SUMMARY RESEARCH PROGRESS REPORT FOR 1992
AND RESEARCH PROPOSAL FOR 1993-1994
Submitted to: San Luis Valley Research Center Committee
and the Colorado Potato Administrative Committee (Area II)

Title: Innovative Strategies for the Detection and Control of Bacterial Ring Rot

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Project Justification:

Bacterial ring rot (BRR) is an economically significant disease affecting seed and table stock production of potatoes in Colorado. It is widely accepted that economic losses due to bacterial ring rot would be much greater if it were not for the success of current certification programs aimed at production and commercial distribution of disease-free seed. Unfortunately, the availability and use of disease-free seed has not eradicated the pathogen, Clavibacter michiganense subsp. sepedonicum (CMS), which is often present in the absence of disease symptoms. There are no chemical control measures for limiting the pathogen in seed used in table stock production, or for preventing certified seed from becoming contaminated by any of several possible sources. Chemical disinfestation of cutting equipment and storage facilities helps to limit spread, but is inadequate to prevent recontamination of seed by bacteria carried in irrigation water or on weeds. The primary objective of this proposal is the development of innovative strategies for the detection and control of BRR in Colorado.

Project Status:

This is an ongoing project. In 1990, the SLV Research Center Committee allotted funds for the development of a biological control system for CMS. In 1991 and 1992, the Committee allotted funds for a continuation of an expanded program aimed at the ultimate goal of managing BRR. These objectives reflect my overall bacterial ring rot program were stated in my 1991 and 1992 proposals. In 1993, I propose to extend and expand previous studies on 1) the development of biological control agents for bacterial ring rot; 2) mapping of resistance and immunity genes in Solanum spp., and 3) evaluate methods for detecting CMS. The status of each of the objectives is summarized below under significant accomplishments.

Significant Accomplishments 1992-1993:

Objective 1: Continue work on the biological control of bacterial ring rot. This project was initiated in 1990 and has been continued to the present date. Funds provided by the SLV Research Center Committee were matched in 1991-1992 by a grant awarded from the Colorado Advanced Technology Institute/Colorado Institute for Research in Biotechnology. To be eligible for this award, an awardee had to have prior funding from an industry in Colorado. The overall goal of the project is the development of biocontrol agents that can be applied to seed prior to planting to limit the spread of bacterial ring rot in commercial production and prevent the recontamination of generation seed.

Bacteria that live inside healthy or diseased potato plants are called endophytic bacteria. Over 700 endophytic bacteria were isolated from potatoes grown in Colorado. Preliminary screening of these bacteria as potential biological control agents for BRR was initiated in greenhouse studies with inoculated potato plants. Of 117 bacterial strains screened to date, 35 strains were selected for further study. Of these, six reduced incidence of disease to zero when rescreened in the greenhouse. All six strains had

antimicrobial activity against CMS in laboratory tests. Biocontrol activity of these six strain was evaluated in 1992 in field trials planted at the SLV Research Station and at the Bay farm in Fort Collins. Significant differences due to strain, inoculation method, and interactions between inoculation methods and strain were detected in foliar symptoms and ELISA values in some cases. A more thorough explanation of these results can be found in the long report of 1992. The results were encouraging, although not striking. Disease incidence at the Bay farm was nearly 100% by the end of the season regardless of treatment. Disease incidence generally was less in the SLV plots than in the Bay farm plots and highest when seed was inoculated by vacuum infiltration.

Objective 2: Evaluate immunity and resistance of Solanum spp. in a potato bioassay. During 1991 and 1992, true seeds, tissue culture plantlets, or tubers of 54 different Solanum species were obtained from investigators in Idaho, Virginia, Wisconsin and Colorado. A subset of these, containing 26 accessions and representing nine species of Solanum, was evaluated for sources of BRR resistance or immunity. To map the genes for resistance or immunity, accessions were established in tissue culture (completed), screened for reaction to bacterial ring rot in potato (completed), and grown in a greenhouse to obtain leaves from which DNA was extracted (completed). Restriction fragment length polymorphism (RFLP) and randomly amplified polymorphic DNA (RAPD) analyses also were completed. The most exciting finding of these studies is the identification and verification of immunity in some of the wild Solanum species and the presence of DNA polymorphisms that can be used to map the immunity gene(s).

Objective 3: Compare methods for detecting and quantifying the ring rot bacterium. In 1990, spontaneous antibiotic-resistant mutants of CMS were selected from strains isolated from potatoes grown in Colorado. In 1991, preliminary studies of the relative virulence of these mutants under field conditions indicated that several of the mutants were as virulent as their parental strain. The goal was the production of marked strains of the pathogen that could be isolated and quantified by direct colony counts on agar media containing the antibiotic to which the mutant was resistant. Other organisms present in potato would not grow on the medium while the antibiotic-resistant mutant would grow in the presence of the antibiotic. However, attempts to isolate CMS from infected eggplants and potatoes during 1990 - 1992 were unsuccessful. Recently, we discovered that the nutrient broth yeast extract medium used to grow CMS contains concentrations of phosphate that inhibit the growth of the pathogen, whether antibiotics are present or not. By omitting phosphates from the medium, recovery of CMS from plant material or from cultures of CMS improved by 1000-fold. Also in 1992, we perfected the immunofluorescent serological detection assay (IFAS) and use it routinely. IFAS was especially valuable for identifying immunity to BRR in Solanum.

Objectives for 1993-94:
1) Continue greenhouse and laboratory screening of endophytic bacteria as biocontrol agents of BRR, and field evaluations of potential biocontrol agents, with an emphasis on determining the best way to apply biocontrol agents. 2) Continue studies to map the genes in Solanum spp. involved in immunity and resistance to BRR. 3) Continue evaluations of methods to detect and quantify C. sepedonicum.

Funding: 1991-1992 Allocation $12,000 1992-1993 Allocation $12,500
1993-1994 Request $15,000

Budget Summary 1993:			
	Labor	$12,000	($9,000 for research assistant and $3,000 for part-time student help)
	Supplies	$ 3,000	(media, enzymes, petri dishes, IFAS and ELISA kits)
	Total	$15,000	