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Detection of *Phytophthora erythroseptica* in Above-ground Potato Tissues, Progeny Tubers, Stolons and Crop Debris Using PCR Techniques

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Abstract Pink rot of potatoes caused by *Phytophthora erythroseptica* is known to infect all underground potato (*Solanum tuberosum*) tissues such as roots, stolons, tubers and basal stems. Potato leaves and stems do not normally exhibit symptoms except when extensive infection of underground plant parts results in plant wilt, chlorosis and necrosis.

This is the first study to investigate the spread of *P. erythroseptica* in above-ground potato tissues using both traditional isolation and PCR methods originally developed to detect the pathogen in tubers. *P. erythroseptica* was detected in 66% of leaf and stem tissue samples originated from artificially-inoculated and naturally-infected tubers of 'Yukon Gold' and 'Shepody' by PCR methods. However, it was only recovered in pure cultures from 38% of stem and leaf tissue samples. The pathogen was also detected in leaf and stem tissues and aerial tubers of plantlets grown in potting mixtures infested with *P. erythroseptica*. Pure cultures of *P. erythroseptica* obtained from stem and leaf tissues, together with the 95% of the detection with the real-time PCR assay and 45% with the conventional PCR assay confirmed the viability of the pathogen in above-ground potato tissues. Furthermore, the pathogen was detected in progeny tubers and stolons produced by the infected potato plants. *P. erythroseptica* was also detected in a few samples of debris taken from naturally senesced above-ground potato tissue after harvest.

Resumen Se conoce que la pudrición rosada de la papa causada por *Phytophthora erythroseptica* infecta todos los tejidos subterráneos de papa (*Solanum tuberosum*), tales como raíces, estolones, tubérculos y tallos de la base. Las hojas y tallos aéreos generalmente no exhiben síntomas excepto cuando hay una infección generalizada de las partes subterráneas de la planta, lo cual da como resultado marchitez, clorosis y necrosis.

Este primer estudio es para investigar la diseminación de *P. erythroseptica* en las partes aéreas del tejido de papa usando ambos métodos de aislamiento, el tradicional y el método por PCR originalmente desarrollado para detectar patógenos en tubérculos. *P. erythroseptica* fue detectado en 66% de las muestras de tejido de tallo y hojas artificialmente inoculadas y tubérculos naturalmente infectados de ´Yukon Gold´ y ´Shepody´ por métodos PCR. Sin embargo fue recuperado solamente en cultivos puros del 38% de las muestras de tejido de tallo y hojas. El patógeno fue detectado también en el tejido de tallos y tubérculos aéreos de las plántulas crecidas en mezcla de substrato de macetas infestadas con *P. erythroseptica*. Cultivos puros de *P. erythroseptica* obtenidos de tejidos de tallos y hojas junto con el 95% de detección con la prueba de PCR de tiempo real y 45% con la prueba convencional, confirmaron la viabilidad del patógeno en las partes aéreas del tejido de papa. Más aún el patógeno fue detectado en tubérculos de la progenie producidos de tubérculos y estolones de plantas infectadas. *P. erythroseptica* también fue detectada en unas pocas muestras de deshecho tomadas después de la cosecha del tejido aéreo de plantas naturalmente envejecidas.

Keywords *Solanum tuberosum* · Plantlets · Methods

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Introduction

Pink rot, caused by *Phytophthora erythroseptica* (Pethyb.), is found in most major potato-growing regions of the world (Vargas and Nielsen 1972). The disease causes serious losses in stored potatoes world-wide. All underground potato tissues such as roots, stolons, tubers and basal stems can become infected with the pink rot pathogen (Peters et al. 2004). Potato leaves and stems do not normally exhibit symptoms, except when extensive infection of underground plant parts results in plant wilt, chlorosis and necrosis (Peters et al. 2004). Lonsdale et al. (1980) reported that the pathogen spread from an infected mother tuber, stem, stolon or root into adjacent organs, but growth upwards into the stem was slight. It is well known that infection of above-ground potato tissue with any pathogen can significantly compromise plant vigor and in turn the tuber bulking process thereby reducing yields (Vargas and Nielsen 1972). However, there is a general lack of knowledge on the spread of *P. erythroseptica* within the infected plant and the importance of tissues other than tubers in the pink rot disease cycle.

In recent years, polymerase chain reaction (PCR) has emerged as a powerful tool in plant disease diagnostics. It does not require the isolation of pure fungal cultures from infected tissue and is often more sensitive, accurate, robust and rapid, less labor intensive and more economical than conventional diagnostics (Tooley et al. 1997). Several conventional and real-time PCR protocols with different levels of specificity and sensitivity have been developed to detect *P. erythroseptica* from tuber tissue based on the primers designed from internal transcribed spacer (ITS) regions of ribosomal DNA (Tooley et al. 1997; Cullen et al. 2007). These methods have not been adapted to detect *P. erythroseptica* in plant tissues other than potato tubers. The objective of this study was to investigate the spread of *P. erythroseptica* within infected potato plants using traditional and molecular protocols.

Materials and Methods

Detection of *Phytophthora erythroseptica* in Above-ground Potato Tissues Grown from Artificially-Inoculated and Naturally-Infected Seed Tubers

Twenty potato tubers of 'Shepody' and 'Yukon Gold' without blemishes were tested for *P. erythroseptica* using PCR methods described below. The tubers were washed with tap water and air dried before a small piece (~100 mg) was removed from the stem end of each tuber. DNA was extracted using a Qiagen DNeasy plant mini-kit (Qiagen Sciences, Maryland, USA) following the manufacturer's instructions. Only 50 µl of elution buffer was used in the final step of the protocol in order to increase DNA concentration. The presence of *P. erythroseptica* was determined using conventional and real-time PCR methods described below. Of the 'Shepody' tubers tested, sixteen tubers were disease-free while *P. erythroseptica* was detected in four tubers. Of the 'Yukon Gold' tubers that were tested, nineteen tubers were disease-free while *P. erythroseptica* was detected in one tuber.

From the tubers tested above, fifteen disease-free potato tubers of 'Shepody' and 'Yukon Gold' were chosen. These tubers were then surface sterilized by immersing in a 0.6% NaClO solution for 10 min and rinsing twice with sterile distilled water (SDW). The tubers were blotted dry with sterile filter paper in a laminar flow hood. An approximately 2 cm cut was made on all tubers close to an eye using a sterile scalpel. The tubers were inoculated with zoospore suspensions (1.75×10^5 zoospores/ml) using two methods. The zoospore suspension was prepared in sterile pond water (isolate PE-9913-2DSZ1 from Prince Edward Island, Canada) using the protocol described by Peters et al. (2001). In the first inoculation method, five tubers from each cultivar were spray-inoculated until run-off. In the second method, five tubers from each cultivar were dipped in a zoospore suspension for 24 h and drained. The inoculated tubers were then placed on moist filter paper in plastic containers and incubated in darkness for four days at room temperature (22ºC). Five tubers from each cultivar were sprayed with SDW until run-off (control) and incubated in separate containers. The tubers were arranged in a split-plot design with cultivar as the main plot factor and inoculation method as the sub-plot factor.

After incubation, the tubers were planted in plastic pots [diameter 19 cm] filled with soilless potting mixture (ASB-Greenworld Ltd., Pointe Sapin, New Brunswick, Canada) and placed in the greenhouse maintained at 25–30ºC. The four naturally-infected 'Shepody' tubers and the 'Yukon Gold' tuber were also planted as above. Plants were watered daily and were fertilized with 10-52-10 Plant-Prod water soluble fertilizer (Plant Product Co. Ltd., Brampton, Ontario, Canada) every three weeks. A single, entire plant stem from each inoculated tuber was harvested eight weeks after planting (seven-week-old potato plants). DNA was extracted in three replicates from stem pieces sampled approximately 2.5, 5.0, 7.5 and 10.0 cm from the soil level and from one randomly picked leaf. In addition, DNA was extracted in three replicates from two leaves randomly chosen from the upper and lower part of the potato plants with the approximate height at which the leaves were picked recorded. Approximately 100 mg of plant tissue was ground using a pre-chilled mortar and pestle with a pinch of acid washed sea sand (Fischer Scientific, Fairlawn, New Jersey, USA) for each sample.

DNA was extracted using a Qiagen DNeasy plant mini-kit (Qiagen Sciences, Maryland, USA) following the manufacturer's instructions. Only 50 µl of elution buffer was used in the final step of the protocol in order to increase DNA concentration. Extracted DNA were either processed immediately for PCR or frozen at −20ºC for future use. All progeny tubers and stolons were harvested after natural senescence of above-ground tissue. DNA was extracted in three replicates from the stolons (separated from the tubers) and from tissue taken from the stem end of progeny tubers. Above-ground potato debris from naturally senesced plants was also collected for each individual plant. DNA was extracted in three replicates from the debris using the methods described above. The presence of *P. erythroseptica* was determined using conventional PCR and real-time PCR methods described below.

Detection of *Phytophthora erythroseptica* from Potato Plantlets Grown in Potting Mixtures Amended with *P. erythroseptica* Zoospores

Sixteen disease-free 'Shepody' micro plantlets, 6–7 cm tall (maintained as disease free plant lines by Plant Propagation Centre, New Brunswick Department of Agriculture and Aquaculture, Fredericton, New Brunswick) were transplanted into plastic inserts with eight cells filled with sterilized potting mixture (ASB-Greenworld Ltd., Pointe Sapin, New Brunswick, Canada). The inserts were placed in a growth chamber (Conviron; CMP 3244) at 18ºC with 16 h day/night light cycles. The plantlets were watered as required. After 7 days, each cell of one insert was amended with 1 ml of zoospore suspension (23×10^4 zoospores/ml). Each cell of the other insert received 1 ml of SDW. The inserts were then placed in plastic containers and the containers were filled with distilled water to flood the soils. After 3-days, the inserts were removed from plastic containers and allowed to drain. The plantlets were watered as required thereafter. Four weeks after inoculation, the plantlets were harvested. DNA was extracted from leaves, aerial tubers, roots and stem pieces sampled approximately 5 cm above soil level. Approximately 100 mg of plant tissues were used for each DNA extraction as described previously. Extracted DNA were either processed immediately for PCR or frozen at −20ºC for future use. The presence of *P. erythroseptica* was determined using conventional PCR and real-time PCR methods as described below.

Traditional Recovery of *P. erythroseptica*

Attempts were made to isolate *P. erythroseptica* in pure cultures from stem pieces taken approximately 2.5, 5.0, 7.5 and 10.0 cm above soil level, and from leaf sections of seven-week-old potato plants. Stem and leaf sections were surface sterilized with 0.6% NaClO for 30 s and washed twice with SDW. The surface sterilized tissues were blotted dry on sterilized filter paper and plated on water agar. Cultures were then transferred onto clarified V-8 agar medium and incubated in the dark at 20ºC for one week. Microscopic identifications were made based on colony morphology and spore characteristics (Newhook et al., 1978). When the isolated culture had colony characteristics of *P. erythroseptica*, it was transferred to a 50 ml plastic tube filled with 15 ml of V-8 broth and incubated in the dark at 18ºC for three days. The tubes were centrifuged (Sorvall RC-5B Refrigerated Superspeed Centrifuge, DuPont Instruments, DuPont, Canada) at 5,000 rpm at 17°C for 5 min and excess V-8 broth was decanted. The mycelial pellet was washed twice with SDW followed by centrifugation and then squeezed to remove excess water. It was ground using a pre-chilled mortar and pestle with a pinch of acid washed sea sand (Fischer Scientific, Fairlawn, New Jersey, USA). DNA was extracted using a Qiagen DNeasy plant mini-kit (Qiagen Sciences, Maryland, USA) following the manufacturer's instructions. Only 50 µl of elution buffer was used in the final step of the protocol in order to increase DNA concentration. Attempts were also made to isolate *P. erythroseptica* in pure cultures from stem pieces and leaf sections of plantlets grown in soils amended with *P. erythroseptica* zoospores. Number of samples from which *P. erythroseptica* was recovered in pure cultures given as a ratio of total number of samples cultured was reported as % recovery.

Conventional PCR Method

The PCR method developed by Tooley et al. (1997) was modified to increase the sensitivity of the assay. PCR amplification of samples was based on an initial denaturation at 94°C for 30 s, followed by 36 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 1 min, and a final elongation at 72°C for 4 min in a reaction volume of 25 µl using a PTC-200 Peltier thermal cycler (MJ Research, Waltham, MA). Optimal conditions for PCR contained a master mix of the following components: 1 x PCR buffer, 4 mM $MgCl_2$, 0.5 units of *Taq* Polymerase (AmpliTaq DNA polymerase with GeneAmp, Applied Biosystems, Foster City, CA, USA), 140 µM each dNTPs (Promega, Madison, USA) and 0.2 µM PERY2 and ITS4 primers (Applied Biosystems, Foster City, CA, USA). Nucleotide sequences of the primers PERY2 and ITS4 are given in Table 1. Undiluted DNA (2 µl) was used as the template in 23 µl of master mix. DNA extracted from pure cultures of *P. erythroseptica* was used as the positive control while DNAse free water served as the negative control. PCR products were analyzed by electrophoresis

Table 1 Nucleotide sequences of the PCR primers and probes used in this study for detecting *Phytophthora erythroseptica*

Primer	Sequence	Source
PERY2	CTGTTCCGGCGTAAGCTGG	Tooley et al. 1997
ITS4	TCCTCCGCTTATTGATATGC	Tooley et al. 1997
99F	TGTGCTAGGCTTGGCGTTT	Cullen et al. 2007
177R	CCTCGTCCACCCCAGCTTA	Cullen et al. 2007
133T[a]	TGCGAAGTAGGGTGTGTTCCGGC	Cullen et al. 2007

[a] Probe 133 T was labeled at the 5′-end with FAM (6-carboxy-fluorescein) and 3′-end with the quencher dye TAMRA

on 2% agarose gels stained with ethidium bromide and photographed under UV illumination (FisherBiotech Electrophoresis System, Fisher Scientific, Pittsburgh, PA, USA). Low DNA mass ladder was used as the molecular weight standard. The PCR assay yielded a 136 bp product in the presence of the target template.

Real-Time PCR Method

The real-time PCR method developed by Cullen et al. (2007) was performed in MicroAmp optical 96-well plates using ABI 7000 sequence detectors (Applied Biosystems, Foster City, USA) following the recommended generic (three stage) thermal cycle protocol. Real-time PCR reaction involved the addition of 1 µl of target template to an optimized mix consisting of TaqMan Buffer (1x), 3.5 mM $MgCl_2$, 0.5 units Ampli*Taq* Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA), 0.2 mM each of dNTPs (Promega, Madison, USA), 100 nM probe 133 T and 300 nM each of forward 99F and reverse primers 177R (Applied Biosystems, Foster City, CA, USA). Nucleotide sequences of the probe 133 T and the primers 99F and 177R are given in Table 1. PCR products were analyzed by electrophoresis on 2% agarose gels stained with ethidium bromide and photographed under UV illumination. Low DNA mass ladder was used as the molecular weight standard. The PCR assay generated a 78 bp product in the presence of the target template.

Results and Discussion

Detection of *P. erythroseptica* in Potato Tissues Other Than Tubers

P. erythroseptica was detected in stem and leaf tissues of seven-week-old ‘Shepody’ and ‘Yukon Gold’ potato plants grown from artificially-inoculated seed tubers regardless of the method of inoculation (Table 2). The pathogen was detected in leaves taken from both upper (average height of 62.5 cm above soil level) and lower (average height of 22.5 cm above soil level) parts of the potato plants grown from artificially-inoculated tubers. *P. erythroseptica* was also detected in stem and leaf tissues grown from naturally-infected seed tubers. Number of samples in which *P. erythroseptica* was detected by conventional PCR or/and real-time PCR assay as a ratio of total number of

Table 2 Detection of *Phytophthora erythroseptica* from above-ground tissues of potato plants grown from artificially-inoculated and naturally-infected seed tubers

Cultivar	Method of tuber inoculation				Samples tested positive by PCR/real-time PCR						
	Sterile water inoculated	Dip inoculated	Spray inoculated	Naturally infected	Stem piece 1*	Stem piece 2*	Stem piece 3*	Stem piece 4*	Random Leaf**	Upper Leaf***	Lower Leaf****
Yukon Gold		*N*=5			1/4	3/5	2/3	1/3	4/4	0/1	-
			N=5		2/2	0/3	1/2	1/2	1/1	0/1	0/1
				N=1	1/1	0/1	1/1	0/1	0/0	1/1	-
	N=3				0/0	0/0	0/0	0/0	0/0	-	-
Shepody		*N*=5			1/3	0/3	1/2	1/4	0/1	1/3	1/1
			N=5		1/2	4/4	4/5	1/3	1/2	0/0	-
				N=4	1/4	0/2	1/2	0/1	1/2	0/1	0/0
	N=5				0/0	0/0	0/0	0/0	0/0	0/0	-

*Stem pieces above 2.5, 5.0, 7.5 and 10.0 cm above soil level.

** Leaf chosen randomly from each harvested stem

*** Leaves were selected from upper part of the stem above the midpoint of total height

**** Leaves were selected from lower part of the stem below the midpoint of total height

N=number of potato plants tested for each inoculation method

-=no testing done

leaf and stem samples processed was reported as % detection. *P. erythroseptica* was detected in 66% of leaf and stem tissue samples originated from artificially-inoculated and naturally-infected tubers of 'Yukon Gold' and 'Shepody'. *P. erythroseptica* were recovered from 38% of stem and leaf tissue samples that originated from artificially-inoculated and naturally-infected seed tubers. The identity of the causal agent *P. erythroseptica* was confirmed using conventional PCR and real-time PCR assays.

Pethybridge (1914) noted that it was difficult to identify *P. erythroseptica* based on foliar symptoms unless the infection was extreme. In our study, we noted that few infected plants from both cultivars exhibited chlorosis and pre-mature abscission of basal leaves. The margins of the leaflets were rolling upwards and inwards and were brown, dry and crisp. However, symptoms were generally absent. Subsequent detection in stem and leaf tissues by PCR and traditional recovery of the pathogen indicated that stem infection may progress above ground without any apparent symptoms for the majority of the plants originated from artificially-inoculated or naturally-infected seed tubers. All artificially-inoculated tubers, except one, produced plants that in turn produced progeny tubers. According to Vargas and Nielsen (1972), when stem infection progresses above ground, the advancing margin becomes water soaked, and the necrotic tissue below turns brown and shrivels. These water-soaked stem lesions just above the soil line also have been noted in greenhouse and field studies in Prince Edward Island (R. D. Peters, unpublished data). However, we did not observe water soaked or necrotic stem tissue growing from artificially-inoculated or naturally-infected tubers in our experiments.

In our study, some potato plants may not have developed pink rot even though the seed tubers were inoculated. It is possible that some of the tubers became infected but the pathogen remained confined to the below-ground plant parts and did not move to the above-ground potato tissue. Another possibility is that the pathogen moves to above-ground tissue only in some stems and not in all stems that originate from an infected seed tuber and simply did not spread to the stem that was harvested. Pethybridge (1914) reported that although all the stalks of a plant are frequently affected in a similar manner there were a few cases where some of the stalks were diseased while others remained healthy.

P. erythroseptica was detected in stem pieces, leaf tissue, aerial tubers and roots (Table 3) of "Shepody" plantlets grown in potting mixtures amended with *P. erythroseptica* zoospores. The plantlets were on average 23 cm in height at the time of harvest with the leaves confined to the upper 4 cm. Therefore, the presence of the pathogen in a randomly selected leaf indicated that it had spread to the upper portion of the plantlet. The pathogen was also isolated in pure cultures from the leaves of the plantlets grown in amended potting mixtures and its' identity was confirmed using conventional PCR and real-time PCR assays. Infected plantlets formed aerial tubers in leaf axils, and exhibited symptoms including chlorosis, wilting and plant death. Production of aerial tubers in leaf axils has been reported as evidence of disruption of translocation of photosynthate to developing tubers (Lonsdale et al. 1980).

A correlation between the resistance of above-ground tissue and the resistance of tubers to pink rot has been observed for some cultivars, but not for others (Peters and Sturz 2001). Resistant above-ground tissue may restrict pathogen spread within the infected plant. The methods used in this study could be used to evaluate non-tuber germplasm such as above-ground tissue for resistance to *P. erythroseptica* and complement current established protocols (Peters and Sturz 2001). The ratio between the total numbers of samples in which the pathogen was detected by one PCR assay to total number of samples in which *P. erythroseptica* was detected by either one or both PCR assays was computed as percent detection by each assay. About 95% of the detections were made with the real-time PCR assay while only 45% were detected with the conventional PCR assay (Fig. 1). The real-time PCR assay was clearly more sensitive than the conventional PCR assay. Cullen et al. (2007) reported that the real-time PCR

Table 3 Detection of *Phytophthora erythroseptica* in plantlets grown in potting mixture amended with *P. erythroseptica* zoospores or sterilized potting mixture

Treatment	Samples tested positive by PCR/real-time PCR				
	Stem piece*	Leaf**	Aerial Tubers	Below-ground Tubers	Roots
Plantlets grown in infested potting mixture n=8	5/5	5/8	5/6	0/2	3/2
Plantlets grown in sterilized potting mixture n=5	0/0	0/0	0/0	0/0	0/0

* Five cm above soil level

** Leaf chosen randomly from each plantlet

n=number of plantlets tested

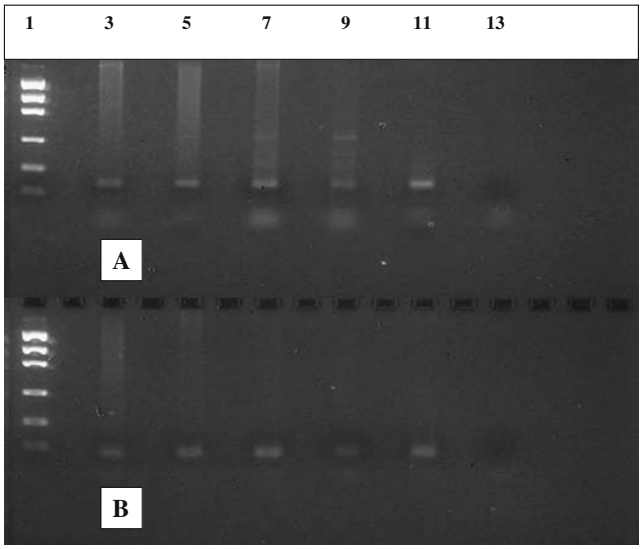

Fig. 1 Detection of *P. erythroseptica* in stem and leaf tissue samples taken from potato plants grown from artificially-inoculated seed tubers with **A:** PCR assay Lane1. DNA mass ladder; Lanes 3 & 5. Yukon Gold (spray inoculated) and Shepody (dip inoculated) stem samples 7.5 cm above soil level; Lanes 7 & 9. Yukon Gold (dip inoculated) leaf and stem sample 5.0 cm above soil level; Lane 11.$+^{ve}$ control (DNA from pure cultures of *P. erythroseptica)*; Lane 13.$-^{ve}$ control: and **B:** Real-time PCR assay Lane 1. DNA mass ladder; Lane 3. Shepody (dip inoculated) stem sample 5.0 cm above soil level; Lane 5. Yukon Gold (dip inoculated) leaf sample; Lane 7. Yukon Gold (spray inoculated) stem sample 2.5 cm above soil level; Lane 9. Shepody (spray inoculated) stem sample 2.5 cm above soil level; Lane 11.$+^{ve}$ control- (DNA from pure cultures of *P. erythroseptica*); Lane 13.$-^{ve}$ control

assay reliably detected atto gram (10^{-18} g) levels of target DNA from pure cultures.

Detection of *P. erythroseptica* in Crop Debris of Above-ground Plant Parts Grown from Artificially-Inoculated and Naturally-Infected Seed tubers

P. erythroseptica was detected in a few samples of debris that originated from plants grown from artificially-inoculated and naturally-infected seed tubers (Table 4). However, these tissues were neither examined for oospores nor cultured. PCR assays may detect mycelia that may no longer be viable. Therefore, further studies are warranted to determine if above-ground tissues of infected plants could re-introduce the pathogen into soil after harvest.

Detection of *P. erythroseptica* in Progeny Tubers and Stolons

P. erythroseptica was also detected in progeny tubers and their stolons (Table 4). Varying amount of decay was observed in some progeny tubers while others appeared healthy. These results are in agreement with the findings by Cunliffe et al. (1977) who reported that tubers which become superficially contaminated produced rotting daughter tubers when grown. *P. erythroseptica* was not detected in all progeny tubers. Also most stolons were already decayed and therefore could not be tested. Based on these results, if infected seed tubers are planted, infected potato

Table 4 Detection of *Phytophthora erythroseptica* in progeny tubers, stolons and debris of potato plants grown from artificially-inoculated and naturally-infected tubers

Cultivar	Method of inoculation				Samples tested positive by PCR/real-time PCR		
	Sterile water inoculated	Dip inoculated	Spray inoculated	Naturally infected	Progeny Tubers	Stolons	Debris
Yukon Gold					0/7	0/1	0/1
		N=5			*n*=12	*n*=2	*n*=5
					3/5	0/1	
			N=5		*n*=11	*n*=4	0/0
					0/0		*n*=4
	N=5				*n*=5	-	0/0
					0/3	0/0	*n*=5
Shepody		*N*=5			*n*=9	*n*=1	0/0
					1/7		*n*=3
			N=5		*n*=13	-	0/2
					0/2	0/0	*n*=2
				N=4	*n*=5	*n*=2	1/2
					0/0		*n*=2
	N=5				*n*=7	-	0/0
							n=2

N=number of replicates; *n*=number of samples tested; -=not tested

plants may grow without obvious symptoms of the disease and produce infected but symptomless progeny tubers. These seemingly healthy progeny tubers may rot in storage or may remain asymptomatic. Asymptomatic seed tubers then can become the source of primary inoculum in non-infested soils and also spread the pathogen from region to region (Cunliffe et al. 1977). Lonsdale et al. (1980) reported that in non-infested soil, infected mother tubers can apparently give rise to infected plants by mycelial invasion of the stem base and stolons. Progeny tubers are usually infected via the growth of mycelium from diseased stolons (Peters and Sturz 2001).

Conclusions

Phytophthora erythroseptica was detected in leaf and stem tissues of potato plants grown from artificially-inoculated and naturally-infected seed tubers of 'Yukon Gold' and 'Shepody' and from plantlets grown in potting mixtures amended with *P. erythroseptica* zoospores. In addition, the pathogen was also detected in aerial tubers and roots of some of the plantlets. It appears that the pathogen entered the plantlets through the roots and spread to above-ground stem and leaf tissues. PCR assays and culture isolations confirmed the actual presence of *P. erythroseptica* in above-ground potato tissues originating from artificially-inoculated and naturally-infected seed tubers even when symptoms were not apparent. Pure cultures of *P. erythroseptica* isolated from above-ground tissues also indicated that the propagules in leaves and stems are viable. *P. erythroseptica* was also detected in a few samples of naturally senesced above-ground plant tissue that originated from artificially-inoculated and naturally-infected seed tubers. Furthermore, *P. erythroseptica* was detected in some progeny tubers and stolons. The pathogen presence in above-ground potato tissues may have several implications for pink rot management. Planting infected seed tubers which do not exhibit pink rot symptoms may result in infected plants that also do not exhibit obvious symptoms of the disease and produce infected but symptomless progeny tubers. These asymptomatic seed tubers may become the source of primary inoculum in non-infested soils and also spread the pathogen from region to region.

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