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A Simple Technique to Test for the Presence of *Phytophthora erythroseptica* in Soil

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ABSTRACT

Pink rot of potato, caused by *Phytophthora erythroseptica*, is one of the most important potato tuber diseases worldwide. To help identify fields with soils infested with *P. erythroseptica*, a simple and cost-effective technique was developed to test for the presence of this organism in soil. Disease-free potato tubers were surface disinfested with 0.6% NaClO, rinsed in sterile distilled water, and then left to dry in a laminar hood. A potato core, 5 mm in diameter and 20 mm deep, was removed from the stem end of a tuber, filled with 1 g of soil, and then covered by inserting a portion of the core that had been removed. Positive (vermiculite and sterilized field soil mixed with *P. erythroseptica* mycelium) and negative (vermiculite and sterilized field soil free of *P. erythroseptica*) controls were included. Tubers were then stored in clear polyethylene containers padded with a sheet of moistened cheese cloth. Tubers were misted with distilled water and the containers were covered and incubated at 10°C and 95% RH. After three weeks of incubation, each tuber was cut longitudinally and then exposed to air for 30 min. Tubers with pink-black flesh were considered positive for pink rot.

Keywords: disease assay, pink rot, potato, *Solanum tuberosum*

INTRODUCTION

Pink rot, caused by *Phytophthora erythroseptica* Pethyb., is a disease of economic importance to the Canadian potato industry and worldwide, and is also responsible for severe yield losses in the field and storage facilities (Powelson *et al*. 1993; Secor and Gudmestad 1999). In the province of New Brunswick, losses caused by this disease amounted to 20 million dollars in 2004 (Al-Mughrabi *et al*. 2007). The recovery of metalaxyl-resistant strains and increase in the incidence of this disease in certain potato growing areas of North America has led to a renewed interest in studying the causal pathogen (Lambert and Salas 1994; Goodwin and McGrath 1995; Salas *et al*. 2000a; Taylor *et al*. 2002). The incidence and severity of this disease are favored by warm and wet climatic conditions at harvest, although potato infection could occur immediately after emergence (Rowe and Nielsen 1986). Tuber infection can occur through stolons, lenticels, tuber eyes, and wounds (Salas *et al*. 1997; Salas *et al*. 2000b; Taylor *et al*. 2004). The pink rot pathogen can be introduced into uninfested soils through infected seed tubers (Cunliffe *et al*. 1977) and can survive in soil in the form of oospores for many years (Vujicic and Park 1964). Crop rotation, planting potato crops in well drained soils, avoiding excessive irrigation, allowing sufficient time between vine killing and harvest for periderm development, modifying tuber handling procedures to reduce wounding and harvesting when tuber temperatures are below 21°C are some of the management practices undertaken to reduce pink rot infection and disease development (Powelson *et al*. 1993; Secor and Gudmestad 1999; Lambert and Salas 2001; Salas and Secor 2001). Knowing the *P. erythroseptica* status of the soil could be helpful to the grower in planning crop rotations for subsequent seasons. The present study describes a new procedure which was developed to test soil samples collected from potato fields for the presence of *P. erythroseptica*.

MATERIALS AND METHODS

Potato tubers

Potato cv. 'Red Norland' (Elite 1 class; Bon Accord Elite Seed Potato Centre, New Brunswick, Canada) was used in this study.

Field soil samples

Forty eight soil samples were collected randomly from potato fields of 5 farms in New Brunswick, Canada and were used in this study. Testing of the soil samples for the presence of *P. erythroseptica* was repeated three times.

Generation of inoculum for use as controls

Tubers exhibiting pink rot symptoms were collected from potato storages in New Brunswick, Canada. Small tuber tissue samples (10 mm × 5 mm × 3 mm) were taken from the margins of the internal necrotic areas with a sterile scalpel, surface sterilized in 0.6% sodium hypochlorite (NaClO) for 30 seconds, rinsed twice in sterile distilled water, and blotted dry on sterile filter paper (Whatman No. 4). Tissue pieces were then plated onto clarified V8 agar in Petri plates (100 × 15 mm, Fisher Scientific Co., Nepean, Ontario, Canada). Petri plates were incubated at 22°C for 4 days in darkness. After incubation, hyphal tips from the margins of actively growing cultures were removed using a sterile probe and plated onto clarified V8 medium to generate pure cultures.

A metalaxyl-m-sensitive isolate of *Phytophthora erythroseptica* (No. 364-2) from New Brunswick, Canada (Al-Mughrabi 2006) was purified as described above and was used in this study. The isolate was subcultured on V8 agar and incubated at 22°C for two weeks under reduced light. Twenty five Petri plates containing *P. erythroseptica* mycelium and oospores were added to 250 mL distilled water and blended for two minutes into a thin gel (Al-Mughrabi *et al*. 2007).

The blended fungal material (500 mL) was mixed thoroughly with 1 Kg of autoclaved horticultural vermiculite (Holiday VIL Vermiculite, Inc., Division of Normiska Corporation, Montréal,

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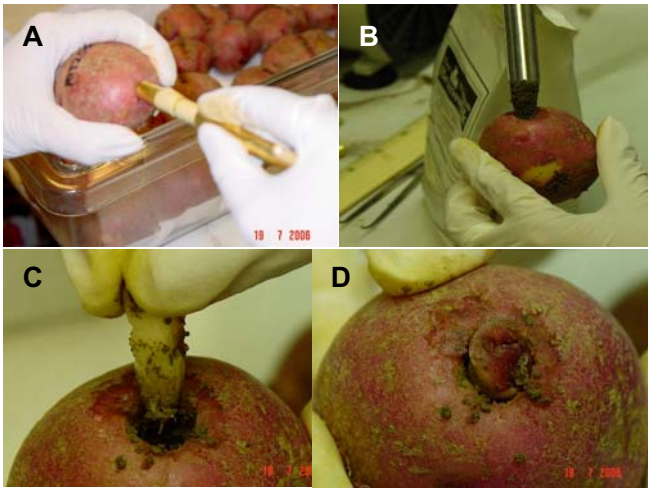

Fig. 1 Schematic representation of steps involved in testing soil for the presence of *Phytophthora erythroseptica*. (**A**) removing a core from the stem end of a potato tuber; (**B**) addition of 1 g of soil in the cored end; (**C, D**) covering the cored end containing soil with the excised tuber plug.

QC) or soil from a field until the final texture of the inoculum became crumbly. Vermiculite or sterilized soil samples fortified with *P. erythroseptica* were used as positive controls to test the efficiency of this technique.

Test assay, inoculation and disease evaluation

Disease-free tubers (~ 100 g) were surface disinfested with 0.6% NaClO, rinsed in sterile distilled water, and then left under a laminar hood (Thermo Forma, model no. 1845, class 100, Forma Scientific, Inc., Ohio, USA) in order to air dry under aseptic environment. A potato core, 5 mm in diameter and 20 mm in depth was removed from the stem end of each individual tuber (**Fig. 1A**), filled with 1 g of soil or vermiculite (**Fig. 1B**), and then covered by inserting a portion of the core that had been previously cut (**Fig. 1C, 1D**). Controls (2 positive: vermiculite and sterilized field soil mixed with *P. erythroseptica* mycelium prepared as described above; and 2 negative: vermiculite and sterilized field soil) were included. The potato tubers were then stored in clear polyethylene containers (Rubbermaid Commercial Products Inc., Virginia, USA) padded with a sheet of moistened cheese cloth (VWR International, Ontario, Canada). Tubers were then misted with distilled water and the containers were covered and incubated at 10°C in order to slow bacterial growth. After three weeks of incubation, each tuber was cut longitudinally and then exposed to air for 30 min. Tubers with pink-black flesh were considered positive for pink rot.

Grow-out studies

To validate the results obtained from the assay, seed potatoes (cv. 'Red Norland'; E1 class) were planted in a subsample of the soil samples that are under study. Soil samples were placed in pots (3 pots per sample) and one seed tuber was planted in each pot. The experiment was set out in a growth room at 18°C, under 400 W sodium lamps (P. L. Light Systems, Beamsville, ON, Canada) that were located 85 cm above the foliage (14-h photoperiod). Plantlets were watered daily and fertilized as needed. Tubers were harvested 60 days after planting. Tubers were cut longitudinally and left exposed to air for 30 min. Tubers exhibiting pink rot symptoms were considered positive for pink rot. The experiment was repeated twice.

RESULTS AND DISCUSSION

Currently there is no easy method for testing for the presence of the pink rot pathogen in soil. In the present study an attempt was made to establish a practical and inexpensive technique to test for the presence of *P. erythroseptica* in soil. Forty eight soil samples randomly collected from potato fields were tested for the presence of *P. erythroseptica*. In comparison to the soil and vermiculite samples that were fortified with mycelium of *P. erythroseptica*, 37 samples tested negative for the presence of the pink rot pathogen using both soil and grow-out assays (**Table 1**). The two controls namely, vermiculite and sterilized soil mixed with *P. erythroseptica* mycelium were heavily infected with pink rot (**Fig. 2**). The method described in this manuscript is easy to conduct, inexpensive, and efficient. However, tubers require 2 to 3 weeks of incubation to allow for disease development and symptoms expression. This is much faster than a grow-out study which takes up to 10 weeks.

Several molecular methods are currently available for the detection and quantification of various potato pathogens including *P. erythroseptica*, *P. infestans*, *Pythium ultimum*,

Table 1 Presence or absence of pink rot (*Phytophthora erythroseptica*) in different soil samples tested using an assay and tuber grow out in the soil samples tested*.

Sample No.	Pink rot incidence		Sample No.	Pink rot incidence	
	Assay	Grow-out		Assay	Grow-out
1	+	+	25	-	-
2	-	-	26	-	-
3	-	-	27	+	+
4	-	-	28	-	-
5	-	-	29	-	-
6	+	+	30	-	-
7	+	+	31	-	-
8	-	-	32	-	-
9	-	-	33	+	+
10	-	-	34	-	-
11	-	-	35	-	-
12	-	-	36	-	-
13	-	-	37	+	+
14	-	-	38	-	-
15	-	-	39	+	+
16	+	+	40	+	+
17	-	-	41	+	+
18	-	-	42	-	-
19	-	-	43	-	-
20	-	-	44	-	-
21	-	-	45	-	-
22	-	-	46	-	-
23	+	+	47	-	-
24	-	-	48	-	-
Vermiculite fortified with *P. erythroseptica*				+	+
Sterilized soil fortified with *P. erythroseptica*				+	+

* Each study was repeated three times. Each test sample was replicated three times.

Fig. 2 Pink rot symptoms expression and disease development in tubers receiving soil fortified (right) and un-fortified (left) with *Phytophthora erythroseptica* mycelium.

Fusarium sambucinum, *Erwinia carotovora* subsp. *carotovora* and *Erwinia carotovora* subsp. *atroseptica* (Tooley *et al*. 1997; Trout *et al*. 1997; Ristaino *et al*. 1998; Tooley *et al.* 2002; Wangsomboondee and Ristaino 2002; Atallah and Stevenson 2006; Cullen *et al*. 2007). Although these molecular-based assays are reliable, rapid, sensitive and accurate, the costs incurred for equipment, primers and other supplies needed to conduct such tests are high. In some cases, visual observations of tubers can lead to misdiagnosis (Tooley *et al*. 1997; Wangsomboondee and Ristaino 2002). The present method eliminates such problem as the pink rot symptoms (salmon color) exhibited in the infected tuber tissue after exposure to air for 20 to 30 min (Vujicic and Park 1964) are indicative of the presence of the disease. There are also views which state that coupling molecular techniques with traditional diagnostics should provide rapid, accurate diagnosis of potato pathogens ultimately leading to efficient management of disease and minimization of losses caused by the disease (Trout *et al*. 1997).

It should be noted that the pink rot fungus has the capability to survive as oospores in soil for many years (Vujicic and Park 1964). The pathogen propagules of this fungus are prevalent in potato cultivated soils (Lambert and Salas 2001) and the introduction of this pathogen into uninfested soils could be possible by use of infected seed tubers (Cunliffe *et al*. 1977). The method developed here for detection and identification of pink rot from soil could be a reliable, simpler, cost effective and useful tool for potato growers.

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