

**SUMMARY RESEARCH PROGRESS REPORT FOR 1994
AND RESEARCH PROPOSAL FOR 1995**

Submitted to:
SLV Research Center Committee
and the
Colorado Potato Administrative Committee (Area II)

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

Project Leader: C. A. Ishimaru, Department of Plant Pathology and Weed Science

Project Justification:

Bacterial ring rot (BRR) is an economically significant disease affecting seed and table stock production of potatoes in Colorado. Seed certification programs have helped reduce losses from BRR, but the disease still occurs and efforts to eradicate the pathogen, Clavibacter michiganensis subsp. sepedonicus (Cms) have failed. The long term goal of my research is to eliminate BRR in the seed and commercial potato industries in Colorado. Four approaches divided into short term annual objectives are being taken: 1) improve host resistance by incorporating genes for BRR immunity into cultivated potato; 2) prevent seed infection by seed treatments with biocontrol agents; 3) improve assays for detecting the pathogen, and 4) identify the key ways that Cms causes disease and use this information to design strategies to prevent disease.

Project Status:

This is an ongoing project, funded since 1990. The funds have been used to help support technical assistants and to purchase supplies such as media, potting soil, chemicals, Cms detection kits, petri dishes, pipets, and disposable glassware.

A major portion of the funding has been used to evaluate biological control as a means of reducing BRR in the field. I began by isolating potential biocontrol agents from potato and then developed a BRR assay to screen for biocontrol agents in the greenhouse. After identifying strains that reduced BRR in greenhouse studies, field trials were conducted in the SLV and Fort Collins in 1992, 1993, and 1994. The overall field performance has been poor, and at this time I do not intend to pursue biocontrol in the field further.

Funds from the SLV Research Committee have also supported greenhouse and tissue culture experiments to identify species that are immune to BRR. The screening method developed for biocontrol studies has worked very well for these studies. Two wild relatives of cultivated potato were shown to be immune to BRR in the greenhouse. Nora Lapitan and I have designed a strategy for mapping the genes for immunity. My project has paid for propagating plants in tissue culture and in the greenhouse, for inoculating plants to screen for immunity, and for detecting Cms by immunofluorescent antibody staining (IFAS). I plan to continue my collaboration with Nora in 1995. I also plan on verifying that immunity, which has been demonstrated in greenhouse trials only, holds up under field conditions. Additionally, I will test the effect of strain on immunity, as all studies to date have been conducted using only one strain of Cms.

Funds were also used for detecting Cms by IFAS, ELISA, polymerase chain reaction (PCR), and selective growth on antibiotic media. I have obtained my goal of incorporating IFAS, PCR and

selective media into my program. All of these methods are used routinely in the immunity and biocontrol research.

Finally, funds in 1994-95 were used to pay for supplies to examine the role of sucrose in disease. A post-doctorate research associate funded by the Agriculture Experiment Station has conducted these studies. Preliminary results are consistent with the idea that foliar symptoms of BRR are associated with changes in a plant's balance of sucrose and glucose. I plan to continue this study, as it may lead to a way of detecting disease before the appearance of visible symptoms.

Significant Accomplishments 1994-1995:

- Completed the third year of field trials of biocontrol agents in the SLV and in Fort Collins. A sticking agent (carboxymethylcellulose) was used to apply the biocontrol agent CICA90. Disease levels were not reduced by the biocontrol agent regardless of how it was applied. I also found that CICA90 colonizes roots and stems of potato in inoculated potato plants grown in the greenhouse.
- Identified sources of immunity to BRR in wild relatives of potato.
- Completed greenhouse and IFAS experiments that, together with N. Lapitan's genetic analyses, showed two genes are required for immunity, and that a genetic marker from tomato segregates with immunity.
- Evaluated a tissue culture method to screen for immunity. Cells of Cms were detected in the immune accession, *Solanum acaule* 7-8; however, growth of the pathogen population was not observed. Cms cells detected in 7-8 were clumped, unlike those in non-immune accessions.
- Determined that a tissue culture assay is not suitable for rapid immunity screening, but may be suitable for studies on tissue specificity and the biochemical basis of immunity.
- Collaborated with A.S.N. Reddy to evaluate a quantitative PCR method for detecting Cms. A manuscript summarizing these results has been prepared for publication.

Objectives for 1994-95:

I will focus my efforts in 1995 on evaluating immunity under field conditions, summarizing results from biocontrol studies, completing comparative studies on detection of Cms by PCR and IFAS, and examining the role of sucrose in BRR symptom development.

Funding:

1994-1995 Allocation:	\$15,000
1995-1996 Request:	\$20,000*
Budget Summary 1995:	\$15,000 personnel: \$10,000 for research assistant and \$ 5,000 for hourly help
	<u>\$ 5,000</u> supplies: media, enzymes, petri dishes, IFAS kits, and PCR reagents
	\$20,000 TOTAL

*Increase reflects a higher cost for skilled technical assistance and additional costs for routine IFAS and PCR testing.