Report to the Area II Potato Administrative Committee:

Methods and Materials Used in Determining the Presence of *Clavibacter michiganense* subsp. *sepedonicum* in True Potato Seed

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Introduction

Relatively little is known about how the ringrot bacterium, *Clavibacter michiganense* subsp. *sepedonicum* (*Corynebacterium sepedonicum*), persists in 'healthy' potato seed stocks and causes new infections. However, a recent development has been the discovery that the ringrot bacterium can be recovered from sugar-beet seed. The ringrot bacterium is also known to be present in true seed recovered from the fruit harvested from infected tomato plants.

Because of the close similarities in the physiology of potato and tomato plants, it is also possible that true seed recovered from potato 'seed balls' could also carry the bacterium. Determining the presence or absence of the ringrot bacterium in true potato seed would be a significant contribution to understanding the nature of the disease. The planting and handling of true potato seed is a necessary part of all potato breeding programs and true potato seed is even being developed for use by home gardeners. If ringrot is to be eradicated, all sources of the pathogen must be identified and if effective management strategies are to be developed.

Materials and Methods

Seed balls were harvested from mature potato plants in the 1990 clone testing plots. Seed balls came from flagged plants previously characterized for bacterial ringrot symptom development during the growing season. Seed balls were also harvested from healthy (uninoculated) plants.

Seed balls were surface disinfested in 0.5% sodium hypochlorite (1 bleach:9 water v:v) and placed in labelled paper bags. Bags were stored at 4°C until assay. Although considerable decay of the seed balls occurred in storage, it was still possible to work with a range of seed balls harvested from both infected and healthy plants. The remainder of the seed balls were combined into two composite samples (see Table 1). The weight of the seed extracted from the seed balls and weight of the seed macerated for assay is shown in Table 1.

The seed balls were macerated in a blender with 250 ml of DI water and 1 g of activated yeast at a temperature of 38°C. The mixture was allowed to blend for 1 minute. The suspension was poured out of the blender into 14 oz. clear plastic cups and stored on a lighted shelf at 27°C for 3 days. Seeds (true potato seeds) were separated from the suspension by pouring the suspension through a 250 (sq./cm) screen and running warm water over the screen to remove extraneous tissues. The seeds were then air dried by removing them from the screen, and placing them on paper towels.

Each seed sample was macerated in a sterile mortar and pestle using 3 ml of sterile DI water. The macerate was washed out of the mortar into 40 ml centrifuge tubes using 7 ml of sterile water. The samples were covered and allowed to stand over night in a 4°C refrigerator.

Centrifuge tubes were removed from the refrigerator and vortexed to mix the sample. Each sample was then centrifuged at 200 g for 20 minutes to pellet seed coats and large tissue pieces. The superannuate was decanted into a new sterile centrifuge tube and centrifuged for 20 minutes at

12,000 x G to pellet bacterial cells. The superannuate in the new tube was discarded and the pellet was re-suspended in 100 ul of sterile water.

The eggplant bioassay was used to determine if CMS cells were present in the macerated seed tissues. Nine eggplant (cv. Black Beauty), 3 plants in each 3 pots were wounded for inoculation. A 120 ul aliquot of the sample suspension was applied to the wound of each of the nine eggplants.

Eggplants will be watered and fertilized as required. After seven days, daily observation of the plants will be done to detect CMS symptoms in the foliage of the eggplants. The plants will be maintained for up to 40 days. After 40 days each plant will be cut off 1 cm above the inoculation scar and a Gram smear obtained to determine if infection occurred in the absence of symptom development.

Table 1. True-potato seed (TPS) samples assayed for the presence of CMS bacterium, seed weight obtained from each sample and weight of each sample assayed; G.D. Franc and J. Zizz, Center, CO, 1990-1991.

	Weight in Grams	
Sample Identification	Seed Obtained[1]	Seed Assayed[2]
Clone 17 Healthy	0.78	0.30
Clone 17 Infected	0.01	0.01
Clone 13 Healthy	0.08	0.08
Clone 13 Infected	0.20	0.20
Clone 7 Infected	0.58	0.30
Clone 11 Healthy	0.16	0.16
Clone 2 Healthy	0.41	0.30
Clone 14 Infected	0.45	0.30
Composite Sample #1	0.60	0.30
Composite Sample #2	2.16	0.30

[1] TPS was harvested from the BRR clone testing plot during Sept. 1990.
[2] TPS was assayed using the eggplant (cv. Black Beauty) bioassay.

Results

Inoculation of the eggplants was done during early February, 1991 and development of symptoms may require up to 40 days. A final report will be prepared after the procedures outlined in the materials and methods are completed.

Acknowledgment

This work was done with the assistance of Mr. Jim Zizz.