Annual Report 1987
Fungus and Bacterial Diseases
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Summary: Studies during 1987 primarily involved work primarily on blackleg and ringrot. Proposals were written to obtain funding for work on early dying and chemical disinfection of seed and machinery. Both proposals were successful and work will start in 1988.

Work on the effects of irrigating crops produced from Erwinia-free seed with contaminated water showed that productivity was not decreased during the first year. Tuber weight, tuber number and foliar growth was the same regardless of the amount of contamination in the water. The percentage of plant stems and daughter tubers invaded by Erwinia increased as the amount of water contamination increased.

Work on factors affecting the expression of ringrot symptoms in eggplants and tissue cultured plantlets has shown that several factors including inoculum concentration, light temperature and fertility may affect symptom expression. Under some combinations of environmental conditions plants may not show symptoms even though invaded by the bacteria. These results may eventually help to explain the problem of latency in the San Luis Valley.

Methods, Materials and Results:

Effect of contaminated irrigation water on productivity of Erwinia-free seed. Seed (G-1 or G-2 stocks) of four cultivars, Norgold Russet, Centennial Russet, Russet Burbank and Sangre, was cut aseptically and planted in plots at the San Luis Valley Research Center on May 18, 1987. Five hills of each cultivar were planted in each of four rows in each of four replications. Shortly after emergence a plastic ring approximately 12 in. diameter and 4 in. high was placed around each plant and buried in the soil. The rings served to retain water in the root and tuber zone when it was poured into the ring. Within each replicate, 5 hills of each cultivar were watered 6 times at 10-14 days intervals beginning on June 27 with approximately 1 acre inch of water containing 0, 10^2, 10^3, or 10^4 *Erwinia carotovora* subsp. *carotovora* serogroup XXIX cells per ml of water.

At regular intervals during the season, plants were examined for blackleg expression, foliage height and width were measured for each plant to determine vigor and growth rate. Maturity readings were also recorded and tubers from each hill were hand dug, counted and weighed at harvest.

Isolations were made from sap extracted from surface sterilized stems pulled from 60% of the hills in each plot to determine the percentage of invasion by *Erwinia* and the population of the organism inside the stems. Isolations from tuber peels will also be completed before the 1988 season to determine if the amount of tuber contamination is related to the level of *Erwinia* contamination in irrigation water.

A separate study funded by the Area III Potato Administrative Committee at Greeley was carried out to determine how *Erwinia*-free seed performed relative to non-*Erwinia*-free seed when watered with naturally contaminated water.

Results of the study are summarized in figures 1-5. Data (Figures 1 and 2) show that tuber yield when measured as either average tuber weight per hill or average number of tubers per hill was not significantly affected by *Erwinia* inoculum in the irrigation water.

Figure 1: Tuber Weight

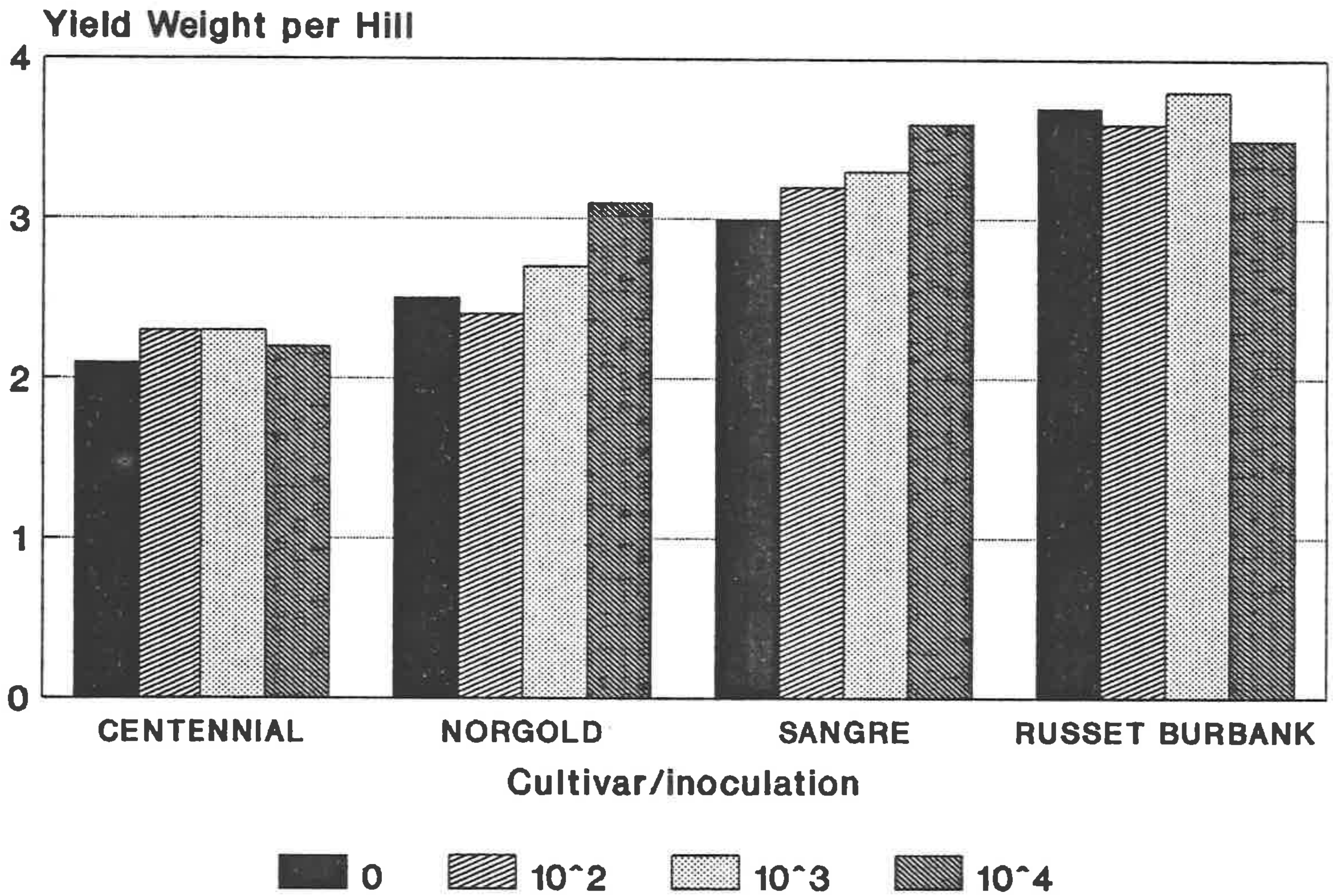
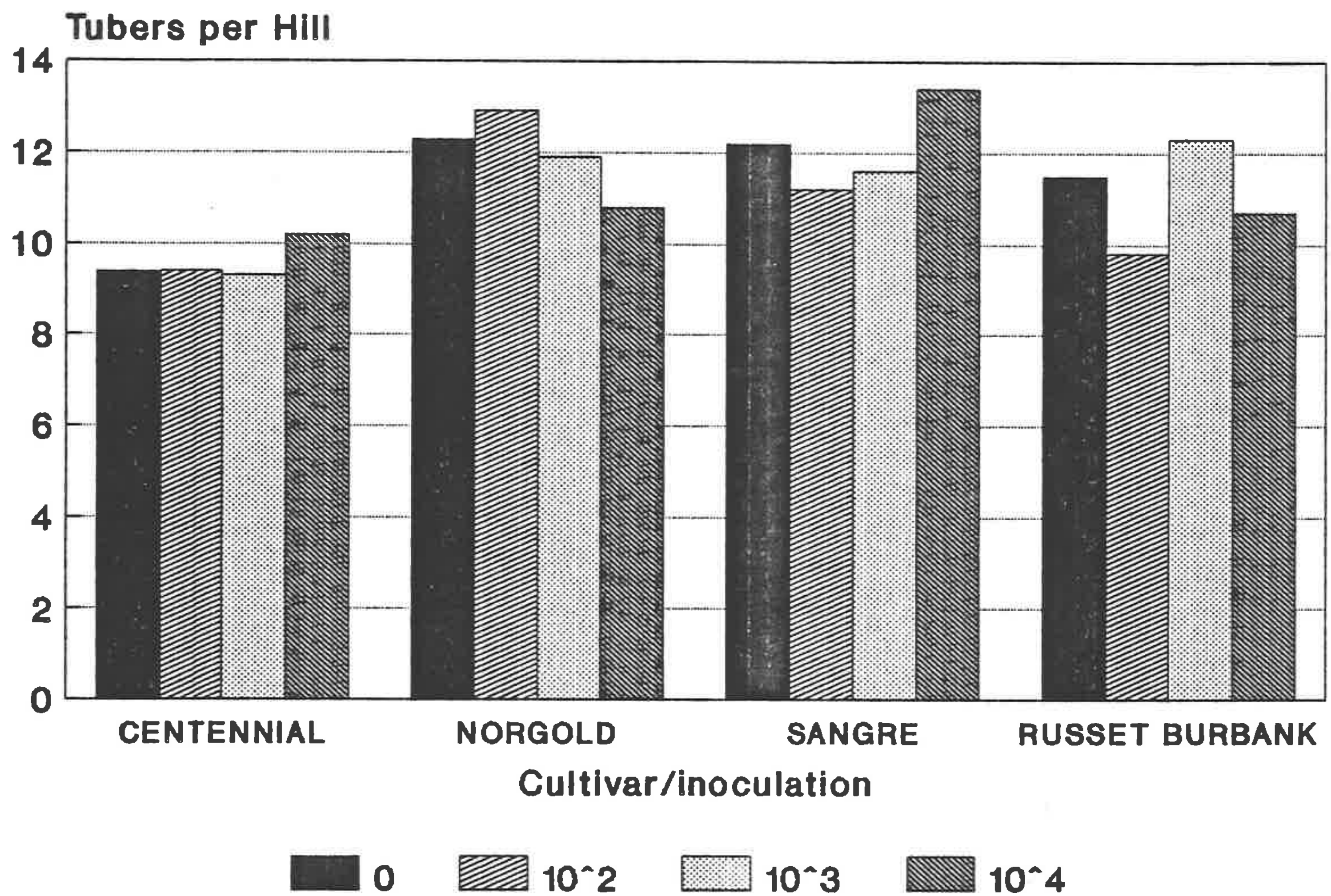


Figure 2: Number of Tubers



There were differences among cultivars, as expected, but among treatments within each cultivar no significant differences were found.

Likewise, growth, as measured and converted to a canopy index (Height x Diameter/1000) (Figure 3), was not affected by the four inoculum levels applied in the irrigation water. There were differences among cultivars; Centennial and Norgold had the smallest canopies and Sangre and Russet Burbank the largest.

Invasion of potato stems by Erwinia carotovora was related to the amount of inoculum applied in the irrigation water. The percentage of stems in which the organism could be detected in stem sap extracted from surface sterilized stem pieces increased as the amount of inoculum in the water increased. Percentage of stems invaded ranged from 0 (Norgold, Russet Burbank and Sangre) to 8.8% (Centennial Russet) when non-infested water was used to irrigate the plants. These plants received only the very low number of cells found naturally in irrigation water from the well at the Research Center. Plants which received 10^2 cells/ml had from 0 (Sangre) to 33.3% (Norgold) of their stems invaded by the pathogen. Plants receiving 10^3 or 10^4 cells/ml of water had from 33.3% to 58.3% of the stems invaded. In general the rapid increase in the percentage of stems invaded occurred between the 10^2 and the 10^3 inoculum densities. Statistically significant ($P = 0.05$) differences were found among the four inoculum levels in both Russet Burbank and Sangre. In both cultivars treatments 3 and 4 (10^3 and 10^4 Erwinia cells/ml) were significantly higher than treatments 1 and 2 (0 and 10^2 cells/ml). No significant differences were found among treatments in Centennial Russet and Norgold Russet although the trend was toward increasing infection as inoculum levels increased.

Figure 3: Plant Growth Rate

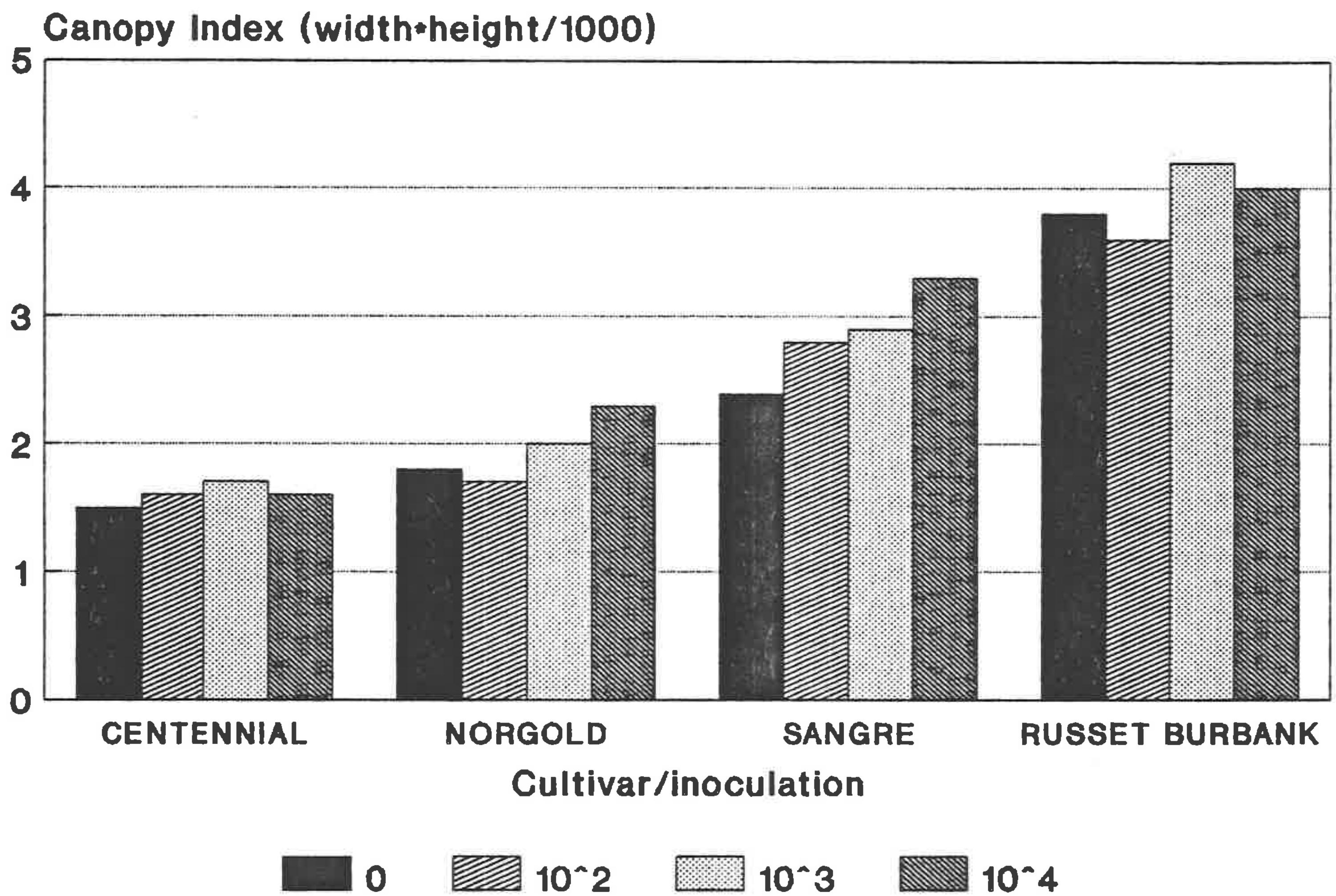
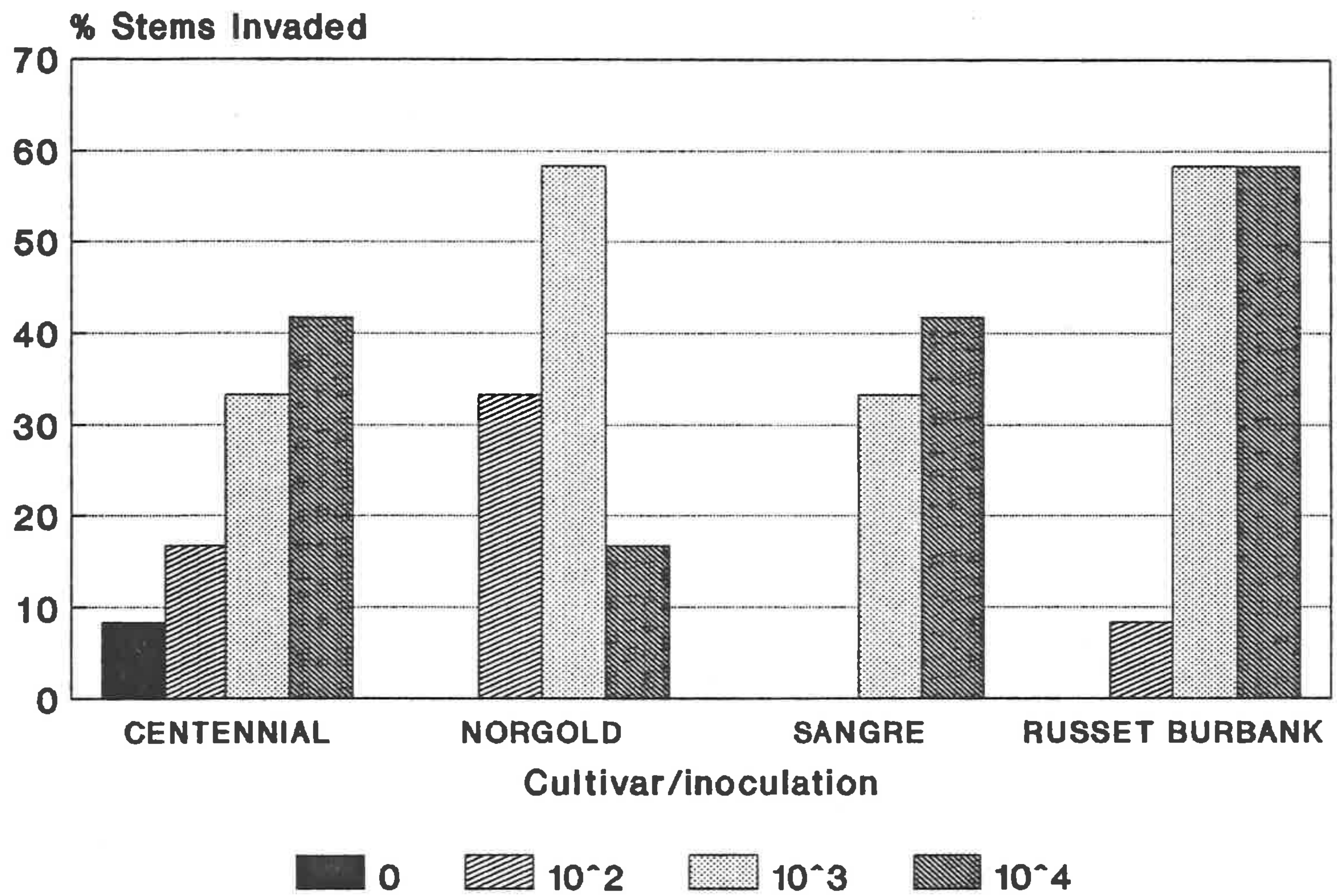


Figure 4: Stems Invaded by Erwinia

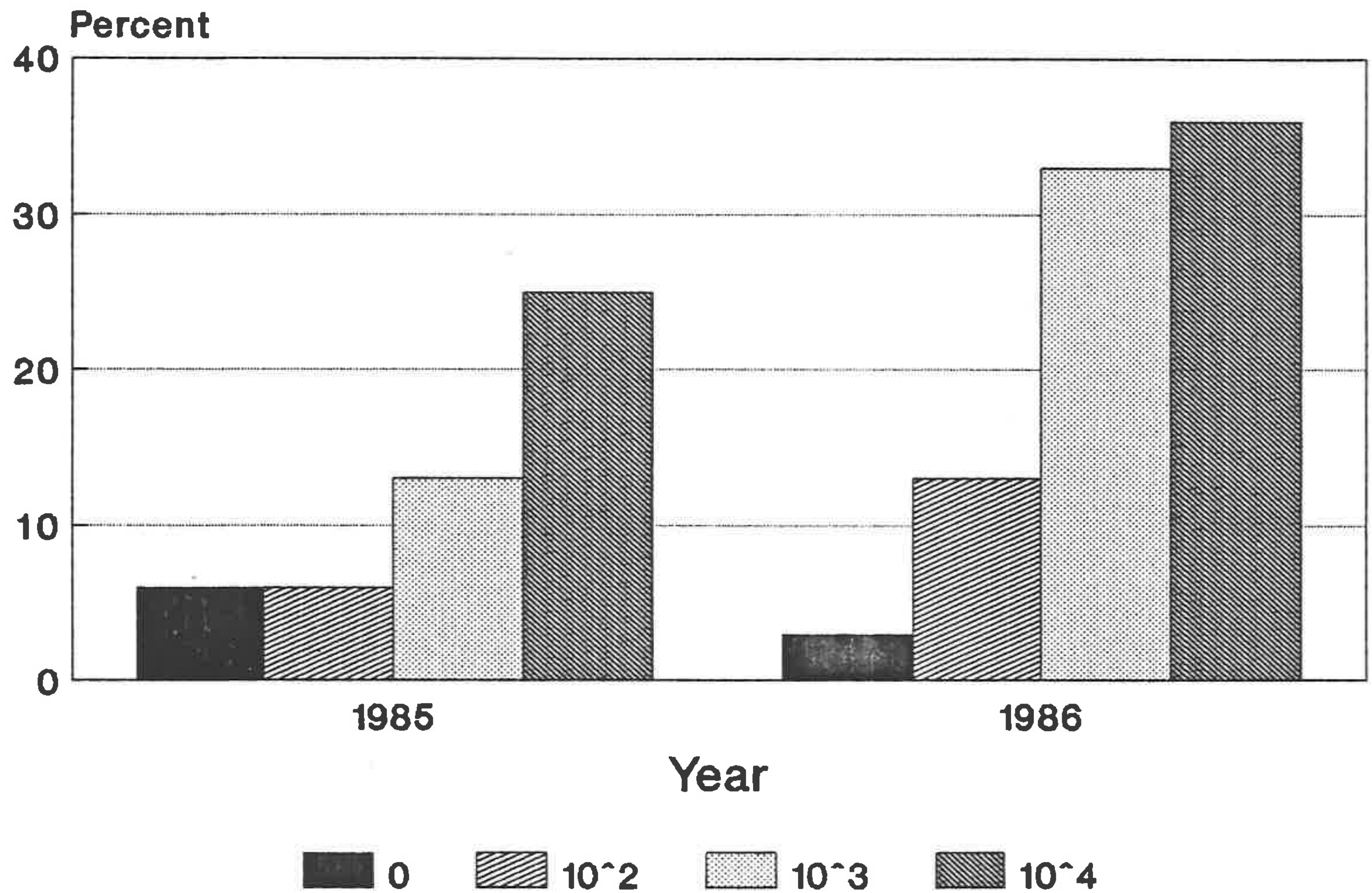


Data on tuber contamination in 1987 are still being collected, however, Figure 5 shows results from 1985 and 1986 tests. The results show that the percentage of tubers invaded by Erwinia applied in irrigation water increased as the level of contamination in the water increased. The greatest increase appeared to be between inoculum levels 10^2 and 10^3. In this regard, the tuber data closely resembled the stem data from 1985, 1986 and 1987.

The results from three experiments have shown that although stem invasion and tuber contamination from Erwinia cells applied in irrigation water increase, no decrease in productivity occurs during the first year of exposure. Also, the data indicate that the amount of stem and tuber invasion is relatively slow when populations of Erwinia in irrigation water are below 100 cells/ml of water. In fact the increases in contamination do not appear to increase rapidly until cell numbers are between 100 and 1000 cells/ml. Erwinia populations above 100 cells/ml have only rarely been found in irrigation water in the San Luis Valley and numbers in the 0-10/ml range are much more common than those in the 50-100/ml. This suggests that significant recontamination of blackleg-free seed from irrigation water will be relatively slow in the San Luis Valley while in areas with populations of Erwinia in water the recontamination rate may be much higher.

The data indicate that even in areas with very high water populations of E. carotovora performance of Erwinia-free seed in terms of vigor and yield will not be adversely affected at least in the first year of production. Stem and tuber infection would be expected to increase, however, and perhaps result in reduced performance in subsequent years if seed from the crop was saved and replanted.

Figure 5: Percent Tubers Infested



Data from a study done in the Gilcrest/Plattville in Northeastern Colorado in 1987 indicate that the data from the San Luis Valley studies are valid. Norgold (G-2) stocks grown in the San Luis Valley were planted side-by-side in replicated plots with North Dakota certified stocks and their productivity compared. Even though natural Erwinia populations in the irrigation water in the area ranged as high as 350 cells/ml performance of the G-2 stocks was superior to the non-Erwinia-free stocks in terms of vigor, longevity of healthy plants, blackleg infection and yield.

It is not known how many generations of exposure to low levels of water born inoculum would be required for vigor and yield to be adversely affected. This should be determined in subsequent studies in which seed stocks are exposed to specific levels of Erwinia in contaminated water for several generations and serial data collected on growth rates and yield data.

Ringrot epidemiology in Colorado. A series of greenhouse, laboratory and field experiments have been made during the past year to help understand the factors which affect the severity of the ringrot problem in the San Luis Valley and to develop methods to detect the ringrot bacteria and hopefully find ways to reduce infection.

Limited water and weed samples were collected and assayed for the presence of Corynebacterium sepedonicum. This work will be expanded in 1988 with the addition of a new graduate student, James Zizz, who will work exclusively on the sources of ringrot inoculum. Also a preliminary study was done in cooperation with Dr. Steve Slack, University of Wisconsin, to determine how well ringrot symptoms are expressed in the San Luis Valley relative to expression in other areas.

Seedpieces of Norgold Russet, Norland and Norchip were inoculated with a range of inoculum levels, planted in the field and observed for symptom expression.

This work will be expanded in Colorado in 1988 to determine how cultivar, inoculum density and environment interact to determine whether or not symptoms are expressed. A new graduate student, Anne Van Buren, will be working on this project.

Intensive laboratory and greenhouse work has been carried out during the past year by graduate student Beverly Schuld to determine ways to improve the eggplant bioassay test for detecting Corynebacterium sepedonicum and to study the persistence of the organism in tissue culture and conditions which favor symptom expression in tissue cultured plantlets.

Studies with eggplants have been made to determine effects of methods and sites of inoculation, plant age, fertility level, light and temperature on symptom expression, time required for symptoms to appear after inoculation, and the percentage of inoculated plants which show symptoms.

In tissue culture, studies have been made to determine if and under what conditions inoculated plantlets express ringrot symptoms and if bacterial cells can persist undetected in inoculated plantlets through several generations.

Results to date have shown that ringrot expression in inoculated plants is poorer by far in the San Luis Valley than in any other place in the United States where identical materials have been planted. This includes Maine, Wisconsin, North Dakota, Montana, Oregon, and Idaho. The reason for such poor expression in the San Luis Valley will be the object of extensive research during the next two years. Limited testing of weed roots and irrigation water has failed to detect Corynebacterium sepedonicum, however, improved methods and the assistance of a new graduate student who will work on the problem of the possible existence of other sources of ringrot bacteria besides infected tubers and contaminated equipment associated with the potato operation in the San Luis Valley.

Considerable progress has been made on determining factors which affect susceptibility and development of ringrot symptoms in eggplants and tissue cultured plantlets. A new method has been developed whereby small amounts of inoculum suspension containing specific numbers of C. sepedonicum cells can be precisely placed into various infection courts. This has allowed us to determine the minimum numbers of cells required to produce symptoms and the relative susceptibility of various host tissues. Inoculation into axillary buds is more effective than inoculating into the petiole scar below the buds. Bud inoculation produces more severe symptoms perhaps more quickly than petiole scar inoculations. Also it has been shown that inoculation into different nodes at different positions on the plants produces different percentages of plants with symptoms.

Dilution tests have shown that there is a threshold number of C. sepedonicum cells required to produce symptoms in 100% of inoculated plants. Currently studies are underway to determine a dose response curve, i.e. the dosage (cells/plant) required to produce certain percentages of infected plants.

Studies on plant age and fertility levels have shown that both of these factors affect susceptibility of eggplants to infection (symptom expression) by C. sepedonicum.

Greenhouse and growth chamber studies have shown that temperature and photoperiod both affect the percentage of plants which show symptoms and the intensity of symptom expression. There appears to be an interaction between photoperiod and some other factors which determine relative susceptibility as measured by symptom expression. Certain combinations of environmental factors render inoculated plants almost completely symptomless even though they are invaded by the pathogen. These studies may provide clues to reasons why inoculated potato plants often remain symptomless in the San Luis Valley.

There seem to be two factors involved in symptom expression, one which keeps the plants in a susceptible state over an extended period of time and another which allows the pathogen to invade and produce symptoms. Work is continuing in this area of ringrot epidemiology.

In tissue culture, inoculated plantlets have failed to show ringrot symptoms when grown under environmental conditions similar to those normally used for propagating plantlets for commercial use. This suggests that plantlets in tissue culture can indeed carry undetected C. sepedonicum cells. Modifying the chemical composition of the growth medium and the environment show promise of rendering inoculated plants susceptible to symptom expression and thus allow the presence of the ringrot bacterium to be more readily detected.

It has been found that tissue cultured plantlets transplanted to soil in the greenhouse and inoculated with C. sepedonicum will show symptoms nearly as fast (7-10 days) as eggplants and much faster than potato plants grown from seedpieces (ca 90 days). Studies are now underway using these findings to determine if C. sepedonicum cells

Table 1. Effects of environmental conditions on expression of ringrot symptoms in inoculated eggplants

	Environment Pre-Inoculation	Environment Post-Inoculation	Symptoms
1.	Constant light	Constant light	
	25°	25°	Very poor
	15°	15°	Poor
	15°	25°	Very poor
	25° day, 15° night	25° day, 15° night	None
2.	Constant light	Light 16 hr, Dark 8 hr	
	25°	25°	Good, 11-12 days
	15°	25°	Good, 12 days
3.	Light 16 hr, Dark 8 hr	Light 16 hr, Dark 8 hr	
	25°	25°	Good, 11-12 days
4.	Light 16 hr, Dark 8 hr	Constant light	
	25°	25°	Fair

present in tissue cultured plantlets can be carried undetected through normal tissue culture procedures then show symptoms when they are grown in the field or greenhouse.

All of these studies are providing the basic information necessary to: 1) use the eggplant test more effectively to detect ringrot bacteria present in unexpected sources and in potato seed before it produces visible symptoms; 2) successfully use tissue culture to eliminate ringrot bacteria from seedlots by providing growing conditions conducive to symptom expression in culture and to find means to insure that tissue cultures are free from the bacteria.

This work will continue for at least one more year with new refinements being implemented to maximize the efficiency of the eggplant test and to increase the probability of detecting ringrot bacteria in tissue cultured plantlets.