Summary Research Progress Report for 1989
and Research Proposal for 1990

Submitted to:

San Luis Valley Research Center Committee
and the
Area II Potato Administrative Committee

Title: Fungus and Bacterial Diseases of Potatoes

Project Leader: Monty Harrison

Project Justification: Ringrot, blackleg, and early dying continue to be important problems in the San Luis Valley. The determination of factors which affect ringrot expression, sources of the pathogen which may contaminate clean seed lots and means for detecting infections early before they may be visible in the field are critical to effective control of the ringrot disease. Factors which affect survival of the blackleg/soft rot organism from season to season need to be determined in order to effectively devise strategies to delay or eliminate recontamination of Erwinia - free seed derived from tissue culture. Early dying is becoming an increasingly serious problem in the San Luis Valley especially with the introduction of very susceptible cultivars like Russet Norkotah and the warmer than normal growing seasons experienced during the last two or three years. Our previous work has shown that the major organism (Verticillium dahliae) involved in early dying is commonly present in San Luis Valley soils. However, we need to know what factors affect the incidence and severity of the disease in order to take steps to minimize losses.

Project Status: This project which has been continuing for 28 years will be terminated in 1990 due to the retirement of the project leader. However, two parts of the project being carried out by graduate students, Rob Davidson and Lisa Harrison will continue for one more season. Lisa will continue her study of factors affecting survival of the blackleg/soft rot bacteria in soil and Rob will continue his work on factors affecting incidence and severity of the early dying disease. Both are funded by the Western Regional IPM project.

Significant Accomplishments 1988-1989

Ringrot: Considerable progress has been made toward better understanding the behavior of the ringrot disease in the San Luis Valley. It has been shown for the second field season that cultivar, numbers of ringrot bacteria in seed tubers, location and planting date, all affect ringrot expression in field grown potatoes. Generally, warmer areas and higher inoculum levels produce more visible infections. Cultivars vary in terms of expression depending upon inoculum levels and environment. It has also been found that ringrot bacteria can be isolated from certain weeds and water exposed to infected plants or contaminated water. Also the organism has been detected in symptomless seed tubers from certified seed lots grown on farms where ringrot has been found.

Sangre mini-plants may be good bioassay hosts for detecting ringrot bacteria. They show excellent symptoms as quickly as eggplants. The presence of

Verticillium in plant extracts may interfere with detection of ringrot bacteria when the eggplant bio-assay is used.

Certain weeds, particularly grasses, appear to extend the length of survival of the blackleg organisms in soil in the field based upon one year's research. This work will continue in 1990 to determine if the bacteria can overwinter in soil where specific weed species have been grown.

Work with early dying has indicated that three factors probably affect the severity of the problem in the San Luis Valley. They are, high soil moisture amount of Verticillium in the soil and presence of the blackleg/soft rot bacterium in seed tubers.

Objectives for 1990-1990: The project will be terminated except for a final season of work on survival of the blackleg bacteria in association with weeds and factors affecting development of early dying. Both of these studies will be funded by funds from the Western Regional IPM project.

Funding:

1989 allocation	$6900
1990 budget request	-0-

Annual Report

Fungus and Bacterial Diseases of Potatoes

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INTRODUCTION

A number of studies on the epidemiology of bacterial ring rot and potato blackleg/bacterial soft rot in the San Luis Valley were carried out in 1989. Studies included work on the effect of weed species on survival of the blackleg/softrot organisms (*Erwinia carotovora*) in soil; effects of various factors including bacterial numbers, environment, planting date and cultivars on ring rot expression; potential sources of ring rot inoculum; persistence and detection of ring rot bacteria in tissue culture; factors affecting expression of ring rot in eggplants; ring rot expression in mini plants in the greenhouse and the interaction between *Erwinia*, *Verticillium* and environment on early dying in the San Luis Valley. These studies are described and summarized briefly in this report.

EPIDEMIOLOGY OF BACTERIAL RING ROT

Potential Sources of Ring Rot Bacteria (James Zizz)

For two seasons, possible sources of ring rot bacteria besides those commonly known normally expected have been studied. Possible sources which have been investigated include irrigation water, weeds, insects, and symptomless tubers from certified seed lots.

Laboratory studies have shown that viable cells of *Clavibacter sepedonicum*, the ring rot bacterium, can survive in raw water collected from the Rio Grande river in sufficient numbers to cause infections in eggplants for as long as 32 days when relatively high numbers (10^6 cfu/ml) are initially present in water held at 20°C during the day and 15°C at night. When lower numbers of bacteria are introduced into the water, survival time in sufficient numbers to cause visible infections in eggplants is considerably shorter.

Samples of irrigation water collected in the field from rows in which infected Sangre plants were growing have produced infection in eggplants after the water has been filtered to concentrate the bacteria present and inoculated into eggplants. The fact that the infections were caused by the ring rot organism has been confirmed by Grams stain and serological tests.

These data suggest that water may carry sufficient ring rot bacteria to contaminate ring rot-free seed lots if it has been contaminated by tail water from a field where ring rot-infected plants are present.

Insects, aphids, grasshoppers, and psyllids have not been shown to transmit the ring rot bacterium from infected to healthy plants in the greenhouse or the field.

Weeds exposed to contaminated irrigation water in the field and some merely planted in soil where ring rot-infected potatoes were grown the previous season have been shown to carry the ring rot organism. Hairy Nightshade plants collected 69-97 days after planting by extracting sap and injecting it into eggplants, have been shown to have the organism present in or on their stems. This may be a way the ring rot bacterium could be perpetuated from season to season in the soil protected by mature weed stem tissue which is not rapidly decayed by the organism.

Tubers collected from certified seed lots grown on a farm where other lots were rejected because of ring rot infection have been shown to carry the ring rot bacterium in symptomless tubers. Of ten tuber samples collected from one certified field, one was shown to carry the ring rot bacterium based upon the development of ring rot symptoms in eggplants inoculated with macerated tissue from the stem ends of the tubers. The presence of the ring rot bacterium was verified by Grams staining and serological tests.

These studies have suggested that irrigation water, infected weeds and latently-infected seed tubers all may be sources of ring rot bacteria from which re-infection of clean seed lots could occur..

Interaction of Verticillium and Clavibacter sepedonicum in Eggplants (James Zizz and Anne Van Buren)

It was discovered during the course of checking potato plants, weeds and irrigation water for the presence of the ring rot bacterium by inoculating eggplants with extracts from these sources that some eggplants developed typical ring rot symptoms without ring rot bacteria being present in the symptomatic tissue.

It was shown that such plants were infected with Verticillium--the same organism that causes verticillium wilt (early dying in potatoes). Experiments have shown that eggplants inoculate with Verticillium show symptoms in about the same time as those inoculated with C. sepedonicum, the bacterium that causes ring rot. Furthermore, it appears that the presence of Verticillium in water, or plant tissue used to inoculated eggplants may reduce the ability of the ring rot bacterium to develop in the plants in sufficient numbers to be detected, especially if low numbers of bacteria are present initially. This may limit our ability to detect the presence of small numbers of ring rot bacteria, using the eggplant test, in fried samples from the areas where Verticillium is often present. This may limit the value of the eggplant bioassay which is, at present, the most sensitive and reliable way to detect the ring rot organism. Certainly, symptoms alone cannot be used to confirm ring rot infections.

Expression of Ring Rot Symptoms in Tissue Culture and in Mini Plants Derived from Tissue Culture (Beverly Schuld)

Concerns about the possible undetected passage of cells of the ring rot bacterium through the tissue culture procedures led to a series of studies on the behavior of the organism in tissue culture.

Tissue culture plantlets were inoculated with C. sepedonicum and carried for several generations in in-vitro culture under various conditions. The basal sections with roots attached were also transplanted to soil in pots and grown in the greenhouse. In onestudy, when plantlets inoculated with ring rot bacteria were grown under normally used tissue culture conditions no visible symptoms of infection were evident but the bacteria survived in the plantlets and remained infective. Microscopic examination of tissues from the plantlets showed that masses of bacteria were present even near the growing tips. When very high numbers of bacteria were used to inoculate plantlets some stunting and wilting in a few plants occurred after the third generation of nodal transfers.

Further studies working with inoculum levels and modification of the growth medium showed that in-vitro symptoms (primarily stunting but sometimes wilting) develop only when inoculum levels are high or the culture medium is devoid of a carbon (sugar) source. Plantlet length was reduced (plantlets stunted) when a "high" inoculum concentration (5000 cfu/ml) was used to inoculate plantlets but not when "low" inoculum (500 cfu/ml) was used. No symptoms were observed when plants were grown on normal culture medium (with sugar) unless inoculation levels were high.

These results show that ring rot bacteria can probably be carried in tissue cultured plantlets at least through several modal transfers without showing

recognizable visible symptoms, particularly if bacterial numbers are low and normal culture media containing sugar are used.

When inoculated plantlets which had shown no symptoms in culture were transplanted to soil in pots in the greenhouse, however, typical ring rot symptoms developed after two to three weeks growth. This suggests that infection will probably be detected if plants are grown in soil for two to four weeks before transplanting into the field. However, considerable spread of infection could go undetected in in-vitro culture unless the material is tested by means other than visual observation.

Based upon the finding that greenhouse-grown mini plants transplanted from tissue culture developed symptoms in the greenhouse much faster than expected (two to three weeks vs two to four months for plants grown from seedpieces), the possibility of using such plantlets instead of eggplants as bioassay hosts was investigated. It was found that mini plants of the cultivars Sangre and Russet Burbank developed ring rot symptoms relatively quickly (11 to 25 days) when inoculated with about 40 to 160 cfu of the ring rot organism. Time from inoculation to symptom expression was not significantly affected by the inoculum levels used but the cultivars differed significantly. Sangre mini plants showed symptoms as fast as eggplants but Russet Burbank required a significantly longer incubation period for symptoms to appear than did either Sangre or eggplant.

These results suggest that Sangre mini plants may be a good substitute for eggplant as a bioassay host for the ring rot bacterium. Also, they suggest that perhaps the ability to detect symptoms even in greenhouse-grown mini plants may be affected by the cultivar involved.

Factors Affecting Expression of Ring Rot in Field Grown Potatoes (Anne Van Buren)

A study on the effects of various factors on expression of ring rot in field grown potatoes begun in 1988 was repeated in 1989. The factors studied, inoculum density (0, 10^2, 10^4, 10^6, and 10^9 cells/seed tuber), cultivar (Sangre, Russet Burbank and Russet Norkotah), location (Weld County and the San Luis Valley) and planting date (early, normal and late at each location) were the same as in 1988. Data collected included emergence, plant height, vigor, symptom expression (timing and amount) and tuber yield.

Data from 1989 are still in the process of being analyzed but some clear trends are evident which appear to closely match those from 1988. Inoculated plants grown in Weld County generally showed more symptoms than those grown in the San Luis Valley. Increasing inoculum density generally increased the percentage of plants which expressed ring rot symptoms regardless of location and planting dates had distinct effects on symptom expression. In Weld County plants in the earliest planting showed the most symptoms followed by those in the latest planting than those planted at the "normal" planting time. In this area Sangre showed the most severe infection followed by Russet Norkotah then Russet Burbank.

In the San Luis Valley plants in the latest planting showed more symptoms than those in the first planting followed by those in the normal planting. Sangre showed the most severe symptoms followed by Russet Burbank and Russet Norkotah which both expressed symptoms about equally well in the Valley in 1989.

The 1989 season was exceptionally warm and Verticillium wilt was unusually severe especially in Russet Norkotah which was killed relatively early in the

season. The appearance of Verticillium infection made ring rot symptoms somewhat difficult to read, especially later in the growing season.

A greenhouse experiment was carried out which was designed to determine whether soil or air temperature had the most effect on ring rot expression. Inoculated seedpieces of the same three cultivars used in the field studies were planted and held at 15°, 25° and 30°C air temperature and at 18°, 21°, 24°, 24°/18° and 18°/16°C for 100 days. Ring rot symptoms failed to develop in any visible ring rot symptoms. Eggplants inoculated with sap taken from random plants showed that the potato stems were infected with the bacteria but symptoms were latent. This suggests that environment can greatly affect the expression of the disease, at least in the three cultivars studied.