RESEARCH PROPOSAL FOR 1993

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

TITLE: EPIDEMIOLOGY OF BACTERIAL RING ROT - SOURCES OF INOCULUM

PROJECT LEADERS: Robert Davidson & Gary Franc

PROJECT JUSTIFICATION: Bacterial ring rot (BRR) of potatoes is caused by *Clavibacter michiganensis* subsp. *sepedonicus* (Cms). Programs designed to clean up seed stocks through the use of tissue culture derived sources of seed and a limited generation approach have become standards in the seed industry over the last decade. While these programs have been quite successful, there still appears to be an inability to totally eradicate BRR from successive generations in the field. This would suggest that unidentified sources of Cms inoculum probably exist. Nematodes as potential vectors of Cms have not been studied, yet they have been targeted on a national level (identified as a research priority during the USDA-ARS Bacterial Ring Rot Workshop held in Denver) as likely candidates for Cms spread. We propose to determine the potential for Cms transmission and BRR development by soil-borne inoculum and to initiate studies to determine if nematodes are involved. In addition, we will continue the studies to determine the potential for Cms transmission through tissue culture sources currently funded by the USDA-ARS project.

PROJECT STATUS: New project for 1993.

1993 OBJECTIVES: Sangre microplants, root inoculated with marked Cms strains, will be planted in field plots at two locations, the SLV and Torrington, Wyoming, representing two distinct environmental areas. Plants will be evaluated for BRR symptom expression during the growing season and additionally tubers will be rated for BRR expression to verify that BRR development occurred. At harvest, rhizosphere soil will be recovered from the roots of both healthy and inoculated plants, sieved through a fine mesh screen and used in two related experiments. If BRR has a soil-borne phase, the greatest concentration of bacteria would most likely be associated with the rhizosphere. Also, any naturally occurring potato-parasitic nematodes would be concentrated in this soil.

A) Healthy (disease-free) Sangre plantlets will be planted and grown in the sieved rhizosphere soil under controlled greenhouse conditions. Plants will be evaluated and tested for the presence of Cms. Tubers from these plants will be harvested and, after dormancy, planted at two field locations. Resultant plants will be rated for BRR symptom expression and assays repeated to determine if Cms transmission occurred.

B) Nematodes will be recovered from the sieved rhizosphere soil using standard methods. A portion of the population will be assayed for Cms by crushing and plating the nematodes onto culture media selective for the Cms strain. A bioassay of the colonies will also be completed. A second subsample of the nematodes will be applied to the roots of healthy Sangre plantlets growing in sterile (pasteurized) soil. These plants will be grown under controlled greenhouse conditions and evaluated, harvested, and handled in a manner similar to the plants in experiment A.

FUNDING REQUEST: 1993 Request: $5500.00

The majority of the funding request will be utilized for hourly labor, materials and supplies and basic plot care. $600.00 is allocated for travel to actually conduct the experiments. Nematode work will be done at the University of Wyoming with greenhouse work at both Fort Collins and Wyoming.

Epidemiology of Bacterial Ring Rot

Robert D. Davidson & Gary D. Franc
CSU & U of WY

Introduction: Bacterial ring rot (BRR) of potatoes is caused by *Clavibacter michiganensis* subsp. *sepedonicus* (CMS). The inability to eradicate BRR, especially from seedlots produced via limited generation programs, suggests that unidentified sources of CMS inoculum probably exist. Nematodes as potential vectors of CMS have not been studied and was identified as a research priority at the Bacterial Ring Rot Workshop held in Denver. The potential for CMS transmission via tissue culture propagation was also considered a high priority research area.

We propose to determine the potential for ring rot development by soil-borne inoculum and to initiate studies to determine if nematodes are involved (Objective 1). The continuation of studies, initiated in 1992, designed to determine the potential for CMS transmission through tissue-culture propagation is also proposed (Objective 2).

Objective 1. Determination of the Potential for Ring Rot Development Via Soil-Borne Inoculum. Sangre transplants, root-inoculated with marked CMS strains, will be planted in field plots at two locations; the San Luis Valley and a warmer production area (Torrington, WY). Plants will be periodically evaluated for ring rot symptom expression. At harvest, tubers will also be rated for symptom expression to verify that ring rot development occurred. At the time of tuber harvest, rhizosphere soil will be recovered from the roots of both inoculated and healthy plants grown in the field plots. If ring rot has a soil-borne phase, the greatest concentration of CMS would most likely be associated with the rhizosphere. Any naturally occurring potato-parasitic nematodes would also be concentrated in this soil. The soil will be sieved through a fine-meshed screen in preparation for use in two related experiments.

A. Healthy (disease-free) Sangre plantlets will be planted and grown in the sieved rhizosphere soil under greenhouse conditions. Plants will be evaluated and tested for the presence of CMS. Tubers from greenhouse grown plants will be harvested and, after dormancy, planted at two field locations. Resultant plants will be rated for symptom expression and assays repeated to determine if CMS transmission occurred via the rhizosphere soil.

B. Nematodes will be recovered from sieved rhizosphere soil using standard methods. A portion of the population will be assayed for CMS by crushing and plating nematodes onto culture media selective for the CMS strain. A bioassay of colonies will be done. A second subsample of the nematodes will be applied to the roots of healthy Sangre plants growing in pasteurized ("sterile") soil. These plants will be grown under greenhouse conditions and tested for the presence of CMS. Tubers from greenhouse grown plants will be harvested and planted at two field locations. Resultant plants will be rated for symptom expression and assays done to determine if CMS transmission occurred via nematodes recovered from rhizosphere soil.

Objective 2. Determination of the Potential for Ring Rot Development Via Tissue-Culture Derived Propagation Material. This project was initiated during 1992. Psyllid injury at Torrington made data on symptom development meaningless. Funding for a similar study will be provided via an USDA-ARS Cooperative Agreement (formal commitment of the funding has <u>not</u> yet been made).

Proposed Budget

A.	Hourly Labor	$2,730.00
B.	Materials & Supplies culture media, petri plates, greenhouse flats, etc.	$1,000.00
C.	Plot Care	$ 700.00
D.	Field Plot Travel	$1,800.00

###

3-8-93

Rob - Some background Info
I prepared for you Gary

Background Information: Diseases caused by several bacteria have been shown to increase in the presence of nematodes. For example, the incidence of bacterial wilt in alfalfa, caused by *C. michiganensis* subsp. *insidiosus*, increased when feeding by *M. hapla* occurred. Also, *M. incognita acrita* feeding increased the incidence of brown rot of potato, caused by *Pseudomonas solanacearum*. Although it was not determined if *Meloidogyne* spp. served as vectors in these examples, several other nematodes are vectors. Nematode vectors either carried bacteria as a contaminant adhered to the surface of the cuticle, (*C. michiganensis* subsp. *insidiosus* in association with *Ditylenchus dipsaci*) or bacteria were present within the digestive tract of the nematode vector (*Erwinia*, *Xanthomonas* and *Pseudomonas* in association with *Helicotylenchus* and *Pratylenchus*).

There are six major nematode parasites of potatoes in the U.S. Three genera selected for this study, and described below, have been implicated as possible vectors of bacterial phytopathogens. The potato rot nematode, *Ditylenchus destructor*, is a migratory endoparasite that enters the tuber through lenticels or the skin near the eyes. This nematode can survive in soils at temperatures as low as -28 C. The lesion nematode, *Pratylenchus* spp., is a migratory endoparasite that attacks the cortical tissues of feeder roots. *P. penetrans*, a common species attacking potato in the United States, is reported to feed on more than 164 different host plants. The northern root-knot nematode, *M. hapla*, is a common potato parasite that feeds on or near the vascular tissues of roots and tubers infected by CMS. It is a sedentary endoparasite with a broad host range. Juveniles may actively move 3 ft laterally when seeking a host plant and can survive adverse winter conditions by migrating downward in the soil profile, re-appearing months later when host crops are planted and conditions become favorable for infection.

Long distance transport of these nematodes among fields is known to occur via contaminated irrigation water, equipment, seed and, to a lesser extent, wind-blown soil. The host ranges of *M. hapla*, *D. destructor* and *P. penetrans* are broad, enabling them to reproduce on crop plants and weeds commonly found in potato growing areas. If parasitic nematodes are able to acquire CMS from infected plant tissue, the potential for survival of the bacterium in association with the nematode during winter months and its spread to healthy plants the following spring, is highly probable. The fact that small amounts of CMS inoculum would be involved increases the chance that latent infections would result.