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Innovative Strategies for the Detection and Control of Bacterial Ring Rot

Submitted by:

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Introduction

Bacterial ring rot (BRR) is an economically significant disease affecting seed and table stock production of potato 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. I have taken four approaches, which are divided into short-term annual objectives, to reach this goal. The approaches are 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. This report summarizes the significant findings of 1994 and is arranged with respect to the four approaches listed above.

Results and Discussion

Approach 1: Improve host resistance by incorporating genes for BRR immunity into cultivated potato.

This is a collaborative project between Nora Lapitan and I. Please refer to the 1994 Annual Report entitled "Molecular Markers for Bacterial Ring Rot and Resistance Genes" for a detailed description of the strategy and progress toward this approach. The major objective was to identify a source of genes for immunity. This was accomplished and our results were published in the American Potato Journal (see attached).

Immunity is a form of resistance which prevents establishment of the pathogen in the plant. This is desirable for BRR control because it would prevent latent infections from occurring in seed or table stock production. We found immunity in two wild relatives of cultivated potato, *Solanum tuberosum*. In the past year, we made crosses of immune and non-immune accessions and screened the progeny for immunity. We found that only two genes are required for immunity. We also found that a genetic marker from tomato segregates with immunity in the F1 progeny. This

success has led us to propose genetic mapping of the immunity genes, as well as field evaluation of immunity.

Approach 2: Prevention of seed infection by seed treatments with biocontrol agents.

Bacteria that live inside potato were used as a source of potential biological control agents (BCAs) for BRR. Over 700 endophytic bacteria isolated from potatoes grown in Colorado have been stored cryogenically in my lab. Two hundred bacterial strains have been screened for activity against BRR in inoculated potato plantlets grown in a greenhouse. Several BCAs reduced disease to 0% and produced antibiotics that killed Cms in petri-dish experiments.

A three year field study of biocontrol conducted in Fort Collins and in the San Luis Valley was completed in 1994. Significant differences in external or internal BRR symptoms, stand counts, plant height, or yield were not detected at either location. Significant differences due to inoculation method were found at most sample dates at both locations. Disease was greatest in plants grown from seed inoculated by vacuum infiltration. Less disease occurred in plants grown from seed inoculated with a contaminated cutting knife. Attempts to improve disease control by applying BCAs in a slurry of carboxymethylcellulose were unsuccessful (Table 1).

Biocontrol under field conditions remains elusive. Overall, field performance of BCAs was very poor. Possibly the disease pressure in the field was greater than that in the greenhouse. A major difference between the two situations is that BCAs in greenhouse studies were applied before inoculating with Cms. BCAs in field studies were applied after inoculating with Cms. Though a recent report from Solke DeBoer on biocontrol of BRR in Canada indicates BCAs can reduce disease under field conditions, the level of control varied markedly from field to field and year to year. I do not think further biocontrol experiments are justifiable at this time and have decided to discontinue biocontrol studies for the time being. I will consider an offer to collaborate with Dr. DeBoer in the future, but do not intend to allocate funds for biocontrol this field season.

Approach 3: Improve assays for detecting the pathogen.

Methods for diagnosis of Cms have been developed in several labs across the United States. This technology has many applications in seed certification and can aid id understanding the ecology and epidemiology of BRR. I have incorporated these methods into my research and currently use IFAS, ELISA, polymerase chain reaction (PCR), and growth on antibiotic media for detecting and enumerating Cms.

In 1994, a collaboration with A.S.N. Reddy, Department of Biology, CSU, was initiated to evaluate quantitative PCR as a means of estimating the population size of Cms in infested tissues. We found that the number of Cms cells in inoculated eggplants estimated by IFAS was positively correlated with the intensity of the PCR signal (Table 2, Figures 1 and 2). A manuscript on this work has been prepared for

publication.

Also in 1994, a tissue culture assay to screen for immunity was evaluated by IFAS and PCR (Figure 3). Cells of Cms were detected in the immune accession, *Solanum acaule* 7-8; however, the population of Cms in the tissue did not increase over time. As visualized by IFAS, *S. acaule* contained aggregates of Cms cells, while non-immune accessions contained single non-aggregated cells. It is concluded that a tissue culture assay cannot be used to screen for immunity, probably because IFAS and PCR detect both living and dead cells; however, a tissue culture assay may be useful in studying the biochemical basis and the timing of the immune response.

Approach 4: Identify the key ways that Cms causes disease and use this information to design strategies to prevent disease.

This is a new approach, which has the short term objective of evaluating the role of sucrose in BRR symptom development. A post-doctorate research associate funded by the Agriculture Experiment Station has conducted studies on sucrose levels in symptomatic and asymptomatic tissues from eggplant and potato. 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 pursue this approach, as it may lead to a way of detecting BRR before visible symptoms appear in the field.

Table 1. Summary of 1994 BRR biocontrol field trials[a] conducted at the San Luis Valley Agriculture Experiment Station and at ARDEC in Fort Collins, CO.

Disease Variable Measured	Significant differences detected in San Luis Valley field trial	Significant differences detected in ARDEC field trial
Plant emergence (%)	inoculation method BCA x inoculation method	BCA x inoculation method
Plant height (average of 1-3 readings, cm)	inoculation method	n.s.[b]
Incidence of foliar symptoms at harvest (%)	inoculation method BCA x inoculation method	n.s.
Severity of foliar symptoms at harvest (%)	inoculation method	n.s.
Severity of foliar symptoms at harvest (0-10 rating)	inoculation method	n.s.
External tuber symptoms at harvest (%)	inoculation method	inoculation method BCA x sticking agent BCA x sticking agent x inoculation
Internal tuber symptoms at harvest (%)	inoculation method	BCA x sticking agent
yield (kg)	inoculation method	BCA x inoculation method

[a]Seed pieces of cv. Sangre were treated prior to planting with biocontrol strain CICA90 (BCA) applied in a sticking agent (carboxymethylcellulose) or in water. The pathogen was applied by vacuum infiltrating freshly cut seed pieces with a 10^8 cfu/ml cell suspension of CIC31 or by cutting with a knife dipped in a cell suspension of CIC31. The experimental design was a randomized complete block with 12 treatments and 8 replications. Statistical analyses were performed by Jim Zumbrunnen, Dept. of Statistics, CSU. Disease was not detected in any of the negative control plots. Results from negative controls were not included in the data analyses, and the table summarizes only differences due to the present or absence of the BCA.

[b]n.s. = not statistically significant.

Table 2. IFAS[a] and PCR[b] detection of *C. m.* subsp. *sepedonicus* in eggplant and potato plants 65 and 105 days, respectively, after inoculation with CIC31. Major veins of plant leaves were separated from mesophyll tissue. Chlorotic regions (CR) and non-chlorotic regions form symptomatic leaves were analyzed separately.

Host	CMS treatment[c]	Tissue sampled	Detection by PCR	Detection by IFAS
Eggplant	-	basal stem	-	0
	-	major vein of lower, mature leaf	-	0
	-	mesophyll of lower, mature leaf	-	0
	-	major vein of upper, expanding leaf	-	0
	-	mesophyll of upper, expanding leaf	-	0
	+	basal stem	+ +	2.9
	+	major vein of lower, mature leaf	+ +	3.9
	+	NCR mesophyll of lower, mature leaf	+	2.6
	+	CR mesophyll of lower, mature leaf	+ + +	3.3
	+	major vein of upper, expanding leaf	+ + + + +	4.7
	+	mesophyll of upper, expanding leaf	+ + + +	4.3

Host	CMS treatment[c]	Tissue sampled	Detection by PCR	Detection by IFAS
Potato	-	basal stem	-	0
	-	major vein of middle leaf	-	0
	-	mesophyll tissue of middle leaf	-	0
	-	major vein of upper, expanding leaf	-	0
	-	mesophyll of upper, expanding leaf	-	0
	-	basal stem	+ + + +	4.4
	-	major vein of middle leaf	-	1.5
		NCR mesophyll of middle leaf	+	1.5
		CR mesophyll of middle leaf	-	0.7
		major vein of upper expanding leaf	+	2.1
		mesophyll of upper, expanding leaf	+ +	1.5
		tuber	+ +	2.6

[a]IFAS = log(IFAS units)/ sample

[b]PCR = relative intensity of signal: (-) = no signal; (+ to + + + + +) = increasing signal intensity.

c(+) indicates plants were inoculated with cells of CIC31. (-) indicates plants were mock inoculated only.

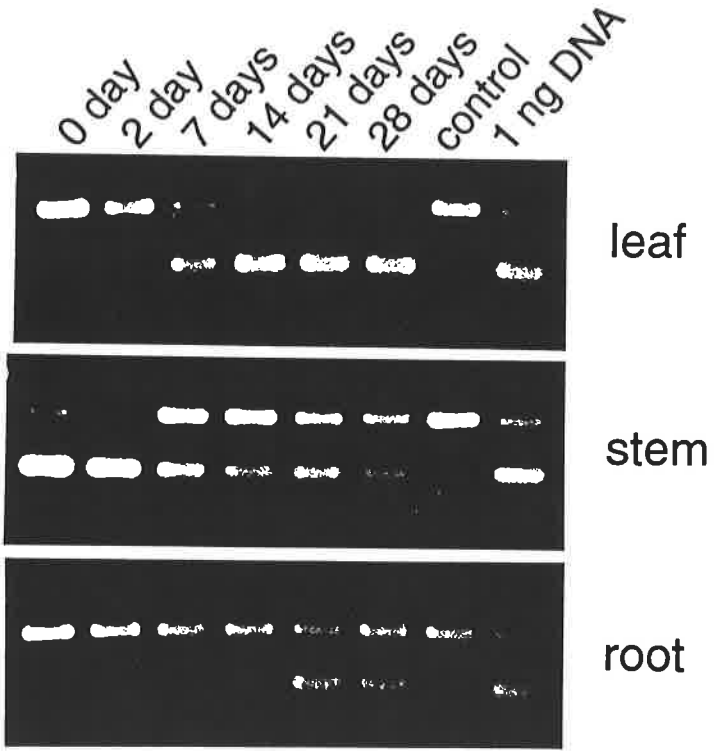

Fig. 1. Quantitative PCR analysis of CMS colonization in susceptible eggplants. Plants inoculated with bacteria were sampled at 0, 2,7,14,21, and 28 days after inoculation from leaf, stem, and root tissues. An equal amount of plant samples (e.e. 0.2 g) was extracted with CTAB buffer to obtain DNA.

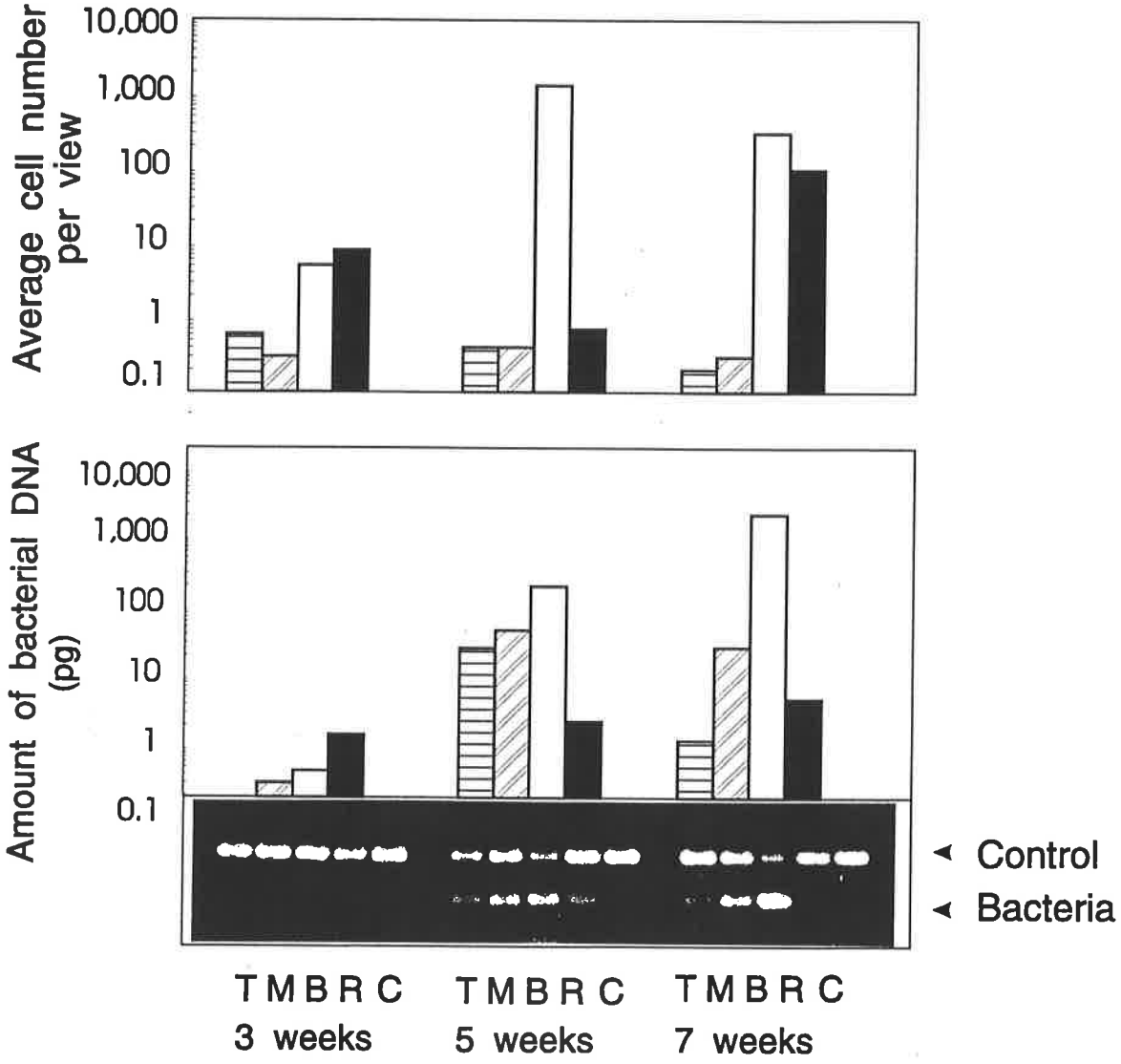

Fig. 2. Quantification of bacterial colonization in potato plants by PCR-based assay and IFAS method. The histograms summarize the quantitative results of IFAS assay (upper panel) and PCR method (lower panel). The average cell number per view was made from 10 independent views. Results from top stem (T), middle stem (M), base stem (B), and root (R) tissues are presented. A control sample (C) was taken from a stem section of an non-inoculated, water-treated plant.

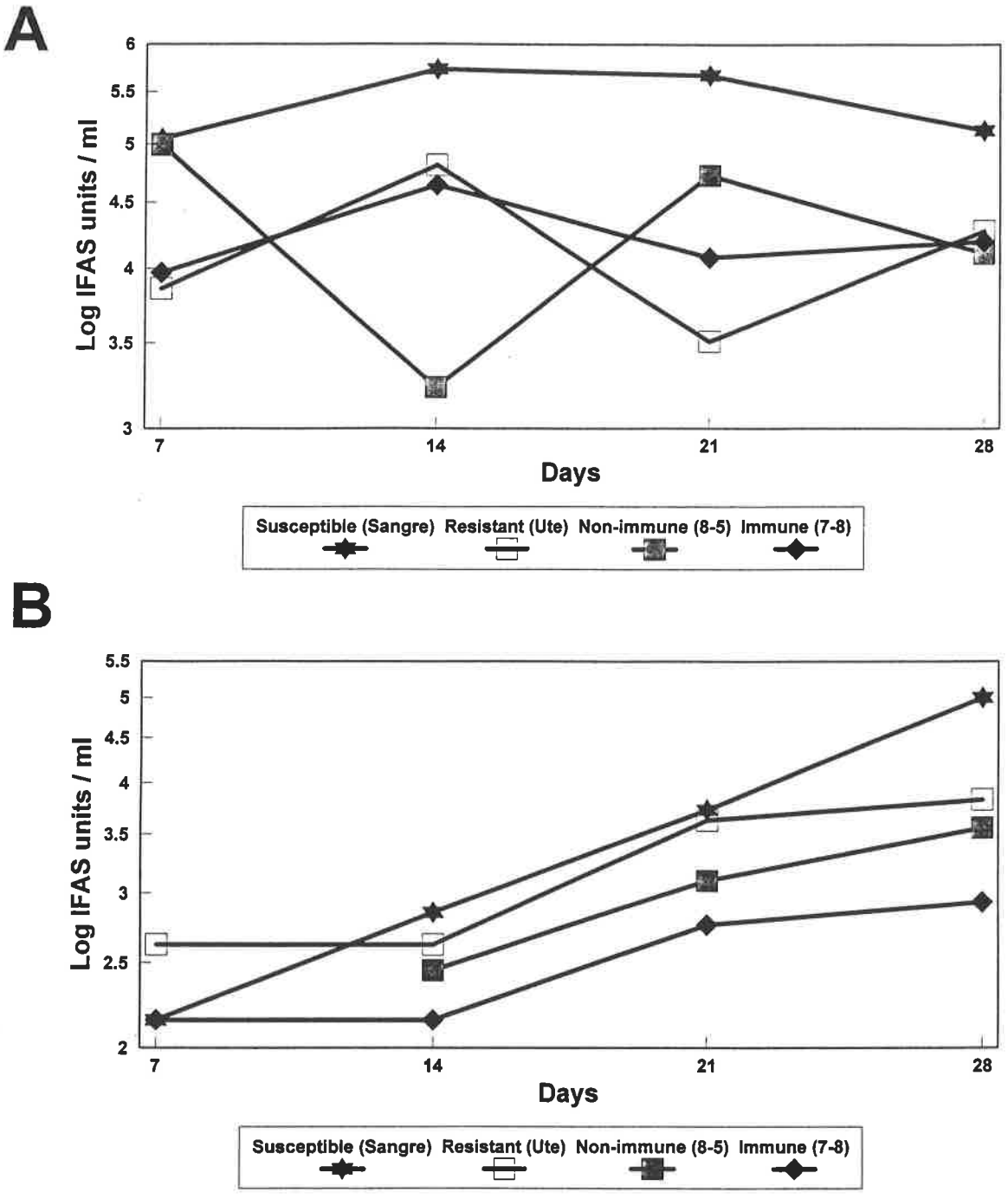

Fig. 3. Tissue culture assay to monitor CMS populations in immune and non-immune potato accessions. Nodal sections of Sangre S14 (susceptible), Ute (resistant), *Solanum acaule* 8-5 (non-immune) and *S. acaule* 7~~0~~8 (immune) were inoculated with about 20 cells of CIC31 before transferring to MS media and incubating in a growth chamber. The first centimeter of growth closest to the node was removed at 7, 14, 21, and 28 days post inoculation. The number of cells of CMS were determined by IFAS. Ten nodal sections of each accession were examined for each time point. The entire experiment was completed three times. Results from two of these replications are presented in panels A and B.