SUMMARY RESEARCH PROGRESS REPORT FOR 1992
AND RESEARCH PROPOSAL FOR 1993

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

TITLE: BACTERIAL RING ROT SYMPTOM DEVELOPMENT ON MICROPLANTS GROWN IN THE FIELD

PROJECT LEADERS: Robert Davidson & Gary Franc

PROJECT JUSTIFICATION: Bacterial ring rot (BRR) of potatoes caused by *Clavibacter michiganensis* subsp. *sepedonicus* (Cms) has been a serious disease in the San Luis Valley for decades. Recently, programs designed to clean up seed stocks by using tissue culture derived sources of seed have been implemented in the Colorado seed potato program as well as most other areas of North America. They have proved extremely successful, however, there have still been many unexplained cases of BRR cropping up in seed stocks derived from these clean sources after two or three years in the field. This inability to eradicate BRR suggests that there are unidentified sources of Cms inoculum which exist. This project was begun to examine the potential for latent Cms infections in basal level tissue culture stocks and examine the actual symptom expression of BRR in these stocks.

PROJECT STATUS: Initial funding in 1992.

1992 SIGNIFICANT ACCOMPLISHMENTS: Four varieties commonly grown in the San Luis Valley; Centennial Russet, Sangre, Russet Norkotah and Russet Burbank, were root inoculated with three levels of Cms (10^0, 10^2, & 10^8 cfu/ml) as microplants. Four replications of between nine to fifteen plantlets of each variety at each inoculum level were planted in a randomized complete block design. These treated plantlets were grown at two locations; the SLV and Torrington, Wyoming, representing two very distinct environmental conditions.

There were significant differences in stands between the varieties and the treatments. Stands of Russet Burbank and Sangre remained at 100% regardless of the treatment or time of the season. Stands of Russet Norkotah and Centennial Russet dropped off incrementally from the control to the highest inoculum levels. As the season progressed, there was a further decrease in overall stands with the most severe decreases in the Cms+ treatments (see Table/figure 1). It is not uncommon for BRR symptoms to be expressed in the field with the death of the infected plant over time and as such, the significant decrease in stands of both Russet Norkotah and Centennial Russet should be considered as a visible symptom of BRR. It is interesting to note that both of these varieties are known for their latent reaction to BRR.

Visual foliar BRR symptoms were seen only in the Russet Burbank plantlets at the highest inoculum level during the growing season (see Table 2). First symptoms were visible at 45 days after planting. Symptoms included early dwarfing, rosette and interveinal chlorosis. This is 15 days earlier than the symptom development normally experienced in the BRR plots.

At harvest, exterior tuber symptoms of BRR were seen in both the Russet Burbank and Sangre, low and high inoculum levels. There were more obviously damaged tubers at the high inoculum levels than at the low levels. Yield was reduced in numbers of tubers produced and size of these tubers in the Cms+ treatments. The control had the highest numbers and largest tuber size while the highest Cms inoculum level had the lowest number and size. Over all varieties the control typically had eight to ten tubers per plant in the 100 to 250 gm. (1-2 oz) range. The Cms+ treatments had one to three tubers per plant with the largest tubers below the 100 gm. size. Both growing areas had similar results with the Wyoming location experiencing severe psyllid damage during the growing season making readings difficult.

1993 OBJECTIVES: This project will be under a USDA-ARS Cooperative Agreement (formal commitment of the funding has not yet been made) for 1993. A copy of the research proposal is included with this summary. All tubers produced from the 1992 plot will be grown and plants examined for symptoms in 1993.

FUNDING REQUEST:

1992 Allocation:	$4000.00
1993 Request:	None

Table 1. Microplant stand counts (San Luis Valley).

Treatment	35 DAP		56 DAP
	% stand	plant condition*	% stand
RB 10^0	100	4	100
RB 10^2	100	4	100
RB 10^8	100	4	100
SG 10^0	100	4	100
SG 10^2	100	4	100
SG 10^8	100	3	100
NK 10^0	100	3	89
NK 10^2	96	2	75
NK 10^8	78	2	28
CR 10^0	87	2	64
CR 10^2	57	1	5
CR 10^8	66	2	16

*Plant condition is a measure of the general appearance of the individual microplants with 1=poor and 4=excellent.
DAP refers to days after planting.
Planting date - 6/23/92, harvest date - 9/15/92.

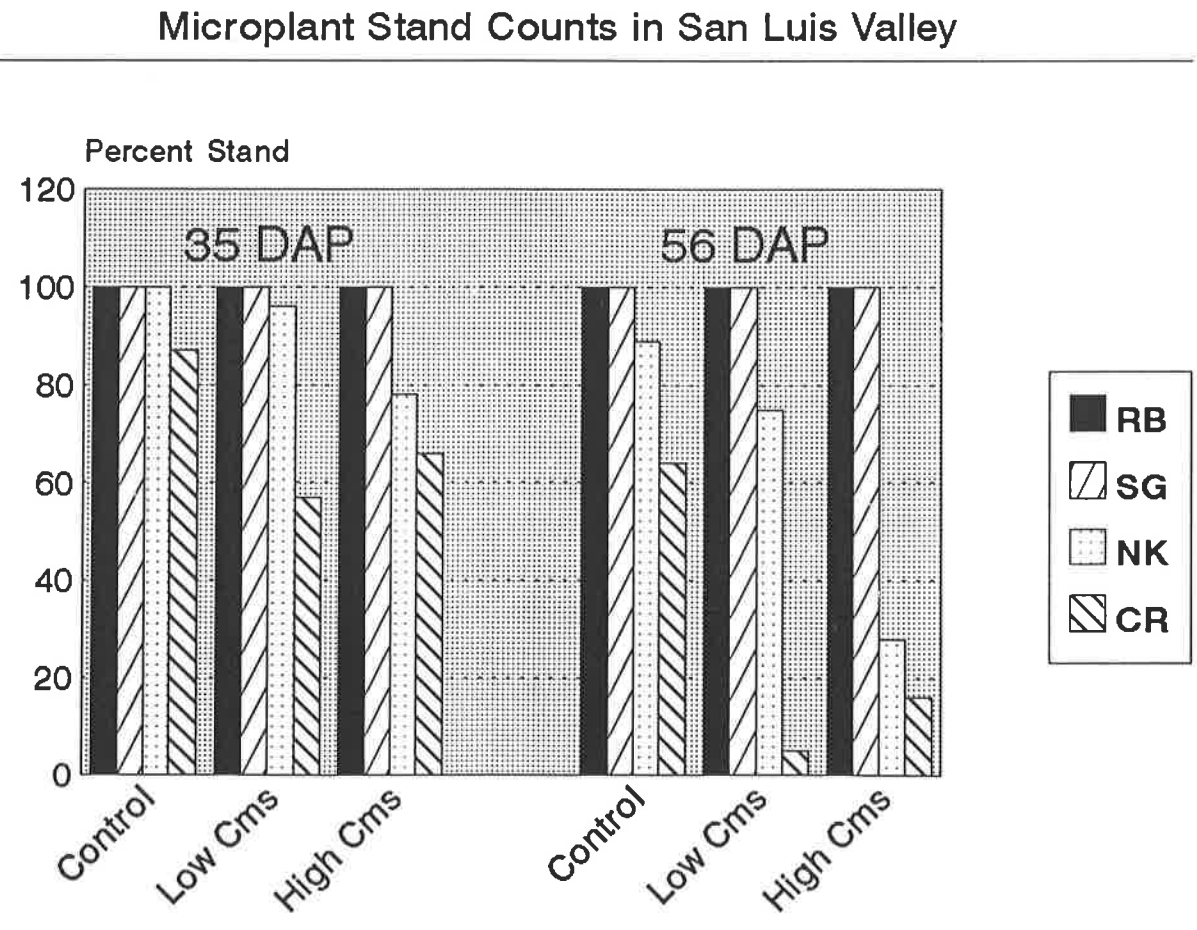

Table 2. BRR symptom expression - (San Luis Valley).

	# positive/total #	symptoms*	
45 DAP			
RB 10^8 -Rep1	5/10	R, ED & IVC	
RB 10^8 -Rep2	6/ 9	" "	
RB 10^8 -Rep3	4/ 9	" "	
56 DAP			
RB 10^8 -Rep1	10/10	R, ED & IVC	(33% affected)
RB 10^8 -Rep2	8/ 9	" "	(50% affected)
RB 10^8 -Rep3	4/ 9	" "	(49% affected)

*R=rosette, ED=early dwarfing & IVC=interveinal necrosis
DAP refers to days after planting.
Planting date - 6/23/92, harvest date - 9/15/92.

Clavibacter michiganensis subsp. *sepedonicus* Transmission via Tissue Culture Derived Microtubers, Plantlets and Minitubers

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Project Justification

Project Summary: The overall objective of this work is to determine the potential for "accidental" dissemination of *Clavibacter michiganensis* subsp. *sepedonicus* via tissue culture derived propagation material. We will use the same methods currently employed by seed potato growers for in vitro, greenhouse and field production of seed potatoes. Materials and procedures proposed for this study are briefly described below. Facilities for conducting this research are presently available at the University of Wyoming and at the Colorado State University San Luis Valley Research Center. Field plot space is available at Center, CO and at Laramie and Torrington, WY. The funding level requested is $34,037.00.

Introduction: Bacterial ring rot (BRR) of potatoes is caused by *Clavibacter michiganensis* subsp. *sepedonicus* (CMS). The adoption of tissue culture methods and limited generation seed potato production programs has reduced the incidence of BRR in certified seed. However, some certification programs still have an unexplained low incidence of BRR in upper level tissue culture derived stocks (one to three years after planting to the field). Therefore, there are indications that BRR may be escaping detection within these tissue culture programs. Furthermore, vine death for most greenhouse crops and first year production occurs at 90 days or less, reducing time available for symptom expression.

The transmission of inoculum through tissue culture derived propagation material has not been characterized. Virtually all certified seed produced today originates from a tissue culture base. Therefore, if infection occurs and remains undetected, the potential for a massive crop failure and substantial monetary loss to the grower exists.

Procedures and Objectives

Bacterial Strains: Antibiotic resistant CMS strains will be used to facilitate detection and identification of living bacterial cells recovered from infected plant tissue. These 'marked' CMS strains were selected in Dr. Carol Ishimaru's laboratory (Colorado State University) and were selected for normal symptom development and marker stability in potato under different environmental conditions at three field locations (Fort Collins and Center, CO and at Torrington, WY) during 1991 and two field locations (Center, CO and Torrington, WY) during 1992.

Host Plants: Disease-tested in vitro plantlets from the CSU clone bank will be the tissue source for all studies. Three cultivars, Sangre, Russet Burbank and Russet Norkotah will be used. Results from field and greenhouse tests showed Sangre foliage and tubers developed BRR symptoms more readily and consistently than Russet Norkotah. Russet Burbank also readily expressed BRR symptoms and, occasionally, had a characteristic "early dwarfing" symptom visible in the field that was not observed in the other cultivars. Thus, these cultivars represent a wide range of symptom expression.

Normal tissue culture methods will be used to generate microtubers and in vitro plantlets; microtubers will be produced in vitro by incubating plantlets in the dark. Plantlets will be transplanted into the greenhouse for minituber production.

Host Plant Inoculation: Nodal cuttings from in vitro plantlets will be inoculated with at least three concentrations of CMS plus a negative check. The inoculum will be quantified using a hemacytometer. Inoculation will be done by placing a drop of an aqueous CMS suspension onto the basal portion of a fresh nodal cutting and then applying a slight vacuum to the apical end to draw the suspension into the stem. This method has worked well in previous studies. Inoculated nodal cuttings will be placed into tissue culture to generate progeny in vitro plantlets using normal tissue culture methods.

CMS Assay: Presence of CMS will be verified by culturing on media, amended with the appropriate antibiotic, to recover marked strains. Cultural methods are needed to detect latent infection and to determine if transmission of living, infectious cells occurred. Bioassay of recovered strains will be done to verify pathogenicity. Immunofluorescence staining (IFAS) will also be done to verify presence or absence of CMS.

Objective 1. To determine the potential for transmission of CMS via infected in vitro plantlets.

Nodal cuttings will be inoculated as described above. Plantlets derived from inoculated nodal cuttings will be subcultured by transferring the apical, intermediate and basal nodes to fresh media. The presence or absence of symptoms in vitro will be recorded and a subsample assayed for CMS. Assays will be done for approximately 5 generations, which simulates commercial operating practices. After 5 generations, plantlets will be transplanted into the greenhouse and two field locations. Plants will be observed for symptom expression and assayed for presence of CMS. Progeny tubers will be harvested, symptom expression recorded and assayed for presence of CMS.

Objective 2: To determine the potential for transmission of CMS via microtubers.

Nodal cuttings will be inoculated as described above, at least two generations of in vitro plantlets generated, and microtubers produced. After dormancy, a subsample of microtubers will be planted in the greenhouse and at two field locations. Plants will be observed for symptom expression and assayed for presence of CMS. Daughter tubers will be harvested, symptom expression recorded and assayed for presence of CMS.

Objective 3: To determine the potential for transmission of CMS via minitubers.

Nodal cuttings will be inoculated as described above and in vitro plantlets produced. After approximately 5 generations, plantlets will be transplanted into the greenhouse for minituber production. Symptom expression will be recorded for plants and a subsample assayed for CMS. Minitubers will be harvested and, after dormancy, planted at 2 field locations. Symptom expression will be recorded and stems assayed for presence of CMS. Progeny tubers will be harvested, symptom expression recorded and assayed for CMS.

Proposed Budget

A.	Salaries and Wages	
	Research Associate	15,876.00
	Hourly	2,400.00
B.	Fringe Benefits	4,861.00
C.	Total Salaries, Wages and Fringe Benefits	**$23,137.00**
D.	Materials & Supplies	5,800.00
E.	Domestic Travel (Field Plots & Reporting)	2,900.00
F.	Publication	500.00
G.	Computer	500.00
H.	Land Rental & Plot Maintenance	1,200.00
I.	Total Direct Costs	**10,900.00**
J.	Indirect Costs	0.00
K.	Total Amount Requested	**$34,037.00**