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Development of a multiplex assay for genus and species-specific detection of *Phytophthora* based on differences in mitochondrial gene order

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ABSTRACT

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A molecular diagnostic assay for *Phytophthora* that is specific, sensitive, has both genus and species specific detection capabilities multiplexed and can be used to systematically develop markers for detection of a wide range of species would facilitate research and regulatory efforts. To address this need, a marker system was developed based on the high copy sequences of the mitochondrial DNA utilizing gene orders that were highly conserved in *Phytophthora* but different in the related genus *Pythium* and plants to reduce the importance of highly controlled annealing temperatures for specificity. An amplification primer pair designed from conserved regions of the *atp9* and *nad9* genes produced an amplicon of approximately 340 bp specific for the *Phytophthora* spp. tested. The TaqMan probe for the genus-specific *Phytophthora* test was designed from a conserved portion of the *atp9* gene whereas variable intergenic spacer sequences were used for designing the species specific TaqMan probes. Specific probes were developed for 13 species and the *P. citricola* species complex. *In silico* analysis suggests species specific probes could be developed for at least 70 additional described and provisional species; the use of locked nucleic acids in TaqMan probes should expand this list. A second locus spanning three tRNAs (*trnM-trnP-trnM*) was also evaluated for genus specific detection capabilities. At 206 bp, it was not as useful for systematic development of a broad range of species-specific probes as the larger 340 bp amplicon. All markers were validated against a test panel that included 87 *Phytophthora* spp., 14

provisional *Phytophthora* spp., 29 *Pythium* spp., 1 *Phytopythium* sp. and 39 plant species. Species specific probes were validated further against a range of geographically diverse isolates to ensure uniformity of detection at an intraspecific level, as well as with other species having high levels of sequence similarity to ensure specificity. Both diagnostic assays were also validated against 130 environmental samples from a range of hosts. The only limitation observed was primers for the 340 bp *atp9-nad9* locus did not amplify *P. bisheria* or *P. frigida*. The identification of species present in a sample can be determined without the need for culturing by sequencing the genus specific amplicon and comparing that with a reference sequence database of known *Phytophthora* species.

Keywords: real-time PCR, molecular detection, diagnostics, *Phytophthora ramorum, Phytophthora kernoviae*

Species in the genus *Phytophthora* have had a significant impact on production of economic crop plants and the health of native plant communities. The importance of the genus as plant pathogens infecting a wide range of host species is shown by the more than 11,500 entries in the Systematic Mycology and Microbiology Lab website listing fungal - host reports (http://nt.ars-grin.gov/fungaldatabases/fungushost/FungusHost.cfm). This number is likely to increase in the future, as the number of newly discovered and described species continues to increase. From 2000 to 2007, the number of described species in this genus essentially doubled (9), and while the recent taxonomic updates of the genus by Kroon et al. (27) lists 105 described species a later review noted 117 species

(32). However, these listings are already out of date as there have been a number of new species described as well as provisional species named since these manuscripts were published.

While the increased availability of sequence data for confirming species identification and conducting phylogenetic analysis has contributed to this increase in species description, perhaps equally important is the increased number of field surveys in forest and commercial nursery environments that have resulted in the identification of new species. An example of this in the USA can be seen with the quarantine species *P. ramorum*, which was initially found in the forest ecosystem of central coastal California but has since spread to other parts of coastal California and southern Oregon (16,39). *P. ramorum* has been found in the nursery industry and been transported on infected nursery stock from west coast to east coast as well as between states within the eastern and western USA (15,18). Significant effort has been put into surveys to determine the extent of pathogen spread and the inspection of plant shipments to prevent introductions into new locations. This has provided a much better picture of other *Phytophthora* species present in the various ecosystems investigated and provided isolates of previously undescribed species. Surveys for *P. ramorum, P. kernoviae* and *P. alni,* which have caused significant disruptions to natural ecosystem in Europe (9-11), have also resulted in the isolation and description of previously unknown species in Europe.

Given the role of international trade in moving plant material around the world, the consequence of the introduction of invasive species on native ecosystems and the regulatory needs surrounding the plant trade industry to prevent movement of quarantine species, having a robust molecular diagnostic capability for detection of *Phytophthora* is

essential. An ideal marker system would be highly sensitive so the pathogen could be detected when present in low amounts, be capable of detecting the pathogen at both a species and genus specific level, and allow for the systematic development of species specific markers. Of particular importance is the availability of a robust genus specific detection capability that can be run concurrently with species specific detection. This would allow for a broader analysis of any species that may be present in a sample or ecosystem rather than just be able to determine if an individual species is present. With the availability of a sequence database of the target locus for the genus it would be possible characterize the species community without the need for culturing.

A variety of markers have been designed for conventional and real time PCR assays for *Phytophthora* (reviewed in 32). While the design of some of these have focused on a particular species using a unique random clone, an amplicon from a random amplified polymorphic DNA assay or a unique sequence differences in a specific gene, several loci that are conserved within a species but variable between them have been used for the systematic development of multiple species specific diagnostic markers for conventional and real time PCRs. The internal transcribed spacer (ITS) region of the rDNA has been commonly used for this purpose, as have introns of the *Ras* related *Ypt1* gene and the spacer region between the *cox1* and *cox2* mitochondrially encoded genes (reviewed in 32). While a genus specific detection capability for real time PCR has been reported for the ITS based diagnostic assay for *P. ramorum* (26) it is not fully genus specific as several *Pythium* species were also detected. Likewise, while there is a genus specific detection capability using the spacer region between the *cox1* and *cox2* genes, this is dependent on control of the annealing temperature for specificity and is not

suitable for real time PCR diagnostic techniques (34). A genus specific detection capability has been described for the *Ras* related *Ypt1* gene, but this is with conventional PCR and the approximately 470 bp amplicon may not be suitable for real time PCR (41,42). A real-time PCR probe for *Phytophthora* has been developed by Bilodeau et al. (5) using the β-tubulin region but has not been fully validated against a range of *Pythium* species.

When developing molecular diagnostic techniques for pathogen detection it is important to keep in mind that the assay must be reproducible and accurate, as well as capable of high throughput sample processing with different operators using different equipment in different locations. For example, a variety of thermal cyclers may be used in different facilities and if a narrow window for annealing temperature is necessary for accurate results, then additional revalidation of thermocyclers to account for variation in temperature calibration and ramping speed among machines may be required. Amplification conditions may also need to be monitored consistently as a means for ensuring the accuracy of results. This requirement increases the amount of work needed to complete the assays, but also the potential for unnecessary false positive and false negative results and problems with reproducibility of results between different diagnostic facilities. The capability of high throughput sample processing is also required for some diagnostic labs, so the ability to obtain accurate results with an assay using either a 96 well plate or single tubes is essential.

In an effort to improve the diagnostic capability for *Phytophthora* this study approached the design of a new marker system with several goals in mind: 1) a desire to limit the importance of precisely controlled annealing temperature for maintaining

specificity by selecting primer annealing sites that were adjacent in *Phytophthora* but separated in *Pythium* and plants, 2) a robust genus specific detection capability was needed that could be multiplexed with the species specific marker, 3) identify a locus that would allow for the systematic development of species specific markers for a wide range of species, 4) the target sequence needed to be high copy number to provide the greatest sensitivity of detection, and 5) the diagnostic marker system needed to include a plant amplicon to serve as a positive control. Due to its high copy number, the mitochondrial genome was selected for designing this new marker system. Having the sequenced mitochondrial genomes for 20 *Phytophthora* and 14 *Pythium* spp. (F. Martin, unpublished) provided the opportunity to identify gene orders that were highly conserved in *Phytophthora* but different in *Pythium* and plants that could be used for the design of genus specific amplification primers. Rather than have an amplicon used for genus specific detection and a second amplicon for species specific detection, the genus specific primers were designed so the amplicon included a highly conserved region that could be used for annealing of a genus specific TaqMan probe as well as variable intergenic spacer sequences for design of species specific TaqMan probes. Sequencing these regions from a wide range of species allowed us to evaluate the feasibility of this location for the design of species specific TaqMan probes. This report describes the design of two marker systems and validation of their specificity with culture and environmental samples.

MATERIALS AND METHODS.

***Phytophthora, Pythium, Phytopythium* and plant species used.** The 667 isolates used in this investigation represented 87 valid and 14 provisional *Phytophthora* species (95 of these were used for the core plate used to evaluate all primers and probes) and were from the World *Phytophthora* Genetic Resource Collection at the University of California, Riverside (Table 1). Cultures were grown and DNA extracted as previously described (8). Some additional isolates of *P. megakarya* and *P. palmivora* recovered from cacao in Ghana were also included in the analysis (DNA samples kindly provided by F. Govers). Twenty nine *Pythium* spp. were used to confirm the specificity of the assay (Table 2), information on their culture and DNA extraction can be found in Martin (30). A range of plant species were also used to evaluate the specificity (Table 2). DNAs were extracted as previously noted (35) or were part of the environmental sample evaluation and were extracted using the USDA-APHIS approved DNA extraction protocol (47). To verify the presence of amplifiable *Pythium* DNA, real-time PCR with SYBR Green was performed using universal primers ITS1 and ITS4 (48). PCR was performed using SYBR Green PCR Master Mix 1X (Applied Biosystems, Carlsbad, CA), Primers ITS1 and ITS4 at 0.4µM. PCR in a reaction volume of 25µl on the ICycler instrument (IQ4; Bio-Rad Laboratories, Hercules, CA). Amplification conditions were: 1 cycle activation at 95°C for 8.5 min, followed by 40 cycles of: 95°C for 30 sec., 60°C for 30 sec and 72°C for 60 sec; finishing with a final cycle of72°C for 10 min. Melt-curve analysis started at 60°C and increased 0.5°C every 2 cycles 70 times with a final incubation at 4°C. To confirm the suitability of plant DNA for PCR amplification the TaqMan internal plant control probe and primers were used as described below.

Identification of MtDNA regions for marker development. Mitochondrial genome data for *P. infestans, P. ramorum* (33), *P. sojae*, and 17 other *Phytophthora* spp. and 14 *Pythium* spp. are part of an ongoing mitochondrial genome sequencing project (F. Martin, unpublished) which provided us the opportunity to use comparative genomic approaches to identify regions of the genome that might be useful for developing diagnostic markers for *Phytophthora* spp. Plant mitochondrial sequences used for this studies were obtained from GenBank. Gene order differences that were conserved among *Phytophthora* spp. but different in *Pythium* spp. and plant were identified. Three regions were the focus of this investigation: *trnM-trnP-trnM*, *atp9-nad9* and *nad11-secY*. In *Pythium,* the gene order *trnM-trnP* was conserved but the last *trnM* was located more than 10 kb away and in the opposite orientation, *atp9* and *nad9* were located 18 to 30 kb apart and *nad11* and *secY* were at least 15 kb apart. This may be observed using comparative genomics of mitochondrial genomes deposited in GenBank (AY894827, AY894828, AY894835, DQ832717, DQ832718 and NC002387 for *Phytophthora* spp. and NC014280 for *Pythium ultimum*).

Amplification and sequencing. For sequencing of the *trnM-trnP-trnM* locus amplification primers were selected from the flanking genes (*lrn* and *rpl14*); amplification primers for the two other loci were in the indicated genes (Table 3). All amplifications were performed using approximately 10 ng of template DNA, 0.4 mM forward and reverse primers, 2 mM $MgCl_2$, 200 µM dNTP, 1× amplification buffer, and 1 unit of AmpliTaq (Applied Biosystems, Foster City, CA) in a volume of 25 µl. Templates were amplified in an ABI 9600 thermal cycler with the following cycling conditions: 1)

for *TrnM-TrnM*, 1 interval of 95°C for 3 min; 35 cycles of 95°C for 1 min, 1 min of annealing 61°C, and extension at 72°C for 2 min; and 1 interval of 72°C for 5 min followed by a 4°C hold; 2) for *atp9-nad9*, 1 interval of 95°C for 3 min; 40 cycles of 95°C for 1 min, 1 min of annealing 61°C, and extension at 72°C for 1 min; and 1 interval of 72°C for 10 min followed by a 4°C hold and 3) for *nad11-secY*, 1 interval of 95°C for 3 min; 40 cycles of 95°C for 30 sec, 45 sec of annealing 51°C, and extension at 72°C for 1 min; and 1 interval of 72°C for 5 min followed by a 4°C hold. After confirming template amplification by running samples on an agarose gel, sequencing templates were treated with ExoSap (USB, Cleveland, OH) according to the manufacturer's instructions and sent to the Penn State Genomics Core Facility of the Huck Institute for Life Sciences (University Park, PA) for sequencing with the amplification primers. Each template was sequenced in both directions to generate a consensus sequence based on complementary strands. Sequencher 4.7 (Gene Codes, Ann Arbor, MI) was used to generate consensus sequences while DS Gene v. 2.5 (Accelrys, San Diego, CA) was used for making alignments.

Marker development. Sequence alignments were examined for highly conserved regions for design of amplification primers and a genus specific TaqMan probe. Intergenic spacer sequences were also examined for GC content and if the level of sequence divergence was appropriate for development of species specific TaqMan probes. The IDT SciTools OligoAnalyser 3.1 (Integrated DNA Technologies Inc., Coralville, IA) was used for primer design. Specific primers were designed so the nucleotides unique to the target were at the 3′ end position of the primer and a TaqMan

probe (20) could be designed in the middle of the amplicon when possible. For TaqMan probe design, the following parameters (6) were used with some exceptions because of the high A-T's content of the these mitochondrial region: T_m of the TaqMan probe was selected to be 10° C higher (when possible, if not then at least 3-5° C degree higher than the amplification primers, 15 to 36 bp in length with the total number of G's or C's in the last five nucleotides at the 3′ end of the primer not exceeding two. Mismatching nucleotides responsible for species specificity were positioned as close as possible to the middle of the probe rather than at the ends while avoiding positions with secondary structure (14). The 5' end of the *atp9-nad9* TaqMan probe for *Phytophthora* genus was labeled with FAM (fluorescein), and other probes tested with CAL Fluor Red, Hex or Cy5 (Table 3). The *trnM-trnM Phytophthora* genus specific probe was labeled with Hex. The 3′ end of all probes was labeled with the Black Hole Quencher-1 (BHQ-1; Biosearch Technologies, Inc., Novato CA) or Black Hole Quencher-2 (BHQ-2) depending on the TaqMan assay. The amplification master mix contained 0.5 µM of the genus-specific primer pair (some primers had a 5'Flap to improve the real-time PCR signal (1)). Depending on the assay the TaqMan probe concentration varied; it was 0.05 µM for *atp9-nad9 Phytophthora*-genus specific, *P. cactorum, P. cambivora P. fragariae* and *P. syringae* species specific probes, 0.1 µM for *trnM-trnM Phytophthora* genus specific probe, *P. alni, P. cinnamomi, P. citricola* complex*, P. kernoviae, P. lacustris, P. nicotianae, P. palmivora, P. pseudosyringae* and *P. rubi* species-specific probes, or 0.025 µM for *P. ramorum* species-specific probe. Other components of the amplification mix included 6 mM of $MgCl_2$, and Real Master Mix without Rox (5 Prime, Fisher Scientific Company, LLC, Waltham, MA) with PCR cycling conditions set at 95°C for 2 min, 50

cycles at 95°C for 15 s, the appropriate annealing temperature for each species-specific TaqMan probe (62°C for the *trnM-trnM* genus specific assay and the temperatures indicated in Table 4 for the remaining markers) for 1 min 30 s, in a reaction volume of 25µl on the ICycler instrument. The primers for the plant internal control were present at 0.0125 µM and the probe, which was labeled with CAL Fluor Red and BHQ-2, at 0.01µM.

Testing of the marker systems for specificity and sensitivity. Two loci, the *trnM-trnM* and *atp9-nad9* regions (Figure 1), were tested for genus specificity on a core of 87 described and 14 provisional *Phytophthora* spp. (101 total, Table 1), 29 *Pythium* spp. (Table 2) and 39 plant species (Table 2). The intergenic spacer regions of the *atp9-nad9* locus was chosen for design of the species specific TaqMan probes because this region contained a higher level of sequence divergence than the *trnM-trnM* locus. Fourteen species-specific TaqMan probes (Table 3) were tested with the core *Phytophthora* species plate (Table 1) for specificity. Probes were tested on multiple isolates of the species for which they were designed and other species that had similar sequences at probe annealing sites. The *P. ramorum* and *P. kernoviae* species specific probes were tested on a panel of additional species with 256 isolates of *Phytophthora* (94 species with 1 to 6 isolates/species). To determine the limit of sensitivity for genus and species specific detection, standard curves using a serial dilution from 1ng to 10 fg of stock DNA of the target species were evaluated.

Evaluation on field samples. Fields samples were received as a blind test for evaluation with the two *Phytophthora* genus specific TaqMan assays and the *atp9-nad9* species-specific assay. All samples were run in multiplex with the plant internal control to confirm the ability to amplify the DNA sample.

California Department of Food and Agriculture (CDFA) field samples: Ninety nine plant samples submitted to the CDFA from different locations in Californian had DNA extracted using the standard USDA-APHIS procedure for *P. ramorum* (47). All samples were tested with both the *atp9-nad9* and *trnM-trnM Phytophthora* genus specific markers. The *atp-9-nad9* assay was multiplexed with *P. ramorum* and *P. kernoviae* species specific probes with the following species specific probes tested individually: *P. syringae, P. cambivora, P. citricola* group and *P. pseudosyringa*e. To confirm the species that were present, the genus specific amplicon was sequenced for all samples that were positive for a *Phytophthora* spp. and the sequence compared to our sequence database by BLAST analysis using BioEdit ver. 7.0.9.0 (19).

Big Sur: Field samples were collected on June 2, 2010, at Pfeiffer Big Sur State Park and Andrew Molera State Park, Monterey County, CA. Leaf pieces with lesions and non-symptomatic leaves were cut with a #3 cork borer, with half of the leaf disk plated on PARP (pimaricin – ampicillin – rifampicin-PCNB agar) V8 medium (24) for selective isolation of *Phytophthora* spp., and half used for DNA extraction using the USDA-APHIS protocol (47) with the Qiagen DNeasy Plant MiniKit (Qiagen, Valencia, CA). Cultures were checked after a few days to determine if a *Phytophthora* sp. or *P. ramorum* were growing and with species identification done by conventional morphological classification. DNA extractions were diluted 1/10 in sterile water and tested with the

atp9-nad9 Phytophthora genus specific probe and *P. ramorum* and *P. kernoviae* species specific probes. Results were also independently validated in an PCR hybridization array system (13).

Oregon raspberry: (*P.rubi*). DNA was extracted from a total of 8 raspberry roots using the previously described USDA-APHIS protocol (47). The DNA was diluted 1/10 and 1/50 and assayed using the *atp9-nad9 Phytophthora* genus specific assay with *P. rubi* and *P. fra*gariae species specific probes. Two DNA samples previously extracted from infected raspberry were also included in the assay.

UK samples: (*P. kernoviae*). DNA from leaves naturally and artificially inoculated with *P. kernoviae* were provided by Jennifer Tomlinson (Food and Environment Research Agency, Sand Hutton, York, UK) and tested in the Martin laboratory using the *atp9-nad9 Phytophthora* genus specific assay and the *P. ramorum* and *P. kernoviae* species specific probes.

RESULTS

Mitochondrial gene order differences in *Phytophthora* vs *Pythium* and plants. Three conserved mitochondrial gene order differences were observed when the organization of the mitochondrial genome of *Phytophthora* was compared to *Pythium* and plants. The *trnM-trnP-trnM* region located between the rRNA-large subunit (*rnl*) and ribosomal protein L14 (*rpl14*) spanned a region of approximately 258 bp (size varied among species) containing the three indicated tRNAs (Figure 1, Table 3). Because of the level of conservation of the tRNA coding sequences it was possible to design highly conserved forward and reverse primers from the *trnM* coding regions (amplicon approximately 206

bp) with a genus specific probe designed from the 3' end of the first *trnM* coding region. Over 170 isolates representing a wide range of species in the genus were sequenced and while there was enough interspecific sequence variation in the coding and spacer sequences to accommodate the design of 38 species specific probes, this region was not variable enough to use for systematically designing markers for a wide range of *Phytophthora* spp. and was used subsequently as a genus specific marker only.

The *atp9-nad9* gene order was highly conserved in *Phytophthora* and the presence of highly conserved sequences allowed for the design of amplification primers in the flanking regions of each gene and a *Phytophthora* genus specific probe in the 3' end of the *atp9* gene (Fig1, Table 3). Amplicons were approximately 340 bp (size varied among species) and contained 110 bp of the 3'- end of the *atp9* gene and 85 bp of the *nad9* gene with the remaining sequences representing intergenic spacer. This region was sequenced for over 720 isolates representing 91 valid and 30 provisional *Phytophthora* species. A total of 84 species specific probes for *Phytophthora* were designed from this locus (70 *in silico*; Table S2 and alignment file in supplement materials).

The *nad11-secY* gene order was also highly conserved in *Phytophthora* but the sequence divergence in these genes precluded the development of conserved primers and probe for genus specific detection in the coding regions. In the spacer region between these two genes there is a *trnL-trnL* tRNA cluster that is suitable for designing a genus specific marker (amplicon approximately 172 bp), and while there was enough sequence divergence for designing species specific TaqMan probes for approximately 65 species, the same *trnL-trnL* gene order was present in the *Pythium* mitochondrial genome and

consequently, a problem with primer specificity leading to background amplification of some *Pythium* spp. Additional work with this locus was not pursued.

Evaluation of specificity of genus specific amplification. The t*rnM-trnM* and *atp9-nad9* genus specific primers and probe did not amplify (verified by running amplification on agarose gel) or have any background detection of *Pythium* or plant species when tested by real-time PCR (Table 2). When evaluated against a wide range of *Phytophthora* spp. (256 isolates representing 87 valid and 14 provisional *Phytophthora* species, culture accessions in italics in Table 1) the t*rnM-trnM* assay detected all species and isolates (data not shown) while the *atp9-nad9* marker also detected all the same isolates and species with the exception of *P. bisheria* and *P. frigida* (Table 4).

Specificity of species-specific TaqMan probes. A total of 14 species specific probes were tested in multiplex with the genus specific TaqMan probe for specificity against the core plate of *Phytophthora* spp.; all probes were highly specific and identified only the intended species (Table 4). The probe for the *P. citricola* complex detected *P. citricola, P. citricola* clade E, *P. multivora, P. pini* and *P. plurivora*. Further testing of the *P. ramorum* and *P. kernoviae* species specific TaqMan probes was done with a wide range of *Phytophthora* spp. (256 isolates representing 87 valid and 14 provisional *Phytophthora* species, culture accessions in italics in Table 1) and in all cases species specificity was observed. To confirm that the plant internal control would not influence the sensitivity or accuracy of detection of these species, the plant amplification primers and TaqMan probe was included in these assays and had no effect on results. When tested against additional

isolates of the species the probe was designed to detect, as well as other species with sequence similarity at the probe annealing site, the 12 remaining species specific TaqMan probes were all species specific (Table 5). This includes the probe specific for the hybrid species *P. alni*, which detected all three subspecies (*P. alni* subsp. *alni*, subsp. *multiformis* and subsp. *uniformis*). Details on specific isolates included in this analysis may be found in the supplementary tables (*P. ramorum* and *P. kernoviae,* Table S1a; *P. alni,* Table S1b; *P. cactorum* and *P. fragariae,* Table S1c; *P. cambivora* and *P. syringe,* Table S1d; *P. citricola* (group), Table S1e; *P. cinnamomi,* Table S1f; *P. nicotianae,* Table S1g; *P. palmivora,* Table S1h; *P. pseudosyringae,* Table S1i; *P. rubi,* Table S1j; *P. lacustris,* Table S1k). Some of these additional validations were run in multiplex to mimic how the markers may be used in diagnostic labs. The tests for *P. ramorum* and *P. kernoviae* were run together with the *atp9-nad9 Phytophthora* genus specific probe. To mimic detection of the pathogen in strawberries, *P. cactorum*, *P. fragariae* and the *P. citricola* complex probes were multiplexed. To mimic detection of a *Phytophthora* species in forest or nurseries samples, probes for *P. syringae* and *P. cambivora* were multiplexed.

Standard curve. To evaluate amplification efficiency and the limit of detection, a standard curve for all genus and species specific assays was determined using a dilution series of target DNA ($E= (10^{(-1/slope)} - 1) *100$) (Table 2). Amplification efficiency was usually close to or slightly more than 100%. In general, amplification of the *Phytophthora* genus specific *atp9-nad9* assay exhibited a linear response until the DNA

concentration was less than 100 fg (10^{-4} dilution) with an amplification efficiency of 103%.

Marker validation with field samples. The two genus specific amplicons and six of the *atp9-nad9* species specific probes were evaluated with 99 field samples collected by the CDFA in Sacramento, CA (Table 6). A total of 16 different taxonomic entities were identified (11 described species, and 5 unnamed phylogenetic species) in a total of 58 samples; no *Phytophthora* was detected with either genus specific marker in 41 of the samples. These results agreed with the findings of the CDFA (based on culturing of the isolate or a positive PCR reaction using the accepted USDA-APHIS *P. ramorum* molecular assay). All probes were confirmed to lack nonspecific background detection with these environmental samples and the species specificity for four of the probes was validated; *P. ramorum* was accurately detected in 11 samples with no false positives for the four *P. hibernalis* and three *P. foliorum* samples; *P. pseudosyringae* was detected in 3 samples with no false positive for the *P. nemorosa* sample; and *P. syringae* was detected in 11 samples with no false positive for the *P. austrocedrae* sample. Three additional samples tested positive for *P. syringae* but these had a sequence identity of 97% when the sequence of the *atp9-nad9* genus specific amplicon was compared with known *P. syringae* isolates. The species specific probe for the *P. citricola* complex detected *P. multivora* in 5 samples, *P. pini* in 1 sample and *P. plurivora* in 3 samples. The only species specific probes that were not validated in these environmental samples were for *P. kernoviae* and *P. cambivora* (all samples were negative for these species).

In addition to formal described species, several undescribed phylogenetic species were detected. *P.* sp. *aff. colocasiae* 1 (three samples) is a phylogenetic species closely related to *P. colocasiae*, but distinct in phylogenetic analysis (F. Martin, unpublished). Likewise, *P.* sp. *aff. brassicae* 1 (two samples from *S. actinophylla*) is another phylogenetic species closely related to *P. brassicae*. Two species were also detected that did not match any of the other sequences in our database or GenBank (samples 1518453-1 and 1556826) and may represent new, undescribed species.

Analysis of the 18 samples collected from Big Sur with the *atp9-nad9* genus specific and *P. ramorum* and *P. kernoviae* species specific probes revealed 5 samples that were culture positive for *P. ramorum*, all of which were also positive with the genus and species specific *P. ramorum* probe (Table 7). One sample that was culture negative had a positive real time PCR result (sample 6) with a relatively low C_t of 26.6. No positive detections were observed for the *P. kernoviae* probe. Two additional samples were tested but because the plant positive control did not amplify they were not included in the analysis (data not shown). Of the ten root samples from *Rubus* sp. seven of them tested positive for *P. rubi* with both the *trnM-trnM* and *atp9-nad9* genus specific markers. All of these samples also tested positive with the *atp9-nad9 P. rubi* probe (Table 8). None of these samples tested positive with the *P. fragariae* probe, confirming the species specificity of detection of this probe. Of the five samples extracted from tissue infected with *P. kernoviae*, four tested positive with the *atp9-nad9* genus and *P. kernoviae* specific probe and one tested positive for *P. ramorum* (data not shown). These results agreed with the collaborator's identifications the samples.

DISCUSSION

Comparative mitochondrial genomics was effective for identifying gene order differences useful for developing genus and species specific markers for *Phytophthora*. With this approach we hoped to improve the specificity of the detection assay and provide a technique that would work under a wider range of amplification conditions with a reduced risk of false positives. The thought being that even if nonspecific primer annealing occurred due to lower stringency (such as reduced annealing temperatures) that the intervening distance between the primers would be too great for amplification to occur under the cycling parameters used. The loci that were the primary focus of this investigation had primer annealing sites that were close enough in *Phytophthora* to be useful for designing a TaqMan real time PCR assay but distant enough (10 kb+) in the related genus *Pythium* and plant species to prevent amplification.

Three regions exhibiting conserved gene order differences in *Phytophthora* were evaluated for developing primers and probes for multiplex real-time PCR detection: *atp9-nad9*, *trnM-trnM* and *nad11-secY*. With the exception of the *nad11-secY* locus, the other loci had highly conserved sequences in the flanking genes for design of amplification primers and exhibited polymorphisms in the intervening spacer sequences useful for design of species-specific TaqMan probes. The *atp9-nad9* region was the most useful region because the amplicon size of approximately 340 bp was suitable for TaqMan real time PCR, a single primer pair was used to generate an amplicon where both a genus and species-specific probe could anneal, and the intergenic spacer region exhibited enough sequence divergence that species-specific probes could be designed for

the widest range of species. Unfortunately, the genus specific primer pair was unable to amplify *P. bisheria* and *P. frigida*. While the reason for this has not been determined conclusively, preliminary data suggests that there is an unusual gene order difference in these species that separates the two target genes (T. Miles and F. Martin, unpublished). The second region examined spanned three tRNAs (*trnM-trnP-trnM*) located between the *rnl* and *rpl14* coding regions. A single primer pair generated an amplicon from all species of approximately 206 bp that contained annealing sites for a *Phytophthora* genus specific probe and for some species, a species-specific probe. Species specific probes could be designed for approximately half the number of species as the *atp9-nad9* locus due to a lower level of sequence divergence than the 340 bp amplicon. For this reason work with this locus focused only on developing an additional *Phytophthora* genus specific detection assay. The *nad11-secY* region was the third region examined and due to the inability to design truly genus-specific primers, work using this locus was discontinued.

The *atp9-nad9* and *trnM-trnM* markers were validated for genus specificity by testing against 29 *Pythium* and 39 plant species as well as a total of 256 isolates representing 87 described and 14 provisional *Phytophthora* species. Consistent results were also observed between the two different markers when used to evaluate the presence of a *Phytophthora* spp. in the 130 environmental samples evaluated. In an effort to improve the fluorescent signal of the *atp9-nad9* detection, and hence the sensitivity of the assay, amplification primers were modified with a 5'flap (tail of AATAAATCATAA) that did not anneal to target sequences in a similar fashion as reported by Afonina et al. (1) and Arif et al. (2). Using these modified primers improved the amplification

efficiency of the reaction, increased the fluorescence signal compared to using unmodified primers and thereby increased sensitivity of detection.

Due to the level of sequence divergence observed in the spacer region between the *atp9* and *nad9* genes, this region was useful for the systematic design of species-specific TaqMan probes that could be multiplexed with the *atp9-nad9* genus-specific assay. Species-specific probes were validated for 13 species, including the quarantine pathogens *P. ramorum* and *P. kernoviae,* and one species complex. As part of the validation process specificity was tested against 1) multiple isolates of the target species collected from different geographic regions, 2) other species that exhibited sequence similarity at the probe annealing site and 3) environmental samples. Since the work on the specific primer for the *P. citricola* complex was validated in this study, this species complex has been separated into *P. citricola, P. multivora* (43)*, P. plurivora* (25), *P. capensis* (3), *P. pini* (21) and *P. mengei* (22); consequently, the TaqMan probe is specific only for the species complex. The genus specific amplicon was sequenced for over 720 isolates representing 114 species (90 recognized, 17 provisional and 7 phylogenetically distinct species; F. Martin, unpublished). *In silico* analysis of this data reveled sequence differences that should support the development of TaqMan probes specific for an additional 58 described and 12 provisional species (supplementary table S2), including some species in the *P. citricola* complex (*P. citricola, P. multivora* and *P. plurivora*). This means that species specific markers should be able to be developed for more than 75% of the species evaluated in this study, and with the use of locked nucleic acids in the probe the number species specific probes may increase.

A number of real time PCR assays have been developed for detection of *Phytophthora* spp., most of which have used primarily SYBR Green or TaqMan technology and either the ITS or *Ypt1* locus as a target for systematically developing primers/probes (reviewed in 32). The mitochondrial assays described herein offers several advantages over those previously available, perhaps most important of which is the genus specific detection capability. Although a real time PCR *Phytophthora* genus specific detection capability has been reported for an ITS based detection assay, background detection of *Pythium* spp. was noted (26). The use of loci spanning conserved mitochondrial gene order differences for *Phytophthora* has eliminated this problem for the two diagnostic markers described in this work. While there were two species that were not amplified by the *atp9-nad9* primer pair, all species were amplified and detected by the *trnM-trnM* detection assay. The ability to confirm if a *Phytophthora* species is present with the use of the genus specific amplicon rather than just test for a particular species will improve diagnostic capabilities and facilitate a better understanding of the involvement of *Phytophthora* spp. in agricultural and natural ecosystems. With the *atp9-nad9* locus is it will be possible to identify unknown species without culturing by sequencing the amplicon and comparing the data with the sequence database representing over 720 isolates of 114 distinct phylogenetic entities (F. Martin, unpublished). When more than one species is present, such as with samples recovered by baiting from streams, cloning may be necessary to generate useful sequence data.

Like the ITS region, the mitochondrial genome is high copy number, thereby improving the sensitivity of detection. However, unlike the nuclear genome it is

uniparentally inherited so caution should be exercised when using these mitochondrial markers to detect hybrid species because the mitochondrial background of a hybrid isolate may differ depending on which species is the maternal parent (7,17,29,36). Therefore the assay may not provide an accurate detection unless coupled with a nuclear marker assay. A species specific TaqMan probe was developed for one hybrid species, *P. alni*, and found to be specific for all three subspecies (*P. alni* subsp. *alni*, subsp. *multiformis* and subsp. *uniformis*) even though *cox1* and *nadh1* gene sequences were different for *P. alni* subsp. *multiformis* and *P. alni* subsp. *uniformis* (*P. alni* subsp. *alni* had sequences that grouped with either of these subspecies, (23)). Sequence alignment of the *Phytophthora* genus specific amplicon for multiple isolates of this species revealed a highly conserved TaqMan probe annealing site (supplemental alignment file). The parental lineages of this species has yet to be identified (23) so it is unknown if these would cause a nonspecific background detection when using the *P. alni* specific TaqMan probe.

The appropriateness of using this marker system for pathogen quantification has yet to be fully explored. It needs to be experimentally determined if there are different amounts of mitochondria present depending on the age and condition of the lesion, which would influence the linear relationship between C_t and level of pathogen colonization. Likewise, if the markers are going to be used for pathogen quantification in the soil, the linear relationship between propagule densities and C_t must be characterized. An internal control has been developed with the *atp9-nad9* amplification primers spanning unique internal sequences using the same approach as the internal control for a *Verticillium*

dahliae soil quantification assay (4) to evaluate the suitability of this marker system for soil quantification (Bilodeau, unpublished). Although the ITS region has been used as a target for real time PCR quantification assays, the suitability of this locus for this purpose may also be compromised due to variation in copy number. While variation in copy number of the rDNA repeat has been observed in several Eumycotan fungi (4,12,28,37, 38,40) and there is evidence to suggest this occurs with the related genus *Pythium* (31) and *Phytopythium* (45), it has not been experimentally verified for *Phytophthora*.

The multiplexed assay includes a plant positive control, confirming if the extracted DNA is amplifiable and reducing the chance of false negatives. The C_t of the plant amplification can also give some idea if PCR inhibitors are affecting amplification efficiency and reducing the sensitivity of pathogen detection. Due to the high amount of plant relative to pathogen DNA in most samples, if the C_t for plant amplification is higher than expected a sample dilution or additional DNA purification steps may be necessary to be able to accurately detect if the pathogen is present.

Isolating a *Phytophthora* sp. from some environmental samples can be difficult, sometimes requiring multiple attempts at different times of year to obtain a viable isolate. One approach diagnosticians use to evaluate if a *Phytophthora* spp. is present is an ELISA based test. This has the advantage of giving reliable results year round, even if the tissue has some decay. However, the ELISA based tests are not entirely genus specific, they can react with some *Pythium* spp. (reviewed in 32) so positive results must be scrutinized carefully. It is always useful, and sometimes necessary, to know what

Phytophthora spp. is causing damage on a particular host even when a live culture is unobtainable. For example, overseas shipment of raspberry mother plants must be tested for *Phytophthora rubi* before phytosanitary certification can be obtained for export. If an ELISA test for a *Phytophthora* species is positive for one of these plants, a genus-specific PCR test, such as the ones described in this manuscript, can then be used to determine the species infecting the plant by sequencing the amplicon. A species-specific PCR test can also be used to quickly eliminate *P. rubi* from the list of possibilities. Sequencing is now often less expensive and always faster for preliminary identification of a *Phytophthora* isolate, especially if it is an unfamiliar species.

The multiplexed diagnostic assays described herein provide a useful assay for detection and identification of *Phytophthora* species in environmental samples. The genus specific detection capability will significantly increase diagnostic capabilities over current real time PCR assays by providing a broader view of the involvement of this genus in causing disease. It will also provide a useful tool for evaluating the presence of this genus in natural ecosystems in the absence of disease symptoms (eg. stream baiting assays) and with the extensive sequence database of the *atp9-nad9* locus available, will facilitate species identification by BLAST analysis without the need for culturing. The screening completed to date indicates genus level specificity when tested against *Phytopythium vexans* and a range of plant and *Pythium* spp., however, additional validation against other oomycetes would be advisable when conducting ecological studies to ensure specificity. While species specific detection capabilities was demonstrated for 13 species and one species complex, *in silico* analysis suggests species

specific probes can be developed for approximately 75% of the species for which sequence data was collected. Given this level of sequence divergence and the available sequence database for the genus, this locus should be useful for the systematic development of markers for newly described species. The design of the marker system with a single pair of amplification primers and different TaqMan probes for genus and species specific detection reduces the potential of affecting amplification efficiency when multiple loci are amplified simultaneously. It also simplifies development of multiplex assays since labeling dyes on the TaqMan probe can be adjusted to provide the detection capabilities needed. The *atp9-nad9* and *trnM-trnM* diagnostic assays both support high throughput sample processing and will provide sequence data that will be useful for development of other molecular diagnostic assays such as macro (13) or micro arrays (44) for pathogen detection. The alignments used for designing species specific probes may be found in the supplementary material, *atp9-nad9* sequences for additional isolates may be found on the *Phytophthora* Database (http://www.phytophthoradb.org) and GenBank.

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TABLE 1. *Phytophthora*, *Pythium* and *Phytopythium* spp. isolate numbers and origins included in this investigation

Species ***Phytophthora* species :**	Isolate number [a]	Country
alni[d]	***P10564*** [b], *P11193* [c], *P11318, P16202*[***], P10563, P10565, P10566, P10567, P10568, P10569, P16203[*], P16206[**]	France, Poland, Netherlands, Hungary, Sweden
andina	***P13660***	Ecuador
asparagi n.i.[e]	***P10690***, *P10693, P10707*	New Zealand
austrocedrae	***P15132***, *P16040*	Argentina
bisheria	***P7191***, *P11311, P10117*	Netherlands, USA
boehmeriae	*P1257, P1378,* ***P6950,*** *P3963, P3964, P3968, P3969, P7460, P7472, P7790, P13823, P3967, P3970*	Papua New Guinea, Argentina, Taiwan
botryosa	*P1044, P3425, P6944,* ***P6945***	Malaysia , Vietnam
brassicae	***P3273***, *P10155*	Netherlands
cactorum	***P0714***, *P10365, P11184,* P3138, P3139, P3405, P6186, P6187, P6472, P0715, P10193, P10194, P10195, P10371, P10372, P10373, P10374, P10770, P10773, P10774, P10775, P11095, P11096, P11272, P11281, P11293, P11317, P11322, P1235, P1615, P1721, P1724, P1725, P3468, P3482, P3730, P6681, P8349, P0472, P1258, P1354, P15078, P15079, P15138, P15142, P15290, P15296, P15687, P3132, P3219, P6224, P6486, P6625, P6677, P6690, P6838	Netherlands, Argentina, Poland, Germany, France, USA, UK, Zimbabwe, Japan, New-Zealand, South Africa, French Polynesia, India, Taiwan, Australia, Canada
cajani	***P3105***	India
cambivora	***P0592***, *P1431*, P1432, P3465, P3671, P7140, P6359, P6358, P6360, P10196, P10197, P1995, P1996, P11155, P11556	USA, Australia, Japan, UK, Poland, Germany
sp. canalensis	***P10456***	USA
capsici	***P10386***, *P1319, P3375, P3605, P6522*	USA, Italy
captiosa	***P10719***, *P10720*	New Zealand
castaneae (*P. katsurae n.i.*)	***P10187***, *P6921*	Japan, USA
cinnamomi	*P2100, P2121, P2160, P2301, P3232,* ***P6305,*** P3664, P3665, P10933, P3237, P6492, P6493, P11558, P10781, P15837, P6304, P15821, P15822, P15824, P2183, P3656, P3657, P3658, P3659, P3660, P3662, P11307, P11312, P11320, P15881, P15887, P10162, P10140, P2159, P2370, P2371, P2425, P15314, P15332, P15347, P15348, P15349, P15378, P15883, P2284, P15838, P15839, P6379, P10203, P11596, P11600, P2096, P2138, P2288, P2399, P2424, P2428, P2475, P2400, P2110	USA, Madagascar, South Africa, China, Indonesia, Australia, Germany, Japan, Netherlands, Papua New Guinea, Poland, Portugal, Puerto Rico, Spain, Switzerland, Taiwan, West Sumatra
cinnamomi var robiniae	***P16351,*** P16350	China
citricola	*P0716,* P0845, P1579, P10782	Australia, Taiwan, USA
citricola clade E[f]	P10338, P10366, P6624	Argentina, Ireland, Taiwan
citrophthora	***P10341***, *P10368, P1212,* P0318	USA, UK (England), Argentina, Brazil, Australia
clandestina	***P3942***, *P3943*	Australia
colocasiae	***P6317***, *P6290, P6102*	Indonesia, India
cryptogea	*P10705,* ***P1088***, *P11822, P16165, P1739, P3103, P3700*	New Zealand, USA, Colombia, Ecuador
sp. cuyabensis	***P8213***, *P8218*	Ecuador
drechsleri	***P10331***, *P1087, P11638*	USA
erythroseptica	***P0340***, *P10382, P1693*	Tasmania, USA, Ireland
europea	P10324, P10325, P10326	France, Germany
fallax	*P10722, P10723,* ***P10725***	New Zealand
foliorum	***P10969***, *P10971*	USA
fragariae	*P11808, P1435,* ***P3820***, *P6406,* P10737, P10739, P10743, P10746, P10749, P10752, P10948, P11200, P11804, P11806, P3570, P3821, P11805, P3570, P6368	USA, UK (England, Scotland), France, Canada, Poland
frigida	***P16051,*** *P16053,* ***P16054***, *P16059*	South Africa
glovera	*P10618,* ***P10619***	Brazil
gonapodyides	***P7050,*** , P7186, P6993, P6988, P6135, P7189, P7002, P6137, P7188, P6986, P6992, P6765, P6998, P6989, P6985, P6990, P7187, P6996, P7000, P7171, P6999, P7006, P6872	UK (England), New Zealand, USA

hedraiandra	***P11678,*** P11052, P11060, P11061, P11093	Italy, Spain, USA
heveae	*P0578, P1000,* ***P3428****, P10167, P8240*	Malaysia, Guatemala, Malaysia, Ecuador
hibernalis	***P3822****, P7298, P0647*	Australia, USA
humicola	***P3826****, P6701*	Taiwan
idaei	***P6767***	UK (Scotland)
ilicis	***P3939****, P6098, P6099, P6860*	Canada, USA, UK (England)
infestans	***P10650****, P12022, P13198, P15168, P15938, P1594,* P10110, P12038	Mexico, Russia, Ecuador, Netherlands, UK (England), USA
insolita	*P6703,* ***P6195***	Taiwan
inundata	***P8478****, P8479, P8619*	UK (England), Iran
ipomoeae	***P10225****, P10226, P10227,* P10145, P10150	Mexico, USA
iranica	***P3882***	Iran
sp. kelmania	***P10613****, P1810, P10614*	USA
kernoviae	***P10681****, P10956, P10958, P10957, P10671*	New Zealand, UK (England)
lacustris	*P10337,* P10283, P10284	UK
sp. lacrimae	***P15880***	Netherlands
sp. lagoariana	*P8220,* ***P8223***	Ecuador
lateralis	*P3361,* P1728, P3888	USA
macrochlamydospora	***P10267****, P8017*	Australia
meadii	***P6128***	India
medicaginis	*P0127,* ***P10683****, P7029*	Australia, USA
megakarya[g]	*P1664, P1672,* ***P8516,*** GH-AR 06, GH-AR 08, GH-AR 15, GH-AR 16, GH-AR 18, GH-BAR 17, GH-BAR 21, GH-BAR 26, GH-BAR 28, GH-VR 04, GH-VR 09, GH-VR 10, GH-VR 13, GH-WR 21, GH-WR 47, GH-WR 51, GH-WR 56, GH-WR 60, 327, 328	Nigeria, Cameroon, Sao Tome, Ghana
megasperma	*P10340, P1679,* ***P3136****, P3600, P6957*	Australia, Japan, USA
melonis	***P3609****, P6870,* ***P1475****, P1748*	Japan, China
mengei	***P127****,* P1275	USA
mexicana	P0646	Mexico
mirabilis	*P10231,* ***P3005****, P3010,* P3007	Mexico
multivesiculata	***P10670***	New Zealand
multivora	***P7902***, P10300, P10458, P10977, P11094, P11569, P11832, P1817	South Africa, Spain, USA
sp. napoensis	*P8225, P8221,* ***P8222***	Ecuador
nemorosa	***P10288****, P16352*	USA
nicotianae	***P10297****, P10381, P6915, P7146 ,* P1325, P10381, P6915, P7146, P3458, P10802, P10297, P6915, P1494, P7665, P7387, P7330, P1751, P1753, P1752, P7346, P6113, P7522, P0700, P3815, P1577, P6115, P1955, P7622, P16870, P16883, P1083, P1452, P16824, P3813, P1350, P1495, P0583, P1333, P1334, P1335, P0582, P16823, P3234, P6832, P3549, P3456, P7449, P3461, P3118, P7561	USA, China, Germany, Mexico, Venezuela, Japan, Australia, Ponape, South Africa, Spain, Greece, Pakistan, India, UK, Philippines
sp. niederhauserii	*P10279,* ***P10617****, P10616, P10976, P16237,* P16384, P7377	Hungary, USA, Netherlands
sp. novaeguineae	***P3389****, P1256*	Papua New Guinea
sp. ohioensis	***P16050***	USA
palmivora	*P6390,* ***P0255****, P0113,* P0376, P0633, P0739, P10212, P10213, P10272, P10296, P10336, P10366, P10420, P10422, P10423, P10425, P10769, P10817, P10818, P11005, P11007, P11009, P11010, P11011, P11012, P11013, P11026, P11099, P11175, P1182, P11851, P15825, P16385, P3249, P3502, P6213, P6218, P6375, P7090, P7537, P7551, P8690, P8702, P8766, GH-WR 61, GH-WR 38, GH-ER 18, GH-CR 15, GH-BAR 13, GH-BAR 12, GH-AR 22, 329, ***P10213***	Indonesia, Costa Rica, USA, American Samoa, Argentina, Ghana, Guam, Indonesia, Malaysia, Philippines, Windward Island
parsiana	***P21282****, P21281*	Iran, USA
parvispora	***P8495****, P7154,*P8494, P6378, P2404	Germany, Israel, Taiwan
sp. personii	***P11555***	USA
sp. PgChlamyo	P10669, P6133, P6983, P6997, P6134, P6138	New Zealand, UK, USA
phaseoli	*P6609, P10150,* ***P10145***	USA
pini	*P0767,* P10204, P10762, P10763, P10764, P10765, P11154, P1632, P1801, P1806	Canada, Poland, USA
pinifolia	***P16100***	Chile
pistaciae	***P6197****, P6196*	Iran
plurivora	*P1805*, P0316, P0768, P10185, P10189, P10623, P10627, P10679, P11058, P11100, P11386, P11425, P11426, P11427, P11500, P11833, P11834, P15137, P3543, P6810, P7491	New Zealand, Poland, Slovenia, UK, USA
polonica	***P15005***	Poland
porri	*P6207, P7899,* ***P10728****, P7518*	Switzerland, Denmark, France, Netherlands
primulae	*P10220,* ***P10333***	Germany
pseudosyringae	***P10443****, P16355,* P10437, P10444, P16354	Germany, USA
pseudotsugae	***P10339****, P10218*	USA
psychrophila	*P10434,* ***P10433***	France, Germany

quercetorum	***P15555***	USA
quercina	***P10334***, *P10441*	Germany, Serbia
quininea	***P3247***, *P8488*	Peru
ramorum	*P10102,* ***P10301,*** *P10084, P10090, P10130, P11047, P11122, P11333, P10343, P11051*	Germany, Netherlands, USA, Poland, Belgium
richardiae	***P6875***, *P3876*	USA
rubi	*P3316,* ***P3289***, P6404, P15596	USA, UK(Scotland), Germany
sansomea	***P3163***	USA
siskiyouensis	***P15122***, *P15123, P16301*	USA
sojae	*P10704,* ***P3114***, *P6497, P7061, P0405*	New Zealand, USA, Canada
sp. sulawesiensis	***P6306***	Indonesia
syringae	***P10330***, *P10332, P2004,* P6903, P6901, P11836, P11835, P15090, P15093, P15092, P15094, P7018, P6208, P3013, P3014, P3016, P3015, P3012	Germany, UK (Scotland), Australia, Switzerland
tentaculata	*P10363,* ***P8497***	Argentina, Germany
sp. thermophilum	***P10457***	USA
trifolii	***P1462***	USA
tropicalis	***P10329***	USA
uliginosa	***P10328***, *P10413*	Germany, Poland
vignae	***P3019***, *P7471*	Australia

Outgroup **species :**

Pythium sp.	*P8209, P8201*	Ecuador
Pythium sylvaticum	***P15580***	Canada
Phytopythium vexans	***P3980***	n.d.

[a] Additional information on isolates (hosts) may be obtained at the website for the World Phytophthora Genetic Resource Collection at the University of California,
Riverside (available online : http://phytophthora.ucr.edu/databasemain.html); isolates starting with the letter "P" are from this collection.
[b] Isolates of all species used for the core plate are in bold face.
[c] All italic isolates number were tested with the *P. ramorum* and *P. kernoviae*, ATP9-NAD9 *Phytophthora* genus and TrnM *Phytophthora* genus probes.
[d] *subspecies *alni* (type), **subspecies uniformis (type), ***subspecies multiformis (type).
[e] *n.i. = noma invalidum*, is a well characterized species but the name is not valid.
[f] *P. citricola* clade E refers to the classification of Jung and Burgess (25).
[g] Isolates starting with a GH are from Ghana and DNA was provided by F. Govers.

TABLE 2. *Pythium* and plant species tested with the *Phytophthora* genus specific markers and the plant internal control.

Species DNA	Sample #	Phy Genus ATP9 probe	Phy Genus TrnM probe [e]	Plant probe	ITS SYBR Green[g]
Pythium[a]					
P. oligandrum	81-10	N/A[b]	N/A	N/A	30.4
P. splendens	85-3	N/A	N/A	N/A	26.6
P. spinosum	79-4	N/A	N/A	N/A	27.4
P. ultimum (HS isolate)	23-1	N/A	N/A	N/A	24.9
P. graminicola	1986-1	N/A	N/A	N/A	29.1
P. catenulatum	1986-8	N/A	N/A	N/A	26.7
P. mamillatum	1986-37	N/A	N/A	N/A	33
P. sylvaticum	1987-73	N/A	N/A	N/A	30.4
P. paroecandrum	1987-60	N/A	N/A	N/A	26.1
P. sylvaticum	1987-14	N/A	N/A	N/A	27.6
P. sulcatum	1987-98	N/A	N/A	N/A	26.6
P. nunn	1987-58	N/A	N/A	N/A	28.6
P. myriotylum	1987-134	N/A	N/A	N/A	25.9
P. ultimum	1987-69	N/A	N/A	N/A	26.8
P. irregulare	1987-68	N/A	N/A	N/A	24.6
P. ultimum (HS isolate)	1987-78	N/A	N/A	N/A	27.5
P. ultimum	1987-65	N/A	N/A	N/A	26.8
P. ultimum	1987-92	N/A	N/A	N/A	24.4
P. ultimum spor	1993-25	N/A	N/A	N/A	26
P. aristosporum	1994-15	N/A	N/A	N/A	21.5
P. volutum	1995-105	N/A	N/A	N/A	32.5
P. coloratum	1999-20	N/A	N/A	N/A	25.2
P. dissimile	1999-23	N/A	N/A	N/A	35.7
P. aristosporum	1999-19	N/A	N/A	N/A	32.6
P. vanterpoolii	1999-31	N/A	N/A	N/A	29.7
P. pyrilobum	1999-28	N/A	N/A	N/A	26.4
P. acanthicum	A-6	N/A	N/A	N/A	33.4
P. deliense	1989-1a	N/A	N/A	N/A	24.9
P. arrhenomanes	1991-12	N/A	N/A	N/A	26.1
P. irregulare	1991-15	N/A	N/A	N/A	26.3
Plant species					
Cucumus sativa[c]		N/A	N/A	23.3	16.7
Citrullus lantanus[c]		N/A	N/A	21.1	12.9
Sequois sempervirens[c]		N/A	N/A	33.4	N/A
Prunus sp.[c]		N/A	N/A	30.2	21.6
Quercus agrifolia[c]		N/A	N/A	20.5	15.6
Citrus sp.[c]		N/A	N/A	18.3	14.7
Fragaria x ananassa[c]		N/A	N/A	25.1	20.5
Ipomoea batatas[c]		N/A	N/A	20.6	N/A
Lithocarpus densiflorus[d]		N/A	N/A	23.5	NT[f]
Umbellularia californica[d]		N/A	N/A	24.5	NT
Acer sp.[d]		N/A	N/A	23.3	NT
Poplar sp.[d]		N/A	N/A	22.8	NT
Rubus sp.[d]		N/A	N/A		NT
Rhododendron sp.[d]		N/A	N/A	17.5	NT
Mahonia aquifolium[d]		N/A	N/A	18.4	NT
Magnolia grandiflora[d]		N/A	N/A	16.7	NT
Xylosma sp.[d]		N/A	N/A	18.4	NT

Pieris japonica [d]	N/A	N/A	14.7	NT
Osmanthus fragrans [d]	N/A	N/A	16.3	NT
Osmanthus heterophyllus [d]	N/A	N/A	16.8	NT
Heteromeles arbutifolia [d]	N/A	N/A	19.1	NT
Rhamnus californica [d]	N/A	N/A	20.0	NT
Kalmia sp. [d]	N/A	N/A	20.0	NT
Laurus nobilis [d]	N/A	N/A	16.5	NT
Psidium cattleianum [d]	N/A	N/A	21.3	NT
Loropetalum chinensis [d]	N/A	N/A	19.1	NT
Arbutus unedo [d]	N/A	N/A	33.2	NT
Photinia fraseri [d]	N/A	N/A	15.7	NT
Schefflera actinophylla [d]	N/A	N/A	16.3	NT
Viburnum tinus [d]	N/A	N/A	18.1	NT
Sapium sebiferum [d]	N/A	N/A	18.8	NT
Camellia japonica [d]	N/A	N/A	19.5	NT
Olea sp. [d]	N/A	N/A	17.3	NT
Mahonia aquifolium [d]	N/A	N/A	19.1	NT
Ficus microcarpa [d]	N/A	N/A	16.1	NT
Rhus integrifolia [d]	N/A	N/A	28.7	NT
Osmanthus fragrans [d]	N/A	N/A	15.3	NT
Tristania sp. [d]	N/A	N/A	19.7	NT
Frangula californica [d]	N/A	N/A	18.5	NT
Controls				
Phytophthora ramorum P10301	22.2	23.2	N/A	21.1
Phytophthora palmivora P0255	22.9	21	N/A	21
H2O	N/A	N/A	N/A	N/A

[a] *Pythium* isolates DNA concentration used was under 1ng/µl, *Pythium* isolate information may be found in Martin (30). *Pythium ultimum* HS isolate, HS = hyphal swelling, no oospores produced.
[b] N/A, No amplification.
[c] Plant DNA concentration ranged from 0.5 to 16ng/µl,
[d] DNA for plant species from field sample.
[e] For the TrnM assay, the baseline was 6-15 cycles and threshold at 10.
[f] NT Not tested.
[g] Amplification using the ITS1 and ITS4 primers (48).

TABLE 3. Primers and probes tested in this study.

Primer-probe name	Sequence 5' to 3'	Modification	Target	Notes	Std curve equation	Efficiency %	R^2
Sequencing primers							
Nad9-F	TACAACAAGAATTAATGAGAAC		ATP9-NAD9 spacer				
Nad9-R	GTTAAAATTTGTACTACTAACAT		ATP9-NAD9 spacer				
Lrn-F	CTGAAAGCATCTAAGTAAGA		lrn-TrnM region				
TrnM-R	GAACCTACATCTTCAGATTA		lrn-TrnM region				
PhyG_ATP9_nested_F	TTYTGTTTAATGATGGCTTT		ATP9-NAD9 spacer	Nested primer			
Real-time PCR primers							
Genus primers and probe							
ATP9-NAD9 region[b]					y=-3.24x+18.79	103	0.9939
PhyG_ATP9_2FTail	**AATAAATCATAA**CCTTCTTTACAACAAGAATTAATG		*Phytophthora*	5' Flap			
PhyG-R6_Tail	**AATAAATCATAA**ATACATAATTCATTTTTATA		*Phytophthora*	5' Flap			
ATP9_PhyG2_probeR	AAAGCCATCATTAAACARAATAAAGC	Fam/BHQ1	*Phytophthora*	Probe			
TrnM region					NA[a]		
PhyG-F2	CGTGGGAATCATAATCCT		*Phytophthora*				
PhyG-Rb	CAGATTATGAGCCTGATAAG		*Phytophthora*				
TrnM_PhyG_probe2	ATRTTGTAGGTTCAARTCCTAYCATCAT	Hex/ BHQ1	*Phytophthora*	Probe			
Species specific probes							
PfraVf_nad9sp_TaqMan2	ATCTCGTAATAGATATATATGTATATTTAATACGT	Hex/BHQ1	*P. fragariae*	Probe	y = -3.21x + 19.52	105	0.9985
Pcact_nad9sp_probe2	TTACATGTTATATAATTATTAACACTATTTATAAAA	Quasar670/BHQ2	*P. cactorum*	Probe	y = -3.14x + 20.94	108	0.9953
Pker_nad9sp_1Fb	TTTATATTATTCACAGATTTATTAATTTTTTTCTA	Quasar670/BHQ2	*P. kernoviae*	Probe	y = -3.11x + 21.91	110	0.9982
Pram_nad9sp_1F	ACGTTACGTCTAGACTTGTATTATGCATTG	Hex/BHQ1	*P. ramorum*	Probe	y = -3.21x + 21.56	105	0.9727

Palni_nad9sp_probe1	AATAGATATATACGTATATTTAACGCATAATTAGC	Quasar670/BHQ2	*P. alni*	Probe	y = -2.996x + 22.006	116	0.9952
Ppalm_nad9sp_probe2	TATAATTACTTAGRCYTGAGTATTTAAATTGAAA	Quasar670/ BHQ2	*P. palmivora*	Probe	y = -3.252x + 19.657	103	0.996
Psyr_nad9sp_probe1	TACTTTTARCTAAATGTTAACTATTTTTCTAA	Quasar670/ BHQ2	*P. syringae*	Probe	y = -2.952x + 21.067	118	0.9949
Pcambi_nad9sp_probe1	ATCCTATAATAGGTATATATGTACATTTAATGCA	Hex/ BHQ1	*P. cambivora*	Probe	y = -3.34x + 22.973	99	0.9917
Pcinn_nad9sp_probe1	AAGAAATATTTAGTTTATTAATATATAATATAACT	Quasar670/ BHQ2	*P. cinnamomi*	Probe	y = -3.063x + 20.498	112	0.9824
PfraVrubi_Atp9_TaqMan1	ATATATACGTGTATTTAATGCATAATCAGCTA	Quasar670/BHQ2	*P. rubi*	Probe	y = -3.1631x + 17.334	107	0.993
Pcit_nad9sp_T1F	AATAATAGTTTATTTTTTTGATATATAAATATTTAT	CALFluorRed 610/BHQ2	*P. citricola group*	Probe	y = -3.043x + 22.058	113	0.9936
Pnicot_ATP9_Probe1	ATGTTATATCATTATTTTTTATTATATATACAAAT	Quasar670/ BHQ2	*P. nicotianae*	Probe	NA[a]		
Ppssyr_ATP9_Probe	TTAGATATGTAAGTACTTATAGTGTTTATATT	Quasar670/ BHQ2	*P. pseudosyringae*	Probe	y = -3.362x + 20.457	98	0.9918
Pgona_nad9sp_probe2	ATAATACACGTATACTTAAACCCTTTTTAGTA	Quasar670/ BHQ2	*P.lacustris*	Probe Salix-soil	y = -3.546x + 17.936	91	0.9984
Plant primers and probe[c]							
FMPl2b	GCG TGG ACC TGG AAT GAC TA		Plant				
FMPl3b	AGG TTG TAT TAA AGT TTC GAT CG		Plant				
Plant CAL-Red probe	CTT TTA TTA TCA CTT CCG GTA CTG GCA GG	CALFluorRed 610/BHQ2	Plant	Probe	NA[a]		
ITS universal primers used with SYBR Green.							
ITS1[d]	TCCGTAGGTGAACCTGCGG		Universal	SYBR Green			
ITS4[d]	TCCTCCGCTTATTGATATGC		Universal	SYBR Green			

[a] NA mean not available
[b] Sequences in bold at the start of the amplification primers are the 5' flaps as described by Afonina et al. (1) and Arif et al. (2).
[c] Tooley et al. (46)
[d] White et al. (48)

TABLE 4. *Phytophthora* species core plate tested with the *atp9-nad9 Phytophthora* genus and target species probes.

Target probes tested [a]	*Phy.Genus*	*P.alni*	*P.cactorum*	*P.cambivora*	*P.cinnamommi*	*P.citricola* group	*P.fragariae*	*P.lacustris*	*P.kernoviae*	*P.nicotianae*	*P.palmivora*	*P.pseudosyringae*	*P.ramorum*	*P.rubi*	*P.syringae*
Annealing Temp. EC	57	60	57	60	57	60	57	57	57	53	57	57	57	60	60
Phytophthora species [b]	Ct value														
alni [c]	22.8	23.9	-	-	-	-	-	-	-	-	-	-	-	-	-
andina	20.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-
asparagi n.i. [c]	21.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
austrocedrae [c]	21.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
bisheria [c]	- [d]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
boehmeriae [c]	20.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
botryosa [c]	21.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
brassicae [c]	21.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
cactorum [c]	22.7	-	21.9	-	-	-	-	-	-	-	-	-	-	-	-
cajani	24.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
cambivora [c]	25.4	-	-	26.9	-	-	-	-	-	-	-	-	-	-	-
sp. canalensis	22.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
capsici [c]	21.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-
captiosa [c]	29	-	-	-	-	-	-	-	-	-	-	-	-	-	-
castaneae[c]	21.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
cinnamomi [c]	22.7	-	-	-	22	-	-	-	-	-	-	-	-	-	-
cinnamomi var robiniae [c]	19.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-

citricola complex [c]	20.2	-	-	-	-	20.9	-	-	-	-	-	-	-	-	-
citrophthora [c]	21.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
clandestina [c]	22.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
colocasiae [c]	28.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
cryptogea [c]	21.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
cuyabensis [c]	19.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
drechsleri [c]	24	-	-	-	-	-	-	-	-	-	-	-	-	-	-
erythroseptica [c]	22.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
fallax [c]	32.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
foliorum [c]	22.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-
fragariae [c]	19.4	-	-	-	-	-	18.9	-	-	-	-	-	-	-	-
frigida	- [d]	-	-	-	-	-	-	-	-	-	-	-	-	-	-
glovera [c]	22.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
gonapodyides [b, c]	25.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-
hedraiandra [c]	22.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-
heveae [c]	21.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
hibernalis [c]	22.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
humicola [c]	21.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
idaei	23.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ilicis [c]	25.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-
infestans [c]	23.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
insolita [c]	26.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
inundata [c]	22.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
ipomoeae [c]	21.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-
iranica	23.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
kelmania [c]	22.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
kernoviae [c]	23.3	-	-	-	-	-	-	-	23	-	-	-	-	-	-
lacrimae [c]	20.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
lagoariana [c]	22.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-

macrochlamydospora [c]	23.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
meadii	21.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
medicaginis [c]	30.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
megakarya [c]	22.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
megasperma [c]	20.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
melonis [c]	21.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
mengei	21.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
mirabilis [c]	21.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
multivesiculata	22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
napoensis [c]	24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
nemorosa [c]	29.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
nicotianae [c]	27.7	-	-	-	-	-	-	-	22.6	-	-	-	-	-		
niederhauserii [c]	21.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
novaeguineae [c]	18.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
ohioensis	22.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
palmivora [c]	20.2	-	-	-	-	-	-	-	-	21	-	-	-	-		
parsiana [c]	22.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
parvispora [c]	26.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
personii	22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
phaseoli [c]	23.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
pinifolia	21.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
pistaciae [c]	20.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
polonica	32.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
porri [c]	29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
primulae [c]	21.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
pseudosyringae [c]	22.4	-	-	-	-	-	-	-	-	-	-	21.8	-	-	-	
pseudotsugae [c]	22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
psychrophila [c]	21.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
quercetorum [c]	22.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

quercina [c]	24.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
quininea [c]	23.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
ramorum [c]	22.3	-	-	-	-	-	-	-	-	-	-	-	25	-	-	
richardiae [c]	22.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
rubi [c]	21.2	-	-	-	-	-	-	-	-	-	-	-	-	20.2	-	
sansomea	19.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
sinensis [c]	23.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
siskiyouensis [c]	20.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
sojae [c]	24.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
sulawesiensis	21.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
syringae [c]	22.8	-	-	-	-	-	-	-	-	-	-	-	-	-	23.7	
tentaculata [c]	35.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
thermophilum	19.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
trifolii	22.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
tropicalis	27.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
uliginosa [c]	20.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
vignae [c]	22.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Pythium sylvaticum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Phytopythium vexans	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

[a] All baseline were set up at 8-16 cycles, excepted for *P. fragariae* (6-18), *P. pseudosyringae* and *P. ramorum* were "auto" set-up. The threshold was set at 50 for all assays excepted for *P. cactorum* (25), *P. kernoviae* (100) and *P. palmivora* (75).

[b] Not tested with the core plate but tested with other isolates (S1-tables), *P. lateralis* P3361, *P. lacustris* P10337, P10283, P10284, *P. mexicana* P0646, *Pythium* sp. P8209, P8201 and *P. megasperma* P1679.

[c] Tested again with all isolates of this specific species and closely related species with the same results for specificity (S1-tables).

[d] The DNA preparation could be amplified with primers for other loci, but these species were not detected by this genus specific primer and probe combination

TABLE 5. Listing of species and number of isolates on which the *Phytophthora* primers and species-specific TaqMan probes were tested on to fully validate specificity.

Species specific probe[a]	Species (number of isolates) tested[b]
P. alni	*P. alni* subsp. *alni* (7), subsp. *multiformis* (1), subsp. *uniformis* (2), *P. fragariae* (19), *P. rubi* (4)
P. cactorum	*P. alni* subsp. *alni* (2), subsp. *multiformis* (1), subsp. *uniformis* (1), *P. cactorum* (48), *P. fragariae* (20), *P. rubi* (5), *P. hedrianda* (11)
P. cambivora	*P. cambivora* (15), *P. syringae* (18)
P. cinnamomi	*P. cinnamomi* (60), *P. parvispora* (5), *P. cinnamomi* var *robinae* (2), *P.* sp. *niederhauseri* (7)
P. citricola group	*P. citricola* (7), *P. citricola* clade E (3), *P. multivora* (8), *P. pini* (4), *P. plurivora* (23), *P. citrophthora* (1), *P. europea* (3), *P. lateralis* (3), *P. mengei* (3), *P. mexicana* (1)
P. fragariae	*P. alni* subsp. *alni* (2), subsp. *multiformis* (1), subsp. *uniformis* (1), *P. cactorum* (53), *P. fragariae* (20), *P. rubi* (5), *P. hedrianda* (8)
P. lacustris	*P. lacustris* (3), *P. gonapodyides* (24), *P.* sp. PgChlamydo (5)
P. nicotianae	*P. nicotianae* (46), *P. infestans* (2), *P. cactorum* (1), *P. ipomoeae* (1), *P. mirabilis* (2), *P. phaseoli* (2)
P. palmivora	*P. palmivora* (52), *P. megakarya* (20)
P. pseudosyringae	P. *pseudosyringae* (4)
P. rubi	*P. alni* subsp. *alni* (7), subsp. *multiformis* (1), subsp. *uniformis* (2), *P. fragariae* (19), *P. rubi* (4)
P. syringae	*P. cambivora* (15), *P. syringae* (18)

[a] Probes for *P. ramorum* and *P. kernoviae* were tested against 231 isolates in italics in Table 1 representing 87 described and 14 provisional *Phytophthora* spp.
[b] Isolates used in this validation were different from the core plate of isolates tested in Table 4 unless only a single representative isolate was available.

TABLE 6. Results for real time PCR assays with environmental samples collected from Californian and tested with *Phytophthora* genus and species specific probes for *P. ramorum, P. kernoviae, P. syringae*, *P. cambivora*, *P. pseudosyringae*, and the *P. citricola* group.

	Dilution		**undiluted**	**1/10**	**undiluted**	**undiluted**	**1/10**	**1/10**	**1/10**	**1/10**	
	Probes	**Plant probe**	**TrnM-PhyG**	**PhyGenus Atp9**	***P.ramorum***	***P. kernoviae***	***P. syringae***	***P. cambivora***	***P. citricola* gr.**	***P. pseudosyr.***	**Sequence confirmed[b]**
Sample number	**Host, County**	**Ct**	**Ct**	**Ct**	**Ct**	**Ct**	**Ct**	**Ct**	**Ct**	**Ct**	
1470622-8	*Rhododendron* sp. Sacramento	19.6	29.3	28.3	26	N/A	N/A	N/A	N/A	N/A	*P. ramorum*
1556828	*U. californica,* Alameda	18.3	19.6	21.4	18.9	N/A	N/A	N/A	N/A	N/A	*P. ramorum*[c]
1504926-5C	*U. californica,* Monterey	23.7	31.8	29.7	27.7	N/A	N/A	N/A	N/A	N/A	*P. ramorum*[c]
1504927-4	*U. californica,* Monterey	18.8	20.5	21.2 (und)[a]	21	N/A	N/A	N/A	N/A	N/A	*P. ramorum*[c]
1527809	*U. californica,* San Mateo	18.9	34.2	32.2	29.1	N/A	N/A	N/A	N/A	N/A	*P. ramorum*[c]
1527818	*U. californica,* San Mateo	18.9	33.3 (1/20)[a]	30.7 (und)[ab]	30.2	N/A	N/A	N/A	N/A	N/A	*P. ramorum*[c]
1527813	*U. californica,* San Mateo	19	34.4	24.3	28.4	N/A	N/A	N/A	N/A	N/A	*P. ramorum*[c]
1536190	*U. californica,* Napa	17.3	37.1	36.9 (und)[a**]31.2	31.3	N/A	N/A	N/A	N/A	N/A	*P. ramorum*
1441167	*U. californica,* Santa Cruz	18.4	27.7	25.4	24.8	N/A	29.2	N/A	N/A	N/A	*+C +PCR*[c]
1481745	*U. californica,* Sonoma	19.5	33.6 (1/20)[a]	37.3 (und)[a**] 30.9	41.9 1/20[a**] 30.2	N/A	N/A	N/A	N/A	N/A	*+pcr*[c]
1481747	*U. californica,* Sonoma	19.4	44	36.1	29.6	N/A	N/A	N/A	N/A	N/A	*+C +PCR*[c]
1504296-34	*Arbutus* sp., Contra Costa	20.3	28.8 (1/20)[a]	29.8	N/A	N/A	30.7	N/A	N/A	N/A	*P. syringae* like
1543967-8	*Ficus microcarpa,* Orange	16.1	33.4	29.6	N/A	N/A	44.2	N/A	N/A	N/A	*P. syringae*
1368945-23	*Leucothoe fontanesiana,* Humboldt	17.1	24.1	24.5	N/A	N/A	27.3	N/A	N/A	N/A	*P. syringae*[c]
1426059-6	*M. grandiflora,* San Diego	27.9	37.4	28.1	N/A	N/A	32.9	N/A	N/A	N/A	*P. syringae*
1426065-17	*M. grandiflora,* San Diego	18.9	29.5	28	N/A	N/A	32.5	N/A	N/A	N/A	*P. syringae*
1426059-7	*M. grandiflora,* San Diego	18.2	35.3	28.1	N/A	N/A	32.3	N/A	N/A	N/A	*P. syringae* like (97% identity)
1345363-58	*Magnolia grandiflora,* San Diego	18.8	44.2	25.5	N/A	N/A	29.1	N/A	N/A	N/A	*P. syringae*
1345363-53	*Photinia fraseri,* San Diego	15.7	21	23.1	N/A	N/A	26.4	N/A	N/A	N/A	*P. syringae*
1413764-2	*Psidium cattleianum,* Solano	21.3	28.8	29.1	N/A	N/A	30.9	N/A	N/A	N/A	*P. syringae*
1290387-23	*Rhamnus californica,* Butte	20.5	22	23.5	N/A	N/A	26.7	N/A	N/A	N/A	*P. syringae*[c]
1555575	*Rhododendron* sp., Lake	19	28.5	27	N/A	N/A	29.8	N/A	N/A	N/A	*P. syringae*[c]
1458992-31	*Rhododendron* sp., San Luis Obispo	17.5	24.6	25	N/A	N/A	28.3	N/A	N/A	N/A	*P. syringae*[c]
1458992-32	*Rhododendron* sp., San Luis Obispo	17.3	25.4	26	N/A	N/A	29	N/A	N/A	N/A	*P. syringae*[c]
1459346-10	*Sapium sebiferum,* San Luis Obispo	18.8	26.1	25.8	N/A	N/A	28.8	N/A	N/A	N/A	*P. syringae* like[c]
1353896-26	*L. chinensis,* Los Angeles	20.5	42.4	29.4 (und)[a]	N/A	N/A	N/A	N/A	37.7 (und)[a]	N/A	*P. multivora*
1353896-20	*Loropetalum chinensis,* Los Angeles	19.1	43.3	31	N/A	N/A	N/A	N/A	33.4	N/A	*P. multivora*

1404765-4	*Osmanthus heterophyllus,* Sacramento	16.8	21.8	23	N/A	N/A	N/A	N/A	26.6	N/A	*P. multivora*
1543960-14	*Rhododendron* sp., Orange	28.7	26.5	25.3	N/A	N/A	N/A	N/A	29.7	N/A	*P. multivora*
1503028-1	*U. californica,* Contra Costa	18.1	21.6	22.8	N/A	N/A	30.6	N/A	28.7	N/A	*P. multivora*
1556829	*U. californica,* Alameda	20.4	29.3	34.3	N/A	N/A	N/A	N/A	N/A	N/A	*P. nemorosa*
1470622-10	*Osmanthus fragrans* ,Sacramento	16.3	34.6 (1/20)[a]	27.1	N/A	N/A	N/A	N/A	31.8	N/A	*P. pini*
1309588-15	*Rhododendron* sp., Los Angeles	19.2	23.1	23.1	N/A	N/A	N/A	N/A	26	N/A	*P. plurivora*
1543961-25	*Rhododendron* sp., Orange	21.1	28	26.5	N/A	N/A	N/A	N/A	30.1	N/A	*P. plurivora*
1426752-6	*Rhododendron* sp., San Diego	21.1	24.7	24.9	N/A	N/A	N/A	N/A	29.4	45.9	*P. plurivora*
1404765-11	*Laurus nobilis,* Sacramento	16.5	29.9	27.8	N/A	N/A	N/A	N/A	N/A	N/A	*P.* sp. *aff. colocasiae* 1[d]
1470622-9	*Pieris japonica,* Sacramento	14.7	22	23.4	N/A	N/A	N/A	N/A	27.8	N/A	*P.* sp. *aff. colocasiae* 1[d]
1290221-19	Unknown, Amador	19.8	24.6	26.7	N/A	N/A	N/A	N/A	N/A	N/A	*P.* sp. *aff. colocasiae* 1[d]
1537555	*Sequoia sempervirens,* Madera	22.2	27.8	27.6	N/A	N/A	N/A	N/A	N/A	N/A	*P. austrocedrae*
1556835-2	*Umbellularia californica,* Alameda	19.3	22.7	24.9	N/A	N/A	31.9	N/A	N/A	26.8	*P. pseudosyringae*[c]
1504925-1	*U. californica,* Monterey	17.8	22.5	24.5	N/A	N/A	N/A	N/A	N/A	26.6	*P. pseudosyringae*
1504925-19	*U. californica,* Monterey	19.9	43.3	39.3	N/A	N/A	N/A	N/A	N/A	42.1	*P. pseudosyringae*
1543964-18	*Rhododendron* sp., Orange	22.1	31.1 (1/20)[a]	32.9	N/A	N/A	N/A	N/A	N/A	N/A	*P. foliorum*[c]
1543964-19	*Rhododendron* sp., Orange	21	44.7	37.2	N/A	N/A	N/A	N/A	N/A	N/A	*P. foliorum*
1483418-8	*Rhododendron* sp., Stanislaus	20.1	25.1	26.2	N/A	N/A	N/A	N/A	N/A	N/A	*P. foliorum*[c]
1322287-3	*Camellia japonica,* Santa Clara	19.5	36.9	25.8	N/A	N/A	N/A	N/A	N/A	N/A	*P. hibernalis*
1543964-25	*Rhododendron* sp., Orange	20.7	39.7	25.5	N/A	N/A	N/A	N/A	N/A	N/A	*P. hibernalis*
1413767-4	*Rhododendron* sp., Solano	21.3	21.9	22	N/A	N/A	N/A	N/A	N/A	N/A	*P. hibernalis*
1504971	*Xylosma* sp., Marin	18.4	24.5	25.1	N/A	N/A	N/A	N/A	N/A	N/A	*P. hibernalis*
1309587-25	*Schefflera actinophylla,* Los Angeles	16.3	22.8	21.9	N/A	N/A	N/A	N/A	N/A	N/A	*P.* sp. *aff. brassicae* - 1 like (93% identity)[d]
1309587-26	*S. actinophylla,* Los Angeles	17.2	34.8	25.9	N/A	N/A	N/A	N/A	N/A	N/A	*P.* sp. *aff. brassicae* - 1 like (93% identity)[d]
1470362-41	*Kalmia* sp., Sacramento	20	33.9	27	N/A	N/A	31	N/A	N/A	N/A	*P. tropicalis*[c]
1556826	*Osmanthus fragrans,* Alameda	15.3	21.1	22.1	N/A	N/A	N/A	N/A	27.6	N/A	No homology in database
1518453-1	*Quercus* sp., Merced	18.6	44	32.4	N/A	N/A	N/A	N/A	N/A	N/A	No homology in database
1364175-13	*R. californica,* Santa Clara	19	31.4 (1/20)[a]	31.6	N/A	N/A	N/A	N/A	38.5 (und)[a]	43.1	PS[e]
1364175-12	*Rhamnus californica,* Santa Clara	17.7	47.2	30.6	N/A	N/A	N/A	N/A	N/A	N/A	PS
1422489	*Rhododendron* sp., Alameda	16.1	19.1	21.7	N/A	N/A	25.8	N/A	N/A	N/A	PS - *CDFA + P. syringae culture*
1261274-2	*Tristania* sp., Riverside	19.7	46	33.1	N/A	N/A	N/A	N/A	N/A	N/A	PS

[a] Different amounts of DNA was used in the amplification than indicated in the column heading.
A 1/20 refers to using this dilution rather than the 1/10 and "und" refers to using undiluted DNA.
In some cases two Ct values with one of the alternative DNA concentrations previously mention, are listed, in these examples two different DNA dilutions were tested.

[b] Genus specific amplicons were sequenced and the results compared against a sequence database generated from DNA from purified cultures of all isolates listed in Table 1.
[c] Species identification was confirmed by culturing the pathogen and/or a positive amplification with the USDA-APHIS approved *P. ramorum* molecular diagnostic assay. Cultures were not obtained for all samples.

[d] Based on DNA sequence analysis of the *cox2, nad9, rps10* and *secY* genes isolates representing this group are phylogenetically distinct and are closely related to the indicated formally described species (F. Martin, unpublished).

[e] PS = Poor sequence data, not able to determine species

TABLE 7. Results of real-time PCR assays for samples collected from Big Sur, California using the *Phytophthora* genus, *P. ramorum* and *P. kernoviae* species specific and plant-positive control TaqMan assays.

Isolate [a]	Host	Symptoms	*P. ramorum*		Phy-Genus	*P. ramorum*	*P. kernoviae*	Plant
			culture PARP-V8	dilution	Ct	Ct	Ct	Ct
BS1	Tan oak	Leaf spots	-	1/10	N/A	N/A	N/A	23.5
BS 2	Bay laurel	Leaf spots	+	1/10	30.4	29.6	N/A	24.5
BS 3	Bay laurel	Leaf spots	+	1/10	31.2	30.1	N/A	26.5
BS 4	Maple	Leaf spots	-	1/10	N/A	N/A	N/A	23.3
BS 5	Bay laurel	Leaf spots	+	1/10	26.8	26.7	N/A	24.4
BS 6	Bay laurel	Leaf spots	-	1/10	26.8	26.6	N/A	22.7
BS 7	Live oak	Leaf spots	-	1/10	N/A	N/A	N/A	23
BS 10	Redwood	Red leaves, leaf spots	-	1/10	N/A	N/A	N/A	33.4
BS 11	Bay laurel	Leaf spots	+	1/10	30	29.5	N/A	24.8
BS 12	Tan oak	Leaf spots	-	1/10	N/A	N/A	N/A	20.2
BS 13	Tan oak	Leaf spots	-	1/10	N/A	N/A	N/A	20.5
BS 14	Maple	No symptoms	-	1/10	N/A	N/A	N/A	22.1
BS 15	Bay laurel	No symptoms	-	1/10	N/A	N/A	N/A	21.8
BS 16	Live oak	Few leaf spots	-	1/10	N/A	N/A	N/A	22.9
BS 17	Bay laurel	Leaf spots	+	1/10	25.3	24.6	N/A	23.7
BS 18	Poplar	Leaf spots	-	1/10	N/A	N/A	N/A	22.8
BS 19	Live oak	Few leaf spots	-	1/10	N/A	N/A	N/A	22.7
BS 20	Live oak	Leaf spots	-	1/10	N/A	N/A	N/A	22.1
Controls								
Isolate number	**Species**		**ng/µl**	**dilution**				
P10130	*P. ramorum*		0.39	10^{-1}	23.7	23.4	N/A	N/A
P10130	*P. ramorum*		0.039000	10^{-2}	28.1	28	N/A	N/A

P10130	*P. ramorum*	0.003900	10^{-3}	36	32.3	N/A	N/A
P10130	*P. ramorum*	0.000390	10^{-4}	N/A	N/A	N/A	N/A
P10130	*P. ramorum*	0.000039	10^{-5}	N/A	N/A	N/A	N/A
P10130	*P. ramorum*	0.000004	10^{-6}	N/A	N/A	N/A	N/A
1571	*P. kernoviae*	1	10^{0}	21.7	N/A	23.2	N/A
H_2O				N/A	N/A	N/A	N/A

[a] Location of the samples BS1 to BS13 were collected in Pfeiffer Big Sur State Park and BS14 to BS20 in Andrew Molera State Park, Big Sur, CA.

TABLE 8. Results of real-time PCR assays for root samples collected from *Rubus* sp. in Oregon using the *Phytophthora* genus, *P. rubi* and *P. fragariae* species specific and plant-positive control TaqMan assays.

Samples (dilution)	TrnM-PhyG	ATP9-PhyGenus	*P. rubi*	*P. fragariae*	Plant assay
	Ct	Ct	Ct	Ct	Ct
Raspberry root DNA (dil. 1/50)- r1DNA	26.9	29.8	29.6	N/A	22.9
Raspberry root DNA (dil. 1/50)- r2DNA	26.8	31.9	30.4	N/A	22.7
Raspberry root DNA (dil. 1/50)- r3DNA	27.1	32.1	30.7	N/A	26.6
Raspberry root DNA (dil. 1/50)- r4DNA	N/A	N/A	N/A	N/A	24.3
Raspberry root DNA (dil. 1/50)- r5DNA	26.9	30.9	29.6	N/A	24.5
Raspberry root DNA (dil. 1/50)- r6DNA	25.3	29.1	28.1	N/A	24
Raspberry root DNA (dil. 1/50)- r7DNA	24.2	28.2	28	N/A	22.8
Raspberry root DNA (dil. 1/50)- r8DNA	N/A	N/A	N/A	N/A	23.1
Raspberry root DNA (dil. 1/50)- DNA #1	N/A	N/A	N/A	N/A	25.6
Raspberry root DNA (dil. 1/50)- DNA #2	28.9	34.4	30.4	N/A	32.6
P. rubi isolate (1/10)	- [a]	23.1	22.3	N/A	N/A
P. fragariae var frag. (1/100)	-	25.9	N/A	25.5	N/A
H_2O	N/A	N/A	N/A	N/A	N/A

[a] "-" means samples were not tested

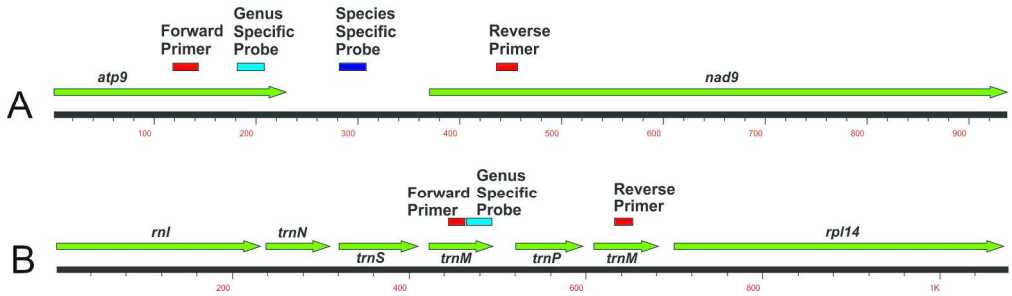
Supplemental files of sequence alignments used in this investigation:

atp9-nad9

http://www.scientificsocieties.org/PHYTOXtras/PHYTO-09-13-0263-R_Phytophthora_atp9-nad9.msf

trnM-trnP-trnM

http://www.scientificsocieties.org/PHYTOXtras/PHYTO-09-13-0263-R_Phytophthora_trn_alignment.msf



Phytophthora genus specific primer and probe location on mitochondrial genome. A) atp9-nad9 region; B) rnl-rpl14 region.
223x63mm (300 x 300 DPI)

Supplementary material:

TABLE S1 (S1a to S1k): All species target tested on multiples isolates and related species with the real-time PCR assay.

TABLE S1a- Species specificity of *P.ramorum* and *P.kernoviae* TaqMan probes when tested against other *Phytophthora* species in a multiplex real-time PCR assay.

	Baseline set up	**"8-15"**	**auto**	**"8-16"**
	threshold	**50**	**50**	**100**
	Probe tested	**PhyG**	**Pram**	**Pker**
***Phytophthora* species**	**Isolates #**	**Ct**	**Ct**	**Ct**
alni subsp. *alni*	P10564	21.5	N/A	N/A
alni subsp. *alni*	P11193	17	N/A	N/A
alni subsp. *alni*	P11318	18.4	N/A	N/A
alni subsp. *multiformis*	P16202	21.3	N/A	N/A
andina	P13660	21.3	N/A	N/A
arecae	P10213	18.5	N/A	N/A
asparagi	P10690	18.6	N/A	N/A
asparagi	P10693	23.7	N/A	N/A
asparagi	P10707	25.8	N/A	N/A
austrocedrae	P15132	20	N/A	N/A
austrocedrae	P16040	23.1	N/A	N/A
bisheria	P7191	N/A	N/A	N/A
bisheria	P11311	N/A	N/A	N/A
bisheria (Type)	P10117	N/A	N/A	N/A
boehmeriae	P1378	20.2	N/A	N/A
boehmeriae	P6950	20.4	N/A	N/A
boehmeriae	P1257	19	N/A	N/A
bohemeria	P3963	15.7	N/A	N/A
bohemeria	P3964	18.1	N/A	N/A
bohemeria	P3968	18	N/A	N/A
bohemeria	P3969	16.4	N/A	N/A
bohemeria	P7460	18.8	N/A	N/A
bohemeria	P7472	14.2	N/A	N/A [a]
bohemeria	P7790	17.2	N/A	N/A
bohemeria	P13823	18.8	N/A	N/A [a]
bohemeria	P3967	19	N/A	N/A [a]

botryosa	P6945	21.2	N/A	N/A
botryosa	P1044	18.9	N/A	N/A
botryosa	P6944	17.7	N/A	N/A
botryosa	P3425	15.8	N/A	N/A
brassicae	P3273	23.1	N/A	N/A
brassicae	P10155	22.2	N/A	N/A
cactorum	P10365	18	N/A	N/A
cactorum	P0714	20.8	N/A	N/A
cactorum	P11184	18.8	N/A	N/A
cajani	P3105	24	N/A	N/A
cambivora	P0592	26.9	N/A	N/A
cambivora	P1431	24	N/A	N/A
canalensis	P10456	20.2	N/A	N/A
capsici	P10386	22.9	N/A	N/A
capsici	P3375	18.9	N/A	N/A
capsici	P1319	20	N/A	N/A
capsici	P6522	20.6	N/A	N/A
capsici	P3605	19.8	N/A	N/A
captiosa	P10719	28.3	N/A	N/A
captiosa	P10720	33.3	N/A	N/A
castaneae	P10187	21.8	N/A	N/A
castaneae	P6921	18.7	N/A	N/A
cinnamomi	P6305	17.8	N/A	N/A
cinnamomi	P2160	20.9	N/A	N/A
cinnamomi	P3232	21.7	N/A	N/A
cinnamomi	P2121	18.6	N/A	N/A
cinnamomi	P2301	20.2	N/A	N/A
cinnamomi	P2100	19	N/A	N/A
parvispora	P7154	23.4	N/A	N/A
parvispora	P8495	26.8	N/A	N/A
robiniae	P16351	19.7	N/A	N/A
citricola	P0767	22.3	N/A	N/A
citricola	P7902	17.8	N/A	N/A
citricola	P1805	21.5	N/A	N/A
citricola	P0716	20.9	N/A	N/A
citrophthora	P10341	24.6	N/A	N/A
citrophthora	P10167	23.5	N/A	N/A
citrophthora	P10368	17.1	N/A	N/A
citrophthora	P1212	20.7	N/A	N/A
clandestina	P3942	21.1	N/A	N/A
clandestina	P3943	22.6	N/A	N/A

colocasiae	P6317	26.8	N/A	N/A
colocasiae	P6290	26.4	N/A	N/A
colocasiae	P6102	26.6	N/A	N/A
cryptogea	P1088	18	N/A	N/A
cryptogea	P10705	28.8	N/A	N/A
cryptogea	P11822	19.4	N/A	N/A
cryptogea	P1739	17.7	N/A	N/A
cryptogea	P3700	20	N/A	N/A
cryptogea	P1810	18.3	N/A	N/A
cryptogea	P3103	22.2	N/A	N/A
cryptogea	P16165	18	N/A	N/A
cuyabensis	P8213	18.6	N/A	N/A
cuyabensis	P8218	18.8	N/A	N/A
drechsleri	P10331	22.8	N/A	N/A
drechsleri	P11638	19	N/A	N/A
drechsleri	P1087	20.6	N/A	N/A
erythroseptica	P0340	17.4	N/A	N/A
erythroseptica	P10382	21.5	N/A	N/A
erythroseptica	P1693	18.5	N/A	N/A
fallax	P10725	29	N/A	N/A
fallax	P10722	27.5	N/A	N/A
fallax	P10723	25.5	N/A	N/A
foliorum	P10969	20.4	N/A	N/A
foliorum	P10971	23.3	N/A	N/A
fragariae	P3820	18.5	N/A	N/A
fragariae	P6406	28.9	N/A	N/A
fragariae	P1435	20.4	N/A	N/A
fragariae	P11808	20.7	N/A	N/A
fragariae	P3820	18.9	N/A	N/A
frigida	P16051	N/A	N/A	N/A
frigida	P16054	N/A	N/A	N/A
frigida	P16059	N/A	N/A	N/A
frigida	P16059	N/A	N/A	N/A
frigida	P16053	N/A	N/A	N/A
glovera	P10618	21.7	N/A	N/A
glovera	P10619	20.1	N/A	N/A
gonapodyides	P7050	23	N/A	N/A
hedraiandra	P11678	21.8	N/A	N/A
heveae	P8240	21.3	N/A	N/A
heveae	P0578	18	N/A	N/A
heveae	P1000	20.2	N/A	N/A

heveae	P3428	19.1	N/A	N/A
hibernalis	P3822	18.3	N/A	N/A
hibernalis	P7298	17.6	N/A	N/A
hibernalis	P0647	15	N/A	N/A
humicola	P6701	25.3	N/A	N/A
humicola	P3826	21	N/A	N/A
idaei	P6767	19.6	N/A	N/A
ilicis	P6860	21.6	N/A	N/A
ilicis	P6098	20.6	N/A	N/A
ilicis	P6099	21	N/A	N/A
ilicis	P3939	18.7	N/A	N/A
infestans	P10650	22.2	N/A	N/A
infestans	P12022	26.9	N/A	N/A
infestans	P13198	23.3	N/A	N/A
infestans	P15938	20.3	N/A	N/A
infestans	P15168	26.9	N/A	N/A
infestans	P15941	21.3	N/A	N/A
insolita	P6703	20.1	N/A	N/A
insolita (Types)	P6195	28.3	N/A	N/A
inundata	P8478	21.5	N/A	N/A
inundata	P8479	26.7	N/A	N/A
inundata	P8619	20.7	N/A	N/A
ipomoeae	P10227	19.7	N/A	N/A
ipomoeae	P10225	24.7	N/A	N/A
ipomoeae	P10226	18.7	N/A	N/A
iranica	P3882	23.7	N/A	N/A
kelmania	P10613	19.8	N/A	N/A
kelmania	P10613	18.2	N/A	N/A
kelmania	P10614	21.6	N/A	N/A
kernoviae	P10681	19.9	N/A	23
kernoviae	P10958	19.8	N/A	22.8
kernoviae	P10671	20.9	N/A	23.6
kernoviae	P10957	15.8	N/A	18
kernoviae	P10956	16	N/A	17.8
lacrimae	P15880	21.7	N/A	N/A
lagoariana	P8220	22.5	N/A	N/A
lagoariana	P8223	22.9	N/A	N/A
lateralis (Type)	P3361	16.8	N/A	N/A
macrochlamydospora	P8017	21.7	N/A	N/A
macrochlamydospora	P10267	24.2	N/A	N/A
meadii	P6128	22.7	N/A	N/A

medicaginis	P7029	26.9	N/A	N/A
medicaginis	P10683	35.8	N/A	N/A
medicaginis	P0127	25.8	N/A	N/A
megakarya	P8516	23.4	N/A	N/A
megakarya	P1664	22.5	N/A	N/A
megakarya	P1672	24.1	N/A	N/A
megasperma	P1679	20.2	N/A	N/A
megasperma	P3600	22.5	N/A	N/A
megasperma	P10340	17.2	N/A	N/A
megasperma	P3136	17.7	N/A	N/A
megasperma-type	P6957	16.9	N/A	N/A
melonis	P3609	22.9	N/A	N/A
melonis	P6870	21.9	N/A	N/A
mengei	P1273	19.2	N/A	N/A
mirabilis	P3010	23.2	N/A	N/A
mirabilis	P10231	26.1	N/A	N/A
mirabilis	P3005	20.5	N/A	N/A
multivesiculata	P10670	22.1	N/A	N/A
napoensis	P8225	23.2	N/A	N/A
napoensis	P8221	19.1	N/A	N/A
napoensis	P8222	22.9	N/A	N/A
nemorosa	P10288	27.8	N/A	N/A
nemorosa	P16352	21.5	N/A	N/A
nicotianae	P10297	29.6	N/A	N/A
nicotianae	P10381	23.3	N/A	N/A
nicotianae	P6915	26.4	N/A	N/A
nicotianae	P7146	25.9	N/A	N/A
niederhauserii	P10279	18.3	N/A	N/A
niederhauserii	P10617	17.6	N/A	N/A
niederhauserii	P10616	22.8	N/A	N/A
niederhauserii	P10976	21.3	N/A	N/A
niederhauserii	P16237	22	N/A	N/A
novaeguineae	P3389	16.4	N/A	N/A
novaeguineae	P1256	20.7	N/A	N/A
ohioensis	P16050	20.1	N/A	N/A
palmivora	P6390	23.7	N/A	N/A
palmivora	P0255	17.9	N/A	N/A
palmivora	P0113	22.2	N/A	N/A
parsiana	P21282	20	N/A	N/A
parsiana	P21281	18.7	N/A	N/A
personii	P11555	21.9	N/A	N/A

phaseoli	P6609	21.3	N/A	N/A
phaseoli	P10150	21.2	N/A	N/A
phaseoli	P10145	22.1	N/A	N/A
pinifolia	P16100	23.3	N/A	N/A
pistaciae	P6197	19	N/A	N/A
pistaciae	P6196	19.3	N/A	N/A
polonica	P15005	21.1	N/A	N/A
porri	P6207	24.9	N/A	N/A
porri	P7899	21.4	N/A	N/A
porri	P10728	26.6	N/A	N/A
porri	P7518	19.4	N/A	N/A
primulae	P10220	18.2	N/A	N/A
primulae	P10333	22.8	N/A	N/A
pseudosyringae	P10443	20.7	N/A	N/A
pseudosyringae	P16355	20.3	N/A	N/A
pseudotsugae	P10339	20.3	N/A	N/A
pseudotsugae	P10218	18.8	N/A	N/A
psychrophila	P10434	18.7	N/A	N/A
psychrophila (Type)	P10433	24.5	N/A	N/A
quercetorum	P15555	20.4	N/A	N/A
quercina	P10334	25.1	N/A	N/A
quercina	P10441	22.1	N/A	N/A
quininea	P3247	24.1	N/A	N/A
quininea	P8488	19.7	N/A	N/A
ramorum	P10102	19.6	21.4	N/A
ramorum	P10301	23.1	25	N/A
ramorum	P10084	19.8	20.1	N/A
ramorum	P10090	20.2	20.8	N/A
ramorum	P10130	17.7	19.1	N/A
ramorum	P11047	24.2	25.3	N/A
ramorum	P11122	18	18.4	N/A
ramorum	P11333	16.4	16.8	N/A
ramorum	P10343	16.1	16.3	N/A
ramorum	P11051	16.1	16.4	N/A
richardiae	P6875	25	N/A	N/A
richardiae	P3876	23.5	N/A	N/A
rubi	P3316	19	N/A	N/A
rubi	P3289	19.7	N/A	N/A
sansomea	P3163	18.2	N/A	N/A
sinensis	P1475	21	N/A	N/A
sinensis	P1748	18.1	N/A	N/A

siskiyouensis	P15122	20.4	N/A	N/A
siskiyouensis	P15123	20.1	N/A	N/A
siskiyouensis	P16301	20.7	N/A	N/A
sojae	P10704	23.1	N/A	N/A
sojae	P3114	23.4	N/A	N/A
sojae	P6497	21.8	N/A	N/A
sojae	P7061	21.1	N/A	N/A
sojae	P0405	19.6	N/A	N/A
sulawesiensis	P6306	16.8	N/A	N/A
syringae	P10330	20.1	N/A	N/A
syringae	P10332	26.1	N/A	N/A
syringae	P2004	18.6	N/A	N/A
tentaculata	P10363	24.7	N/A	N/A
tentaculata	P8497	21.8	N/A	N/A
thermophilum	P10457	19.9	N/A	N/A
trifolii	P1462	20.1	N/A	N/A
tropicalis	P10329	22.1	N/A	N/A
uliginosa	P10328	20.6	N/A	N/A
uliginosa	P10413	18.2	N/A	N/A
vignae	P3019	23.1	N/A	N/A
vignae	P7471	18.1	N/A	N/A
Pythium species				
Pythium sp.	P8209	N/A	N/A	N/A
Pythium sp.	P8201	N/A	N/A	N/A
Pythium sylvaticum	P15580	N/A	N/A	N/A
Phytopythium vexans	P3980	N/A	N/A	N/A
Controls				
H_2O		N/A	N/A	N/A
P. ramorum	Pr2[b]	22.7	24.7	N/A
P. kernoviae	Pker 1571[b]	19.6	N/A	23

[a] = Negative but Ct over 40 cycles.
[b] = Control isolate from Martin et at (2004)

TABLE S1b- *Phytophthora* genus and *P. alni* species specific probes tested on multiple isolates of this and closely related species

	Baseline	**"6-18"**	**"6-18"**
	Threshold	**50**	**50**
	Probe tested	**PhyG**	**Palni**
***Phytophthora* species**	**Isolate #**	**Ct**	**Ct**
alni subsp. *alni*	P10564	20.4	21.4
alni subsp. *uniformis*	P10565	18.6	19.5
alni subsp. *alni*	P10566	18.2	19
alni subsp. *alni*	P10567	19.3	20.2
alni subsp. *alni*	P10568	21.1	22.2
alni	P10569	18.3	19.1
alni subsp. *multiformis*	P16202	18.8	19.7
alni subsp. *alni*	P10563	20.6	21.7
alni subsp. *alni*	P16203	18.7	19.7
alni subsp. *uniformis*	P16206	18.3	19
fragariae	P10737	20.2	N/A
fragariae	P10739	18.4	N/A
fragariae	P10743	17	N/A
fragariae	P10746	18.3	N/A
fragariae	P10749	17.9	N/A
fragariae	P10752	18.2	N/A
fragariae	P10948	18.8	N/A
fragariae	P11200	18.7	N/A
fragariae	P11804	18.4	N/A
fragariae	P11805	19.2	N/A
fragariae	P11806	17.5	N/A
fragariae	P11808	20.6	N/A
fragariae	P1435	19.3	N/A
fragariae	P3570	18	N/A
fragariae	P3820	17.7	N/A
fragariae	P3821	18.7	N/A
fragariae	P6368	18.4	N/A
fragariae	P6404	26.5	N/A
fragariae	P6406	26.3	N/A
rubi	P15596	19.3	N/A
rubi	P3289	19.1	N/A
rubi	P3316	21.3	N/A

Control H_2O	-	N/A	N/A

TABLE S1c- *Phytophthora* genus and *P. cactorum* and *P. fragariae* species specific probes tested on multiple isolates of these and related species

	Baseline	"2-10"	"6-17"	"auto"
	Threshold	50	35	50
	Probe tested	PhyG	*P.cactorum*	*Pfragariae*
***Phytophthora* species**	**Isolates #**	**Ct**	**Ct**	**Ct**
alni subsp. *alni*	P16202	16	N/A	N/A
alni subsp. *alni*	P10563	18.1	N/A	N/A
alni subsp. *alni*	P16203	16	N/A	N/A
alni subsp. *uniformis*	P16206	15.3	N/A	N/A
cactorum	P3138	25.6	27.6	N/A
cactorum	P3139	15.9	18.3	N/A
cactorum	P3405	16	18.5	N/A
cactorum	P6186	17.8	20.1	N/A
cactorum	P6187	17.8	19.6	N/A
cactorum	P0714	16	18.2	N/A
cactorum	P0715	21.7	23.5	N/A
cactorum	P10193	16.6	19	N/A
cactorum	P10194	15.1	17.5	N/A
cactorum	P10195	15	17.5	N/A
cactorum	P10365	16.1	18.1	N/A
cactorum	P10371	17.2	19.1	N/A
cactorum	P10372	18	19.9	N/A
cactorum	P10373	16	18	N/A
cactorum	P10374	15.5	17.7	N/A
cactorum	P10770	15.7	19.5	N/A
cactorum	P10773	18	23.9	N/A
cactorum	P10774	21.7	23.1	N/A
cactorum	P10775	18	22.9	N/A
cactorum	P11095	16.8	18.7	N/A
cactorum	P11096	16.5	18.5	N/A
cactorum	P11272	18.5	20	N/A
cactorum	P11281	16.3	18.2	N/A
cactorum	P11322	16.5	18.5	N/A
cactorum	P1235	16.8	18.7	N/A
cactorum	P1615	16.7	18.3	N/A

cactorum	P1721	16.2	18.2	N/A
cactorum	P1724	19.4	21.2	N/A
cactorum	P1725	17.5	19.4	N/A
cactorum	P3468	18.1	20.3	N/A
cactorum	P3482	16.7	18.8	N/A
cactorum	P3730	24.9	26.8	N/A
cactorum	P6681	28.4	29.1	N/A
cactorum	P8349	17.9	19.4	N/A
cactorum	P0472	17.3	19	N/A
cactorum	P1258	17.6	19.3	N/A
cactorum	P1258	15.5	17.9	N/A
cactorum	P1258	19.8	21.4	N/A
cactorum	P1354	16.6	18.6	N/A
cactorum	P15078	18.2	19.9	N/A
cactorum	P15079	16.9	18.4	N/A
cactorum	P15138	17.9	19.5	N/A
cactorum	P15142	17.9	19.3	N/A
cactorum	P15290	15.9	17.8	N/A
cactorum	P15296	19.1	21.5	N/A
cactorum	P15687	16.2	18.2	N/A
cactorum	P3219	17.3	19.2	N/A
cactorum	P6224	16.3	18	N/A
cactorum	P6486	17.1	18.8	N/A
cactorum	P6625	19.3	20.4	N/A
cactorum	P6677	27.6	29.2	N/A
cactorum	P6690	16.7	18.6	N/A
cactorum	P6838	18.7	20.5	N/A
fragariae	P10737	18.5	N/A	19.7
fragariae	P10739	17.2	N/A	18.9
fragariae	P10743	14.3	N/A	16
fragariae	P10746	16.8	N/A	18.2
fragariae	P10749	15.8	N/A	17.2
fragariae	P10752	15.5	N/A	16.9
fragariae	P10948	16.8	N/A	18.3
fragariae	P11200	17.1	N/A	18.5
fragariae	P11804	15.7	N/A	16.9
fragariae	P11806	14.4	N/A	16.3
fragariae	P11808	18.7	N/A	20
fragariae	P1435	16.9	N/A	18.1
fragariae	P3570	15.2	N/A	16.4
fragariae	P3820	14.9	N/A	15.7

fragariae	P3821	15.3	N/A	16.7
fragariae	P3821	14.2	N/A	16
fragariae	P11805	16.8	N/A	18.3
fragariae	P3570	15.2	N/A	17.4
fragariae	P6368	16.3	N/A	17.8
fragariae	P6406	24.1	N/A	26.2
hedraiandra	P11052	19.5	N/A	N/A
hedraiandra	P11060	17	N/A	N/A
hedraiandra	P11061	17.2	N/A	N/A
hedraiandra	P11093	16.2	N/A	N/A
hedraiandra	P11678	19.3	N/A	N/A
hedraiandra	P11184	16.7	N/A	N/A
hedraiandra	P11293	18.1	N/A	N/A
hedraiandra	P11317	20.7	N/A	N/A
rubi	P6404	24.6	N/A	N/A
rubi	P15596	16.5	N/A	N/A
rubi	P3289	18	N/A	N/A
rubi	P3316	16.8	N/A	N/A
Control H_2O	-	N/A	N/A	N/A

TABLE S1d-*Phytophthora* genus and *P. cambivora* and *P. syringae* species specific probes tested on multiple isolates of these species.

	Baseline	**"6-18"**	**"6-18"**	**"6-18"**
	Threshold	**50**	**50**	**50**
	Probe tested	**PhyG**	***P.cambivora***	***P.syringae***
***Phytophthora* species**	**Isolates #**	**Ct**	**Ct**	**Ct**
cambivora	P0592	20.5	22	N/A
cambivora	P10196	20.6	21.2	N/A
cambivora	P10197	17.8	18	N/A
cambivora	P11155	18.7	19.1	N/A
cambivora	P11556	19.5	20.6	N/A
cambivora	P1431	23	23.5	N/A
cambivora	P1432	18.5	19.1	N/A
cambivora	P1995	22.1	22.4	N/A
cambivora	P1996	27.5	28.6	N/A
cambivora	P3465	18.8	19.1	N/A
cambivora	P3671	17.7	18.3	N/A
cambivora	P6358	18.9	20	N/A
cambivora	P6359	19.2	19.9	N/A
cambivora	P6360	19.2	19.8	N/A
cambivora	P7140	18.1	18.7	N/A
syringae	P10330	19.4	N/A	22
syringae	P10332	20.8	N/A	23.3
syringae	P11835	19.3	N/A	21.7
syringae	P11836	21	N/A	24
syringae	P15090	20.1	N/A	22.1
syringae	P15092	20.5	N/A	23.4
syringae	P15093	19.2	N/A	21.6
syringae	P15094	19.6	N/A	22.4
syringae	P2004	18	N/A	20.7
syringae	P3012	18.6	N/A	21
syringae	P3013	19.7	N/A	22.3
syringae	P3014	22.1	N/A	24.8
syringae	P3015	17.8	N/A	19.9
syringae	P3016	20.2	N/A	22.5
syringae	P6208	20	N/A	22.9
syringae	P6901	19.9	N/A	22.4

syringae	P6903	18.8	N/A	21.1
syringae	P7018	19.3	N/A	22.1
Control H2O	-	N/A	N/A	N/A
Control *cambivora*	Pos. control 1/100	18	N/A	20.6
Control *syringae*	Pos. control 1/100	26.2	26.2	N/A

TABLE S1e- *Phytophthora* genus and *P. citricola* clade specific probe tested on different isolates of related species.

	Baseline	**"6-18"**	**"6-18"**
	Threshold	**50**	**50**
	Probe tested	**PhyG**	***P.citricola***
***Phytophthora* species**	**Isolate #**	**Ct**	**Ct**
citricola	*P0716*	*19.8*	*21.7*
citricola	*P0845*	*20.3*	*21.6*
citricola	*P1579*	*20.9*	*22.7*
citricola	*P1632*	*20.8*	*22.6*
citricola	*P1770*	*19.2*	*20.3*
citricola	*P1801*	*19.5*	*21*
citricola	*P1806*	*22.1*	*23.3*
citricola	*P1807*	*18.9*	*N/A*
citricola	*P3294*	*20.8*	*22.2*
citricola	*P10204*	*19.4*	*20.9*
citricola	*P10300*	*26.7*	*28.2*
citricola	*P10765*	*19.6*	*20.9*
citricola	*P10782*	*19.5*	*20.8*
citricola	*P11154*	*20.6*	*22.3*
citricola clade E	*P6624*	*18.8*	*20*
citricola clade E	*P10338*	*21.1*	*22.5*
citricola clade E	*P10366*	*18.3*	*19.3*
citrophthora	*P0318*	*19.6*	*N/A*
europaea	*P10324*	*19.8*	*N/A*
europaea	*P10325*	*30.1*	*N/A*
europaea	*P10326*	*21.9*	*N/A*
lateralis	*P1728*	*22.6*	*N/A*
lateralis	*P3361 type*	*18.4*	*N/A*
lateralis	*P3888*	*20.2*	*N/A*
mengei	*P1273*	*19.5*	*N/A*
mengei	*P1275*	*18.5*	*N/A*
mexicana	*P0646*	*18*	*N/A*
multivora	*P1817*	*18.2*	*18.6*
multivora	*P7902*	*20.8*	*22.5*
multivora	*P1233*	*19*	*20*
multivora	*P10300*	*27*	*28.7*
multivora	*P10458*	*19.3*	*20.6*

multivora	*P10977*	*18.8*	*20.2*
multivora	*P11094*	*18.5*	*19.5*
multivora	*P11569*	*18.1*	*19.1*
multivora	*P11832*	*20.4*	*22.4*
pini	*P0767*	*19.5*	*20.8*
pini	*P10762*	*20.6*	*22.5*
pini	*P10763*	*21.2*	*22.9*
pini	*P10764*	*20.5*	*22.2*
plurivora	*P0316*	*18.2*	*19.4*
plurivora	*P0768*	*18.3*	*19.2*
plurivora	*P1805*	*18.6*	*19.6*
plurivora	*P3543*	*19.5*	*20.9*
plurivora	*P6810*	*18.2*	*19.3*
plurivora	*P7491*	*19.7*	*20.9*
plurivora	*P10185*	*19.5*	*20.8*
plurivora	*P10189*	*19*	*20.2*
plurivora	*P10623*	*18.7*	*19.7*
plurivora	*P10627*	*19.8*	*21*
plurivora	*P10679*	*18.1*	*18.8*
plurivora	*P11058*	*18.5*	*19.1*
plurivora	*P11100*	*19*	*20.4*
plurivora	*P11386*	*19.3*	*20.7*
plurivora	*P11425*	*20.6*	*21.7*
plurivora	*P11426*	*19.9*	*21.9*
plurivora	*P11427*	*17.9*	*19.3*
plurivora	*P11500*	*20*	*21.8*
plurivora	*P11833*	*17.9*	*18.5*
plurivora	*P11834*	*17.3*	*17.3*
plurivora	*P15137*	*19.5*	*20.5*
Control H_2O	-	N/A	N/A

TABLE S1f- *Phytophthora* genus and *P. cinnamomi* tested on different isolates of *P. cinnamomi*.

	Baseline	**auto**	**"6-16"**
	Threshold	**50**	**50**
	Probe tested	**PhyG**	***P. cinnamomi***
***Phytophthora* species**	**Access #**	**Ct**	**Ct**
cinnamomi	P2021	17.2	18.2
cinnamomi	P2096	17.7	18.6
cinnamomi	P2100	16.3	17.6
cinnamomi	P2110	17.7	18.8
cinnamomi	P2121	18	18.9
cinnamomi	P2138	17.6	18.6
cinnamomi	P2159	18.6	19.2
cinnamomi	P2160	17.7	18.8
cinnamomi	P2183	17.6	18.9
cinnamomi	P2284	17.9	18.7
cinnamomi	P2288	16.8	18
cinnamomi	P2301	17.1	18.2
cinnamomi	P2370	16.7	17.7
cinnamomi	P2371	17.9	18.8
cinnamomi	P2399	16.6	17.4
cinnamomi	P2400	18.2	19
cinnamomi	P2424	18.1	19
cinnamomi	P2425	17.7	18.3
cinnamomi	P2428	17.4	18.7
cinnamomi	P2475	16.7	17.7
cinnamomi	P3232	17.5	18.6
cinnamomi	P3237	16.7	17.9
cinnamomi	P3656	16.3	17.6
cinnamomi	P3657	17	18
cinnamomi	P3658	15.3	16.8
cinnamomi	P3659	17.8	18.6
cinnamomi	P3660	16.6	17.4
cinnamomi	P3662	18.7	19.9
cinnamomi	P3664	15.2	16.7
cinnamomi	P3665	17.4	18.3
cinnamomi	P6304	16.4	17.5

cinnamomi	P6379	19.3	19.3
cinnamomi	P6492	15.8	17.1
cinnamomi	P6493	18.8	19.9
cinnamomi	P10140	18.1	19.1
cinnamomi	P10162	18.7	19.6
cinnamomi	P10203	17.4	18.7
cinnamomi	P10781	17.8	19
cinnamomi	P10933	13.8	15.8
cinnamomi	P11307	18.1	19.1
cinnamomi	P11312	19.3	20.2
cinnamomi	P11320	18	18.8
cinnamomi	P11558	17.7	18.6
cinnamomi	P11596	14.9	15.9
cinnamomi	P11600	16.4	17.7
cinnamomi	P15314	17	18.2
cinnamomi	P15332	18.8	19.7
cinnamomi	P15347	15	16.8
cinnamomi	P15348	16.3	17.6
cinnamomi	P15349	15.8	17.3
cinnamomi	P15378	16	17.2
cinnamomi	P15821	18	18.3
cinnamomi	P15822	18.9	18.8
cinnamomi	P15824	16.7	17.7
cinnamomi	P15837	17.2	17.9
cinnamomi	P15838	20.1	20.3
cinnamomi	P15839	15.6	16.6
cinnamomi	P15881	21.6	20.9
cinnamomi	P15883	16.8	17.6
cinnamomi	P15887	21.3	21.5
parvispora	P2404	14.3	N/A
parvispora	P6378	16.7	N/A
parvispora	P8494	21.1	N/A
parvispora	P8495	21.4	N/A
cinnamomi var *robiniae*	P16350	17.9	N/A
cinnamomi var *robiniae*	P16351	18	N/A
SP. NOV. niederhauserii	P7377	18.2	N/A
SP. NOV. niederhauserii	P16384	17.8	N/A
SP. NOV. niederhauserii	P16237	18.2	N/A
SP. NOV. niederhauserii	P10279	16.4	N/A
SP. NOV. niederhauserii	P10616	17	N/A
SP. NOV. niederhauserii	P10617	14.6	N/A

SP. NOV. niederhauserii	P10976	19	N/A
Control H_2O	-	N/A	N/A

TABLE S1g- *Phytophthora* genus and *P. nicotianae* species specific probes tested on isolates of *P. nicotianae*.

	Baseline	**"6-16"**	**"6-16"**
	Threshold	**50**	**50**
	Probe tested	**PhyG**	***P. nicotianae***
***Phytophthora* species**	**Isolate #**	**Ct**	**Ct**
nicotianae	P0582	19.9	20.8
nicotianae	P0583	16.1	16.6
nicotianae	P0700	17.3	18.1
nicotianae	P1083	18.9	19.8
nicotianae	P1325	23.2	21
nicotianae	P1333	16	16.5
nicotianae	P1334	16	16.4
nicotianae	P1335	17.5	18.3
nicotianae	P1350	16.1	16.7
nicotianae	P1452	17.1	17.8
nicotianae	P1494	17.6	18.4
nicotianae	P1495	17.9	18.7
nicotianae	P1569	18	18.7
nicotianae	P1577	18.7	19.6
nicotianae	P1751	18.6	19.3
nicotianae	P1752	17.4	18.2
nicotianae	P1753	16.5	17.2
nicotianae	P1955	18.1	19.5
nicotianae	P3118	16.2	16.8
nicotianae	P3234	19.7	20.6
nicotianae	P3456	17.2	17.9
nicotianae	P3458	17.5	18.1
nicotianae	P3461	16.2	16.7
nicotianae	P3549	17.2	17.9
nicotianae	P3813	17.9	18.8
nicotianae	P3815	16.6	16.9
nicotianae	P6113	17	17.8
nicotianae	P6115	16.9	17.7
nicotianae	P6832	17.9	18.9
nicotianae	P6915	16.3	17.1
nicotianae	P7146	19	18.9
nicotianae	P7330	17	17.7
nicotianae	P7346	17.1	18

nicotianae	P7387	16.7	17.5
nicotianae	P7449	17.1	17.6
nicotianae	P7522	18.8	19.7
nicotianae	P7561	18	18.8
nicotianae	P7622	21.5	22.5
nicotianae	P7665	16.6	17.1
nicotianae	P10297	17.6	18.6
nicotianae	P10381	15.7	15.8
nicotianae	P10802	16.8	17.6
nicotianae	P16823	17	17.8
nicotianae	P16824	18.2	19
nicotianae	P16870	17.1	17.9
nicotianae	P16883	17.9	18.6
cactorum	P0714	16.7	N/A
infestans	P10110	15.8	N/A
infestans	P12038	16.8	N/A
ipomoeae	P10225	15.4	N/A
mirabilis	P3005	17.5	N/A
mirabilis	P3007	16.7	N/A
phaseoli	P10145	18.3	N/A
phaseoli	P10150	16.9	N/A
Control H_2O	-	N/A	N/A

TABLE S1h- *Phytophthora* genus and *P. palmivora* specific probes tested on isolates of *P. palmivora*.

	Baseline	**"auto"**	**"8-15"**
	Threshold	**50**	**75**
	Probe tested	**PhyG**	**P. palmivora**
***Phytophthora* species**	**Isolate #**	**Ct**	**Ct**
palmivora	329	18.9	21
palmivora	P3249	19.3	21.6
palmivora	P3502	16.2	18.4
palmivora	P6213	17.5	19.4
palmivora	P6218	16.3	18.3
palmivora	P6375	18.6	20.8
palmivora	P6390	17.1	19.2
palmivora	P7090	16.2	18.2
palmivora	P7537	17.2	19.6
palmivora	P7551	17.6	19.7
palmivora	P8690	17.5	19.5
palmivora	P8702	21	23.1
palmivora	P8766	16	18.1
palmivora	P0113	16.7	19.3
palmivora	P0255	16.8	19.3
palmivora	P0376	16.2	18.7
palmivora	P0633	16.9	19.1
palmivora	P0739	17.6	19.8
palmivora	P10212	16.9	19.3
palmivora	P10213	16.8	19.3
palmivora	P10272	16.6	18.6
palmivora	P10296	19.7	22
palmivora	P10336	14.6	16.9
palmivora	P10366	15.1	17.3
palmivora	P10420	18	20
palmivora	P10422	15.8	18.6
palmivora	P10423	17.5	20.1
palmivora	P10425	19.8	22.3
palmivora	P10769	16.7	18.8
palmivora	P10817	18.4	20.7
palmivora	P10818	17.2	19.8
palmivora	P11005	15.9	18

palmivora	P11007	16.8	18.8
palmivora	P11009	17.6	19.5
palmivora	P11010	17	19.4
palmivora	P11011	19.2	21.3
palmivora	P11012	17.7	20
palmivora	P11013	18.3	20.8
palmivora	P11026	18.7	20.9
palmivora	P11099	21.4	23.7
palmivora	P11175	17.6	19.9
palmivora	P1182	16.3	18
palmivora	P11851	15.8	17.8
palmivora	P15825	20.3	22.8
palmivora	P16385	16.2	18.2
palmivora	GH-WR 61 [a]	19.2	21.4
palmivora	GH-WR 38	16.1	18.1
palmivora	GH-ER 18	14.4	16.9
palmivora	GH-CR 15	13.9	16.3
palmivora	GH-BAR 13	14.5	16.7
palmivora	GH-BAR 12	18	19.8
palmivora	GH-AR 22	14.6	16.9
megakarya	327	18.4	N/A
megakarya	328	23.6	N/A
megakarya	GH-AR 06	15	18.2[b]
megakarya	GH-AR 08	15.3	N/A
megakarya	GH-AR 15	20.1	N/A
megakarya	GH-AR 16	13.9	N/A
megakarya	GH-AR 18	17.3	N/A
megakarya	GH-BAR 17	16.5	N/A
megakarya	GH-BAR 21	16	N/A
megakarya	GH-BAR 26	19.4	N/A
megakarya	GH-BAR 28	22.1	N/A
megakarya	GH-VR 04	15.6	N/A
megakarya	GH-VR 09	18.9	N/A
megakarya	GH-VR 10	19.5	N/A
megakarya	GH-VR 13	15.7	N/A
megakarya	GH-WR 21	17.2	N/A
megakarya	GH-WR 47	19.6	N/A
megakarya	GH-WR 51	14.1	N/A
megakarya	GH-WR 56	18.6	N/A
megakarya	GH-WR 60	20	N/A
Control H_2O	-	N/A	N/A

[a] = GH isolates from Guana
[b] = Sequenced, mixed sequences present, so will need to resequence for confirmation of species

TABLE S1i-*Phytophthora* genus and *P. pseudosyringae* species specific probes tested on isolates of *P. pseudosyringae*.

***Phytopthora* species**	**Baseline Threshold Probe tested isolate #**	**"8-16" 50 PhyG Ct**	**"8-16" 50 *P. pseudosyringae* Ct**
pseudosyringae	P10437	17.6	18.3
pseudosyringae	P10444	17.1	17.8
pseudosyringae	P16354	17.9	18.7
pseudosyringae	P16355	17.3	18
Control H_2O	-	N/A	N/A
pseudosyringae	Pos. control 484 1/100	15.9	16.4

TABLE S1j -*Phytophthora* genus and *P. rubi* specific probes tested on isolates *P. rubi* and close related species.

	Baseline	**"8-16"**	**"6-18"**
	Threshold	**50**	**50**
	Probe tested	**PhyG**	***P. rubi***
***Phytophthora* species**	**Isolate #**	**Ct**	**Ct**
alni subsp. *alni*	P10563	20.2	N/A
alni subsp. *alni*	P10564	19.4	N/A
alni subsp. *alni*	P10565	18.8	N/A
alni subsp. *alni*	P10566	17.9	N/A
alni subsp. *alni*	P10567	18	N/A
alni subsp. *alni*	P10568	20.9	N/A
alni subsp. *alni*	P10569	19.3	N/A
alni subsp. *multiformis*	P16202	18.9	N/A
alni subsp. *alni*	P16203	17.7	N/A
alni subsp. *uniformis*	P16206	18.4	N/A
fragariae	P10737	20.5	N/A
fragariae	P10739	17.7	N/A
fragariae	P10743	16.7	N/A
fragariae	P10746	18.6	N/A
fragariae	P10749	17.9	N/A
fragariae	P10752	18.3	N/A
fragariae	P10948	18.8	N/A
fragariae	P11200	18.3	N/A
fragariae	P11804	18.3	N/A
fragariae	P11805	19.5	N/A
fragariae	P11806	16.8	N/A
fragariae	P11808	21	N/A
fragariae	P1435	19.3	N/A
fragariae	P3570	18	N/A
fragariae	P3570	18.1	N/A
fragariae	P3820	18	N/A
fragariae	P3821	17.9	N/A
fragariae	P6368	18.5	N/A

fragariae	P6406	26.4	N/A
rubi	P3289	19.5	20.5
rubi	P3316	20.2	22
rubi	P6404	26.8	28
rubi	P15596	19.3	21.1
Control H_2O	-	N/A	N/A

TABLE S1k-*Phytophthora* genus and *P. lacustris* species specific probes tested on different isolates and related species.

	Baseline	**"auto"**	**"6-18"**
	Treshold	**50**	**50**
	Probe tested	**PhyG**	***P gona*-salix-soil (*P. lacustris*)**
***Phytophthora* species**	**Isolate #**	**Ct**	**Ct**
lacustris	P10283	13.76	14.56
lacustris	P10284	12.93	13.79
lacustris	P10337	15.83	16.58
gonapodyides	P6134	15.54	N/A
gonapodyides	P6135	15.94	N/A
gonapodyides	P6137	17.22	N/A
gonapodyides	P6765	18.67	N/A
gonapodyides	P6872	14.67	N/A
gonapodyides	P6985	15.22	N/A
gonapodyides	P6986	15.31	N/A
gonapodyides	P6988	14.68	N/A
gonapodyides	P6989	17.92	N/A
gonapodyides	P6990	18.35	N/A
gonapodyides	P6992	15.86	N/A
gonapodyides	P6993	16.14	N/A
gonapodyides	P6996	16.33	N/A
gonapodyides	P6998	18.48	N/A
gonapodyides	P6999	15.64	N/A
gonapodyides	P7000	14.31	N/A
gonapodyides	P7002	16.54	N/A
gonapodyides	P7006	15.07	N/A
gonapodyides	P7050	17.92	N/A
gonapodyides	P7171	14.80	N/A
gonapodyides	P7186	16.35	N/A
gonapodyides	P7187	15.30	N/A
gonapodyides	P7188	17.86	N/A
gonapodyides	P7189	14.25	N/A
sp. PgChlamydo/gonapodyides	P6133	17.49	N/A
sp. PgChlamydo/gonapodyides	P6138	14.25	N/A
sp. PgChlamyd/ogonapodyides	P6983	15.21	N/A
sp. PgChlamydo/gonapodyides	P6997	15.51	N/A
sp. PgChlamydo/gonapodyides	P10669	15.81	N/A
Control H_2O		N/A	N/A

TABLE S2: Putative *in silico* designed TaqMan probes for additional *Phytophthora* species; not validated for specificity.

Target	Probe name	Sequences	Notes[a]
Phytophthora sp. “brasilienlis”	Pcaps_nad9sp_probe1	ATT YAT TWA AAT TAT ATA TAT ACT GAT AAA TAA T	Low TM
Phytophthora sp. "cuyabensis"	Pcuyab_nad9sp_probe	TACCATTAATATAAAAAATAAAATCTTATATAAGA	
Phytophthora sp. " aff.erythroseptica "	Peryt-rich_probe1	TCGGTACTAATTCGATAATCTATCCTATTTTTAG	One SNPs with the described *P. erythroseptica*
Phytophthora sp. "kelmaniae"	Pkelm_nad9sp_probe	TACTAATAYGACAATCTACACTATTTTTAG	
Phytophthora sp. "lagoariana"	Plago_nad9sp_probe	TAATATTATACTGAAAAATAAAATCTTATATAAGA	Low Tm
Phytophthora sp. "niederhauserii"	Pniederh_nad9sp_probe	ACCACCCATTTTGATATAYGGATACAGGGGTATTT	
Phytophthora sp. "ohioensis"	Pohio_nad9sp_probe	ATGCTATATAATCACTAATATTAAATTCTTA	
Phytophthora sp. "personii"	Pperso_nad9sp_probe	TAAATATTAAAACATATTTTTAATATTTATTATCA	Low Tm
Phytophthora sp. "sulawesiensis"	Psulaw_nad9_probe	TAATACTACAACCATATTTATACATTATTTATAGG	
Phytophthora PgChlamydo	PgC_nad9sp_probe	TATGTTATACGGGTACTTCCCGCTT	
Phytophthora asparagi	Paspar_nad9sp_probe	TTCTATGTTATAGATATACTTAAATGTACTTATAA	
Phytophthora austrocedrae	Paustr_nad9sp_probe	ACAGTGTTACGTATGTACTTTAGGGTAAT	Based on only one sequence available
Phytophthora boehmeriae	Pboehm_nad9sp_probe	TATTTTAATTTTTATTATATTTTTAATAAAAATTA	Low Tm
Phytophthora botryosa	Pbotry_nad9sp_probe	TTATTATTCATGTTATGTGTATACTATTATATAAA	
Phytophthora brassicae	Pbrass_nad9sp_probe	ATTTTATTATAAAGCTATATCTGTACTTTAACAAA	
Phytophthora cajani	Pcajani_nad9sp_probe	ATTTACTACGTATATTTTTGATATATGTATACCT	
Phytophthora capensis	Pcapen_probe1	TTATATTGTAGTATTATTTATATATATACTGC	
Phytophthora capsici	Pcaps_nad9sp_probe2	ATTTATYTWAAATTATATATATACTRTTAAATAAA	
Phytophthora captiosa	Pcaptiosa_nad9sp_probe	ATAAAATATATAAATACTGCAGTAAAATTATAATA	
Phytophthora cinnamomi var robiniae	Probin_nad9sp_probe	ATACAGGTACTRGGTAGGTTTGAATA	
Phytophthora citricola SS	PcitSS_probe	CAGGTTATATAACTACTGATATTAGGAATTAAACT	

Phytophthora aff. colocasiae 1	P aff coloc_nad9sp_probe1	TTTATCATTTA+CGTTATATATATACTATTATATA	intraspecific variability,need to add LNA (+) [a]
Phytophthora clandestina	Pcland_nad9sp_probe	ATATAAATTTTTATTATATTTTATATAACTGTTA	Low Tm
Phytophthora colocasiae	Pcoloc_nad9sp_probe	TATCAATGTTATATAAATACTGTTATAAAAAATTA	
Phytophthora cryptogea	Pcryp_probe1	TACTGTTATATCAGTACTGGTGCAATAATCTATCC	
Phytophthora cryptogea like GII	PcrypGII-probe1	ACACTGTTATATTAGTACTGGTGCGGTAATTTACT	
Phytophthora drechsleri	Pdresh_nad9sp_probe	TCAATACTAATACTATAATATGTACTATTTTTAGT	
Phytophthora erythroseptica	Peryth_nad9sp_probe	TCGGTACTAATGCGATAATCTATCCTATTTTTAG	
Phytophthora europea	Peuro_nad9sp_probe1	ATTGATATATACGTATAYTYGATTTTTAATTAGAT	
Phytophthora fallax	Pfalax_nad9sp_probe	ATAAAATATATGGGTACTTAAATAGGGTTATAATA	
Phytophthora foliorum	Pfol_nad9sp_probe2	ATATTTATACACAAAGGTAAAAATACATTAT	
Phytophthora gallica	Pgali_probe1	ACATTTATATTTAAAGTATTTCCAGAAATCCT	
Phytophthora glovera	Pglovera_nad9sp_probe	AAATTATATAT+GTACTGTTAAATAAAATATAT	Few mistmatch with tropicalis, capsici, and low Tm, may need to add LNA (+) [a]
Phytophthora gonapodyides	Pgona_probe3	ACTCCTCTTTAAACTTCTTAGTTTTTATTAAGT	
Phytophthora hedraiandra	Phed_probe1	TCATTATTAATATTATTTATAAAAATAATTTAGAA	Low Tm
Phytophthora hibernalis	Phiber_nad9sp_probe1	TATATCTGAACTCTGATCTTATTATAAATTAT	
Phytophthora humicola	Phumic_nad9sp_probe	ATACATACTTAAYCATTTTTATAAATTA	
Phytophthora idaei	Pidaei_nad9sp_probe	CATAATTATTAATACTGTTTATAAAAATGTTTTAT	
Phytophthora ilicis	Pilicis_nad9sp_probe	TAAGACACGTAAGTACTTATCACAGTTTTA	
Phytophthora infestans	Pinfest_nad9sp_probe1	TTGTTACGTAATTATTTATAGTAAATATC	Difficult, Tm low, only one possibility and do not distinguish between *P. andina,* complex Clade 1c [b]
Phytophthora insolita	Pinsolita_nad9sp_probe	ATATATATGTATTACCGTTTTATTATTATTTTTAA	
Phytophthora inundata	Pinund_nad9sp_probe	TGTTATACRTATACTTAACYGTTTTTAGAAATTA	
Phytophthora ipomoeae	Pipom_nad9sp_probe	TTATATTTTTACCTTGTTACGTAATTATTTATAG	Only 1 SNP with P. infestans and few with P. mirabilis[b]
Phytophthora iranica	Piran_nad9sp_probe	TATTTTATATATAATTGTTATATAATTATTTATAA	Very difficult, too low Tm [b]
Phytophthora irrigate	Pirri_probe1	TCGCTTTTTAAAATTAAATAAACCTTGTATTTATT	
Phytophthora lateralis	Plat_nad9sp_probe1	ACGTCTGCACTGAAAGACGTATAAAAT	
Phytophthora medicaginis	Pmedica_nad9sp_probe	CTATGTTACATTAATACTAACCCATTAATATACAC	

Phytophthora megakarya	Pmegak_nad9sp_probe	TAATATAAAAATATTTATATTTATTTTTTATGAAAA	Low Tm
Phytophthora megasperma	Pmegasperm_nad9sp_probe	CGTATATACCCYCCCGTTTTTATGAATTATTTTTC	
Phytophthora melonis	Pmelon_nad9sp_probe	TGATATATAAATACCGTTACGTATTAAGTA	
Phytophthora mirabilis	Pmira_nad9sp_probe	CTGTTACATAATTATTTATAGTAAATATCTAAAT	Some *P. infestans* and *P. andina* with similar sequences [b]
Phytophthora multivesiculata	Pmultiv_nad9sp_probe	TATAGGAATATATAGTTACTGTTAACTAAAAATAA	
Phytophthora multivora	Pmult_probe1	AATTATATGTATACTGGTATTAAATTTTAAATTAT	Low Tm
Phytophthora multivora	Pmult_probe2	ATATTAATGAAAATTTTCGTTAATATATTTTATTT	Low Tm
Phytophthora nemorosa	Pnem_nad9sp_probe1	TAAGACACGTAAATACTTACTATAATTTTA	
Phytophthora parvispora	Pparvis_nad9sp_probe	TACTTTTTWAAAAAACTCGAATATTTTTGTA	
Phytophthora pinifolia	Ppinif_nad9sp_probe1	AGGTGTTATACGTATACTTAACCCCTTTTAG	
Phytophthora pistaciae	Ppist_nad9sp_probe	TAAATACAAATATACTGTAAGTAAATATTTTTATA	
Phytophthora plurivora	Ppluri_probe1	AGGTTATATACTTACTGATACTGAAAATTAAATTA	
Phytophthora polonica	Ppolonica_nad9sp_probe	TATAAATACCGTAAAACATGTATATATTATTATTT	
Phytophthora primulae	Pprimulae_nad9sp_probe	TTATGAAGTCACATGTGTACTTTATAAAAAAT	
Phytophthora pseudotsugae	Ppseudotsug_nad9sp_probe	ATTTTTTATATGTTATATAATTATTAATACTATTT	Low Tm
Phytophthora quercetorum	Pquercet_nad9sp_probe	ATGTTACATATATACTTATATTTTAATATATAAAT	Low Tm
Phytophthora quercina	Pquercina_nad9sp_probe1	ATTATATTCTTATGTTATATAAYCACTAATACTG	
Phytophthora quininea (macrochlamydospora)	Pquininea_nad9sp_probe	ATCACCTTAATTTAATATATTCCTATTTTAATAAT	Same as *P. macrochlamydospora*
Phytophthora richardiae (macrochlamydospora)	Prichard_nad9sp_probe	TATTATTAAAGACTATATATAATATGTATTATATT	Low Tm
Phytophthora roseacearum	Prosac_probe1	ATGCTATATACGTACTTAWCCCCTTTTAGA	
Phytophthora sansomea	Psansom_nad9sp_probe	TATTAGTACTAACTACTAATATGCATTATTTTTAG	
Phytophthora sojae	Psojae_nad9sp_probe1	TTGATATATGAATACAAAGATAGATTTAAGTAAAT	
Phytophthora trifolii	Ptrifolii_nad9sp_probe	ATTAATACTAATTATGGGATATACACTATTTTTAG	
Phytophthora uliginosa	Puligi_nad9sp_probe	CCAATAAAAATAATTGGTATATATGTATATCTA	
Phytophthora vignae	Pvign_nad9sp_probe	TGATATACATATACCTATACAGGTTAGGTAGA	

[a] Would need to add a Lock Nucleic Acid (LNA) to be specific and increase the annealing temperature (Many low Tm probe could also be improve with LNA added). "Low Tm" mean this could be tried without LNA modifications, Tm around 60°C.

[b] Very difficult, could be due to species complex or possibility to amplified a closely related species or low Tm but not impossible to use.

Table S3: Primers used for amplification of the region *nad11-secY* and primers used for the *atp9-nad9 Phytophthora* genus primers without the 5'flap.

Primer-probe name	Sequence 5' to 3'	Target	Notes
Sequencing primers			
PhyG-nad11-1915F	TGTAAATTTTCYTGAAAACARAATTTAGG	*nad11-secY*	For PCR and sequencing
PhyG-SecY-2596R	AATTYTCAWATTTATRTAAACCTT	*nad11-secY*	For PCR and sequencing
PhyG_ATP9_2F	CCTTCTTTACAACAAGAATTAATG	*atp9-nad9*	For real-time PCR
PhyG-R6	ATACATAATTCATTTTTATA	*atp9-nad9*	For real-time PCR