial Russet certified seed potatoes (1983 crop) were used as a seed source. Seed tubers were cut by hand into ca. 57 g (2 ounces)

seedpieces using clean knives. Knives were dipped in ethanol and flamed prior to cutting seed for each replication. Freshly cut seed for each replication (6.35 kg) was placed into a sterile paper bag and 63.5 g of seedpiece treatment dust was slowly added while agitating the bag. This resulted in a net application rate of 0.01 kg dust per 1 kg cut seed (1.0 lbs per cwt). Uniform seedpiece coverage was achieved by rotating and shaking the bag and caking of the seedpieces did not occur. The maximum time between cutting and seedpiece treatment was 1 hr. Seed tubers were warmed prior to cutting and treated seedpieces were planted within 24 hr after treatment. Recommended rates of fertilizer were applied "in-row" at the time of planting.

Each seedpiece treatment plot was planted with a tractor drawn assist-feed 2-row potato planter. Each treatment plot consisted of two planted rows 13.7 m (45 ft) long with blank rows on each side and a 2.1 m (7 ft) buffer on each end. Treatment plots were planted in a randomized complete-block design of four replications and five treatments. Soil moisture was good at the time of planting and plots were irrigated by a center pivot overhead sprinkler throughout the growing season.

Total stand and emergence rates were determined by counting the total number of hills emerged in each treatment plot. The total number of emerged hills was determined on 10 June and 19 June.

Plant vigor was estimated in each plot throughout the growing season using a scale of 1-10. The control treatment plot (fir bark alone) was arbitrarily assigned a value of 5 and more vigorous plots were rated at values greater than 5 and less vigorous plots at values less than 5. Vigor ratings were based on general treatment plot appearance and considered plant color, uniformity, canopy fullness, height, etc. Vigor ratings were

made on 17 July, 7 August and 27 August. Actual stem height (soil line to stem apex) was also determined on 17 July and 7 August. Ten stems per treatment plot were measured.

Ratings for defoliation (estimated percentage of plant canopy detached) were done on 27 August. A Barratt-Horsfall (BH) rating scale (0-11) was used to estimate percentages.

The percentages of mother seedpiece decay and Rhizoctonia stem cankering were estimated on 17 July. Five hills (plants) in each treatment plot were randomly selected and uprooted and the amount of decay for each seedpiece estimated using the BH scale. The amount of stem cankered below the soil line was estimated for each stem in the hill using the same scale.

Verticillium infection was measured directly by assaying four stems per treatment plot in the laboratory. Stems were collected in the field on 7 August; leaves were stripped from the stems and then stems were surface disinfested in 10% chlorox for several minutes. Stems were rinsed in cool tap water and a cross section from the top, middle and bottom portion of each stem was aseptically excised and placed onto water agar. Culture plates were incubated at room temperature for 7-10 days and the presence of Verticillium determined by viewing stem sections for the presence of characteristic Verticillium conidiophores.

Treatment plots were harvested with a two-row potato digger on 18 September. Tubers were picked by hand into baskets and then weighed to determine total yield. A 23 kg (ca. 50 lb) sample from each plot was graded to determine the proportion of the total yield present in each grade. 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. If the F ratio was significant ($P \leq 0.05$), an LSD value was calculated and used to

compare seedpiece treatment means to that of the fir bark control. This is considered a conservative use of the LSD value (Fisher's protected LSD).

## Results

Seedpiece treatment with fir bark + TOPS 2.5 did not significantly increase early stand counts (10 June) but did significantly increase the final stand when compared to the fir bark control (Table 1) ($P = 0.05$).

Seedpiece treatment with fir bar + TOPS 2.5 did not affect the number of stems per hill (Table 1), plant height (Table 2), treatment plot vigor (Table 3), the percentage of stems infected by *Verticillium* (Table 4) and the estimated percentage of mother seedpiece decay and *Rhizoctonia* stem cankering observed in the field (Table 4) ($P = 0.05$).

The effect of seedpiece treatment with fir bark + TOPS 2.5 on yield and tuber grade was statistically equivalent to that of the fir bark control (Table 5) ($P = 0.05$).

Table 1. The effect of seedpiece treatment on cv. Centennial Russet plant stand and the average number of stems per plot - Center, CO, 1984.

| Treatment | Average plant stand[1] determined on: 10 June | 19 June | Number of stems per hill ($\bar{X}$)[2] |
|---|---|---|---|
| 1) Fir Bark | 16.4 | 37.9 | 4.95 |
| 2) Fir Bark + TOPS 2.5 | 14.0 | 41.3*[3] | 5.15 |
| | NSD | P = 0.05 | NSD |

[1] Average stand for four replications of two rows for each treatment. Perfect stand is 45 plants (45 ft of row at 1 ft seedpiece spacing).

[2] Five hills per treatment plot were evaluated on 17 July 1984.

[3] Means in columns designated by "*" differ significantly from the fir bark control (P = 0.05) (Fisher's protected LSD). NSD = no significant differences.

Table 2. The effect of seedpiece treatment on cv Centennial Russet potato plant height - Center, CO, 1984.

| Treatment | Average plant height[1] (cm): 17 July | 7 August |
|---|---|---|
| 1) Fir Bark | 40.3 | 40.5 |
| 2) Fir Bark + TOPS 2.5 | 40.1 | 40.0 |
| | NSD[2] | NSD |

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

[2] Means designated by "*" differ significantly (P = 0.05) from the fir bark control (LSD test). NSD = No significant differences were observed.

Table 3. The effect of seedpiece treatment on cv Centennial Russet potato plant vigor and defoliation - Center, CO, 1984.

| Treatment | Relative vigor ($\bar{X}$)[1] as determined on: | | | Estimated percentage of defoliation: |
|---|---|---|---|---|
| | 17 July | 7 Aug. | 27 Aug. | 27 Aug. |
| 1) Fir Bark | 5.0 | 5.0 | 5.0 | 37.0 |
| 3) Fir Bark + TOPS 2.5 | 4.5 | 4.8 | 4.5 | 37.0 |
| | NSD[2] | NSD | NSD | NSD |

[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] Means designated by "*" are significantly different from the fir bark control (P = 0.05). Mean separation was done using the LSD test. NSD = no significant differences were observed.

Table 4. The effect of seedpiece treatment on seedpiece decay, Rhizoctonia stem canker, and Verticillium wilt infection in the field - Center, CO, 1984.

| Treatment | Seedpiece decay[1] (% of seedpiece decayed) | Rhizoctonia stem canker[2] (% stem cankered) | Verticillium wilt[3] (% of stems infected) |
|---|---|---|---|
| 1) Fir Bark | 61.5 | 1.2 | 68.8 |
| 2) Fir Bark + TOPS 2.5 | 31.0 | 1.6 | 62.5 |
| | NSD[4] | NSD | NSD |

[1] Seedpieces (5 per treatment plot) were rated (0-11) on 17 July to estimate the percentage of the seedpiece decayed.

[2] Stems from 5 hills per treatment plot were rated (0-11) on 17 July to estimate the percentage of stem cankered below the soil line.

[3] Stems (4 per treatment plot) were plated onto water agar to detect internal stem infection by Verticillium. Stems were collected on 7 August.

[4] Column means designated by "*" differ significantly (P = 0.05) from the fir bark control. NSD = no significant differences were observed.

Table 5. The effect of seedpiece treatment on total yield and grade (cwt/A) of cv Centennial Russet tubers - Center, CO, 1984.

| Treatment | Tuber yield (cwt/A)[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Total yield | Market-able[2] | US #1 | | Total US #1 | US #2 | Grade B | Culls |
| | | | <10 oz | >10 oz | | | | |
| 1) Fir Bark | 167 | 93 | 87 | 2 | 86 | 6 | 63 | 2 |
| 2) Fir Bark + TOPS 2.5 | 141 | 81 | 74 | 1 | 75 | 6 | 55 | 2 |
| | NSD[3] | NSD | NSD | NSD | NSD | NSD | NSD | NSD |

[1] Treatment plot size was 90 ft of row. 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 designated by "*" differ significantly (P = 0.05) from the fir bark control (Treatment 1). Fisher's protected LSD test was used for comparison of means. NSD = no significant differences were observed.

# Final Report: Schall Chemical Company

## The Effect of Seedpiece Treatment on *Solanum tuberosum* cv. "Centennial Russet" Performance

Gary D. Franc, Monty D. Harrison and Darrell A. Maddox


## Abstract

Cut "Centennial Russet" potato seedpieces were treated with fir bark, fir bark + Zineb and fir bark + Mertect. Treated seedpieces were planted in the field in the San Luis Valley, CO, and plots were evaluated throughout the growing season.

Although both seedpiece treatments significantly increased total stand ($P = 0.05$) when compared to the fir bark control there were no significant effects on the average number of stems per hill, plant height (stem length), plant vigor, defoliation observed near the end of the growing season, seedpiece decay, *Rhizoctonia* stem cankering, the incidence of *Verticillium* stem infection and, lastly, no effect on tuber yield and grade ($P = 0.05$).


## Materials and Methods

A field with sandy loam soil cropped continuously to potatoes for ca. 23 years was selected for the study. The field was located near Center, CO in the San Luis Valley (SLV). The SLV is a high desert valley (2316 m) that receives ca. 18 cm rain each year. Therefore, successful crop production requires irrigation throughout the growing season.

Centennial Russet certified seed potatoes (1983 crop) were used as a seed source. Seed tubers were cut by hand into ca. 57 g (2 ounces) seedpieces using clean knives. Knives were dipped in ethanol and flamed

prior to cutting seed for each replication. Freshly cut seed for each replication (6.35 kg) was placed into a sterile paper bag and 63.5 g of seedpiece treatment dust was slowly added while agitating the bag. This resulted in a net application rate of 0.01 kg dust per 1 kg cut seed (1.0 lbs per cwt). Uniform seedpiece coverage was achieved by rotating and shaking the bag and caking of the seedpieces did not occur. The maximum time between cutting and seedpiece treatment was 1 hr. Seed tubers were warmed prior to cutting and treated seedpieces were planted within 24 hr after treatment. Recommended rates of fertilizer were applied "in-row" at the time of planting.

Each seedpiece treatment plot was planted with a tractor drawn assist-feed 2-row potato planter. Each treatment plot consisted of two planted rows 13.7 m (45 ft) long with blank rows on each side and a 2.1 m (7 ft) buffer on each end. Treatment plots were planted in a randomized complete-block design of four replications and five treatments. Soil moisture was good at the time of planting and plots were irrigated by a center pivot overhead sprinkler throughout the growing season.

Total stand and emergence rates were determined by counting the total number of hills emerged in each treatment plot. The total number of emerged hills was determined on 10 June and 19 June.

Plant vigor was estimated in each plot throughout the growing season using a scale of 1-10. The control treatment plot (fir bark alone) was arbitrarily assigned a value of 5 and more vigorous plots were rated at values greater than 5 and less vigorous plots at values less than 5. Vigor ratings were based on general treatment plot appearance and considered plant color, uniformity, canopy fullness, height, etc. Vigor ratings were made on 17 July, 7 August and 27 August. Actual stem height (soil line to

stem apex) was also determined on 17 July and 7 August. Ten stems per treatment plot were measured.

Ratings for defoliation (estimated percentage of plant canopy detached) were done on 27 August. A Barratt-Horsfall (BH) rating scale (0-11) was used to estimate percentages.

The percentages of mother seedpiece decay and Rhizoctonia stem cankering were estimated on 17 July. Five hills (plants) in each treatment plot were randomly selected and uprooted and the amount of decay for each seedpiece estimated using the BH scale. The amount of stem cankered below the soil line was estimated for each stem in the hill using the same scale.

Verticillium infection was measured directly by assaying four stems per treatment plot in the laboratory. Stems were collected in the field on 7 August; leaves were stripped from the stems and then stems were surface disinfested in 10% chlorox for several minutes. Stems were rinsed in cool tap water and a cross section from the top, middle and bottom portion of each stem was aseptically excised and placed onto water agar. Culture plates were incubated at room temperature for 7-10 days and the presence of Verticillium determined by viewing stem sections for the presence of characteristic Verticillium conidiophores.

Treatment plots were harvested with a two-row potato digger on 18 September. Tubers were picked by hand into baskets and then weighed to determine total yield. A 23 kg (ca. 50 lb) sample from each plot was graded to determine the proportion of the total yield present in each grade. 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. If the F ratio was significant ($P \leq 0.05$), an LSD value was calculated and used to compare seedpiece treatment means to that of the fir bark control. This is considered a conservative use of the LSD value (Fisher's protected LSD).

## Results

There was no effect of seedpiece treatment on early stand counts determined on 10 June ($P = 0.05$) (Table 1). However, seedpiece treatment with fir bark + Zineb and fir bark + Mertect significantly increased the final stand counts determined on 19 June when compared to the fir bark control ($P = 0.05$).

Seedpiece treatment did not affect the average number of stems per hill (Table 1), plant height (Table 2), plant vigor and the amount of defoliation observed near the end of the growing season (Table 3), the amount of seedpiece decay, *Rhizoctonia* stem cankering and incidence of *Verticillium* infection (Table 4), and, lastly, no effect on tuber yield or grade (Table 5) ($P = 0.05$).

Table 1. The effect of seedpiece treatment on cv. Centennial Russet plant stand and the average number of stems per plot - Center, CO, 1984.

| Treatment | Average plant stand[1] determined on: 10 June | 19 June | Number of stems per hill ($\bar{X}$)[2] |
|---|---|---|---|
| 1) Fir Bark | 16.4 | 37.9 | 4.95 |
| 3) Fir Bark + Zineb | 17.4 | 44.0*[3] | 4.25 |
| 4) Fir Bark + Mertect | 15.3 | 41.8* | 5.30 |
| | NSD | P = 0.05 | NSD |

[1] Average stand for four replications of two rows for each treatment. Perfect stand is 45 plants (45 ft of row at 1 ft seedpiece spacing).

[2] Five hills per treatment plot were evaluated on 17 July 1984.

[3] Means in columns designated by "*" differ significantly from the fir bark control (P = 0.05) (Fisher's protected LSD). NSD = no significant differences.

Table 2. The effect of seedpiece treatment on cv Centennial Russet potato plant height - Center, CO, 1984.

| Treatment | Average plant height[1] (cm): 17 July | 7 August |
|---|---|---|
| 1) Fir Bark | 40.3 | 40.5 |
| 3) Fir Bark + Zineb | 43.2 | 43.8 |
| 4) Fir Bark + Mertect | 44.2 | 43.0 |
| | NSD[2] | NSD |

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

[2] Means designated by "*" differ significantly (P = 0.05) from the fir bark control (LSD test). NSD = No significant differences were observed.

Table 3. The effect of seedpiece treatment on cv Centennial Russet potato plant vigor and defoliation - Center, CO, 1984.

| Treatment | Relative vigor ($\bar{X}$)[1] as determined on: | | | Estimated percentage of defoliation: |
|---|---|---|---|---|
| | 17 July | 7 Aug. | 27 Aug. | 27 Aug. |
| 1) Fir Bark | 5.0 | 5.0 | 5.0 | 37.0 |
| 3) Fir Bark + Zineb | 6.3 | 6.8 | 5.0 | 46.0 |
| 4) Fir Bark + Mertect | 5.5 | 5.8 | 4.8 | 28.0 |
| | NSD[2] | NSD | NSD | NSD |

[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] Means designated by "*" are significantly different from the fir bark control (P = 0.05). Mean separation was done using the LSD test. NSD = no significant differences were observed.

Table 4. The effect of seedpiece treatment on seedpiece decay, Rhizoctonia stem canker, and Verticillium wilt infection in the field - Center, CO, 1984.

| Treatment | Seedpiece decay[1] (% of seedpiece decayed) | Rhizoctonia stem canker[2] (% stem cankered) | Verticillium wilt[3] (% of stems infected) |
|---|---|---|---|
| 1) Fir Bark | 61.5 | 1.2 | 68.8 |
| 3) Fir Bark + Zineb | 17.0 | 2.0 | 87.5 |
| 4) Fir Bark + Mertect | 59.5 | 1.2 | 87.5 |
| | NSD[4] | NSD | NSD |

[1] Seedpieces (5 per treatment plot) were rated (0-11) on 17 July to estimate the percentage of the seedpiece decayed.

[2] Stems from 5 hills per treatment plot were rated (0-11) on 17 July to estimate the percentage of stem cankered below the soil line.

[3] Stems (4 per treatment plot) were plated onto water agar to detect internal stem infection by Verticillium. Stems were collected on 7 August.

[4] Column means designated by "*" differ significantly (P = 0.05) from the fir bark control. NSD = no significant differences were observed.

Table 5. The effect of seedpiece treatment on total yield and grade (cwt/A) of cv Centennial Russet tubers - Center, CO. 18 September, 1984.

| Treatment | Tuber yield (cwt/A)[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Total yield | Market-able[2] | US #1 | | Total US #1 | US #2 | Grade B | Culls |
| | | | <10 oz | >10 oz | | | | |
| 1) Fir Bark | 167 | 93 | 87 | 2 | 86 | 6 | 63 | 2 |
| 3) Fir Bark + Zineb | 181 | 110 | 101 | 0 | 101 | 8 | 66 | 1 |
| 4) Fir Bark + Mertect | 179 | 114 | 107 | 1 | 107 | 7 | 61 | 1 |
| | NSD[3] | NSD | NSD | NSD | NSD | NSD | NSD | NSD |

[1] Treatment plot size was 90 ft of row. 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 designated by "*" differ significantly (P = 0.05) from the fir bark control (Treatment 1). Fisher's protected LSD test was used for comparison of means. NSD = no significant differences were observed.

Final Report: J.R. Simplot Company

# The Effect of Talc + TBZ Seedpiece Treatment on *Solanum tuberosum* cv "Centennial Russet" Performance

Gary D. Franc, Monty D. Harrison and Darrell A. Maddox

## Abstract

Cut "Centennial Russet" potato seedpieces were treated with fir bark alone and talc + TBZ. Seedpieces were planted in the field in the San Luis Valley, CO, and plots were evaluated throughout the growing season.

Seedpiece treatment with talc + TBZ significantly increased the final stand counts and the average number of stems per hill when compared to the fir bark control ($P = 0.05$). Although tuber yields for marketable grades were unaffected ($P = 0.05$), talc + TBZ seedpiece treatment resulted in significantly more grade "B" tubers ($P = 0.05$). This effect is probably due to the increased number of stems per hill.

There was no effect of seedpiece treatment on the average plant height (stem length), plant vigor, estimated percentage of defoliation observed in the plots near the end of the growing season, the amount of mother seedpiece decay and *Rhizoctonia* stem cankering observed in the field and, lastly, no effect on the incidence of stem infection by *Verticillium* ($P = 0.05$).

## Materials and Methods

A field with sandy loam soil cropped continuously to potatoes for ca. 23 years was selected for the study. The field was located near Center, CO

in the San Luis Valley (SLV). The SLV is a high desert valley (2316 m) that receives ca. 18 cm rain each year. Therefore, successful crop production requires irrigation throughout the growing season.

Centennial Russet certified seed potatoes (1983 crop) were used as a seed source. Seed tubers were cut by hand into ca. 57 g (2 ounces) seedpieces using clean knives. Knives were dipped in ethanol and flamed prior to cutting seed for each replication. Freshly cut seed for each replication (6.35 kg) was placed into a sterile paper bag and 63.5 g of seedpiece treatment dust was slowly added while agitating the bag. This resulted in a net application rate of 0.01 kg dust per 1 kg cut seed (1.0 lbs per cwt). Uniform seedpiece coverage was achieved by rotating and shaking the bag and caking of the seedpieces did not occur. The maximum time between cutting and seedpiece treatment was 1 hr. Seed tubers were warmed prior to cutting and treated seedpieces were planted within 24 hr after treatment. Recommended rates of fertilizer were applied "in-row" at the time of planting.

Each seedpiece treatment plot was planted with a tractor drawn assist-feed 2-row potato planter. Each treatment plot consisted of two planted rows 13.7 m (45 ft) long with blank rows on each side and a 2.1 m (7 ft) buffer on each end. Treatment plots were planted in a randomized complete-block design of four replications and five treatments. Soil moisture was good at the time of planting and plots were irrigated by a center pivot overhead sprinkler throughout the growing season.

Total stand and emergence rates were determined by counting the total number of hills emerged in each treatment plot. The total number of emerged hills was determined on 10 June and 19 June.

Plant vigor was estimated in each plot throughout the growing season using a scale of 1-10. The control treatment plot (fir bark alone) was arbitrarily assigned a value of 5 and more vigorous plots were rated at values greater than 5 and less vigorous plots at values less than 5. Vigor ratings were based on general treatment plot appearance and considered plant color, uniformity, canopy fullness, height, etc. Vigor ratings were made on 17 July, 7 August and 27 August. Actual stem height (soil line to stem apex) was also determined on 17 July and 7 August. Ten stems per treatment plot were measured.

Ratings for defoliation (estimated percentage of plant canopy detached) were done on 27 August. A Barratt-Horsfall (BH) rating scale (0-11) was used to estimate percentages.

The percentages of mother seedpiece decay and Rhizoctonia stem cankering were estimated on 17 July. Five hills (plants) in each treatment plot were randomly selected and uprooted and the amount of decay for each seedpiece estimated using the BH scale. The amount of stem cankered below the soil line was estimated for each stem in the hill using the same scale.

Verticillium infection was measured directly by assaying four stems per treatment plot in the laboratory. Stems were collected in the field on 7 August; leaves were stripped from the stems and then stems were surface disinfested in 10% chlorox for several minutes. Stems were rinsed in cool tap water and a cross section from the top, middle and bottom portion of each stem was aseptically excised and placed onto water agar. Culture plates were incubated at room temperature for 7-10 days and the presence of Verticillium determined by viewing stem sections for the characteristic Verticillium conidiophores.

Treatment plots were harvested with a two-row potato digger on 18 September. Tubers were picked by hand into baskets and then weighed to

determine total yield. A 23 kg (ca. 50 lb) sample from each plot was graded to determine the proportion of the total yield present in each grade. 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. If the F ratio was significant ($P \leq 0.05$), an LSD value was calculated and used to compare seedpiece treatment means to that of the fir bark control. This is considered a conservative use of the LSD value (Fisher's protected LSD).

## Results

Seedpiece treatment with talc + TBZ did not significantly affect early stand counts determined on 10 June (Table 1) ($P = 0.05$). However, seedpiece treatment did significantly increase final stand counts determined on 19 June ($P = 0.05$). Treatment with talc + TBZ also significantly increased the average number of stems per hill when compared to the fir bark control ($P = 0.05$). There was no effect of seedpiece treatment with talc + TBZ on the average plant height (stem length), plant vigor, estimated percentage of defoliation observed near the end of the growing season, the amount of mother seedpiece decay and *Rhizoctonia* stem cankering observed in the field and the percentage of stems infected by *Verticillium* ($P = 0.05$) (Tables 2-4).

Although seedpiece treatment with talc + TBZ did not significantly increase total yield, marketable yield or yield of tuber grades US #1 or US #2, it did significantly increase yield of "B" grade tubers (tubers less than ca. 4 ounces) (Table 5) ($P = 0.05$).

Discussion

Seedpiece treatment with talc + TBZ significantly increased the stand in the treatment plots, but there was no measurable effect on total yield or marketable yield.

Seedpiece treatment with talc + TBZ increased the average number of stems per hill when compared to the fir bark control which accounts for the concomitant significant increase in the yield of smaller (B grade) tubers observed in the field. It is generally accepted that an increased number of stems per hill is correlated with an increased number of tubers of a smaller average tuber size. This apparently is the result of tubers produced by each stem competing for limited physical space and nutrients which is undesirable in the San Luis Valley where the growing season is relatively short (ca. 120 days). Under conditions of a longer growing season an increased average number of stems per hill may not be as detrimental since tubers would have sufficient time to "size up" to one of the more desirable grades.

The increased stem numbers per hill each year in the San Luis Valley due to talc + TBZ may not be "real" since a formulation of fir bark plus Mertect also included in the same field trials in 1984 did not significantly increase the average number of stems per hill. However, Mertect treatment of an experimental S. tuberosum clone (WNC230-14) in separate field trials (San Luis Valley) did result in an undesirable increase in the number of small tubers (M. Workman, personal communication). There is also a report that seedpiece treatment with Mertect in Idaho resulted in a reduction in the number of tubers greater than 10 ounces suggesting, once again, that Mertect (TBZ) seedpiece treatments reduced tuber size.

Table 1. The effect of seedpiece treatment on cv. Centennial Russet plant stand and the average number of stems per plot - Center, CO, 1984.

| Treatment | Average plant stand[1] determined on: 10 June | 19 June | Number of stems per hill ($\bar{X}$)[2] |
|---|---|---|---|
| 1) Fir Bark | 16.4 | 37.9 | 4.95 |
| 2) Fir Bark + TOPS 2.5 | 14.0 | 41.3*[3] | 5.15 |
| | NSD | P = 0.05 | NSD |

[1] Average stand for four replications of two rows for each treatment. Perfect stand is 45 plants (45 ft of row at 1 ft seedpiece spacing).

[2] Five hills per treatment plot were evaluated on 17 July 1984.

[3] Means in columns designated by "*" differ significantly from the fir bark control (P = 0.05) (Fisher's protected LSD). NSD = no significant differences.

Table 2. The effect of seedpiece treatment on cv Centennial Russet potato plant height - Center, CO, 1984.

| Treatment | Average plant height[1] (cm): 17 July | 7 August |
|---|---|---|
| 1) Fir Bark | 40.3 | 40.5 |
| 2) Fir Bark + TOPS 2.5 | 40.1 | 40.0 |
| | NSD[2] | NSD |

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

[2] Means designated by "*" differ significantly (P = 0.05) from the fir bark control (LSD test). NSD = No significant differences were observed.

Table 3. The effect of seedpiece treatment on cv Centennial Russet potato plant vigor and defoliation - Center, CO, 1984.

| Treatment | Relative vigor ($\bar{X}$)[1] as determined on: | | | Estimated percentage of defoliation: |
|---|---|---|---|---|
| | 17 July | 7 Aug. | 27 Aug. | 27 Aug. |
| 1) Fir Bark | 5.0 | 5.0 | 5.0 | 37.0 |
| 5) Talc + TBZ | 5.8 | 6.0 | 4.8 | 46.0 |
| | NSD[2] | NSD | NSD | NSD |

[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] Means designated by "*" are significantly different from the fir bark control (P = 0.05). Mean separation was done using the LSD test. NSD = no significant differences were observed.

Table 4. The effect of seedpiece treatment on seedpiece decay, Rhizoctonia stem canker, and Verticillium wilt infection in the field - Center, CO, 1984.

| Treatment | Seedpiece decay[1] (& of seedpiece decayed) | Rhizoctonia stem canker[2] (% stem cankered) | Verticillium wilt[3] (% of stems infected) |
|---|---|---|---|
| 1) Fir Bark | 61.5 | 1.2 | 68.8 |
| 5) Talc + TBZ | 79.0 | 2.0 | 56.2 |
| | NSD[4] | NSD | NSD |

[1] Seedpieces (5 per treatment plot) were rated (0-11) on 17 July to estimate the percentage of the seedpiece decayed.

[2] Stems from 5 hills per treatment plot were rated (0-11) on 17 July to estimate the percentage of stem cankered below the soil line.

[3] Stems (4 per treatment plot) were plated onto water agar to detect internal stem infection by Verticillium. Stems were collected on 7 August.

[4] Column means designated by "*" differ significantly (P = 0.05) from the fir bark control. NSD = no significant differences were observed.

Table 5. The effect of seedpiece treatment on total yield and grade (cwt/A) of cv Centennial Russet tubers - Center, CO, 18 September, 1984.

| Treatment | Tuber yield (cwt/A)[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Total yield | Market-able[2] | US #1 <10 oz | US #1 >10 oz | Total US #1 | US #2 | Grade B | Culls |
| 1) Fir Bark | 167 | 93 | 87 | 2 | 86 | 6 | 63 | 2 |
| 5) Talc + TBZ | 179 | 90 | 83 | 0 | 83 | 7 | 85*[3] | 1 |
| | NSD[3] | NSD | NSD | NSD | NSD | NSD | P = 0.05 | NSD |

[1] Treatment plot size was 90 ft of row. 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 designated by "*" differ significantly (P = 0.05) from the fir bark control (Treatment 1). Fisher's protected LSD test was used for comparison of means. NSD = no significant differences were observed.

# 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 was to determine if whole potato tubers inoculated with different numbers of *Corynebacterium sepedonicum* (ringrot) cells can produce plants and tuber progeny that appear visually healthy in the field but are, nevertheless, infected (latent infection). The occurrence of latent ringrot infection could 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 1984. It will be continued through 1986.

Whole Russet Burbank and Centennial Russet tubers (mother 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 $6.3 \times 10^8$ cells.

Daughter tubers harvested from DEP81 plots were planted back in the field during 1982. Plants with foliar ringrot symptoms (secondary symptoms) were found in Russet Burbank but only when the original mother tuber received the maximum inoculum concentration ($6.3 \times 10^8$ *C. sepedonicum* cells). All Centennial plants were symptomless during 1982. When granddaughter tubers (i.e., with respect to original seedpieces inoculated in 1981 study) were planted in the field in 1983 symptom development

occurred only in Centennial plants whose grandmother seedpieces received 10 C. sepedonicum cells in 1981. All other plants in both cultivars appeared healthy. Plant-back of great granddaughter 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 when mother tubers had received $10^4$ cells (Russet Burbank) or $10^6$ cells (Centennial). Tuber assays in 1983 showed infection only in Centennial treatments that had received $10^6$ cells. All tuber assays in 1984 were negative.

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 (i.e., the first year in the field) only occurred when Russet Burbank mother tubers received inoculum concentrations of $10^6$ and $10^9$ cells. Primary symptoms did not occur in Centennial. Secondary foliage symptoms (in 1983) occurred in Russet Burbank only in the treatment receiving the plants from $10^4$ cells per seed tuber. Both the higher and lower inoculum loads produced healthy appearing plants. Secondary symptoms in Centennial occurred at the two highest inoculum concentrations ($10^9$ and $10^6$ cells). This corresponded with tuber bioassays done in the greenhouse during the springs of 1983 and 1984. Bioassays of Russet Burbank tubers only detected infection when the original mother tubers received $10^9$ C. sepedonicum cells. Tertiary symptoms 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.

In DEP83 studies, plants showed primary symptom development for the

$10^4$, $10^6$, and $10^9$ inoculum concentrations in Russet Burbank and $10^1$, $10^4$, $10^6$, and $10^9$ in Centennial. In treatments where Centennial mother tubers received $10^2$ cells the plants appeared healthy. Visual inspection of daughter tubers at harvest detected symptoms when mother tubers received inoculum concentrations of $10^6$ and $10^9$ cells in Russet Burbank and $10^4$, $10^6$, and $10^9$ in Centennial. Greenhouse assays of 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. Secondary foliar symptom development in Russet Burbank occurred when inoculum levels of $10^2$, $10^4$, $10^6$ and $10^9$ cells per tuber were used and in Centennial when $10^4$, $10^6$ and $10^9$ cells per tuber were applied.

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 which 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 Center 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 isolate of C. sepedonicum (CS43) 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 $10^3$ 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 (Whatman #2 was used for the DEP83 study). Serial dilutions of the filtrate were made and lightly strained with 1-2 drops of crystal violet. Aliquots of stained filtrate were placed in a Petroff-Hauser counting chamber and the number of cells per 2.5 x $10^{-3}$ $mm^2$ square were counted using a microscope at 45 X. Fifty squares were counted to determine the average number of cells per 2.5 x $10^{-3}$ $mm^2$. The chamber was then 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:$\bar{X}$ = 318 cells/2.5 x $10^{-3}$ $mm^2$, S = 0.625). An average of 6.34 x $10^9$ cells per

ml were determined to be present in the undiluted filtrate. Serial dilutions were made to achieve cell suspensions of $6.34 \times 10^8$, $1 \times 10^7$, $1 \times 10^5$, $1 \times 10^3$ and $1 \times 10^2$ 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:$\bar{X}$ = 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 $10^{-8}$ 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 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 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:$\bar{X}$ = 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.

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 was counted (20 squares) (rep. I:$\bar{X}$ = 11.60, rep. II:$\bar{X}$ = 10.20; rep. III:$\bar{X}$ = 11.25, rep. IV:$\bar{X}$ = 10.50). The original suspension had a concentration of $2.1775 \times 10^{11}$ 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.) and May 31, 1984 (Ft. Collins). 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 Certifiction 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 DEP82 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. Tubers were not harvested for the DEP84 plots in Ft. Collins.

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 plantd 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 tubers were inoculated with as many as $6.34 \times 10^8$ *C. sepedonicum* cells per tuber (Table 1).

Daughter tubers 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, daughter 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).

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 whose grandmother tubers had been inoculated with $6.3 \times 10^8$ cells. Foliar symptoms were not observed in similarly treated Centennial plants.

Bioassays and field plantings were again repeated in 1983 using granddaughters 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. 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 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 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 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. Progeny tubers from these treatmentswill be planted once again 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 daughter 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 daughter 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 sewd tubers were inoculated with $10^4$ cells or more in 1983.

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. 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.

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

| 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 spring 1983 | Tertiary foliage symptoms[4] summer 1983 | Quaternary foliage symptoms[5] summer 1984 |
|---|---|---|---|---|---|---|
| Russet Burbank: | | | | | | |
| $6.3 \times 10^8$ | - | + | + | - | - | - |
| $10^6$ | - | + | - | - | - | - |
| $10^4$ | - | + | - | - | - | - |
| $10^2$ | - | - | - | - | - | - |
| $10^1$ | - | - | - | - | - | - |
| Buffer | - | - | - | - | - | - |
| Centennial: | | | | | | |
| $6.3 \times 10^8$ | - | + | - | - | - | - |
| $10^6$ | - | + | - | + | - | - |
| $10^4$ | - | - | - | - | - | - |
| $10^2$ | - | - | - | - | - | - |
| $10^1$ | - | - | - | - | + | - |
| 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.

[5] Granddaughter tubers harvested in 1983 were replanted in the field in 1983 (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, 1984.

| 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 | Grand-daughter tuber infection spring 1984 | Tertiary foliage symptoms[4] summer 1984 |
|---|---|---|---|---|---|---|
| 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.

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

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

| Cultivar | Number of cells per mother tuber | Primary foliage symptoms[1] summer, 1983 | Daughter tuber infection[2] fall, 1983 | Daughter tuber infection[3] spring, 1984 | Secondary foliage symptoms[4] summer, 1984 |
|---|---|---|---|---|---|
| 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 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).

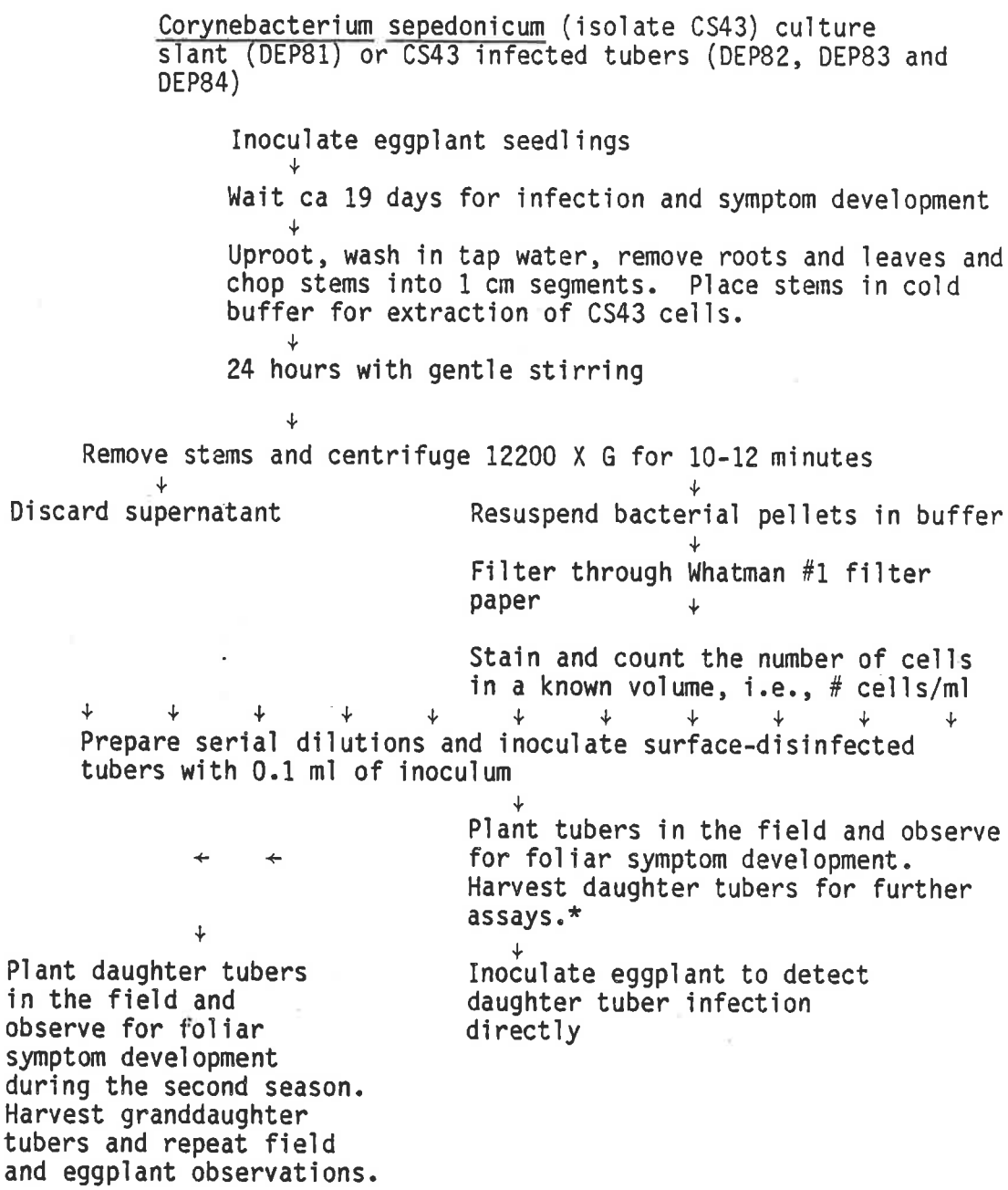
| Serial Dilution Plated ( cells/ml)[1] | Number of C. sepedeonicum cells per ml as determined by viable counts[2] |
|---|---|
| $10^{10}$ | TNTC[3] |
| $10^{7}$ | TNTC |
| $10^{5}$ | TNTC |
| $10^{3}$ | 353 ± 129 |
| $10^{2}$ | 130 ± 39 |
| Buffer | 0 |

[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.




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