A Methodology to Detect and Quantify Five Pathogens Causing Potato Tuber Decay Using Real-Time Quantitative Polymerase Chain Reaction

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ABSTRACT

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Late blight (*Phytophthora infestans*), pink rot (*Phytophthora erythroseptica*), leak (*Pythium ultimum*), dry rot (*Fusarium sambucinum*), and soft rot (*Erwinia carotovora* subsp. *carotovora* and subsp. *atroseptica*) are particularly damaging diseases of stored potato tubers worldwide. In this study, we present a methodology to detect and quantify the causal agents of the five aforementioned diseases from whole potato tubers, using real-time quantitative-polymerase chain reaction. Six primer pairs were designed to amplify targets smaller than 150-bp DNA in single copy protein-coding gene targets of each of the pathogens and the potato host.

Using a large collection of pure culture DNA samples, all primer pairs specifically detected the DNA target in the intended pathogenic species. Amplification efficiencies over a five-log dilution series ranged between 95 and 100% and were unaffected by the presence of large amounts of host DNA. The detection level of the primers reached 0.5 pg of target DNA. Pathogens were detected in 100 pg of total DNA extracted from 170 to 250 g of tubers, 4 days after inoculation, regardless of the presence of symptoms. The presence of *P. erythroseptica*, *Pythium ultimum*, or *E. carotovora* was also detected in 1 ng of DNA extracted from potato tubers collected from a commercial storage facility. This study provides the first step in a methodology to predict the storability of potato tubers following harvest.

Postharvest potato (*Solanum tuberosum*) losses due to plant pathogens in Wisconsin exceeded \$9 million for the 2000 and 2001 stored crops (1). Crops grown during those years experienced above average rainfall and unseasonably warm conditions during harvest and early storage. Such conditions are highly favorable for pathogen dispersal and disease occurrence. Losses in some individual storage facilities exceeded 20%. Nationally, potato storage losses in the United States amounted to 1.3 to 1.9 million tons (30 to 42 million hundred weight $\approx$ 100 lb or 45.4 kg) annually between 1999 and 2001, equivalent to 8 to 9% of the total annual potato production, representing more than \$200 million (1).

Multiple pathogens are capable of infecting potato tubers at various stages through the course of the growing season, at harvest and during handling and storage, and cause a variety of symptoms on the tuber periderm and internal tissues. The most notable oomycetes and fungi that cause tuber rots include *Phytophthora infestans* (late blight), *Phytophthora erythroseptica* (pink rot), *Pythium ultimum* var. *ultimum* (leak), and *Fusarium sambucinum* (dry rot). Bacterial soft rot is caused by *Erwinia carotovora* subsp. *carotovora* (syn. *Pectobacterium carotovorum*) and *E. carotovora* subsp. *atroseptica* (syn. *Pectobacterium atrosepticum*). In Wisconsin, *E. carotovora* subsp. *carotovora* causes the majority of losses (A. Charkowski, *personal communication*). *P. infestans* and *P. erythroseptica* are primary pathogens able to infect tubers directly, through the eyes or the periderm. Conversely, *E. carotovora* subsp. *carotovora*, *E. carotovora* subsp. *atroseptica*, *Pythium ultimum*, or *F. sambucinum* are generally associated with tuber tissues infected by primary pathogens,

physiologically stressed (e.g., periderm immaturity, bruising, and open lenticels) or damaged mechanically during harvest and handling (15,23,26).

Infections with the above-mentioned pathogens occur in field and storage facilities, where management practices affect symptom development and storability of crops. Constant high relative humidity is required during potato storage to prevent tuber dehydration and weight loss, and ventilation air is frequently recirculated. Such conditions favor increases in pathogen populations, distribution of airborne inoculum, and infection of additional tubers. Tubers destined for processing (fries, chips, and dehydration, etc.) are routinely piled to heights of 5.5 to 7.5 m (18 to 20 feet) and maintained at approximately 95% relative humidity at 7 to 13°C (45 to 55°F). Such conditions are favorable for sporulation and infection by pathogens that can penetrate tubers directly through the tuber lenticels, periderm, or eyes, or indirectly through wounds, to cause decay. Nevertheless, such storage conditions are required to preserve the marketing qualities of potato tubers (prolonged dormancy, processing qualities, minimal water loss, and minimal pressure bruising).

Tubers infected with any of the aforementioned pathogens could serve as inoculum sources for adjacent tubers during storage, leading to a rapid spread of disease. Anecdotal reports of collapsed piles and liquid running out storage doors can be a recurrent theme in years of excessive rainfall and elevated temperatures during harvest and the early storage period. Water condensation from tuber respiration and leakage from infected tubers serve as a medium for the spread and germination of all the above-mentioned pathogens.

Reliance on postharvest pesticides, such as hydrogen peroxide, chlorine dioxide, or ozone is frequent, even though such pesticides have not conclusively been shown to impact disease onset or reduce losses under controlled experimental conditions (9,20,35). There is an urgent need to develop an alternative to the reliance

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on pesticides in potato storages with management recommendations that reduce storage losses and to develop storage-expectancy prediction models.

Quantification of pathogen levels prior to and during storage is expected to assist processors in key decisions related to storage options. Such specific information does not exist at this time and needs to be generated using methods that would allow the detection of the smallest possible number of propagules (conidia, sporangia, and oospores, etc.). Real-time quantitative-polymerase chain reaction (Q-PCR) allows for increased sensitivity and target quantification compared with conventional PCR (6,7,27,37–39). Ribosomal DNA (rDNA) sequences, especially the internally transcribed spacer region (ITS), have been repeatedly used as

PCR and Q-PCR primers to detect a number of different fungal pathogens in potato tissue (6,7,16,24,32–34). High target copy numbers in fungal propagules allow for easy amplification and detection. However, the number of rDNA copies fluctuates with age and stage of growth of organisms (21). Furthermore, non-orthologous copies of the ITS region within one species and among strains in the same species are described in the literature (14). Such discrepancies may hamper accurate quantifications of propagule numbers in the starting material, and limit the ability to compare among samples. Conversely, single-copy genes could serve as specific primer sources that allow for constant pathogen quantification regardless of age and growth stage. Quantification of pathogen loads on potato tubers prior to storage

TABLE 1. List of fungi, oomycetes, and bacteria used to design and evaluate specific quantitative-polymerase chain reaction (Q-PCR) primers (threshold cycle [Ct] values of Q-PCR assay are also listed)

Organism	Origin	Source	Crop	Ct values with specific primers				
				<i>Phytophthora infestans</i>	<i>Pythium ultimum</i>	<i>Phytophthora erythroseptica</i>	<i>Fusarium sambucinum</i>	<i>Erwinia carotovora</i>
<i>Alternaria daucii</i> PMR92	Wisconsin	Rogers ^a	Carrot	...	...	...	NA ^b	...
<i>Alternaria solani</i> NR100	Wisconsin	Rozenzweig ^c	Potato	...	...	...	NA	...
<i>Bipolaris maydis</i>	Wisconsin	Nakhla ^d	Corn	...	...	...	NA	...
<i>Colletotrichum coccodes</i>	Wisconsin	Atallah	Potato	NA	NA	NA	NA	...
<i>Erwinia amylovora</i> Ea246	Illinois	Charkowski ^e	Raspberry	...	...	...	...	NA
<i>Erwinia amylovora</i> Ea273	New York	Charkowski	Apple	...	...	...	...	NA
<i>Erwinia carotovora atroseptica</i> Ecc21	Netherlands	Charkowski	Potato	NA	NA	NA	NA	11.4
<i>Erwinia carotovora atroseptica</i> Eca6	British Columbia	Charkowski	Potato	...	...	...	...	11.6
<i>Erwinia carotovora beta-vascularum</i> Ecb1	California	Charkowski	Sugar beet	...	...	...	...	29.2
<i>Erwinia carotovora beta-vascularum</i> Ecb2	California	Charkowski	Sugar beet	...	...	...	...	27.4
<i>Erwinia carotovora carotovora</i> WPP17	Wisconsin	Charkowski	Potato	NA	NA	NA	NA	24.5
<i>Erwinia carotovora carotovora</i> WPP14	Wisconsin	Charkowski	Potato	...	...	...	...	13.5
<i>Erwinia carotovora wasabi</i> SCRI 488	Japan	Charkowski	Wasabi	...	...	...	...	15.4
<i>Erwinia chrysanthemi</i> CUCPB1018	Florida	Charkowski	Philodendron	...	...	...	...	NA
<i>Erwinia chrysanthemi</i> CUCPB1617	California	Charkowski	Harrisia	NA	NA	NA	NA	NA
<i>Erwinia lupincola</i> Elu W3L16	Washington	Charkowski	Lupinus	...	...	...	...	NA
<i>Erwinia nigrifluens</i> Eni 5D312	—	Charkowski	—	...	...	...	...	NA
<i>Erwinia persicinus</i> Epe 9108-82	Japan	Charkowski	Tomato	...	...	...	...	24.9
<i>Erwinia pyrifolia</i> Ep 1/96	South Korea	Charkowski	Pear	...	...	...	...	NA
<i>Erwinia quercina</i> Equ 11D3	—	Charkowski	—	...	...	...	...	NA
<i>Erwinia rhapontici</i> Erh 739	UK	Charkowski	Rhubarb	...	...	...	...	25.8
<i>Erwinia salicis</i> Esa 15712	UK	Charkowski	Willow	...	...	...	...	NA
<i>Erwinia amylovora</i> Ea 246	Illinois	Charkowski	Raspberry	...	...	...	...	NA
<i>Fusarium acuminatum</i> NRRL 28654	—	O'Donnell ^f	—	...	...	...	17.8	...
<i>Fusarium avenaceum</i> NRRL 25892	—	O'Donnell	—	...	...	...	16.6	...
<i>Fusarium coeruleum</i> NRRL 20434	Wisconsin	O'Donnell	Potato	...	...	...	NA	...
<i>Fusarium culmorum</i> NRRL 25475	—	O'Donnell	—	...	...	...	16.4	...
<i>Fusarium eumartii</i> NRRL 22397	—	O'Donnell	—	...	...	...	NA	...
<i>Fusarium graminearum</i> NRRL 34097	—	O'Donnell	—	...	...	...	19.2	...
<i>Fusarium moniliforme</i> A149	California	Rouse ^g	Corn	...	...	...	NA	...
<i>Fusarium oxysporum</i> NRRL 25891	—	O'Donnell	—	...	...	...	NA	...
<i>Fusarium oxysporum</i> FO916	California	Rouse	Barley grains	...	...	...	NA	...
<i>Fusarium oxysporum</i> FO1055	Wisconsin	Rouse	Carrot	...	...	...	NA	...
<i>Fusarium proliferatum</i> D4853	—	Rouse	—	...	...	...	NA	...
<i>Fusarium sambucinum</i> Fsamb Med#2	Wisconsin	Atallah	Potato	NA	NA	NA	16.5	...
<i>Fusarium subglutinans</i> B3852	—	Rouse	—	...	...	...	NA	...
<i>Fusarium venenatum</i> NRRL 22196	—	O'Donnell	—	...	...	...	NA	...
<i>Fusarium ventricosum</i> NRRL 22241	—	O'Donnell	—	...	...	...	NA	...
<i>Phytophthora cactorum</i> 327	—	Tooley ^h	—	NA	NA	NA	...	...
<i>Phytophthora capsici</i>	Wisconsin	Tooley	Pepper	NA	NA	NA	...	...
<i>Phytophthora cryptogea</i> 390	—	Tooley	—	NA	NA	17.4	NA	NA

(Continued on next page)

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^b NA = no amplification.

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would provide growers and storage managers with a decision-making tool regarding the destiny of crops. Crops with high pathogen loads (number of propagules carried by individual tubers) could be processed shortly after harvest, while those with lower pathogen loads could be stored for longer periods. Additionally, storage conditions could be optimized according to pathogen loads.

The objective of the current study was to design high efficiency Q-PCR primers to detect and quantify five major pathogens of potato tubers (*P. infestans*, *P. erythroseptica*, *Pythium ultimum*, *E. carotovora*, and *F. sambucinum*) or closely related organisms associated with the disease. Such primer pairs are aimed at enabling the absolute quantification of minimal amounts of specific pathogens in the presence of high amounts of host DNA.

MATERIALS AND METHODS

Isolate collections. A collection of isolates of *P. infestans*, *P. erythroseptica*, *Pythium ultimum*, *F. sambucinum*, *E. carotovora* subsp. *carotovora*, and *E. carotovora* subsp. *atroseptica* representative of different genotypes (whenever available or identified) and various geographic locations was used to ensure a wide representation of the pathogen (Table 1). Also, collections of fungi, bacteria, and oomycetes associated with potato (tubers and aerial parts), rotational crops, as well as species within the same genus were used in the design and testing of primers.

Primer design. Sequences of functional protein coding genes were generated for the purpose of designing specific primers for *P. infestans*, *P. erythroseptica*, *Pythium ultimum*, *F. sambucinum*,

TABLE 1. (Continued from preceding page)

Organism	Origin	Source	Crop	Ct values with specific primers				
				<i>Phytophthora infestans</i>	<i>Pythium ultimum</i>	<i>Phytophthora erythroseptica</i>	<i>Fusarium sambucinum</i>	<i>Erwinia carotovora</i>
<i>Phytophthora drechslerii</i> 401	—	Tooley	—	NA	NA	NA	...	...
<i>Phytophthora erythroseptica</i> Pery Med#2	Wisconsin	Atallah	Potato	NA	NA	19.6	...	NA
<i>Phytophthora erythroseptica</i> Pery Med#4	Wisconsin	Atallah	Potato	NA	NA	19.9	...	...
<i>Phytophthora erythroseptica</i> Pery 347	Wisconsin	Atallah	Potato	...	...	20.1	...	...
<i>Phytophthora erythroseptica</i> Pery	Wisconsin	Atallah	Potato	...	...	18.5	...	...
<i>Phytophthora erythroseptica</i> Pery ID	Idaho	Atallah	Potato	...	...	21.2	...	...
<i>Phytophthora fragariae</i> 398	—	Tooley	—	NA	NA	NA	...	...
<i>Phytophthora illicis</i> 344	—	Tooley	—	NA	NA	NA	...	...
<i>Phytophthora infestans</i> US-1 US 940501	—	Helgeson ⁱ	Potato	20.9	...	...	...	...
<i>Phytophthora infestans</i> US-6 BIN-16	New York	Helgeson	Potato	19.6	...	...	...	...
<i>Phytophthora infestans</i> US-7 BG-8	New York	Helgeson	Potato	19.9	...	...	...	...
<i>Phytophthora infestans</i> US-8 US 940480	—	Helgeson	Potato	21.1	NA	NA	NA	NA
<i>Phytophthora infestans</i> US-8 126-C-18	North Dakota	Helgeson	Potato	24	...	...	...	...
<i>Phytophthora infestans</i> US-8 693-3	North Dakota	Helgeson	Potato	23.2	...	...	...	...
<i>Phytophthora megasperma</i> 437	—	Tooley	—	NA	NA	NA	...	...
<i>Phytophthora mirabilis</i> 340	—	Tooley	—	17.9	NA	NA	...	...
<i>Phytophthora nicotiana</i> 361	—	Tooley	—	NA	...	...	...	...
<i>Phytophthora palmivora</i> 327	—	Tooley	—	NA	NA	NA	...	...
<i>Phytophthora phaseoli</i> 404	—	Tooley	—	19.7	NA	NA	...	...
<i>Phytophthora sojae</i>	Wisconsin	Nakhla	Soybean	NA	NA	NA	...	...
<i>Pythium aphanidermatum</i> 1987-60	New York	Martin ⁱ	—	NA	NA	NA	...	...
<i>Pythium arrhenomanes</i> 1994-15	New York	Martin	—	NA	NA	NA	...	...
<i>Pythium catenulatum</i> 1986-8	India	Martin	—	NA	NA	NA	...	...
<i>Pythium insidiosum</i> 58643	—	Martin	—	NA	NA	NA	...	...
<i>Pythium irregulare</i> 1987-68	Washington	Martin	—	NA	NA	NA	...	...
<i>Pythium sylvaticum</i> 1987-14	Iowa	Martin	—	NA	NA	NA	...	...
<i>Pythium torulosum</i> 1991-19	Ohio	Martin	—	NA	NA	NA	...	...
<i>Pythium ultimum</i> PultMed#1	Wisconsin	Atallah	Potato	NA	17.2	NA	NA	NA
<i>Pythium ultimum</i> PultMed#2	Wisconsin	Atallah	Potato	NA	20.3	NA	...	...
<i>Pythium ultimum</i> 1987-65	Florida	Martin	—	NA	16.5	NA	...	...
<i>Pythium ultimum</i> 1987-69	New York	Martin	—	NA	19.1	...	...	...
<i>Pythium ultimum</i> 1991-20	Hawaii	Martin	—	NA	18.6	...	...	...
<i>Pythium ultimum</i> 58-1	Florida	Martin	—	NA	16.7	...	...	...
<i>Pythium ultimum</i> HS 26-1	New Mexico	Martin	—	NA	19.4	NA	...	...
<i>Pythium ultimum</i> HS 23-1	Arizona	Martin	—	NA	18.9	...	...	...
<i>Pythium ultimum</i> v. <i>spor</i> 1993-25	Maryland	Martin	—	NA	20.2	NA	...	...
<i>Pythium ultimum</i> v. <i>spor</i> 1993-26	Lebanon	Martin	—	NA	17.8	...	...	...
<i>Pythium volutum</i> 1995-105	Japan	Martin	—	NA	NA	NA	...	...
<i>Rhizoctonia solani</i>	Wisconsin	Atallah	Potato	...	...	...	NA	...
<i>Sclerotinia homeocarpa</i>	Wisconsin	Jung ^k	Turfgrass	...	...	...	NA	...
<i>Sclerotinia sclerotiorum</i>	Wisconsin	Atallah	Bean	...	...	...	NA	...
<i>Streptomyces scabies</i>	Wisconsin	Atallah	Potato	NA	NA	NA	NA	NA
<i>Streptomyces scabies</i>	Wisconsin	Atallah	Potato	NA	NA	NA	NA	NA
<i>Verticillium albo-atrum</i> DV20	Wisconsin	Rouse	Potato	NA	NA	NA	NA	NA
<i>Verticillium albo-atrum</i> LV79	Wisconsin	Rouse	Alfalfa	...	...	...	NA	...
<i>Verticillium chlamydosporium</i> NRRL 13093	—	Rouse	—	...	...	...	NA	...
<i>Verticillium cinnebarinum</i> NRRL A-3665	—	Rouse	—	...	...	...	NA	...
<i>Verticillium dahliae</i> V18	Wisconsin	Rouse	Potato	NA	NA	NA	NA	NA
<i>Verticillium dahliae</i> LV75	Oregon	Rouse	Potato	...	...	...	NA	...
<i>Verticillium lateritium</i> NRRL A-18693	—	Rouse	—	...	...	...	NA	...
<i>Verticillium lecanii</i> NRRL A-18240	—	Rouse	—	...	...	...	NA	...
<i>Verticillium nigriscens</i> Nigriscens	—	Rouse	—	...	...	...	NA	...
<i>Verticillium psaleotae</i> NRRL A-18376	—	Rouse	—	...	...	...	NA	...
<i>Verticillium tricorpus</i> NRRL 13690	—	Rouse	—	...	...	...	NA	...

and *E. carotovora* subsp. *carotovora*, and subsp. *atroseptica* by application of sequence comparative analyses. For this purpose, seven universal primer pairs were used: *rpb1* (ribosomal polymerase B1) (29), *ef1α* (elongation factor 1α), *cal* (calmodulin), *act* (actin), *ras* (RAS protein) (2), *β-tubulin*, and *H3* (histone 3) (11,13). These sequences are typically highly conserved within species, but potentially polymorphic among species and genera. *Rpb1* primers failed to amplify fungal DNA, and their use was restricted to oomycetes. Sequences of the *mpd* gene (mannitol-*p*-dehydrogenase) for various species of the bacterial genus *Erwinia* were obtained from M. Hibbing (UW-Madison, School of Veterinary Medicine). All primer pairs designed in this study are highlighted in Table 2, and represent an excerpt of the total number of primer pairs designed from the various gene sequences obtained. Nonspecific primer pairs or regions exhibiting no polymorphisms among the amplified organisms are not listed in Table 2.

PCR amplifications were conducted in a Bio-Rad iCycler thermocycler (Bio-Rad, Hercules, CA), one half degree lower than the calculated melting temperature for the primers to maximize amplification specificity as predicted by Primer3 (25). The 20-μl amplification reactions included 1 ng of total purified culture DNA and 200 nM of each primer in PCR Master Mix (Promega, Madison, WI; 1.5 mM MgCl₂, 200 nM of each dNTP, and 25 units of *Taq* polymerase per ml). Amplification products were visualized in 0.8% agarose gels. Both DNA strands of products smaller than 700 bp were sequenced directly following PCR amplification without cloning. PCR-amplified products were cleaned using AMPure magnetic beads (Agencourt, Beverly, MA) prior to labeling with ABI BigDye Terminator version 3.1 (Applied Biosystems, Foster City, CA), and excess labeling dye was removed with CleanSEQ magnetic beads (Agencourt). Amplification products in excess of 700 bp were cloned prior to sequencing (in both directions) in the Topo TA Cloning Kit (Invitrogen, Carlsbad, CA). Sequencing was performed at the University of Wisconsin Biotechnology Center with the ABI 3730xl DNA Analyzer (Applied Biosystems). Sequences were visualized in Chromas (Technelysium, Southport, Queensland, Australia), aligned in Clustal W (30,31), and the consensus sequence was edited in BioEdit (12).

Sequences from the target organisms, related species, genera, and other pathogens of potato and rotational crops were aligned in Clustal W. Primers were designed from areas polymorphic among closely related species, yet monomorphic within the target species. Prospective primer sequences were evaluated using Primer3 for potential self-compatibility melt temperatures and GC clamping. Subsequently, candidate primers and amplicons were evaluated in Mfold (40) for potential secondary structures, which would reduce amplification efficiency. All Q-PCR products were designed not to exceed 150 bp in size. A primer pair was also designed to

amplify the *act* gene (actin) in potato (GenBank accession no. X55751) to be used as an internal control.

Evaluation of primer specificity. Primer specificity was tested against the DNA collection described previously. Q-PCR amplifications were conducted in triplicates, in a Bio-Rad iCycler iQ real-time PCR system using the iQ Supermix SYBR-Green (Bio-Rad) with ≈1 ng of DNA and 200 mM of each primer in 20-μl total volume. The following amplification protocol was used: initial denaturation of 3 min at 95°C followed by 40 cycles of 95°C for 10 s and 62.1°C (identified following temperature gradient amplifications) for 35 s. Melt curve analysis was used to distinguish potential primer dimers and nonspecific amplification products.

Primer efficiency. Amplification efficiency (efficiency = $10^{(-1/\text{slope})} - 1$) of all primer pairs was tested on a fivefold serial dilution of the target pathogen DNA, and a sixfold serial dilution of potato tuber DNA for the potato control primers. Amplifications of pathogen DNA targets were performed in the presence and absence of potato DNA. Twenty-five nanograms of potato DNA extracted from a disease-free and surface-disinfested tuber was added to the serial dilution of pathogen DNA. Q-PCR efficiencies were calculated using the Bio-Rad iCycler version 3.1 software. To determine the potential impact of the addition of potato DNA on the amplification of the target DNA, mean threshold cycle (Ct) values generated in the presence and absence of potato DNA were compared using Fisher's *t* tests (PROC *t* test in SAS [SAS Institute, Cary, NC]) for each of the primer pairs. Additionally, the slopes of the efficiency equations were compared in an analysis of covariance (ANCOVA) for each primer pair. Equations with similar slopes result in *F* statistics greater than one, indicating that variability is greater within an equation compared with between equations (3).

Tuber inoculation. Russet Burbank tubers (170 to 285 g [6 to 10 oz.]) that were specifically planted for this study in fumigated field plots, never exposed to fungicides and not showing any disease symptoms, were surface-disinfested (10 min in 10% bleach) and then inoculated with zoospores of *P. infestans* or *P. erythroseptica*. Inoculations were performed using 1×10^4 zoospores per ml for *P. erythroseptica* (isolate Pery347) and zoospores from sporangial suspensions adjusted to 1×10^4 sporangia per ml for *P. infestans* (US 940480, genotype US-8). The number of zoospores generated by the sporangial suspension of *P. infestans* was not quantified (average of eight zoospores per sporangium). Sporangia of *P. infestans* produced on inoculated tuber disks following Porter and Johnson (22) were washed off with autoclaved water, and their concentration was estimated with a hemacytometer and subsequently adjusted to 1×10^4 sporangia per ml. Sporangia of *P. erythroseptica* were produced following Mitchell et al. (19), which includes the use of V8 broth and soil extract. Sporangial suspensions of both *P. infestans* and *P. ery-*

TABLE 2. Primers developed to detect and quantify five potato pathogens and the potato *act* gene (actin)^a

Target organism	Gene target	Primer	Primer sequence (5'→3')	Efficiency (%)	
				No potato DNA added	Potato DNA added
<i>Phytophthora infestans</i>	<i>ras</i>	PinFRAS-F	CAT TAC ATT GCT CAC ATG GCT TTC	99.2	95.6
		PinFRAS-R	ATC ACG CGG GGA CAA ATG		
<i>Pythium ultimum</i>	<i>rpb1</i>	PultRPB-F	GCA GAT TGT CCC GGA TAT TAA C	98.2	98.8
		PultRPB-R	CAC AAT AAC CCG AGA ATC AAA G		
<i>Phytophthora erythroseptica</i>	<i>rpb1</i>	PeryRPB-F	GAT GAA ACT AAG CGC CTT CTC	98.2	99.1
		PeryRPB-R	CGA CAA TAG TCT TCA AGG TGG AT		
<i>Erwinia carotovora</i>	<i>mpd</i>	MpdEc-F	ACC GCC AGC ACA TCG TC	95.0	96.8
		MpdEc-R	CGG AAG GCC ATC AGG AA		
<i>Fusarium sambucinum</i>	<i>β-tubulin</i>	FsBTub-F	CCT CTC CGT CCA TCA ACT GG	97.8	96.5
		FsBTub-R	AGG TAG TTC AAG TCG CCG TAA G		
<i>Solanum tuberosum</i>	<i>act</i>	PotAct-F	TGA ACA CGG AAT TGT CAG CA	99.6	...
		PotAct-R	GGG GTT AAG AGG GGC TTC AG		

^a Examples of primer efficiencies are listed, in the presence and absence of the host DNA. Primer efficiencies were calculated in the Bio-Rad iCycler software in accordance with the formula $E = (10^{(-1/\text{slope})} - 1) \times 100$.

throseptica were placed at 4°C for 2 h to release zoospores, and kept on ice during the inoculation of tubers.

A 50- μ l aliquot from each of the inoculum suspensions was placed on a 1 cm² piece of filter paper and inverted onto an eye on the apical part of tubers. Tubers inoculated with *P. infestans* were placed in a dew chamber at 20°C in the dark for 4 days. Tubers inoculated with *P. erythroseptica* were subjected to a continuous mist at 20°C in the dark for 8 h, and then incubated in the dark at 20°C for 4 days, with intermittent misting (15 min every hour).

Twelve cv. Russet Burbank tubers were inoculated with *Pythium ultimum* (isolate Pult Med#2), 12 tubers were inoculated with *F. sambucinum* (isolate Fsamb Med), and 10 tubers were inoculated with *E. carotovora* subsp. *carotovora* (isolate WPP14). Surface-disinfested tubers were wounded in four locations along a longitudinal transect with a four-screw hole-puncher (hole diameter 0.5 and 0.2 cm deep). A circular plug of PAR (PCNB-ampicillin-rifampicin) culture medium (18) colonized by *Pythium ultimum* (diameter 0.5 cm) and obtained from the edge of a growing colony was inserted into each wound. Tubers were inoculated with *E. carotovora* (1×10^6 CFU/ml) and *F. sambucinum* (1×10^4 conidia per ml) by placing a drop of inoculum suspension in each wound. The inoculum for the latter inoculations was obtained by washing actively growing cultures of *E. carotovora* and *F. sambucinum* with sterile water. The quantification of *F. sambucinum* conidia was performed with a hemacytometer, whereas a spectrophotometer was used to estimate the concentration of *E. carotovora* CFU. Inoculated tubers were placed for 4 days in a dark dew chamber at 15°C. Controls consisted of two noninoculated tubers that were subjected to the same treatments as inoculated tubers, except that the inoculum was substituted with sterile water, or a sterile noncolonized agar plug in the case of *Pythium ultimum*.

DNA extractions from inoculated tubers. Inoculated tubers were disinfested (10 min in 10% bleach) after incubation to eliminate any pathogen propagules remaining on the surface. Juice from the inoculated tubers was extracted with an Acme Juicerator device (Waring Products, New Hartford, CT). A volume of 40 ml of the juice was collected from each tuber into 50-ml conical tubes, and 4 g of polyvinylpyrrolidone (Fisher Scientific, Pittsburgh, PA) was added to each tube as a phenol binding agent and shaken vigorously. Tubes were centrifuged at $11,000 \times g$ for 15 min to precipitate eukaryotic and prokaryotic cells. The supernatant was discarded and DNA was extracted from 0.1 g of pelleted cells. DNA was extracted with the FastDNA Kit (Q-Biogene, Irvine, CA) following the manufacturer's recommendations. Subsequently, DNA extracts were subjected to cleanup using the AMPure magnetic beads to eliminate remaining potential PCR inhibitors. All DNA samples were quantified fluorometrically with Quant-iT PicoGreen dsDNA (Invitrogen) and stored at 4°C until used.

Q-PCR on inoculated tubers. One hundred picograms of total DNA (tuber plus pathogen DNAs) was subjected to Q-PCR amplifications with the same conditions described previously. Each DNA sample (i.e., individual tuber) was amplified in triplicate Q-PCRs.

To test for the absence of PCR inhibitors, each sample was spiked with 100 pg of pure culture DNA of the pathogen under study. Pure culture DNA alone constituted the positive control, and no-template water samples constituted the negative controls. Any statistically significant increase in Ct value between positive control samples and spiked samples indicates a decreased efficiency of the amplification, attributable to PCR inhibitors. Additionally, the potato *act* gene in each sample was amplified using PotAct-F/PotAct-R as internal control.

Field samples. Tubers of cv. Gold Rush exhibiting symptoms of pink rot, leak, and soft rot were retrieved from a commercial storage facility in Almond, WI. No late blight epidemics have occurred in Wisconsin since 2002; hence, no naturally infected

tubers could be found in the state. Disease symptoms were diagnosed visually and DNA was extracted from eight tubers showing definitive disease symptoms of each of the pathogens (24 tubers total). DNA extractions were completed using FastDNA on 0.2 g of tissues contiguous to disease lesions, and further purified using AMPure, as previously described. DNA was quantified fluorometrically and 1 ng of total DNA was amplified using the primer pairs PeryRPB-F/PeryRPB-R, PultRPB-F/PultRPB-R, and MpdEc-F/MpdEc-R to detect and quantify *P. erythroseptica*, *Pythium ultimum*, and *E. carotovora*, respectively (Table 2). An equal DNA amount (1 ng total DNA) was used to quantify the potato host using primers PotAct-F/PotAct-R. In the absence of *P. infestans* from the tested samples, 2 pg of *P. infestans* DNA (control) was added to all 24 tested samples to ensure the absence of PCR inhibitors. Primers PinfRAS-F/PinfRAS-R were used in Q-PCR amplifications. Substantial increases in Ct value from the *P. infestans* control indicate the presence of PCR inhibitor. All Q-PCR amplifications were carried out in triplicate using 20- μ l reaction volumes as previously described. ΔC_t ($\Delta C_t = C_{t_{\text{sample}}} - C_{t_{\text{control}}}$) was computed (10) based on the mean Ct values obtained. In all amplifications, DNA from pure cultures of each of the three pathogens was used as the positive control and no-template water sample as the negative control. The infection coefficient (IC), which is the ratio of $C_{t_{\text{host}}}/C_{t_{\text{pathogen}}}$ (36), was computed for each sample tested.

RESULTS

Primer design. The generated primer pairs were amplified the following target genes: *ras* in *P. infestans*, *rpb1* in *P. erythroseptica* and *Pythium ultimum*, *mpd* in *E. carotovora* subsp. *carotovora* and subsp. *atroseptica*, β -tubulin in *F. sambucinum*, and *act* in potato (Table 2). Only primer pairs exhibiting minimal self-compatibility and secondary structures and amplifying at similar annealing temperatures (62 to 63°C) were retained.

Primer specificity. The selected primer pairs amplified all isolates of the target species collected from various geographic locations, or belonging to different genotypes (Table 1). Nevertheless, two primer pairs (*P. infestans* and *E. carotovora*) also amplified DNA fragments from close relatives not associated with potato. The primer pair targeting *P. infestans* (PinfRAS-F/PinfRAS-R) also amplified DNA fragments from the phylogenetically related species *P. mirabilis* and *P. phaseoli*. The primer pair designed to amplify *E. carotovora* subsp. *carotovora* and subsp. *atroseptica* (MpdEc-F/MpdEc-R) also amplified fragments from subsp. *wasabi* and subsp. *beta-vascularum*, *E. nigri-fluens*, and *E. quercina*. Melt curve analyses and DNA sequencing of the amplified product failed to differentiate between the intended target organisms and closely related ones amplified with the described primer pairs.

Primer pairs for *P. erythroseptica* (PeryRPB-F/PeryRPB-R) and *F. sambucinum* (FsBTub-F/FsBTub-R) were designed to detect close relatives associated with the same disease symptoms on potato. The primer pair targeting *P. erythroseptica* also detected *P. cryptogea*, described to cause pink rot symptoms in certain potato production areas. The primer pair targeting *F. sambucinum* amplified DNA fragments from *F. avenaceum*, *F. culmorum*, *F. graminearum*, and *F. acuminatum*, all associated with potato dry rot. The primer pair designed to amplify the actin gene in potato (PotAct-F/PotAct-R) did not amplify the DNA of 15 different pathogens commonly associated with potato (data not shown).

Primer efficiency. All six primer pairs exhibited primer efficiencies greater than or equal to 95% in the presence or absence of potato DNA (Table 2; Fig. 1). Ct values from the amplification of fivefold serial DNA dilutions of the five pathogens were not significantly different ($P > 0.05$) when 25 ng of potato DNA was added (Table 3; Fig. 1). Furthermore, we found no significant differences in the ANCOVA on the slopes of the equations

generated with each primer pair (Fig. 1). The primer pair PotAct-F/PotAct-R showed amplification efficiency greater than 99% over 6 orders of magnitude (Fig. 1).

Tuber inoculations. All tubers inoculated with *P. erythro-septica* and *E. carotovora* subsp. *carotovora* exhibited minor pink rot and soft rot lesions, respectively, 4 days postinoculation. Conversely, and because of the short incubation period, tubers inoculated with *P. infestans*, *Pythium ultimum*, or *F. sambucinum* did not show symptoms of late blight, leak, or dry rot, respectively. Moreover, a subset of tubers inoculated with *P. infestans*, *Pythium ultimum*, and *F. sambucinum* were incubated until the appearance of late blight, leak, and dry rot symptoms, respectively. In all instances, noninoculated control tubers did not show disease symptoms.

Q-PCR on inoculated tuber DNA. All five target pathogens were detected in 100 pg of total DNA from all respective inoculated tubers, except for one tuber inoculated with *F. sambucinum*. No pathogen DNA target was amplified from noninoculated tubers (Table 4). Furthermore, Ct values were not statistically different between the DNA replicates ($P > 0.05$). No significant PCR inhibition was observed and no significant deviation in Ct values were observed between the positive controls and the posi-

tive control-spiked samples. The potato *act* gene was amplified in all samples.

Field samples. All but one field sample tested positive for the targeted pathogen. *Pythium ultimum* and *E. carotovora* were detected in all eight symptomatic samples tested, respectively, while only seven samples tested positive for *P. erythro-septica* (Table 5). All negative controls failed to amplify any target DNA. Δ Ct values varied between -1.5 and 1.1 for *P. infestans*-spiked samples, indicating minimal to no PCR inhibition. Infection coefficients varied between 0.7 and 1.1.

DISCUSSION

We have developed a Q-PCR methodology for the detection and quantification of five pathogens of stored potato tubers. Currently, decisions by potato storage managers and processors regarding which harvested crops to store and the projected duration of storage are made solely on the basis of field and grower histories and visual observation of tubers at harvest. The intent of this study is to provide a method to develop a decision-making tool for the storage of potato crops sensitive to minute amounts of pathogen DNA associated with tubers (incidence of pathogen), as

Fig. 1. Standard curves showing amplifications using the primer pairs A, PinfRAS for *Phytophthora infestans*, B, PeryRBP for *Phytophthora erythro-septica*, C, FsBtub for *Fusarium sambucinum*, D, PultRBP for *Pythium ultimum*, E, MpdEc for *Erwinia carotovora* subsp. *carotovora*, and F, PotAct for *Solanum tuberosum*. DNA from pure cultures of each pathogen and DNA from potato tuber was diluted over 5 orders of magnitude and amplified with or without the presence of host DNA (25 ng). Efficiency curve equations and correlation coefficients (R^2) are included. P values of analyses of covariance were used to compare equation slopes, but no significant differences were found in any primer pair.

well as a quantification of that DNA (pathogen load). This measurement of the pathogen load could be used to apply different storage management strategies, to avoid disease, or reduce losses.

The six primer pairs designed in this study selectively amplified their intended DNA targets (Table 1). All primer pairs amplified DNA from multiple isolates of the intended species, from various geographic locations, or belonging to different genotypes. It is worth noting that because of the lack of sequence polymorphisms, the primer pair PinFRAS-F/PinFRAS-R targeting *P. infestans* also amplifies DNA fragments from *P. mirabilis* and *P. phaseoli*. These two oomycetes are not pathogens of potato (8) but are taxonomically closely related to *P. infestans* (4,5,17). Furthermore, melt curve analyses and DNA sequencing of the products amplified from *P. mirabilis* and *P. phaseoli* failed to differentiate between those species and *P. infestans* (data not shown). Amplification of *P. mirabilis* or *P. phaseoli*, with primers designed to detect *P. infestans*, was reported in a number of other published studies (24,33,34). Knowing that the likelihood of finding the DNA of *P. mirabilis* and *P. phaseoli* associated with potato is negligible, the primer pair is considered to be specific to *P. infestans* for this application. The primer pair PeryRPB-F/PeryRPB-R targeting *P. erythroseptica* was purposely designed to also detect *P. cryptogea*, which is associated with pink rot in some potato production regions (15). Similarly, the primer pair FsBTub-F/FsBTub-R targeting *F. sambucinum* was specifically designed to also amplify *F. avenaceum*, *F. culmorum*, *F. graminearum*, and *F. acuminatum*, all reported to cause dry rot in potatoes (28). The primer pair FsBTub-F/FsBTub-R does not amplify *F. coeruleum*, which causes decay of potato seed pieces in fields and is usually not associated with tuber decay in storage. Minimal sequence differences among the various subspecies of *E. carotovora* lead to a primer pair, MpdEc-F/MpdEc-R, which amplifies subsp. *wasabi* and subsp. *betavascularum*, in addition to the target soft-rotting subsp. *carotovora* and subsp. *atroseptica*. *E. carotovora* subsp. *wasabi* and subsp. *betavascularum* are not commonly found on potato. Another two species not associated with disease on potato, *E. nigrifluens* and *E. quercina*, were amplified. Encountering the DNA of the latter two species associated with potato DNA is highly unlikely; hence, making the primer pair MpdEc-F/MpdEc-R reasonably specific for the detection and quantification of soft rotting bacteria in/on potato.

Primer efficiencies were consistently between 95 and 100% over 5 orders of magnitude of target DNA concentration (Table 3; Fig. 1). Primer efficiencies remained high in the presence of high amounts of potato DNA in Q-PCRs, and no statistically significant differences were observed between slopes or among Ct values regardless of the presence or absence of potato DNA (Table 3; Fig. 1). In this study, we amplified as low as 0.5 pg of *Pythium ultimum* DNA, 0.7 pg of *P. erythroseptica* DNA, 1.1 pg of *P. infestans* DNA, 7.5 pg of *F. sambucinum* DNA, and 10 pg of *E. carotovora* DNA (Table 3; Fig. 1). Additionally, we were able to consistently amplify 0.5 pg of potato DNA (Table 3; Fig. 1), which we used to compute the infection coefficient values of field-collected and infected tubers. Amplifications of smaller concentrations were possible, but the variability among replicates became large. Such variation is common when dealing with minute amounts of DNA, where small differences are amplified exponentially (36).

Pathogens were successfully detected and amplified from field-collected potato tubers infected with *P. erythroseptica*, *Pythium ultimum*, and *E. carotovora* (Table 5). Confirmation of the presence of the pathogen was successful in eight samples tested for *Pythium ultimum*, eight tubers tested for *E. carotovora*, but only seven out of eight tubers tested for *P. erythroseptica*. Samples used to extract DNA were taken from an area contiguous to the observed lesions and not showing leak, soft rot, or pink rot symptoms, respectively. It is possible that the excised specimen in

the negative assay was taken from an area that the pathogen had failed to colonize at the time of sampling.

We tested several methods to homogenize the potato periderm, but no blender or mixer provided a complete maceration/homogenization in a reasonable amount of time, or number of steps needed. Moreover, sonication failed to dislodge pathogen propagules and/or DNA from potato tissues. We found tuber juicing to be an adequate method to generate a homogenous sample from individual tubers.

The designed primers exhibited high sensitivity to low amounts of pathogen DNA. All described pathogen detections and quantifications were performed using 100 pg of total DNA extracted a few days after tuber inoculation, in the presence or absence of symptoms. Additionally, we were able to detect *P. erythroseptica*, *Pythium ultimum*, and *E. carotovora* from 1 ng of DNA extracted from field-collected tubers showing symptoms of pink rot, leak, and soft rot, respectively. Sensitivity to very low amounts of target DNA is essential to detect small numbers of pathogen propagules, especially in commercial situations where low pathogen incidence and low pathogen loads can potentially lead to substantial economic losses. This study is a first step in a methodology to provide processors and storage managers with the means to identify and quantify potential threats with accuracy.

TABLE 3. Five-point serial dilutions of *Phytophthora infestans*, *Phytophthora erythroseptica*, *Pythium ultimum*, *Fusarium sambucinum*, *Erwinia carotovora* subsp. *carotovora* DNA from pure cultures, and a six-point serial *Solanum tuberosum* (cv. Russet Burbank) tuber DNA

Organism (isolate)	Primer pair	DNA amount (ng)	Potato DNA added (Ct values) ^a	
			No	Yes
<i>P. infestans</i> (US-8)	PinFRAS	1.10E-03	31.5	31.9
		1.10E-02	28.3	28.4
		1.10E-01	24.5	25.2
		1.1	21.3	22.4
		11	18.1	18.9
		<i>t</i> test		0.83
<i>P. erythroseptica</i> (Pery 347)	PeryRPB	7.00E-04	31.5	30.8
		7.00E-03	29.5	28.1
		7.00E-02	26.2	24.4
		7.00E-01	22.8	21.7
		7	18.7	17.3
		<i>t</i> test		0.96
<i>F. sambucinum</i> (Fsamb Med)	FsBTub	7.50E-03	28.8	28.9
		7.50E-02	25.5	25.5
		7.50E-01	22	21.9
		7.5	18.6	18.6
		75	14.9	15.3
		<i>t</i> test		0.98
<i>Pythium ultimum</i> (Pult Med#2)	PultRPB	5.00E-04	33	32.4
		5.00E-03	29.7	29.2
		5.00E-02	26.2	25.5
		5.00E-01	22.7	22.2
		5	19.6	19.1
		<i>t</i> test		0.87
<i>E. carotovora</i> (Ecc WPP14)	MpdEc	1.00E-02	36.5	35.3
		1.00E-01	33.1	32.9
		1	29.6	29.3
		10	26	24.8
		100	23.3	22.1
		<i>t</i> test		0.76
<i>S. tuberosum</i> (tuber)	PotAct	5.10E-04	33.76	
		5.10E-03	31.17	
		5.10E-02	28.47	
		5.10E-01	24.41	
		5.1	21.54	
		51	18.98	

^a Fisher *t* test comparisons of standard curve amplifications in the presence and absence of potato DNA are indicated.

TABLE 4. Amount of DNA (picograms) of five pathogens detected in 100 pg of total DNA extracted from tubers inoculated individually 4 days postinoculation^a

Tuber	<i>Phytophthora infestans</i> ^b	<i>Phytophthora erythroseptica</i> ^c	<i>Pythium ultimum</i> ^b	<i>Fusarium sambucinum</i> ^b	<i>Erwinia carotovora</i> ^c
Tuber 1	2.29	2.16	4.71	1.80	4.85
Tuber 2	1.44	2.13	3.31	1.89	5.11
Tuber 3	2.11	2.10	3.88	1.31	5.38
Tuber 4	2.08	2.22	2.98	2.48	5.35
Tuber 5	2.23	2.16	3.97	1.83	5.53
Tuber 6	1.27	2.22	4.56	1.34	5.34
Tuber 7	2.23	2.19	4.80	0.69	5.11
Tuber 8	1.94	2.22	2.24	1.54	6.50
Tuber 9	1.82	2.22	3.52	1.39	6.58
Tuber 10	2.49	2.37	3.07	1.80	6.67
Tuber 11	2.46	2.46	2.80	—	—
Tuber 12	2.29	2.07	2.74	1.63	—
Healthy ^d	NA ^e	NA	NA	NA	NA
Healthy	NA	NA	NA	NA	NA

^a DNA amounts were computed from the standard curves in Figure 1 and using the mean threshold cycle (Ct) value of triplicate quantitative-polymerase chain reaction amplifications.

^b Tubers inoculated with *P. infestans* (US-8), *Pythium ultimum* (Pult Med#2), and *F. sambucinum* (Fsamb Med#2) showed no symptoms at the time of DNA extraction, four days postinoculation.

^c Tubers inoculated with *P. erythroseptica* (Pery 347) and *E. carotovora* subsp. *carotovora* (WPP14) showed typical pink rot and soft rot symptoms, respectively, at the time of DNA extraction, 4 days postinoculation.

^d Healthy tubers were treated with distilled water and pathogen-free agar plug for *Pythium ultimum*.

^e NA = no amplification.

TABLE 5. Detection and quantification of *Phytophthora erythroseptica* (pink rot), *Pythium ultimum* (leak), and *Erwinia carotovora* (soft rot) in naturally infected potato tubers collected in a storage facility in Almond, WI

Sample	Control ^a	ΔCt^b	Pathogen ^c (Ct)	Host ^d (Ct)	IC ^e
Leak1	28.9	1.1	26.8	21.1	0.8
Leak2	26.7	-1.1	26.1	20.9	0.8
Leak3	27.4	0.4	24.5	20.6	0.8
Leak4	26.3	-1.5	26.2	22.8	0.9
Leak5	26.9	-0.9	34.3	27.5	0.8
Leak6	27.9	0.1	35.4	24.9	0.7
Leak7	27.5	-0.3	18.9	21.5	1.1
Leak8	28.9	1.1	21.2	21.0	1.0
Softrot1	27.4	-0.4	19.7	21.7	1.1
Softrot2	27.0	-0.8	20.3	21.1	1.0
Softrot3	27.1	-0.7	20.6	23.0	1.1
Softrot4	27.1	-0.7	20.9	19.9	1.0
Softrot5	26.8	-1.0	20.0	22.8	1.1
Softrot6	27.3	-0.5	21.4	19.8	0.9
Softrot7	28.3	0.5	32.8	21.6	0.7
Softrot8	27.3	-0.5	25.2	20.2	0.8
Pinkrot1	27.0	-0.8	24.3	27.6	1.1
Pinkrot2	27.1	-0.7	29.6	31.4	1.1
Pinkrot3	26.7	-1.1	25.5	25.2	1.0
Pinkrot4	27.2	-0.6	25.0	25.3	1.0
Pinkrot5	27.1	-0.7	28.1	20.4	0.7
Pinkrot6	26.9	-0.9	22.1	24.6	1.1
Pinkrot7	27.6	-0.2	—	20.9	—
Pinkrot8	27.8	0.0	23.3	22.8	1.0
Control ^f	27.8	—	—	—	—

^a Threshold cycle (Ct) values using *P. infestans* DNA (2 pg) added to each sample DNA to test for the lack of polymerase chain reaction inhibitors.

^b $\Delta Ct = Ct_{\text{sample}} - Ct_{\text{control}}$.

^c Ct values obtained using primers PultRPB-F/PultRPB-R (leak), MpdEc-F/MpdEc-R (soft rot), and PeryRPB-F/PeryRPB-R (pink rot).

^d Ct values obtained using primers PotAct-F/PotAct-R.

^e Infection coefficient: $IC = Ct_{\text{host}}/Ct_{\text{pathogen}}$.

^f Two picograms of *P. infestans* DNA from pure culture, in the absence of host DNA.

The primer pairs developed in this study could be used for the diagnosis of potato diseases in the field and storage facilities. In laboratory-inoculated tubers, we were able to detect the presence of *P. infestans*, *Pythium ultimum*, and *F. sambucinum* prior to symptom appearance, while *P. erythroseptica* and *E. carotovora* were detected at the appearance of the first symptoms on inoculated tubers. We have also used the primers to detect the presence of individual pathogens in mixed infections of pink rot, leak, and

soft rot. While pathogen isolation on culture media only detected the soft rotting bacteria, Q-PCR revealed the presence of *P. erythroseptica* and *Pythium ultimum* (data not shown).

The methodology described in this study may allow for the development of models to predict the fate of crops prior to harvest, where decisions regarding the storage duration would be based on the amount of DNA detected. By incorporating a quantification of phytopathogen amounts on/in potato tubers, a triage based on risk potential becomes possible. This in turn is expected to allow for substantial reductions in postharvest pesticide applications, particularly in crops exhibiting low pathogen amounts. Nevertheless, a model for the prediction of tuber storability is not yet available and necessitates several growing and storage seasons to develop. Furthermore, studies to identify the most appropriate sampling methodologies at the field and tuber levels are not yet available and will be productive opportunities for future research.

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