1985-86

ANNUAL REPORT

# Fungus and Bacterial Disease Research

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## Introduction

This section summarizes work done in 1985 and includes new proposals and a budget request for 1986. Where applicable, detailed reports are attached to the summary for those interested in additional information.

## 1. Blackleg Research

Research on the epidemiology of *Erwinia carotovora* was emphasized during 1985. Our past research has shown that *Erwinia* can be routinely recovered from surface irrigation water in the San Luis Valley and other potato producing areas. Research has also shown that *Erwinia* can be isolated infrequently from natural precipitation in Colorado. Studies in 1985 were made to determine the significance of contaminated irrigation water on blackleg disease incidence in the field and the extent of daughter tuber infection. Studies were also continued to determine factors involved with the long distance transport of *Erwinia* in the atmosphere and its potential relationship to production of *Erwinia*-free potatoes.

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

A study was initiated in 1985 studying the effect of *Erwinia* contaminated irrigation water on seedlot performance and daughter tuber contamination. This research is being funded primarily by the Western

Regional IPM Program. An identical study is being done by Dr. Mary L. Powelson and her associates at OSU in Corvallis, Oregon. The generous donation of land and equipment by Bob Kula in NE Colorado and Jim and Rob Jones in the SLV made it possible for this work to be done. This report is nothing more than a brief introduction to the experiment and its objectives followed by several summary statements on observations made during the growing season. A formal report will be prepared at the end of the study.

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

Results showed that the typical blackleg observed in Colorado did not appear to be obviously associated with contaminated irrigation water used in the study. Erwinia was found to be present on leaf surfaces, but once again, it is not clear at this point if those populations can be attributed to the use of contaminated irrigation water. We observed very little aerial blackleg in the field lots in 1985. Erwinia strains recovered during the course of the experiment consisted of both Ecc and Eca. Strains

are being serologically tested to assist in determining their source. In contrast to Colorado, results for Oregon showed a greater incidence of aerial blackleg (stem soft-rot) and a predominance of Ecc strains recovered from leaves and infected stems. All daughter tuber assays are being made at CSU.

Objective b. **The Relationship of Contaminated Irrigation Water to Daughter Tuber Contamination and Blackleg**

Field plots were established at the Bay Farm, Fort Collins, CO and at the SLVRC, Center, CO. Erwinia-free microplants, provided by Dr. K.W. Knutson, were used to plant the plots. Each plot was irrigated three times during the growing season with natural irrigation wter spiked with known numbers of Ecc per ml. the four treatments applied were, natural water and natural water plus $10^2$, $10^3$ and $10^4$ Ecc $ml^{-1}$, respectively.

Blackleg symptoms were not observed in the plots at any time during the growing season. However, as Ecc concentration in irrigation water increased, the percentage of stems with internal Erwinia populations also increased. Tuber data also show that, as treatment (inoculum) level increased, the number of treatment plots with Erwinia contaminated tubers also increased. More extensive analyses of the data are being made to determine if the extent of tuber contamination can be predicted by measuring populations of Erwinia present in irrigation water.

Erwinia strains recovered from stems and tubers are being serologically tested to further verify their source. It may be possible to determine the contribution of the natural water and soil to the amount of recontamination seed in the field. Data will be analyzed when these assays are completed.

Objective c. **The Presence and Survival of Erwinia carotovora in Natural Snow**

Natural snow was collected and assayed for the presence of Erwinia. Results show that viable Erwinia can be infrequently recovered from snow collected in the Rocky Mountains.

In a separate study, natural snow was inoculated with suspensions of Erwinia carotovora subsp. carotovora (Ecc) and E. c. subsp. atroseptica (Eca) and stored at ca -20°C. Direct plating results showed that Eca populations declined by ca 70% and Ecc by ca 100% after storage for 28 days. Enrichment results showed that viable cells of Ecc and Eca could be readily recovered after 28 days even though Ecc populations were undetectable by direct plating.

2. Ringrot Research

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

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

## DEP81 STUDY (initiated 1981, terminated 1984)

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

Daughter tubers (generation 1 - G1) harvested from DEP81 plots were planted back in the field during 1982. Plants with foliar ringrot symptoms (secondary symptoms) were found in RB but only when the original mother tuber received the maximum inoculum concentration ($6.3 \times 10^8$ C. sepedonicum cells). All CR plants were symptomless during 1982. When generation 2 (G2) tubers were planted in the field in 1983, symptom development occurred only in CR plants when original seed tubers received 10 C. sepedonicum cells in 1981. All other plants for both cultivars appeared healthy. Plant-back of generation 3 (G3) tubers in 1984 produced plants that all appeared healthy.

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

## DEP82 STUDY (initiated 1982, terminated 1985)

Primary symptoms for DEP82 (i.e., the first year in the field) only occurred when RB mother tubers received inoculum concentrations of $10^6$ and $10^9$ cells. Primary symptoms did not occur in CR plants. Secondary foliage

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

DEP83 (initiated 1983, terminated 1986)

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

plants showed infection for treatments $10^2$ and $10^6$ for RB and $10^9$ for CR.

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

## Research Proposal for 1986

M. D. Harrison and G. D. Franc

Emphasis during the 1986 season will be primarily in two main areas of research. First, experiments to complete the current work on blackleg bacteria, especially their long range dispersal and the role of contaminated irrigation water in current season losses and recontamination of seed tuber crops. Second, to significantly expand our work on the epidemiology of bacterial ringrot in the San Luis Valley (and in Colorado generally). A new graduate student has just begun work on ringrot and our main emphasis will be focused on better understanding of the disease. We will finish the work started some time ago on the effects of low numbers of bacteria on ringrot infection and symptom expression. Results indicate that when tubers are exposed to low numbers of cells recognizable symptoms may not appear for several generations. This raises the question of whether or not small numbers of cells may be carried in stem cuttings or tissue cultured plants without being detected due to the lack of visible symptoms. This will be studied in some depth over the next 3-5 years along with work on possible additional sources of the ringrot bacterium and the factors which affect its expression, particularly in the San Luis Valley.

Specific Studies:

A. Blackleg.

1. Continued studies on the effect of the use of Erwinia-contaminated irrigation water on current season losses and recontamination of seed tubers (this study is jointly funded by the Potato Administrative Committee and the Western Regional IPM Project). Crops irrigated with contaminated and non-contaminated water will be compared.
2. Long distance transport of Erwinia cells with storm systems and their deposition in surface water will be continued and hopefully completed by the end of this year. Studies on the replication of Erwinia in water will be continued. Results from this study will be integrated with those from study A1 to help determine if Erwinia cells transported for long distances are finally deposited on potatoes by irrigation water (we hope to supplement Potato Administrative Committee funds with funds from the Environmental Protection Agency to finish this work).

B. Bacterial Ringrot.

1. Unless new information generated in 1986 changes our plans, this will be the final year for the study on the relationship between inoculum concentration and ringrot symptom expression. Data to date show that ringrot bacteria can be present in tuber seedlots for at least 2 or 3 generations without producing visible symptoms.
2. The possibility of ringrot bacteria being present in stem cuttings or microplants without producing symptoms will be studied.
3. The ability of stem cutting/micro-propogation programs to eliminate ringrot bacteria from known infected tubers will be investigated.

4. Studies will be initiated on the possibility of other sources of ringrot bacteria other than infected tubers and contaminated equipment and storage.
5. Studies of factors other than inoculum levels affecting ringrot expression will be initiated.

It is our intent to study ringrot intensively during at least the next 3-5 years in a manner similar to the way the blackleg problem was approached. The study areas outlined above cannot be completed in one year but a start can be made in all of the areas. We believe that ringrot is enough of a threat to the potato industry (both seed and table stock producers) that it is time for a major effort to be made to better understand and, hopefully, control the disease.

Budget Request for 1986
Fungus and Bacterial Disease Research
(Blackleg and Ringrot)

| | |
|---|---|
| Plot Maintenance | $ 300.00 |
| Labor | 2600.00 |
| Travel | 1700.00 |
| Supplies and Equipment | 2000.00 |
| Total | $6600.00 |

# THE RELATIONSHIP OF CONTAMINATED IRRIGATION WATER TO DAUGHTER TUBER CONTAMINATION AND BLACKLEG

G.D. Franc and M.D. Harrison

Abstract

Field plots were established at the Bay Farm, Fort Collins, CO and at the SLVRC, Center, CO. Erwinia-free microplants, provided by Dr. K.W. Knutson, were used to plant the plots. Each plot was irrigated three times during the growing season with natual irrigation water spiked with known numbers of Ecc per ml. The four treatments applied were, natural water and natural water plus $10^2$, $10^3$ and $10^4$ Ecc $ml^{-1}$, respectively.

Blackleg symptoms were not observed in the plots at any time during the growing season. However, as Ecc concentration in irrigation water increased, the percentage of stems with internal Erwinia populations also increased.

Tuber data also show that, as treatment (inoculum) level increased, the number of treatment plots with Erwinia contaminated tubers also increased. More extensive analyses of the data are being made to determine if the extent of tuber contamination can be predicted by measuring populations of Erwinia present in irrigation water.

Erwinia strains recovered from stems and tubers are being serologically tested to further verify their source. It may be possible to determine the contribution of the natural water and soil to the amount of recontamination seen in the field. Data will be analyzed when these assays are completed.

## Materials and Methods

Identical field plots were established at the Bay Farm, Fort Collins, CO and the San Luis Valley Research, Center, CO. *Erwinia*-free microplants, provided by Dr. K.W. Knutson, were used to plant the plots. Three cultivars, Norgold Russet (four replications), Sangre (four replications) and Russet Burbank (eight replications) were planted in each field plot. Each replication consisted of two hills and each hill consisted of two microplants. Both field plots were watered by overhead irrigation on a regular basis during the growing season.

During the growing season, each plot was irrigated with the natural water from each location spiked with known numbers of *Erwinia carotovora* subsp. *carotovora* (*Ecc*). Treatment levels were uninoculated water, 100 cells per ml, 1000 cells per ml and 10,000 cells per ml. The natural water had detectable levels of *Erwinia* present. These inoculations were done three times at each location.

Tubers were harvested at the end of the growing season and assayed for surface populations of *Ecc*. Assay sensitivity was approximately 1 *Ecc* per 10 grams of tuber peel. Stems were also assayed for internal populations of *Erwinia* at the end of the growing season. Above-ground portions of the stem, ca 2 cm long, were surface disinfested by dipping in ethanol and flaming. The stem section was split with a sterile scalpel and enriched to determine if *Erwinia* was present.

A greenhouse experiment, using the same number of replications and field soil collected from both field plots, was also done. The tubers are currently being assayed to determine the level of contamination versus inoculum concentration.

Field Study Results:

Blackleg symptoms were not observed in the plots at any time during the growing season. However, data in Figure 1 show that Erwinia could be recovered from internal stems at both field locations (FC = Fort Collins, SLV = San Luis Valley). As Ecc concentration in water increased, the percentage of stems with internal Erwinia populations also increased.

Tuber data, Figure 2, also show that, as treatment (inoculum) level increased, the number of treatment plots with Erwinia contaminated tubers also increased. More extensive analyses of the data are being made to determine if the extent of tuber contamination can be predicted by measuring populations of Erwinia in irrigation water.

Erwinia strains recovered from stems and tubers are being serologically tested to further verify their source. It may be possible to determine the contribution of the natural water and soil to the amount of recontamination seen in the field. Data will be analyzed when these assays are completed.

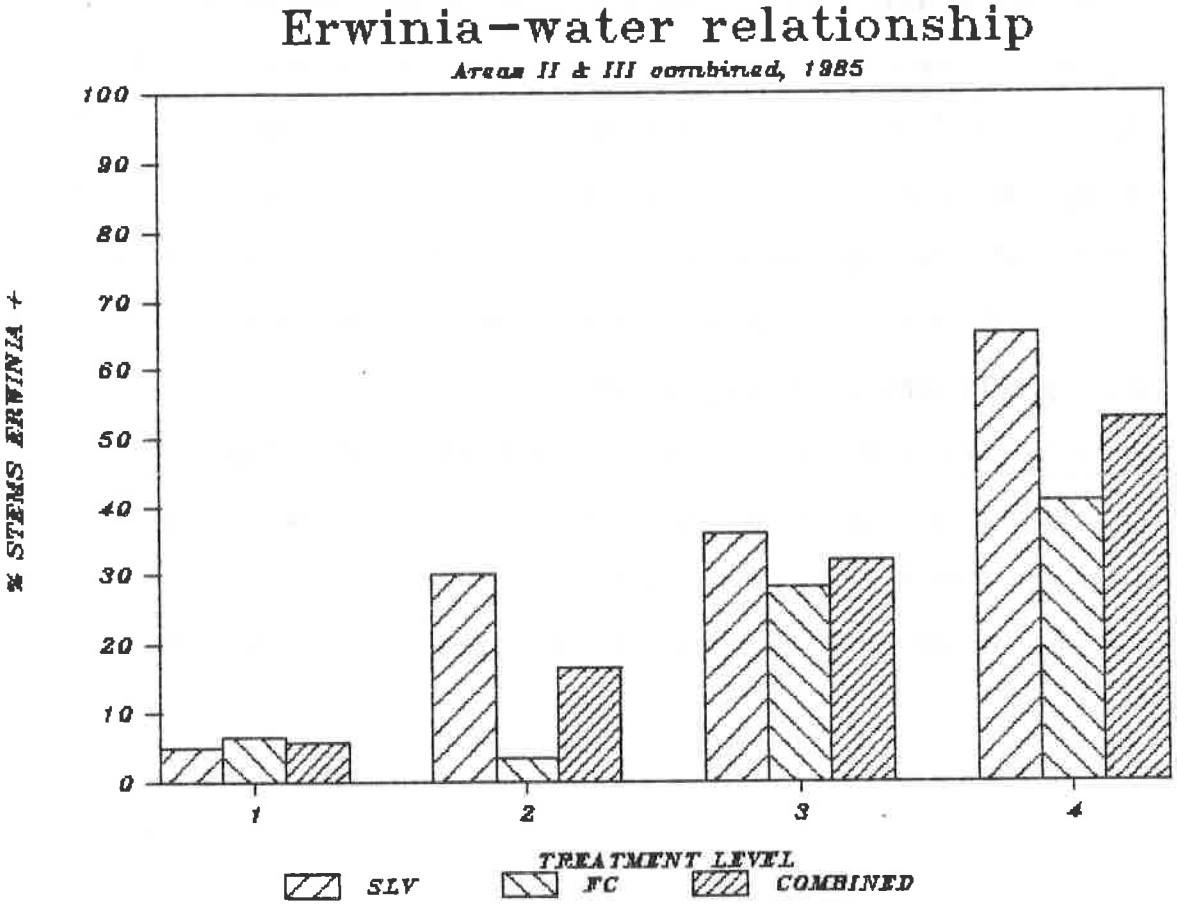



Figure 1. The percentage of stems infected by Erwinia versus the contamination of Ecc in irrigation water. Data are for field plots located in the San Luis Valley (SLV), at Fort Collins (FC) and for combined data from both plots.

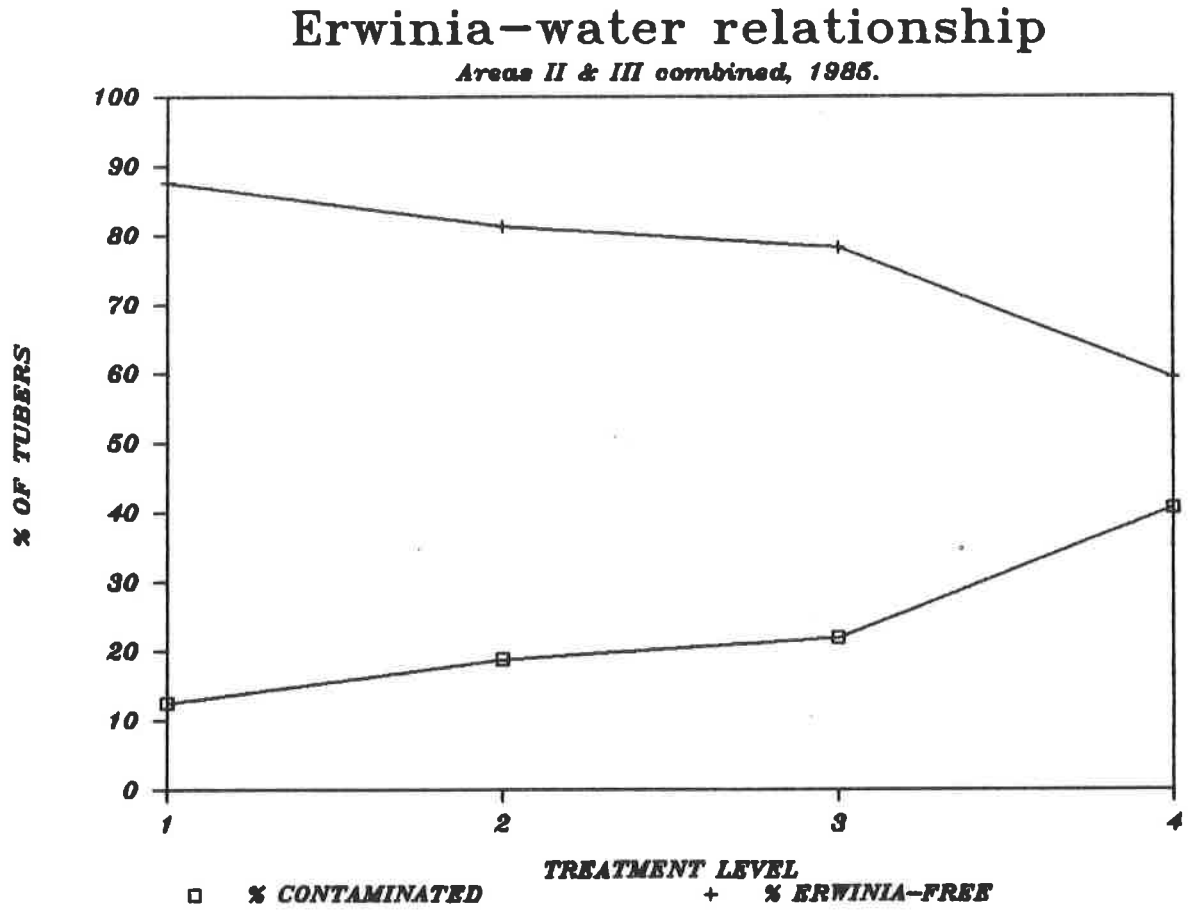



Figure 2. The percentage of tubers contaminated with Erwinia versus treatment level. Treatments are 1) natural water, 2) natural water + 100 cfu/ml, 3) natural water + 1,000 cfu/ml and 4) natural water + 10,000 cfu/ml. Data are for cultivars and locations combined.

# THE PRESENCE AND SURVIVAL OF *ERWINIA CAROTOVORA* IN NATURAL SNOW

Gary D. Franc and Monty D. Harrison

## Abstract

Natural snow was collected and assayed for the presence of *Erwinia*. Results show that viable *Erwinia* can be infrequently recovered from snow collected in the Rocky Mountains.

In a separate study, natural snow was inoculated with suspensions of *Erwinia carotovora* subsp. *carotovora* (*Ecc*) and *E. c.* subsp. *atroseptica* (*Eca*) and stored at ca -20°C. Direct plating results showed that *Eca* populations declined by ca 70% and *Ecc* by ca 100% after storage for 28 days. Enrichment results showed that viable cells of *Ecc* and *Eca* could be readily recovered after 28 days even though *Ecc* populations were undetectable by direct plating.

## Materials and Methods

Snow collected at Fort Collins 1-2 weeks after it fell was placed in a cement mixer and agitated. A suspension of *Erwinia carotovora* subsp. *carotovora* (*Ecc*) or *E. c.* subsp. *atroseptica* (*Eca*) was misted onto the snow while mixing. The snow, after being inoculated with each *Erwinia* strain, was placed in 0.5 gal sterile wide mouth polypropylene jugs and stored at -20°C. Three jugs (replications) were stored for each strain for each time interval. Uninoculated snow was also stored and processed as an internal negative check.

Snow samples were processed at 0, 1, 2, 7, 14 and 28 days after inoculation. Samples were thawed at room temperature and the melted samples were processed by membrane filtration followed by anaerobic

enrichment. Direct enrichment of 50 ml subsamples were also made. The populations of Ecc and Eca in the snow were determined by spread plating directly from the melted snow sample and also by spread plating after filter concentration.

Natural snow was collected from various mountain passes during the winter. Aerosols were collected from natural clouds at Storm Peak Laboratory. Snow samples were processed by filtration through celite or membrane filters followed by anaerobic enrichment.

## Results

The population of Ecc and Eca in snow over time is shown in Figure 1. Results showed that populations of Ecc decayed more quickly than Eca populations. Approximately 30% of the original Eca population was still viable after 4 wk storage at ca -20°C. The Ecc population was undetectable after 7 days storage at ca -20°C. However, enrichment data showed that viable Ecc cells were still present in the snow samples after 4 wk storage. All check samples were negative for Erwinia.

Results for natural snow samples show that Erwinia can be infrequently recovered from snow in the Rocky Mountains during the winter. All aerosol samples were negative.

## Discussion

Although very low numbers of Erwinia are present in natural snow, they appear to be able to survive for prolonged periods of time. During spring runoff, these cells could enter surface water and eventually be applied to potatoes, or other crops, in the irrigation water. Because bacteria can replicate so quickly under the proper environmental conditions, the

presence of low numbers of cells in snow may be an important factor in the production of Erwinia-free potatoes.

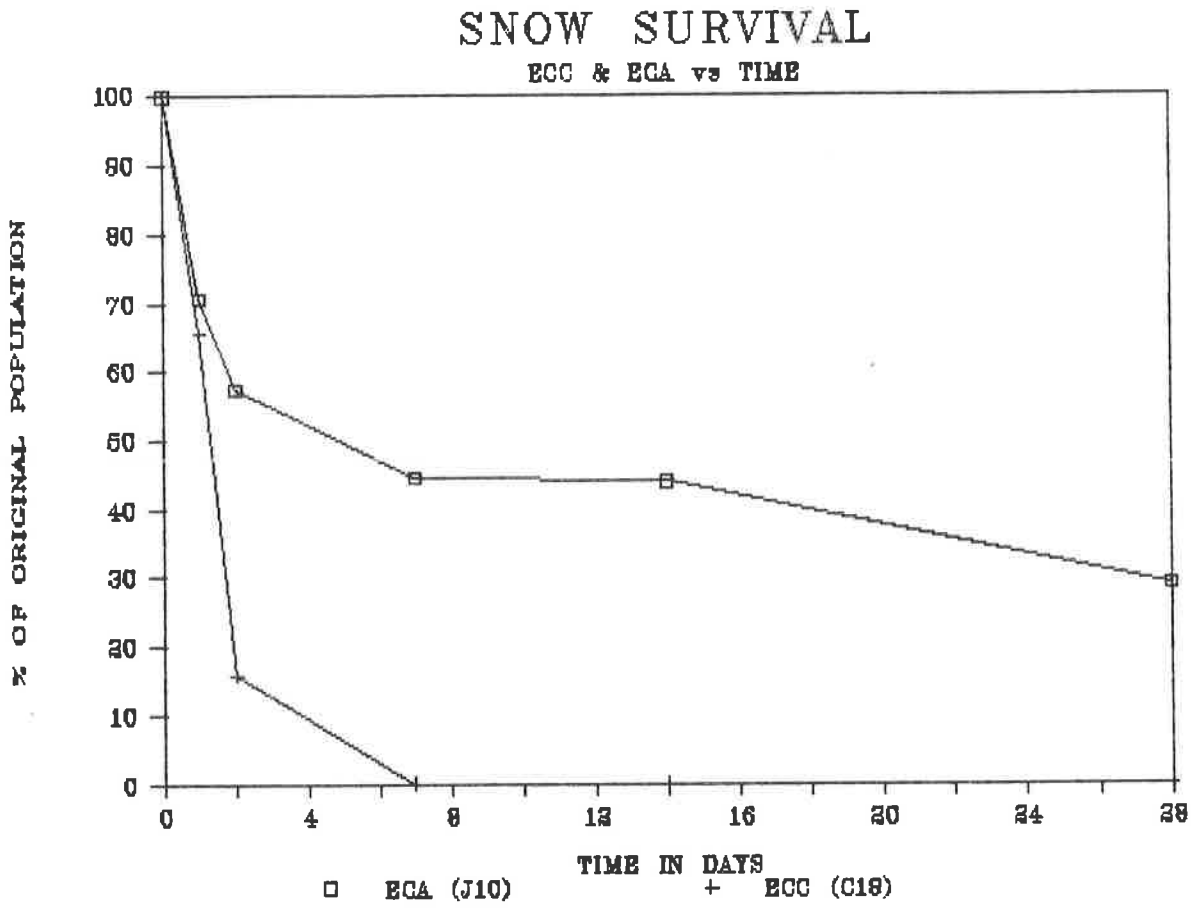



Figure 1. The relative survival of Erwinia carotovora subsp. carotovora (Ecc) and E. carotovora subsp. atroseptica (Eca) in snow stored at -20°C. Each datum entry is the percentage of the $\log_{10}$ of the original population. Populations were determined by spread plating samples after filter concentration.

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

G.D. Franc and M.D. Harrison

## Abstract

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

## DEP81 STUDY (initiated 1981, terminated 1984)

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

Daughter tubers (generation 1 - G1) harvested from DEP81 plots were planted back in the field during 1982. Plants with foliar ringrot symptoms (secondary symptoms) were found in RB but only when the original mother tuber received the maximum inoculum concentration ($6.3 \times 10^8$ C. sepedonicum cells). All CR plants were symptomless during 1982. When generation 2

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

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

DEP82 STUDY (initiated 1982, terminated 1985)

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

## DEP83 (initiated 1983, terminated 1986)

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

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

## Materials and Methods

Foundation grade, 1980, Russet Burbank and Red McClure seed potatoes purchased in the San Luis Valley and Centennial seed potatoes provided by the San Luis Valley Research Center were used in the dilution end-point study established in 1981 (DEP81). Tubers provided by the S.L.V. Research

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 strain was provided by S. Slack, University of Wisconsin-Madison, Madison, WI 53706. Serial dilutions of CS43 cells were prepared for the DEP81 study as follows. On May 6, 1981, eggplant seedlings (Solanum melongena "Black Beauty") in the two-leaf stage were inoculated with the aid of a sterile 1 ml tuberculin syringe and 26G 1/2" needle containing C. sepedonicum (CS43) bacteria suspended in 0.05 M phosphate buffer, pH 7.2. The first foliar ringrot symptoms developed in the eggplants in ca. 12 days after inoculation. On May 25, 1981, the seedlings were uprooted, washed in cold tap water and roots and leaves were removed with a knife previously dipped in ethanol and flamed. The stripped stems (approximately 458.5 g wet weight) were cut into ca. 1 cm lengths and placed in 4-5 l of cold (40°F) 0.05 M phosphate buffer, pH 7.2 and allowed to stand overnight. The bacterial cells were extracted from the stem segments into the cold buffer by this time due to the osmotic pressure differential. Buffer, containing the extracted cells, was passed through cheesecloth to remove the stem segments and large debris. Cells in the strained buffer were concentrated into a pellet by centrifugation using a Servall refrigerated automatic centrifuge and a SS34 rotor at 11- 12 x $10^3$ 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 stained 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:X = 3.16 cells/2.5 x $10^{-3}$ $mm^2$, S = 1.765. Replication II: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 x $10^8$, 1 x $10^7$, 1 x $10^5$, 1 x $10^3$ and 1 x $10^2$ cells/ml buffer.

Inoculum for the DEP82 study was prepared in a similar manner with minor modifications. The CS43 inoculum for eggplant inoculation was prepared by extracting bacteria from infected tubers harvested the previous fall from infected plants in the DEP81 study. Eggplant seedlings (cv. Black Beauty) in the 4-leaf stage were inoculated as described above on May 6, 1982. Symptoms started to develop on May 20 and stems were harvested on May 24. Bacterial cells from eggplant stems were concentrated by centrifugation on May 25 and counted (replication I:X = 0.55 cells/5 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 infection by CS43. Eggplants used in the DEP83 study were planted on March 29, 1983 and transplanted on April 12, 1983. Eggplant seedlings were inoculated on April 26, 1983, as described above, using prepared inoculum from stored DEP81 and DEP82 tubers. Ringrot symptoms were visible in inoculated eggplants on May 6, 1983 and stems were harvested on May 11, 1983. On May

12, cells were concentrated by centrifugation and 20 Petroff-Hauser squares were counted for each replication (replication I:X = 1.10 cells/5 x $10^{-8}$ ml, II:X = 0.70 cells/5 x $10^{-8}$ ml, III: 0.75 cells/5 x $10^{-8}$ ml, IV:X = 1.00 cells/5 x $10^{-8}$ ml, V:X = 1.10 cells/5 x $10^{-8}$ ml, VI:X = 0.95 cells/5 x $10^{-8}$ ml, S = 0.172). Serial dilutions were made, and tubers were inoculated on the same day.

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

Serial dilutions of inoculum for the DEP82, 83 and 84 studies were made to produce inoculum concentrations of $10^{10}$, $10^7$, $10^5$, $10^3$ and $10^2$ C. sepedonicum cells per ml. Spread platings (0.1 ml) of serial dilutions onto nutrient-dextrose agar plates were done in 1983 using DEP83 inoculum to determine viable bacterial counts and their relationship to physical counts determined in the Petroff-Hauser counting chamber (Table 5).

Tubers to be inoculated each year were surface disinfested with 10% chlorox, rinsed with cool tap water and allowed to dry. Tubers were inoculated by scooping out a section of the stolon end with an "EKCO" fruit baller (2.8 cm diameter), pipetting 0.1 ml inoculum directly into the depression and replacing the tuber piece. A small piece of sterile wooden toothpick was inserted to hold the tuber piece in place and the entire

stolon end (approximately 1/4 - 1/3 of the tuber) was immediately dipped into melted paraffin (Gulfwax) twice. An inoculum volume of 0.1 ml was sufficient to coat the cut surface of the tuber with inoculum when the excised tuber piece was replaced and slight pressure applied. The paraffin sealed the excised tuber piece to the intact tuber and prevented the inoculum from drying out. Control tubers, inoculated only with buffer, were treated in the same manner. All inoculations were done in the order of most dilute to most concentrated inoculum preparations.

Inoculated tubers were planted in a field plot ca 33 ft x 120 ft. Plots were planted by hand on May 28, 1981, May 26, 1982, May 17, 1983, May 17, 1984 (S.L.V.), May 31, 1984 (Ft. Collins) and May 22, 1985. A randomized complete block design consisting of two cultivars, six inoculation treatments and four replications (blocks) each for the DEP81, DEP82, DEP83 and DEP84 field plots was used. Individual treatment plots consisted of 10 treated tubers planted at a 14 inch spacing followed by three Red McClure spacers planted at 12 inch intervals. The plots were cultivated by hand and volunteer potatoes were rogued throughout the growing season. The plots were furrow irrigated during the growing season.

The field plots were observed throughout all growing seasons by Potato Virus Lab research personnel and Potato 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, 1984. Tubers were not harvested for the DEP84 plots in Ft. Collins. Tuber harvest for DEP82 and DEP83 was done on September 6, 1985.

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

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

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

## Results

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

Primary foliar ringrot symptoms failed to develop at any time during the first growing season. The plants were water stressed throughout the season and this may be why foliar symptoms did not develop even when mother

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

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

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

Bioassays and field plantings were again repeated in 1983 using granddaughters (generation 2 - G2) tubers harvested during fall 1982. Bioassays detected ringrot infection only in Centennial tuber progeny from original seedpieces receiving $10^6$ cells two generations prior to the assays. No other ringrot infections were detected. Field plots showed development of symptoms in Centennial inoculated with 10 cells in 1981. This showed the first evidence that ringrot infection in plants grown from tubers exposed to low levels of inoculum may not express symptoms until the third growing season after the initial inoculation . Thus, ringrot infection may be latent for that period.

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

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

Primary foliage symptoms in Russet Burbank developed in 1982 when the mother tuber received at least $10^6$ C. sepedonicum cells (Table 2). Foliar symptoms failed to develop in similarly inoculated Centennial plants. G1 tubers harvested in the fall of 1982 were assayed for ringrot infection using the eggplant bioassay during the spring of 1983. Eggplant assays showed detectable ringrot infection in tubers harvested from plants inoculated with $10^9$ cells in Russet Burbank and $10^9$ and $10^6$ cells in Centennial. Ten tubers were also planted in the field. Secondary foliage symptoms developed in the field in treatments which received $10^4$ cells (Russet Burbank) and $10^9$ and $10^6$ cells in Centennial. Tubers (G2) harvested from these plants showed that tuber infection had occurred in plants inoculated with $10^9$ cells (Russet Burbank) or $10^6$ and $10^9$ cells (Centennial). Plants produced by G2 tubers planted back in 1984 failed to show ringrot foliar symptoms in Russet Burbank but Centennial plants derived from treatments inoculated with $10^2$ and $10^9$ cells per tuber did show symptoms. Assays of G3 tubers in 1985 showed infection for inoculum levels of $10^2$ cells for both Russet Burbank and Centennial. No foliar symptoms developed in the field, however. The DEP82 plots were terminated in 1985.

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

Primary foliage symptoms developed in Russet Burbank when mother tubers were inoculated with $10^4$, $10^6$ and $10^9$ cells and in Centennial when mother tubers received $10^1$, $10^4$, $10^6$ and $10^9$ (Table 3). Centennial tubers receiving $10^2$ cells produced plants that were symptomless. Inspection of G1 tubers at harvest showed visible symptoms when mother tubers received $10^6$ and $10^9$ cells (Russet Burbank) or $10^4$, $10^6$ and $10^9$ cells in

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

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

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

A comparison of physical cell counts (determined by use of the Petroff-Hauser counting chamber) versus viable cell counts is shown in Table 5. Viable cell counts were lower than expected based upon physical cell counts (i.e., the cell concentrations in the serial dilutions) for the $10^3$ serial dilution and higher than expected for the $10^2$ serial dilution. An average of the viable cell counts gave an estimate of 827 viable cells per 1000 cells counted in the Petroff-Hauser chamber or a ratio of 83:100. Therefore, the use of the Petroff-Hauser counting chamber appears to be an accurate method for determining viable cell counts for Corynebacterium sepedonicum produced by the method outlined above. The viable estimate may

be slightly low due to the occurrence of cell pairs often observed with coryneform bacteria. Physical counts can distinguish between closely associated cells while viable counts cannot.

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

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

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

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

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

| Cultivar: Number of cells per seed tuber | The Presence (+) or Absence (-) of Detectable Ringrot in: | | | | | | |
|---|---|---|---|---|---|---|---|
| | Mother plants[1] | G1 tubers[2] | G1 plants | G2 tubers[2] | G2 plants | G3 tubers | G3 plants |
| | summer 1982 | spring 1983 | summer 1983 | spring 1984 | summer 1984 | spring 1985 | summer 1985 |
| Russet Burbank: | | | | | | | |
| $10^9$ | + | + | - | + | - | - | - |
| $10^6$ | + | - | - | - | - | - | - |
| $10^4$ | - | - | + | - | - | - | - |
| $10^2$ | - | - | - | - | - | + | - |
| $10^1$ | - | - | - | - | - | - | - |
| Buffer | - | - | - | - | - | - | - |
| Centennial: | | | | | | | |
| $10^9$ | - | + | + | + | + | - | - |
| $10^6$ | - | + | + | + | - | - | - |
| $10^4$ | - | - | - | - | - | - | - |
| $10^2$ | - | - | - | - | + | + | - |
| $10^1$ | - | - | - | - | - | - | - |
| Buffer | - | - | - | - | - | - | - |

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

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

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

| Cultivar: Number of cells per seed tuber | The Presence (+) or Absence (-) of Detectable Ringrot in: | | | | | |
|---|---|---|---|---|---|---|
| | Mother plants[1] | G1 tubers[2] | G1 tubers[3] | G1 plants[4] | G2 tubers | G2 plants |
| | summer 1983 | fall 1983 | spring 1984 | summer 1984 | spring 1985 | summer 1985 |
| Russet Burbank: | | | | | | |
| $10^9$ | + | + | + | + | + | - |
| $10^6$ | + | + | + | + | + | + |
| $10^4$ | + | - | + | + | - | - |
| $10^2$ | - | - | + | + | - | + |
| $10^1$ | - | - | - | - | - | - |
| Buffer | - | - | - | - | - | - |
| Centennial: | | | | | | |
| $10^9$ | + | + | + | + | - | + |
| $10^6$ | + | + | + | + | + | - |
| $10^4$ | + | + | + | + | + | - |
| $10^2$ | - | - | + | - | - | - |
| $10^1$ | + | - | - | - | - | - |
| Buffer | - | - | - | - | - | - |

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

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

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

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

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

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

Table 5. The comparison of Corynebacterium sepedonicum cell counts (Petroff-Hauser chamber) versus viable counts (DEP83).

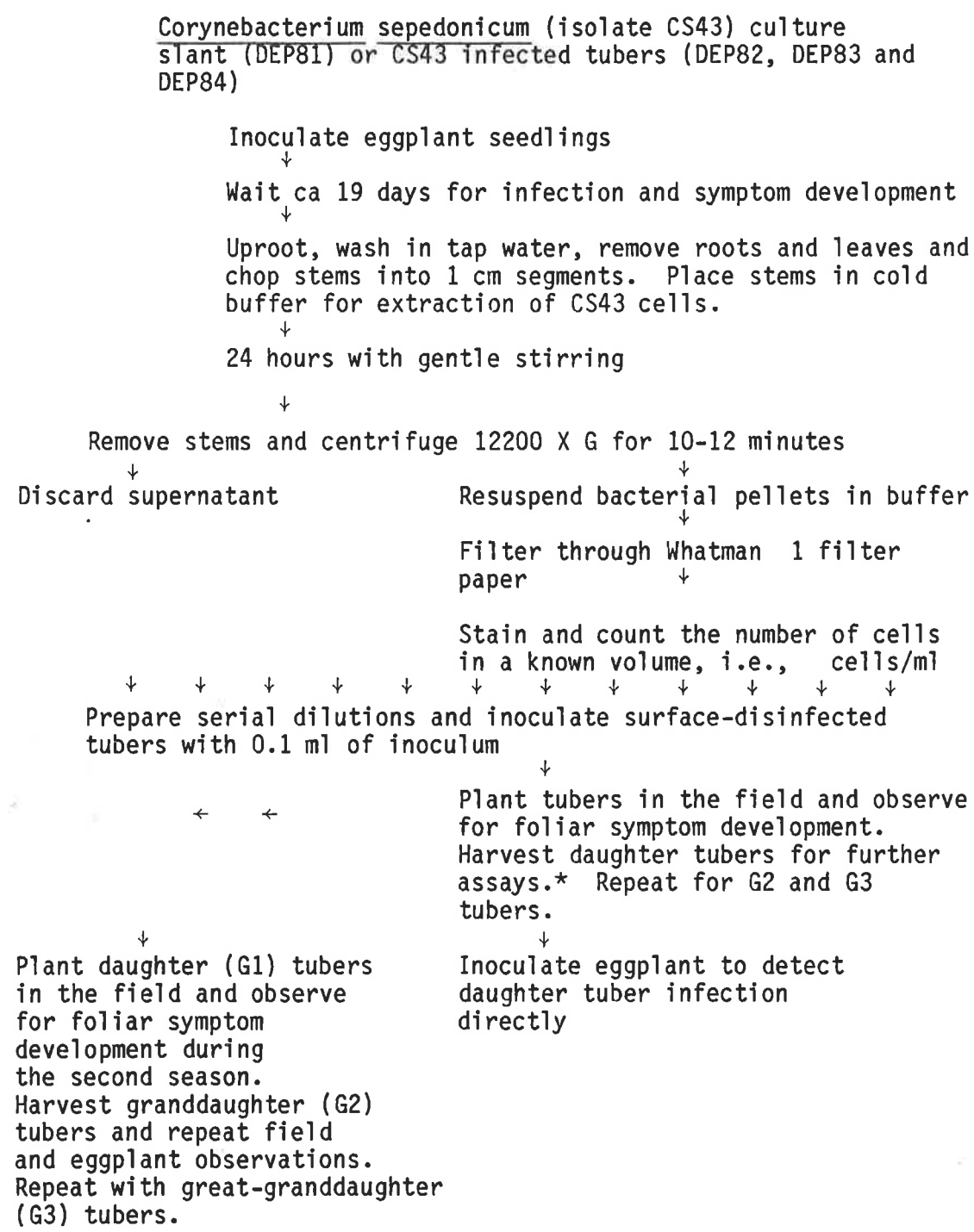
| Serial Dilution Plated ( cells/ml)[1] | Number of C. sepedeonicum cells per ml as determined by viable counts[2] |
|---|---|
| $10^{10}$ | TNTC[3] |
| $10^7$ | TNTC |
| $10^5$ | TNTC |
| $10^3$ | 353 ± 129 |
| $10^2$ | 130 ± 39 |
| Buffer | 0 |

[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.

# The Effect of Ringrot Infection on Symptom Development in Potato Clones

Gary D. Franc and Monty D. Harrison

Summary of 1985 Results and Research Proposal for 1986

## Summary of 1985 Results (see attached report for additional information)

Twelve potato clones were inoculated with Corynebacterium sepedonicum by dipping freshly cut tuber seedpieces in a slurry prepared from ringrot infected tubers. The seedpieces were planted in the field and symptom expression evaluated in the San Luis Valley, CO. Clones tested were AC77652-1, AC77513-1, A74212-1, A72685-2, WNC567-1, WNC230-14, BC9668-1, TC582-1, AC77149-2, Centennial Russet, Russet Burbank and Sangre.

All clones became infected with C. sepedonicum (ringrot) based on foliar and tuber symptom expression as well as stem squeeze results. Russet Burbank expressed foliar symptoms as early as 17 July. All clones, except AC77652-1 and TC582-1, had symptoms evident by 13 August. AC77652-1 and TC582-1 had symptoms evident on 20 August.

Even though all clones expressed foliar ringrot symptoms, those for clone AC77652-1 were considered weak in 1985. Clone A74212-1 had very weak tuber symptom expression even though foliar symptom expression was very evident. Clones BC9668-1 and AC77149-2 were considered weak expressors of typical tuber ringrot symptoms and clones A72685-2 and Sangre were strong expressors.

Research Proposal for 1986

**Ringrot Clone Testing**

G. D. Franc and M. D. Harrison

Results from this testing program continue to show significant variations among clones with regard to ringrot expression and the time required for expression to occur. This information is critical to the variety development program. Should clones be released which express marginal symptoms or express symptoms late in the season, infections could be missed during field inspections and resulting in unwanted spread of ringrot in grower's seed programs.

During 1986, approximately 15 new clones (plus 3 standard check cultivars), for which information about ringrot succeptibility and symptom expression is needed, will be tested in the field. This represents an increase of about 60% from past years. This is due to the increased numbers of advanced clones coming from the variety development program.

As in past years, tubers will be inoculated with the ringrot organism and planted in replicated field plots. Field readings will be made to determine when first symptoms appear, and the types of symptoms expressed in the foliage and tubers. Tissue samples (stems and/or tubers) for clones which do not show recognizable symptoms in the field during the growing season will be tested at the end of the season to determine if they might be symptomless carriers of the bacteria.

Due to the increased numbers of clones planned for testing in 1986, the budget has been increased allow us to accomplish the work.

# Ringrot Clone Testing

## Proposed Budget

| | |
|---|---|
| Plot Maintenance | $ 650.00 |
| Labor | 800.00 |
| Travel | 650.00 |
| Supplies | 150.00 |
| Total | $2250.00 |

# The Effect of Ringrot Infection on Symptom Development in Potato Clones

Gary D. Franc and Monty D. Harrison

Abstract:

Twelve potato clones were inoculated with Corynebacterium sepedonicum by dipping freshly cut tuber seedpieces in a slurry prepared from ringrot infected tubers. The seedpieces were planted in the field and symptom expression evaluated in the San Luis Valley, CO. Clones tested were AC77652-1, AC77513-1, A74212-1, A72685-2, WNC567-1, WNC230-14, BC9668-1, TC582-1, AC77149-2, Centennial Russet, Russet Burbank and Sangre.

All clones became infected with C. sepedonicum (ringrot) based on foliar and tuber symptom expression as well as stem squeeze results. Russet Burbank expressed foliar symptoms as early as 17 July. All clones, except AC77652-1 and TC582-1, had symptoms evident by 13 August. AC77652-1 and TC582-1 had symptoms evident on 20 August.

Even though all clones expressed foliar ringrot symptoms, those for clone AC77652-1 were considered weak in 1985. Clone A74212-1 had very weak tuber symptom expression even though foliar symptom expression was very evident. Clones BC9668-1 and AC77149-2 were considered weak expressors of typical tuber ringrot symptoms and clones A72685-2 and Sangre were strong expressors.

## Materials and Methods

Twelve potato clones were tested for ringrot (Corynebacterium sepedonicum) symptom expression in the San Luis Valley. Clones tested were AC77652-1, AC77513-1, A74212-1, A72685-2, WNC567-1, WNC230-14, BC9668-1, TC582-1, AC77149-2, Centennial Russet, Russet Burbank and Sangre.

Whole tubers were cut and seedpieces were immediately immersed in tap water (treatment A; uninoculated control) or tap water to which a macerate prepared from ringrot infected tubers had been added (treatment B). All treatment A tuber seedpieces were planted before treatment B tuber seedpieces to preclude cross-contamination. Treatment plots were planted on 22 May, 1985.

Treatment plots were visually inspected periodically throughout the growing season for the development of foliar ringrot symptoms. Typical symptoms were wilting (W), interveinal chlorosis (IVC), marginal necrosis (MN) and interveinal necrosis (IVN). Russet Burbank also developed a typical "early dwarfing" (ED) symptom. Plants were visually inspected on 17 and 30 July and on 13, 20 and 27 August. On 13 and 20 August, several stems in ech plot were pulled and a stem squeeze test was also done (a preliminary diagnostic test done in the field).

Tubers harvested from inoculated plants were evaluated for symptom expression on 6 September. Aproximately 14 daughter tubers for each replication (3 replications) were evaluated on this date and the percentage of tubers expressing symptoms was determined. Tubers were also rated on an arbitrary scale (0-3) for the intensity of symptom expression. A "0" rating indicated that tubers had to be cut open before symptoms could be observed; a rating of 1, 2 or 3 indicated that at least 1 tuber for 1, 2, or 3 replications, respectively, had tuber surface-cracking symptoms evident.

## Results

Inoculation of seedpieces did not significantly affect the final stand ($P = 0.05$). The average stand for inoculated plots was 6.3 plants and for control plots was 6.7 plants i.e., 90% and 96% stand, respectively. This

is consistent with observations made in previous years. Data in Tables 2 and 3 showed that ringrot infection occurred in all clones.

The effect of ringrot inoculation on foliar ringrot symptom development and expression is shown in Table 2. Inoculated treatment plots were evaluated for symptom expression by comparing to non-inoculated treatment plots under the same environmental conditions. Plots were evaluated on 17 and 30 July, and on 13, 20 and 27 August. Russet Burbank was the only clone showing foliar ringrot symptoms on 17 July. It was rated +2ED (two out of three replications had at least one plant each with early dwarfing symptoms). Russet Burbank has characteristically expressed foliar ringrot symptoms earlier than other clones in previous studies as well. Clones WNC567-1, BC9668-1 and Sangre all expressed foliar IVC symptoms as well as Russet Burbank by 30 July. All other clones lacked foliar symptoms at this time. However, all clones except AC77652-1 and TC582-1 had expressed foliar symptoms by 13 August. Clone TC582-1 had a positive stem squeeze on 13 August indicating that stem infection had occured in the absense of foliar symptom expression. All clones expressed foliar ringrot symptoms by 20 August. All clones were rated at +2 or greater by this time except for clones AC77652-1 and WNC230-14 which never attained a rating greater than +1 (Table 2).

All clones had at least one daughter tuber with typical ringrot symptoms evident by 6 September. The percentage of daughter tubers with visible symptoms ranged from 1.7% for clone A74212-1 to 28.7% for Sangre. Clones A74212-1, BC9668-1 and AC77149-2 were considered weak expressors, clones A72685-2 and Sangre were considered strong expressors with all other clones being moderate expressors of typical tuber symptoms with surface-cracking (Table 2, column 3).

## Discussion

Clone WNC230-14 was considered to be a weak to moderate expressor of ringrot symptoms during 1985. This is consistent with similar studies done in the past, although there is variation from year to year due to environmental effects and perhaps other causes.

The results in 1985 do show that both AC77652-1 and TC582-1 expressed foliar symptoms later than WNC230-14. Although TC582-1 eventually expressed typical foliar ringrot symptoms later on in the season, the stem squeeze was positive for ringrot infection on 13 August in the absense of any visible foliar symptoms. Eventually, all three replications for TC582-1 had at least one plant with typical foliar symptoms evident by 27 August. Clone AC77652-1 never had more than one replication with visible foliar symptoms evident during the growing season (Table 2) even though tuber data (Table 3) showed that typical tuber symptoms were present in at least two replications at the time of harvest. TC582-1 appeared to be a late maturing clone based on the relative lateness of flowering and the vigor of vines late in the season. This may account for the relatively late onset of foliar symptoms followed by relatively strong symptom expression late in the growing season with good tuber symptom expression. However, clone AC77652-1 did not express foliar symptoms well and, therefore, it may be difficult to detect ringrot in this clone on the basis of foliar symptom expression.

Clone A74212-1 had foliar symptoms expressed during the growing season. Symptoms included a very obvious early dwarf symptom, similar to that of Russet Burbank, in all three replications. However, this clone had weak tuber symptom expression in direct contrast to the extensive foliar symptoms attributed to inoculation with ringrot.

Table 1. The effect of ringrot inoculation of cut seedpieces on total stand in treatment plots - Center, Colorado, 1985.

| Clone Tested | Average Stand Count [1] | |
|---|---|---|
| | Water inoculated | Ringrot inoculated |
| 1) AC77652-1 | 6.3 | 6.7 |
| 2) AC77513-1 | 6.7 | 6.3 |
| 3) A74212-1 | 7.0 | 7.0 |
| 4) A72685-2 | 7.0 | 7.0 |
| 5) WNC567-1 | 6.7 | 6.0 |
| 6) WNC230-14 | 6.3 | 7.0 |
| 7) BC9668-1 | 6.7 | 6.7 |
| 8) TC582-1 | 7.0 | 7.0 |
| 9) AC77149-2 | 7.0 | 5.3 |
| 10) Centennial | 6.7 | 6.0 |
| 11) R. Burbank | 7.0 | 5.0 |
| 12) Sangre | 6.3 | 6.0 |
| $\bar{x}$ = | 6.7 | 6.3 |

[1] Seven tuber seedpieces were planted per treatment plot. Each datum entry represents the average of three replications. Stand counts were determined on 19 June, 1985.

**Table 2.** The effect of ringrot inoculation on foliar symptom development and disease expression in selected potato clones - Center, Colorado, 1985.

| Clone Tested | Dates on which plants were evaluated[1] | | | | |
|---|---|---|---|---|---|
| | 17 July | 30 July | 13 August[2] | 20 August[2] | 27 August[2] |
| 1) AC77652-1 | 0[3] | 0 | 0; sq(-)[2] | +1 wk IVC, MN; sq(+) | +1 ICV, IVN, MNsq(+) |
| 2) AC77513-1 | 0 | 0 | +2 IVC; sq(-) | + IVC; sq(+) | +IVC, MN, LR, W |
| 3) A74212-1 | 0 | 0 | +3 IVC, ED[4]; sq(+) | +3 ED, IVC; sq(+) | +ED, IVC |
| 4) A72685-2 | 0 | 0 | +3 W, IVC; sq(+) | ND[6] | +W, IVC, IVN |
| 5) WNC567-1 | 0 | +1 IVC | +3 W[5], wk IVC; sq(+) | +3[7] IVC, MN, W; sq(+) | +W, IVC, MN, SN |
| 6) WNC230-14 | 0 | 0 | +1 IVC; sq(+) | +1; sq(+) | +1IVC, IVN |
| 7) BC9668-1 | 0 | +1 IVC | +2 IVC, wk W; sq(+) | ND | +IVC, IVN |
| 8) TC582-1 | 0 | 0 | 0; sq(+) | +2 W, IVC; sq(+) | +3 IVC, MN |
| 9) AC77149-2 | 0 | 0 | +3 IVC, W; sq(+) | ND | +MN, W, LR |
| 10) Centennial | 0 | 0 | +3 IVC, W; sq(+) | +3 IVC, IVN; sq(+) | +W |
| 11) R. Burbank | +2[3] ED[3] | +2, ED, IVC | +3 ED[4], IVC, W; sq(+) | +; sq(+) | +W, ED, SN |
| 12) Sangre | 0 | +2 IVC | +3 IVC, W; sq(+) | +; sq(+) | +IVC, IVN, MN |

**Table 2** (continued)

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1 ED = early dwarf symptom; IVC = interveinal chlorosis; IVN = interveinal necrosis; W = wilting; MN = necrosis of leaf margins; LR = leaf rolling; SN = stem necrosis and general necrosis of leaves; wk = weak symptom expression. All inoculated treatments were compared to non-inoculated treatment plots.

2 Stem squeeze results are also shown. Stem squeezes positive for ringrot are designated as "sq(+)" and squeezes not sufficient to enable ringrot diagnosis are designated as "sq(-)".

3 Ratings of foliar symptom expression for ringrot infection are on a scale of 0-3. 0 = inoculated clones appeared healthy; +1, +2, or +3 = at least one plant in 1 replication, 2 replications or 3 replications, respectively, had foliar ringrot symptoms evident.

4 Pictures (color slides) of plants with early dwarfing and rosetting were taken.

5 Wilting in clone WNC567-1 was very pronounced when compared to the non-inoculated treatment.

6 ND = not done.

Table 3. The effect of ringrot inoculation on the incidence of daughter tuber symptom expression in the San Luis Valley - 6 September, 1985, Center, Colorado

| Clone Tested | % of tubers with visible ringrot symptoms[1] | Intensity of ringrot symptom expression (0-3)[2] | Total number of tubers observed |
|---|---|---|---|
| 1) AC77652-1 | 5.7 | 2 | 64 |
| 2) AC77513-1 | 13.3 | 2 | 31 |
| 3) A74212-1 | 1.7 | 1 (wk) | 55 |
| 4) A72685-2 | 19.7 | 3 | 44 |
| 5) WNC567-1 | 10.7 | 2 | 37 |
| 6) WNC230-14 | 20.0 | 2 | 35 |
| 7) BC9668-1 | 4.7 | 1 | 47 |
| 8) TC582-1 | 18.3 | 2 | 45 |
| 9) AC77149-2 | 9.7 | 1 | 42 |
| 10) Centennial | 9.3 | 2 | 35 |
| 11) R. Burbank | 13.7 | 2 | 29 |
| 12) Sangre | 28.7 | 3 | 42 |

[1] Each datum point represents the average of 3 replications.

[2] 0 = tuber symptoms were only visible after cutting open.
1 = at least one tuber in one replication had tuber surface cracking symptoms evident
2 = at least one tuber in each of two replications had tuber surface cracking symptoms evident.
3 = at least one tuber in each of three replications had tuber surface cracking symptoms evident.