Root Rot of Pea and Faba Bean in Southern Sweden Caused by *Phytophthora pisi* sp. nov.

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Abstract

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A root rot disease of pea and faba bean caused by a *Phytophthora* sp. was observed in fields and field soil samples in southern Sweden. Observations of the disease in pea root rot greenhouse assays were systematically recorded, and incidence and geographic distribution data were compared with the pea root rot caused by *Aphanomyces euteiches*. Following one successful isolation of the pathogen, isolation procedures and selective media were optimized to retrieve more isolates. Phylogenetic analysis showed that the isolates belong to a novel lineage, closely related to *Phytophthora sojae*, and proposed here as a new species, *P. pisi* sp. nov. In a collection of 13 isolates from separate

fields, intraspecific variation was detected in both nuclear and mitochondrial loci. Pathogenicity tests on a range of crop plants and wild legumes suggest that the host range of the pathogen is restricted to a group of legumes closely related to pea which, in addition to pea, include the crop species faba bean, lentil, common vetch, and chickpea. Morphology, growth requirements, and pathogenicity traits indicate that the species may be identical to the organism previously described as *P. erythroseptica* var. *pisi*. The work characterizes a novel *Phytophthora* sp. causing root rot of legume crops.

Root rot of pea is a disease complex that can cause extensive yield losses in pea (*Pisum sativum* L.) cultivation worldwide. In Sweden (21) and in other countries, the oomycete *Aphanomyces euteiches* (Drechsler) is considered to be the most destructive and important of the root rot pathogens, capable of causing complete crop failure and with the ability to survive and maintain a high inoculum potential in infested fields over many years. At Findus AB, a vegetable processing company in southern Sweden, the root rot potentials of approximately 1,000 soil samples from contract growers' fields are tested every winter in greenhouse assays, in order to avoid yield losses due to root rot. Each field is assigned a root rot index between 0 and 100, using a procedure standardized in the 1960s (20). The assay is based on visual examination and classification of the pea roots after 4 weeks of growth in the soil sample.

During the last 15 years, atypical root rot symptoms were observed on the test plants in some of the soil samples at Findus AB. If the infection was severe, plants were stunted and, in contrast to root rot caused by *A. euteiches*, symptoms were seldom observed on the epicotyl. Microscope observations of symptomatic roots revealed the presence of oospores with morphology different from that of *A. euteiches*. Similar oospores were also observed in rotted faba bean (*Vicia faba* L.) roots from field trials carried out at Findus AB involving both faba bean and pea, leading to the hypothesis that the same pathogen was able to infect these two hosts. The pathogen causing these symptoms was difficult to isolate but one relatively slow-growing *Phytophthora* isolate (isolate 97603 in this study) was retrieved from symptomatic pea roots in 1997 using a *Phytophthora*-selective medium described by Larsson

and Gerhardsson (16). This isolate was identified by Centraalbureau voor Schimmelcultures (CBS) as *Phytophthora erythroseptica* var. *pisi* Bywater & Hickman, based on morphology and the host from which it was isolated (pea).

To our knowledge, no primary information on this taxon apart from the original reference (2) exists in the literature, nor is there a type culture available. In light of the serious problems caused by *Aphanomyces* root rot, the present study was initiated to characterize the *Phytophthora* root rot of pea and faba bean in southern Sweden.

The objectives of the study were to assess the incidence and distribution of the disease in the region, and to study the host range of the pathogen in order to understand its ecology and potential impact on crop production systems. We also wanted to further characterize a collection of pea-infecting isolates genotypically and phenotypically, to enable comparisons with other *Phytophthora* spp. and determine whether the pea root rot isolates represent a separate species.

Materials and Methods

Observations of *Phytophthora* root rot in field soil samples.

Field soil samples for the root rot greenhouse assays carried out at Findus AB were collected with the purpose of finding fields suitable for commercial pea production the following year. Thus, the selection of fields for sampling was not random but, instead, influenced by factors such as accessibility and previous root rot history of the field. Field soils were sampled by pooling 10 to 15 subsamples (approximately 250 ml each) from the top 20-cm layer collected with a soil sampling auger, where the sampling points represented a W-shaped pattern across the field. Samples were brought to the research station the same day and allowed to dry on a clean surface at room temperature until they had a moisture level suitable for the pot assays.

The assays were based on two 1-liter pots with 10 pea plants in each. When root rot symptoms were observed, a representative piece of symptomatic root was routinely examined under the microscope to identify the resting structures of the disease-causing pathogen which, in the majority of cases, consisted of oospores of *A. euteiches*. Beginning with the samples collected the winter preceding the 2009 growing season, the presence of *Phytophthora*

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*The e-Xtra logo stands for "electronic extra" and indicates that Figure 3 appears in color in the online edition.

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oospores in these samples was systematically recorded, based on distinguishing features such as larger oospore size, aplerotic oospore morphology, and amphigynous antheridia in the pea-infecting *Phytophthora* spp. compared with *A. euteiches*. The data were compiled in a database together with data on the presence of *A. euteiches*, as well as geographic coordinates of the fields. Data from 2009 to 2011 were used for basic statistics of incidence.

Pathogen isolation. Various media and antibiotics mentioned in the literature for isolation of *Phytophthora* spp. were tested in the lab using isolate 97603, in order to improve the isolation procedure. Based on the results, an agar medium with dilute (4%) filtered multi-vegetable juice (Granini juice; Eckes-Granini Group) and 2% agar (GJ agar) was chosen as a nutrient base in isolation media containing antibiotics, and for growth of pure cultures. The selective medium used for isolations contained the following amendments (in GJ agar): benomyl (10 ppm, added in 50% ethanol), vancomycin (200 ppm), pimarin (10 ppm), and hymexazol (20 ppm). Benomyl was added before autoclaving; all other amendments were added as water solutions to autoclaved agar.

Pea plant samples from the Findus AB greenhouse assays diagnosed with *Phytophthora* root rot were stored at 4°C for up to a week before isolation attempts. After initial trials, the procedure selected for isolation was as follows. Fresh pea seedlings were produced by incubation of seed in moist rolled paper towels for 72 h at 24°C in darkness, standing in 2-cm-deep deionized water in a beaker covered with aluminum foil. Infected root material was rinsed in a sieve under running tap water for 1 min and placed at the rootlet of a pea seedling on a new paper towel, which was made into a roll with the seedling and inoculum in the middle, then placed in a 5-ml Falcon tube and incubated in a growth chamber at 24°C under 14 h of light with the Falcon tube two-thirds full with water. The rolls were opened and the seedling was assessed for root rot symptoms at least once between 3 and 7 days. Roots with visible soft rot symptoms were rinsed in tap water, surface sterilized for 3 s in 95% ethanol, rinsed three times in sterile water, and divided with a scalpel into 3-mm segments which were placed on the isolation medium. Mycelia with typical morphology growing out from the root pieces on the selective medium were subcultured on fresh plates of selective medium and eventually transferred to nonamended GJ agar.

In total, 12 isolates were retrieved from soil samples representing separate fields in the provinces of Skåne Län and Hallands Län in southern Sweden (Fig. 1). One isolate (isolate Var) was retrieved from Västra Götalands Län, further north (approximate WGS 84 decimal coordinates 58.261,12.960).

Host range. The pathogenicity test method used was a modification of the inoculum layer method developed for evaluating resistance against root rot caused by *P. sojae* in soybean (29). A 3-week-old GJ agar culture grown at 24°C in a 9-cm petri dish was

placed between two layers of 200 ml of moist vermiculite in square 500-ml disposable plastic pots. Seed of the host plant (usually nine seeds, but varying depending on seed size of the species) were sown in two replicate pots and one noninoculated control. All pots were watered heavily after planting. The pots were incubated in a climate chamber at 24°C with 14 h of light and placed in a deep tray (a separate tray for the noninoculated controls to avoid cross-infection) in which the water level was kept at a 2-cm depth to facilitate infection. The surface of the pots was kept moist until emergence. After 4 weeks, the roots of the plants were rinsed under tap water and examined visually for the presence of root rot, with the noninoculated control used as a comparison. In uncertain cases, root tissue with possible infection lesions was examined under the microscope for the presence of oospores. The host species was scored as either susceptible or resistant. In a few cases, single poorly germinating seedlings became infected while the other plants in the same pot were symptomless; this was attributed to poor seed quality and was not considered evidence of pathogenicity. Pea was included as a positive control host in all experiments. In a first round of experiments, the pathogenicity of isolate 97603 was tested in duplicate on 48 species consisting of various temperate-zone crop plants as well as nondomesticated members of the family Fabaceae. Isolates 97603, 2150, and 3353x were then tested in duplicate on 16 putative host and nonhost legume species in a second experiment which was performed twice.

Morphology. Colony morphology on GJ agar, potato dextrose agar (Oxoid), and 3% bean meal agar (2) was evaluated after growth at 25°C at 17, 12, and 10 days respectively. All isolates were examined on GJ agar; isolates 97603, 3353x, 3370E, 5582A2, and 2150 were also examined on the other two media. Colonies were examined for presence and characterization of aerial mycelium, colony edge shape, and presence of floral patterns in colony morphology. Results are based on at least two separate experiments.

Oospore and oogonium diameter on GJ agar and bean meal agar, oospore wall thickness on GJ agar, and antheridial dimensions on GJ agar were measured on five isolates (97603, 2150, 3353X, 3370E, and 5582A2) using Adobe Photoshop or ImageJ (25) software and digital photomicrographs obtained at $\times 200$ or $\times 400$ magnification. Oospore diameter (isolates 97603, 3353x, and 2150) was also measured in infected pea roots from pathogenicity tests in vermiculite. Sporangial formation was induced in liquid cultures of the pathogen in dilute (0.5%) lima bean broth (28) incubated in sterile cell culture trays. At 65 and 71 h post inoculation at 25°C in darkness, the medium was removed with a pipette from the culture, replaced with autoclaved river water, and further incubated at 25°C. Sporangia were examined at 90 h post inoculation at $\times 200$ magnification under the microscope. Presence of chlamydospores, hyphal swellings, and septa was assessed in the same cultures. Motile zoospores appeared in liquid cultures the same day as the sporangia, encystment was induced by vortexing a sample of the suspension for 30 s, and the cyst diameter was measured under $\times 400$ magnification (isolate 97603 only). The number of observations per isolate used to calculate mean values and ranges of organ dimensions were 24 (oospore and oogonium diameters in GJ agar and bean meal agar), 20 (oospore diameter in pea roots), 48 (sporangia), 11 (antheridia), 8 (oospore walls), and 10 (zoospore cysts).

Temperature limits and optima. The growth rate at 4, 12, 20, 25, 28.5, 32, and 37°C was tested for the same five isolates used for the morphometric measurements, and this experiment was performed twice for each isolate. Plates with 17 ml of GJ agar from the same batch were inoculated with 5-mm plugs from the edge of fresh cultures and incubated at 25°C for 72 h. The perimeter of each colony was carefully marked with a pen under magnification, and the plates were then incubated at the different temperatures in triplicate for each isolate. After 4 days of incubation at the new temperature (7 days post inoculation [dpi]), colony diameter was measured with a ruler, using two measurements of each colony at right angles. The plates were then incubated further at the different temperatures and colony size measured again at 11 dpi, after which

Fig. 1. Geographic origins of 12 of the 13 isolates used for sequencing and phylogenetic analysis. Scale bar: 20 km. The Findus AB processing facility at Bjuv is located close to the cluster of isolates 2, 5, 7, and 8. SI is the type isolate 97603 isolated in 1997.

the plates were further incubated. Growth rates were calculated as the colony size increase between 7 and 11 days in millimeters per day. Plates with no visible growth at 15 dpi were returned to 25°C and checked for resumed growth at 20 dpi. Comparisons of mean growth rate among temperature treatments with experiment as a block factor was done using analysis of variance implemented in JMP 10.0 software (SAS Institute), both individually for each isolate and as a pooled dataset.

Cultures and DNA extraction. Isolates for DNA extraction were grown as liquid cultures in 25 ml of sterile 0.5% lima bean broth in 100-ml Erlenmeyer flasks inoculated with three 5-mm plugs from the edge of a growing agar culture. They were incubated in darkness for 72 h at 24°C. Mycelial mats were decanted, rinsed with sterile water, separated from excess liquid with sterile filter paper, and stored for up to a month at -20°C in Eppendorf tubes before DNA extraction. DNA was extracted from a subsample of the mycelium using 2-by-30-s bead beating and a cetyltrimethylammonium bromide-chloroform extraction protocol (12). DNA samples were stored in ultrapure water solution at -20°C after control of quality and measurement of concentration using a NanoDrop (Thermo Scientific) spectrophotometer.

PCR primers and gene sequences. The nuclear internal transcribed spacer (ITS) and mitochondrial cytochrome oxidase 2 + spacer (cox2) loci were sequenced for all 13 isolates collected. In addition, six nuclear loci were amplified from isolate 97603 for a genus-wide multilocus phylogenetic analysis. Primer sequences

and amplification conditions were obtained from previous studies (1,3,13,17–19,26,31). All polymerase chain reaction (PCR) reactions were performed with the DreamTaq chemistry (Fermentas) with the following final concentrations: Taq polymerase, 0.02 U/μl; MgCl₂, 2.75 mM; forward and reverse primer, 0.67 μM; genomic DNA template, 1 ng/μl. Thermocycling parameters were 5 min at 94°C; followed by 40 s at 94°C, 40 s of annealing at the temperature specific for the primer pair, and 120 s at 72°C, repeated for 35 cycles; followed by a final extension step of 7 min at 72°C. For the cox2 locus, a touchdown protocol was used, starting with 54°C as the initial annealing temperature and decreasing in 1°C increments per cycle to 49°C, which was then used as the annealing temperature for the remainder of the PCR program.

PCR products showing up as single clear bands of the expected length after agarose gel electrophoresis were purified using the AMPURE magnetic bead system (Agencourt) and eluted with ultrapure water. Each PCR product was sequenced from both ends at a commercial facility (Macrogen Inc.), using internal primers when necessary to obtain sufficient sequence quality. Sequences were assembled and manually edited using the SeqMan software (DNASTAR Inc.). Polymorphisms in the ITS or cox2 loci which were only present in one isolate were confirmed by resequencing a new PCR product. Sequence data are available at the National Center for Biotechnology Information (NCBI) GenBank, accession numbers HQ894381 to HQ894412.

Fig. 2. Root rot symptoms from 4-week pathogenicity tests. **A**, Pea; **B**, lentil; **C**, faba bean; **D**, chickpea; **E**, common vetch; and **F**, sweet pea.

Phylogenetic analysis. Three datasets were constructed for phylogenetic analysis. The multi-locus sequence data from isolate 97603 were used together with data from a previous study (1) in a *Phytophthora* genus-wide phylogenetic analysis. The ITS and *cox2* data from all isolates were used to construct datasets, together with sequences obtained from NCBI (Table 1), for a coalescent analysis of the phylogenetic relationships within *Phytophthora* clade 7 (1,14). Sequences were aligned for each locus using ClustalX under default settings (15); alignments were visually inspected and edited for small (single nucleotides or codons) insertions or deletions when necessary. For the ITS and multilocus datasets, the general time-reversible nucleotide substitution model with γ -distributed rate variation, and a proportion of invariable sites (GTR+G+I) was selected based on a hierarchical likelihood ratio test and the Akaike Information Criterion, as implemented in Modeltest version 3.7 (23). For the *cox2* dataset, the HKY substitution model with γ -distributed rate variation and a portion of invariable sites (HKY+G+I) was selected as above.

For the genus-wide dataset, maximum likelihood analysis was performed in GARLI, version 1.0 (33); model parameters were first estimated using two search replicates, then fixed for 1,000 bootstrap replicates. For the maximum parsimony analysis, 500 bootstrap replicates were generated in SeqBoot and analyzed with DNAPars in the PHYLIP package version 3.6 (8). Bayesian analyses were performed in MrBayes, version 3.1.2 (27), with two analyses run simultaneously for 1 million generations. Priors on

nucleotide frequencies and the rate parameters were modeled with flat Dirichlet distributions; uniform priors were used for the proportion of invariable sites (0.01 to 1) and the γ shape parameter (0.1 to 1). A 10% burn-in was determined from trace files, and the majority-rule consensus tree was calculated after removal of the burn-in trees. Alignments and tree files are available on TreeBASE (accession number 11565) or from the authors (J. E. Blair).

Data from multiple isolates were available for the ITS and *cox2* loci; therefore, a multispecies coalescent analysis was performed using the Bayesian method *Beast (9). BEAUTi, version 1.7.1 (5), was used to create the XML-formatted input file; species affiliation was indicated for each isolate and the evolutionary model was specified for each locus. Evolutionary rates were estimated under a

Table 2. Incidence of *Aphanomyces* and *Phytophthora* root rot in greenhouse root rot assays in 2009 to 2011

Year ^a	N	Number of samples with		
		<i>Phytophthora</i> root rot	<i>Aphanomyces</i> root rot	Both ^b
2009	1,079	36 (3.3%)	286 (27%)	25
2010	1,017	20 (2.0%)	249 (25%)	9
2011	1,007	45 (4.5%)	257 (23%)	37

^a Samples collected for growing seasons indicated.

^b Samples included in both the *Phytophthora* and *Aphanomyces* root rot data.

Table 1. National Center for Biotechnology Information accession numbers for *Phytophthora* spp. internal transcribed spacer (ITS) and *cox2* sequences included in this study

Species	Isolate	ITS	cox2
<i>Phytophthora alni</i>	P10564	HQ261497	GU221930
<i>P. alni</i>	P11318	HQ261495	GU221932
<i>P. alni</i>	P11193	HQ261496	GU221931
<i>P. alni</i>	P16202	HQ261494	GU221933
<i>P. cajani</i>	P3105	HQ261515	GU221954
<i>P. cambivora</i>	P1431	FJ802046	GU221955
<i>P. cambivora</i>	P1432	FJ801767	JF771264
<i>P. cinnamomi</i>	P2121	HQ261529	GU221965
<i>P. cinnamomi</i>	P2301	HQ261527	GU221967
<i>P. cinnamomi</i>	P2100	HQ261530	GU221964
<i>P. cinnamomi</i>	P6305	HQ261525	GU221969
<i>P. cinnamomi</i> var. <i>parvispora</i>	P8495	HQ261523	GU221971
<i>P. cinnamomi</i> var. <i>parvispora</i>	P7154	FJ801964	GU221970
<i>P. cinnamomi</i> var. <i>parvispora</i>	P6376	FJ801919	JF771392
<i>P. cinnamomi</i> var. <i>robiniae</i>	P16350	GU259294	GU221972
<i>P. cinnamomi</i> var. <i>robiniae</i>	P16351	GU259118	GU221972
<i>P. europaea</i>	P10324	HQ261556	GU222005
<i>P. europaea</i>	P15554	GU258786	JF771442
<i>P. europaea</i>	P10325	FJ801381	JF771440
<i>P. europaea</i>	P10326	FJ801382	JF771441
<i>P. fragariae</i>	P11808	HQ261565	GU222011
<i>P. fragariae</i>	P3820	HQ261563	GU222013
<i>P. fragariae</i>	P1435	HQ261564	GU222012
<i>P. melonis</i>	P6870	HQ261616	GU222073
<i>P. melonis</i>	P3609	HQ261617	GU222072
<i>P. melonis</i>	P1371	FJ801749	JF771522
<i>P. melonis</i>	P10994	FJ801540	JF771523
<i>Phytophthora</i> sp. “ <i>niederhauserii</i> ”	P10617	FJ801464	GU222092
<i>Phytophthora</i> sp. “ <i>niederhauserii</i> ”	P10616	FJ801463	GU222091
<i>Phytophthora</i> sp. “ <i>niederhauserii</i> ”	P10976	FJ801538	GU222093
<i>Phytophthora</i> sp. “ <i>niederhauserii</i> ”	P10279	FJ801357	GU222090
<i>P. pistaciae</i>	P6196	HQ261645	GU222108
<i>P. pistaciae</i>	P6197	HQ261644	GU222109
<i>P. rubi</i>	P3316	HQ261665	GU222133
<i>P. rubi</i>	P3289	HQ261666	GU222132
<i>P. sojae</i>	P6497	HQ261676	GU222143
<i>P. sojae</i>	P3114	HQ261677	GU222142
<i>P. sojae</i>	P10704	HQ261678	GU222141
<i>P. sojae</i>	P0405	HQ261679	GU222140
<i>P. uliginosa</i>	P10328	HQ261722	GU222155
<i>P. uliginosa</i>	P10413	HQ261721	GU222156
<i>P. vignae</i>	P7471	HQ261723	GU222158
<i>P. vignae</i>	P3019	HQ261724	GU222157

strict clock model, and a Yule process was used as the species tree prior. The analysis was run for 10 million generations in BEAST, version 1.7.1 (5), sampling every 1,000 generations; log files were evaluated in Tracer, version 1.5 (4), for evidence of convergence. The resulting species tree and individual gene trees were calculated using TreeAnnotator, version 1.6 (24), with a burn-in of 1,000 trees (1 million generations).

Results

Occurrence and distribution of *Phytophthora* root rot in field soil samples. Incidence of *Phytophthora* root rot in the greenhouse bioassays performed on soil samples was 2.0 to 4.5% for the years 2009 to 2011. *Aphanomyces* root rot incidence was 23 to 27% (Table 2). The area in southern Sweden from which samples were taken is shown in Figure 1. The sampling intensity and the incidence of *Aphanomyces* root rot was strongly biased to the western part of the area, centered around the Findus AB plant in Bjuv, which has been a center for pea processing since 1950. Sam-

ples further away from Bjuv have a history of less intense pea cultivation, and the southwestern part of the region is also known for a high incidence of calcium-rich till soils suppressive to *Aphanomyces* root rot (10,22). The geographic range of *Phytophthora* root rot roughly followed the pattern for high incidence of *Aphanomyces* root rot and is reflected in the map of the collected isolates shown in Figure 1.

Host range and disease symptoms. The visual examination of root rot symptoms permitted a clear division between susceptible and nonhost species. Roots of nonhost species had no visible symptoms compared with the control. Susceptible species developed a soft rot (Fig. 2), usually starting from root tips of both main and lateral roots. The rotted tissue was flaccid, with abundant oospores observed when viewed under the microscope. The proportion of the root system affected varied among individuals. Either main or lateral roots sometimes ceased elongating after infection, leading to a small rotted portion at the apex, sometimes followed by compensatory growth and branching of noninfected parts of the root system (Fig. 2F). Faba bean plants developed a characteristic black discoloration in infected root parts, whereas rotted root sections in other species took on a light-brown discoloration with time. For all host species, the upper limit of visible soft rot was at the location of the seed, although individual plants with signs of enzymatic breakdown of the lower stem were occasionally observed. Severe infections led to stunting and drying of above-ground parts and partial necrosis starting at the lower leaves or stipules, in some cases leading to plant death within the 4-week growth period. In pea and faba bean, symptoms from naturally infested soil samples were similar to those observed in artificially inoculated vermiculite but usually less severe.

Pea, faba bean, chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medik.), common vetch (*V. sativa* L.), sweet pea (*Lathyrus odoratus* L.), and other *Vicia* and *Lathyrus* spp. showed root rot symptoms when inoculated with isolate 97603 in the first round of experiments. No symptoms were found on any of the non-legumes tested or on legumes of other genera such as alfalfa, soybean, clover, or common bean (Table 3). Pea used as a positive control was severely infected in all experiments. The results from the second round of pathogenicity trials, where 16 legume species were inoculated with three different isolates, were consistent with the first experiments, with no difference in host range seen among the three isolates tested (Table 4).

Morphology. Colony type on GJ agar was smooth, lacking aerial mycelium, and was the same for all isolates collected. Parts of the colony rich in oospores became slightly straw-colored. The colony morphology on bean meal agar was more dense but otherwise similar, with no or very sparse aerial mycelium. Colonies on

Table 3. Pathogenicity of isolate 97603 of *Phytophthora pisi* to various field crop and legume (cultivated and wild growing) species at high inoculum pressure in artificially inoculated greenhouse assays

Species	Symptoms ^a
Non-legumes	
<i>Avena sativa</i> 'Ingeborg'	–
<i>Beta vulgaris</i>	–
<i>Brassica rapa</i> subsp. <i>oleifera</i>	–
<i>Helianthus annuus</i> 'Giganteus'	–
<i>Hordeum vulgare</i> 'Ortega'	–
<i>Linum usitatissimum</i>	–
<i>Phleum pratense</i> 'Lischka'	–
<i>Raphanus sativus</i> 'Adios'	–
<i>Secale cereale</i> 'Amilo'	–
<i>Sinapis alba</i> 'Architect'	–
<i>Triticum aestivum</i> 'Vinjett'	–
<i>Zea mays</i> 'Double Standard'	–
× <i>Triticosecale</i> 'Nilex'	–
Legumes	
<i>Astragalus hamosus</i>	–
<i>A. monspessulanus</i>	–
<i>Cicer arietinum</i> 'Calia'	+
<i>Glycine max</i> 'Hei Nong 48'	+
<i>G. max</i> 'Fiskeby'	–
<i>Lathyrus annuus</i>	+
<i>L. aphaca</i>	+
<i>L. articulatus</i>	+
<i>L. cicera</i>	+
<i>L. odoratus</i> 'Royal'	+
<i>L. vernus</i>	+
<i>L. sativus</i>	+
<i>L. tingitanus</i>	+
<i>Lens culinaris</i>	+
<i>Lotus corniculatus</i>	–
<i>L. tetragonolobus</i> 'Winged bean'	–
<i>Lupinus angustifolia</i>	–
<i>L. luteus</i>	–
<i>Medicago sativa</i> 'Jenny'	–
<i>M. lupulina</i> 'Virgo Pajbjerg'	–
<i>Melilotus officinalis</i>	–
<i>Phaseolus coccineus</i> 'Enorma'	–
<i>P. vulgaris</i> 'Karin'	–
<i>Pisum sativum</i> 'Finulf'	+
<i>Scorpiurus muricatus</i>	–
<i>Trifolium hybridum</i> 'Frida'	–
<i>T. pratense</i> 'Björn'	–
<i>T. repens</i> mixed cultivars	–
<i>T. resupinatum</i> 'Ciro'	–
<i>Vicia faba</i> 'Diana'	+
<i>V. sativa</i> 'Candy'	+
<i>V. benghalensis</i>	+
<i>V. narbonensis</i>	+
<i>Vigna radiata</i>	–
<i>V. unguiculata</i> 'Yard Long'	–

^a Symptoms: + = root rot and – = no symptoms.

Table 4. Pathogenicity of isolates 97603, 2150, and 3353x of *Phytophthora pisi* to 16 legume species

Species	Symptoms ^a
<i>Astragalus alpinus</i>	–
<i>Cicer arietinum</i>	+
<i>Glycine max</i> 'Hei Nong 48'	–
<i>Lathyrus odoratus</i> 'Mammoth Scarlet'	+
<i>Lens culinaris</i>	+
<i>Lotus corniculatus</i>	–
<i>Lupinus angustifolia</i> 'Galant'	–
<i>Medicago sativa</i> 'Jenny'	–
<i>Melilotus officinalis</i>	–
<i>Phaseolus coccineus</i> 'Hestia'	–*
<i>Phaseolus vulgaris</i> 'Speedy'	–*
<i>Pisum sativum</i> 'Finulf'	++
<i>Vicia faba</i> 'Laura'	++
<i>Trifolium pratense</i> 'Titus'	–
<i>V. sativa</i> 'Candy'	+
<i>Vigna unguiculata</i>	–

^a Symptoms: + = root rot detected for all isolates in both replicates in two separate experiments, – = no symptoms, and * = the same host species tested (with the same result) in the description of *P. erythrocephala* var. *pisi* (2).

potato dextrose agar were dense and irregular, with patches of “velvety” aerial mycelium (Fig. 3B). No floral patterns in the colony morphology were formed on any of the media. Measurements of sexual and asexual reproductive organs revealed a large variation within each isolate. Sporangia longer than 100 µm were only found in isolate 3370E. Means and ranges of morphological measurements of sexual organs and sporangia are presented in Table 5. Further qualitative characters are shown in Figure 4 and described in the taxonomy section below.

Temperature limits and optima. No isolates grew at 4 or 37°C. All isolates incubated at 4°C resumed growth after transfer back to 25°C, whereas incubation at 37°C was lethal in all cases. Isolate 2150 was excluded from the statistical comparison of growth rate means among temperature treatments, due to missing data points. For the remaining four isolates, growth at 25°C was significantly (Tukey’s honestly significant difference test, $P < 0.001$) higher than at 20°C. The reduction in growth rate at 28.5 compared with 25°C (Fig. 5) was not significant for any of the isolates analyzed or for all isolates pooled.

Phylogenetic analysis. Analysis of the multilocus nuclear dataset strongly supports *P. pisi* as a novel lineage within molecular clade 7b (Fig. 6); this subclade includes a number of important pathogens of the plant family Fabaceae, including *P. sojae* (soybean), *P. cajani* (pigeon pea), and *P. vignae* (cowpea). The coalescent analysis of the available ITS and cox2 data suggests a close relationship with *P. cinnamomi* var. *robiniae*, the causal agent of stem rot and bark canker on black locust (11). This relationship was strongly supported in the individual gene trees (Fig. 7) as well as the estimated species tree (Fig. 8). Sequencing of the ITS and the cox2 loci for multiple isolates revealed a low level of intraspecific variation (<0.5% polymorphic bases) in both the nuclear ITS (four alleles) and mitochondrial cox2 (two alleles) loci (Table 6).

Taxonomy. *Phytophthora pisi* F. Heyman, sp. nov.; Mycobank number MB801280; etymology: “pisi” refers to the host plant pea, from which it was first isolated.

In diluto (4%) agaro de ‘Granini Juice’, coloniae laeves, mycelio aeris destitutae. Temperatura minima 4–12°C, optima 20–32°C, maxima 32–37°C. Sporangia non caduca et non papillata, 65 (31–169) µm l, 31 (17–45) µm w, interdum interne et externe prolifica.

Fig. 3. Colony morphology of *Phytophthora pisi* on **A**, dilute Granini juice and 2% agar and **B**, Oxoid potato dextrose agar.

Thallus homothallicus; antheridiis amphigyniis, 18 (14–24) µm w, 18 (12–27) µm l. Oogonia levia, 36 (24–47) µm diam. Oosporae apertoticae, globosae, 28 (19–35) µm. diam, pariete 2.6 (1.7–3.9) µm crasso. Parasiticus, radicum variorum Fabacearum (*Pisum sativum*, *Lens culinaris*, *Vicia faba*, *Vicia sativa*, *Lathyrus odoratus* et *Cicer arietinum*) putrefaciens. Typus: Sweden: Skåne Län, Höganäs. Isol. e radicibus *Pisi sativi*, 1997, M. Wikström (97603-holotypus; culturae vivae CBS 130350 et UCR World Oomycete Collection P19612).

Colonies smooth, lacking aerial mycelium on 4% “Granini” vegetable juice agar (GJ agar). On potato dextrose agar, colonies irregular, dense, with patches of velvety aerial mycelium. Growth min. at 4–12°C, opt. 20–32°C, max. 32–37°C. Hyphae septate. Sporangioophores usually bearing a single sporangium, occasionally several, closely sympodially branched. Nested and/or external proliferation occasionally observed. Sporangia noncaducous, lacking papilla, shape and size variable, usually ovoid or ellipsoid, 65 (31–169) µm long and 31 (17–45) µm wide. Hyphal swellings absent on sporangioophores. Encysted zoospores spherical, 12 (11–13) µm diam. Intercalary, globose hyphal swellings observed in liquid cultures. Chlamydospores absent. Homothallic, with gametangia abundantly formed in agar cultures within 4 d. Antheridia amphigynous, 18 (12–27) µm long, 18 (14–24) µm wide. Oogonia spherical and smooth, 36 (24–47) µm diam. Oospores formed within 3 d at 24°C, apertotic, spherical, 28 (19–35) µm. diam. Oospore wall smooth, 2.6 (1.7–3.9) µm thick. Parasitic, with the capacity to cause root rot on various Fabaceae (*Pisum sativum*, *Lens culinaris*, *Vicia faba*, *Vicia sativa*, *Lathyrus odoratus* and *Cicer arietinum*). Habitat: agricultural soils.

Notes. Having amphigynous antheridia and nonpapillate sporangia, *Phytophthora pisi* falls into “group VI” of the identification key of Erwin and Ribeiro in their 1996 monograph (6) which, in turn, was based on the work by Waterhouse (30). Excluding heterothallic species, a current list of recognized *Phytophthora* spp. (14), together with taxa with subspecies status in the Erwin and Ribeiro monograph, contains a number of taxa that fulfill these criteria. Morphological and physiological characters that separate these from *P. pisi* are listed below.

The dominance of apertotic oospores separate *P. pisi* from *P. alni* subsp. *alni*, *P. alni* subsp. *uniformis*, *P. alni* subsp. *multiformis*, *P. astrocedri*, *P. captiosa*, *P. fallax*, *P. polonica*, and *P. richardiae*. Paragynous antheridia are common in *P. brassicae*, *P. glovera*, and *P. uliginosa* and occur in *P. samsomeana*. Caducous sporangia occur in *P. psycrophila*. Ornate oogonia, absent in *P. pisi*, are a characteristic for *P. alni* subsp. *alni*, and are common (approximately 50%) in *P. gibbosa*, a species in which nested proliferation of sporangia is absent. Morphologically similar species with clearly different cardinal temperatures from *P. pisi* are *P. samsomeana* (grows at 3°C) and three species with a growth optimum <20°C: *P. astrocedri*, *P. fallax*, and *P. psycrophila*. Colonies of *P. multivesiculata* are reported to have fluffy aerial mycelium on V8 agar and potato dextrose agar. *P. polonica* displays floral colony patterns on both potato dextrose agar and V8 agar. Intercalary swellings on sporangioophores were reported for *P. multivesiculata*. *P. erythroseptica* var. *erythroseptica* forms evenly shaped colonies

Table 5. Mean values and range of morphometric measurements for *Phytophthora pisi* and comparison with *P. erythroseptica* var. *pisi* with measurements in micrometers

Organism	Substrate ^a	Oospore Ø		Oogonium Ø		Oospore		Antheridium				Sporangium			
		Mean	Range	Mean	Range	Wall	Range	Length	Range	Width	Range	Length	Range	Width	Range
<i>P. pisi</i> ^b	4% GJ	28	19–35	36	24–47	2.6	1.7–3.9	18	12–27	18	14–24	65	31–169	31	17–45
<i>P. pisi</i> ^b	3% BMA	28	19–39	38	24–46	–	–	–	–	–	–	–	–	–	–
<i>P. pisi</i> ^c	Pea roots	24	14–33	–	–	–	–	–	–	–	–	–	–	–	–
<i>P. erythroseptica</i> var. <i>pisi</i> ^d	3% BMA	26	20–35	35	25–43	2.9	1–5	19	14–28	18	14–23	86	54–171	35	25–40
<i>P. erythroseptica</i> var. <i>pisi</i> ^d	Pea roots	26	14–34	–	–	–	–	–	–	–	–	–	–	–	–

^a GJ = Granini juice and 2% agar and BMA = bean meal agar.

^b Mean of five isolates; range is maximum and minimum individual measurements.

^c Mean of three isolates.

^d Data from Bywater and Hickman (2).

on potato dextrose agar and has aerial mycelium on V8 agar. *P. cinnamomi* var. *robiniae* is a primarily heterothallic but occasionally homothallic taxon which is closely related to *P. pisi* according to the present study. Unambiguous morphological characters separating it from *P. pisi* are difficult to distinguish based on the description. The oospores depicted in the original description are

more plerotic than in *P. pisi*, and aerial mycelium was reported in colonies on V8 agar.

Discussion

P. pisi, described in this study, is a root rot pathogen with the capacity to infect four important food crop legumes and representa-

Fig. 4. Morphology of *Phytophthora pisi*. Aplerotic oospores with amphigynous antheridia from the isolates **A** and **B**, 2150; **C** and **D**, 3370E; **E** and **F**, 5582a2; and **G** and **H**, 97603. **I**, Sporangia emerging from an infected root incubated in water. **J**, Variability of sporangial shapes and sizes. **K**, Sporangium with apical protuberance. **L**, External proliferation of sporangia. **M**, Nested proliferation of sporangia. **N**, Globose hyphal swellings in liquid culture. **O**, Oospores formed in pea root tissue. Scale bar = 50 μ m.

tives of the *Vicia* and *Lathyrus* genera which, in addition to a large number of wild species, also include species and varieties domesticated as minor crops and ornamentals. It is widely spread in the agricultural areas of southern Sweden, and probably favored by intense cultivation of pea, because the majority of findings are from soils also infected by the pea root rot pathogen *A. euteiches*.

Although molecular data is essential for modern taxonomy and identification of *Phytophthora* spp., it is, in theory, possible to separate *P. pisi* from all other currently described species in the genus based on host range and morphological and physiological characters. In the original description (2) of *P. erythroseptica* var. *pisi*, the characteristics used in *Phytophthora* identification keys, such as host range and qualitative traits of reproductive organs, match with those presented here for *P. pisi*. Growth-temperature relationships were also similar, with an optimum at 25°C. Dimensions of reproductive organs are in agreement as well, although the large variation makes these less useful for identification. The incidence of unusually elongate sporangia was seen in both studies. The colony morphology is also similar between the studies, with the exception of potato dextrose agar, a medium on which we observed aerial mycelium, whereas Bywater and Hickman observed none. However, this could be due to intraspecific variation or variation between media preparations. Overall, it seems likely that the two taxa represent the same species, and that the identification of isolate 97603 by CBS was, in this sense, correct. The lack of type material for *P. erythroseptica* var. *pisi* means that this cannot be directly resolved, and this name has been considered invalid based on the lack of type material or type illustrations (7). Regardless, it is clear from the phylogenetic analysis that our isolates are not closely related to *P. erythroseptica* and that a new species description is justified.

P. pisi appears to have a strictly soilborne life cycle, because the colonization of host tissue and production of both sexual and asexual spores are restricted to the roots. The sporangia in this group of *Phytophthora* spp. are noncaducous (6) and, hence, do not function as asexual propagules. The possibility of zoospores infecting the shoot of the plant through splash dispersal in the field cannot be excluded, although the absence of stem symptoms in our observations suggests that the aboveground parts of all tested host species might be more resistant to infection compared with the roots. For a root- and soil-dwelling pathogen of crop plants of which the roots are not harvested, it is likely that most dispersal events between fields involve movement of soil. The area from which we have collected our isolates is a busy agricultural district with frequent movement of agricultural machinery and root crops across the landscape (for example, during the sugar beet harvest).

Although genetic variation in both nuclear and mitochondrial loci was found among the 13 isolates collected, we did not observe any difference in pathogenicity spectrum for the three isolates of different genotypes that were examined. No obvious geographic structure could be seen in the distributions of the genotypes. Sampling on different spatial scales and comparisons with populations

Fig. 5. Growth-temperature relationships of *Phytophthora pisi*. Means of five isolates over two experiments are provided. Error bars represent standard deviation of means among isolates based on pooled data from two experiments.

from other regions would shed light on the structure of the global population. Based on the geographical distribution of the soil samples where it has been observed, *P. pisi* seems to occur in the entire area sampled but it is clearly less frequently observed compared with *A. euteiches*. Overall, widespread distribution, genetic variability, and low frequency of observation may suggest that the pathogen has been present in the agricultural fields of Sweden for a long time, despite the lack of earlier records. However, changes in crop

Fig. 6. Maximum likelihood (ML) phylogram of *Phytophthora pisi* sp. nov. and other *Phytophthora* spp. based on nuclear multilocus sequence data (7,346 characters, 2,344 variable sites, and 1,849 parsimony informative sites). Numbers above major nodes represent support values >50% for ML (top), maximum parsimony (MP) (middle), and Bayesian posterior probabilities (bottom). Asterisks (*) indicate >95% support in all analyses. Dashes (-) indicate node not present in analysis.

rotation practices could lead to inoculum accumulation in fields and more frequent disease observations and outbreaks.

Our phylogenetic analyses strongly support the placement of *P. pisi* within molecular clade 7, closely related to other important legume pathogens such as *P. sojae* and *P. cajani*. Interestingly, the

legume species and genera found to be susceptible to *P. pisi* are all found within a subclade of the legume family known as the “Vicioid” clade (32). According to this phylogenetic tree of legumes the genera *Pisum*, *Lens*, *Vicia*, and *Lathyrus* form a monophyletic group, while *Cicer* is on another branch of the tree but still within

Fig. 7. Gene trees for *Phytophthora* clade 7 estimated under a Bayesian multispecies coalescent model for the **A**, internal transcribed spacer (ITS; 833 bp, including the ITS1, 5.8S rRNA, and ITS2 regions) and **B**, cytochrome oxidase 2 (cox2; 940 bp, including the cox2/cox1 spacer region) loci. Numbers on nodes indicate Bayesian posterior probabilities. Isolate identifiers are indicated next to species names (from this study for *Phytophthora pisi*, all others from the World Oomycete Genetics Resource Collection). The root of each topology was arbitrarily placed at the midpoint.

the Vicoid clade. The Vicoid clade is geographically widespread in natural ecosystems (including northern Europe), diverse in the number of species, and contains several ancient crop species thought to have been domesticated in western Asia. Thus, several scenarios are plausible for the evolutionary history of the current pathogen population, which could involve simply a shift of environment from natural grassland to agricultural fields or more complex evolutionary events, including adaptation to new hosts and human-assisted long-distance dispersal after establishment in agroecosystems.

Crop rotation practice is an important tool in the management of soilborne diseases. In a Swedish context, obvious preliminary advice would be to encourage the choice of nonsusceptible legume species when possible. However, for growers in cool temperate climates, there are not many grain legume species to choose from. With *Aphanomyces* root rot being a widespread problem in pea production, faba bean is often seen as a suitable alternate crop in the rotation because it is generally less susceptible to this disease. The acreage of this crop has increased in Sweden over the last decade due, in part, to its reputation as a crop with few disease problems. However, the results of this study illustrate that pea and

faba bean share susceptibility to another soilborne root pathogen widely distributed in Sweden, and should not be regarded as rotational crop partners with respect to this disease.

For this newly discovered disease, more basic knowledge about its biology is needed in order to manage it in an optimal way. Important aspects are pathogen distribution, diagnostics, longevity in the soil, and the feasibility of resistance breeding. The assessment of the disease severity and yield losses under field conditions presents a challenge, because several root rot diseases typically occur in the same field, making it difficult to estimate the relative contribution to yield loss by each pathogen. Studies of these different aspects are planned or under way.

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Literature Cited

1. Blair, J. E., Coffey, M. D., Park, S.-Y., Geiser, D. M., and Kang, S. 2008. A multi-locus phylogeny for *Phytophthora* utilizing markers derived from complete genome sequences. *Fungal Genet. Biol.* 45:266-277.

2. Bywater, J., and Hickman, C. J. 1959. A new variety of *Phytophthora erythroseptica*, which causes a soft rot of pea roots. *Trans Br. Mycol. Soc.* 42:513-524.

3. Cooke, D. E. L., Drenth, A., Duncan, J. M., Wagels, G., and Brasier, C. M. 2000. A molecular phylogeny of *Phytophthora* and related oomycetes. *Fungal Genet. Biol.* 30:17-32.

4. Drummond, A. J., and Rambaut, A. 2009. Tracer version 1.5. <http://tree.bio.ed.ac.uk/software/tracer/>

5. Drummond, A. J., Suchard, M. A., Xie, D., and Rambaut, A. 2012. Bayesian phylogenetics with BEAUTi and the BEAST 1.7. *Mol. Biol. Evol.* 29:1969-1973.

6. Erwin, D. C., and Ribeiro, O. K. 1996. *Phytophthora diseases worldwide*. The American Phytopathological Society, St. Paul, MN.

7. Farr, D. F., and Rossman, A. Y. 2012. *Fungal Databases. Systematic Mycology and Microbiology Laboratory*, U. S. Dep. Agric. Agric. Res. Serv. <http://nt.ars-grin.gov/fungal-databases/>

8. Felsenstein, J. 1989. PHYLIP—Phylogeny Inference Package (version 3.2). *Cladistics* 5:164-166.

9. Heled, J., and Drummond, A. J. 2010. Bayesian inference of species tree from multilocus data. *Mol. Biol. Evol.* 27:570-580.

10. Heyman, F., Lindahl, B., Persson, L., Wikström, M., and Stenlid, J. 2007. Calcium concentrations of soil affect suppressiveness against *Aphanomyces* root rot of pea. *Soil. Biol. Biochem.* 39:2222-2229.

11. Ho, H. H. 2002. *Phytophthora cinnamomi* var. *robiniae* var. *nova* on black locust in Jiangsu Province of China. *Mycotaxon* 82:391-396.

12. Ihrmark, K., Johannesson, H., Stenström, E., and Stenlid, J. 2002. Transmission of double-stranded RNA in *Heterobasidion annosum*. *Fungal Genet. Biol.* 36:147-154.

13. Kroon, L. P. N. M., Bakker, F. T., van den Bosch, G. B. M., Bonants, P. J. M., and Flier, W. G. 2004. Phylogenetic analysis of *Phytophthora* species based on mitochondrial and nuclear DNA sequences. *Fungal Genet. Biol.* 41:766-782.

14. Kroon, L. P. N. M., Brouwer, H., de Cock, A. W. A. M., and Govers, F. 2012. The genus *Phytophthora* Anno 2012. *Phytopathology* 102:348-364.

15. Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J., and Higgins, D. G. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* 23:2947-2948.

16. Larsson, M., and Gerhardson, B. 1990. Isolates of *Phytophthora cryptogea* pathogenic to wheat and some other crop plants. *J. Phytopathol.* 129:303-315.

17. Martin, F. N. 2000. Phylogenetic relationships among some *Pythium* species inferred from sequence analysis of the mitochondrially encoded cytochrome oxidase II gene. *Mycologia* 92:711-727.

18. Martin, F. N., Tooley, P. W., and Blomquist, C. 2004. Molecular detection of *Phytophthora ramorum*, the causal agent of sudden oak death in California, and two additional species commonly recovered from diseased plant material. *Phytopathology* 94:621-631.

19. Moncalvo, J. M., Wang, H. H., and Hseu, R. S. 1995. Phylogenetic relationships in *Ganoderma* inferred from the internal transcribed spacers and 25S ribosomal DNA sequences. *Mycologia* 87:223-238.

20. Olofsson, J. A. N. 1967. Root rot of canning and freezing peas in Sweden. *Acta Agric. Scand.* 17:101-107.

Fig. 8. Species tree for *Phytophthora* clade 7 estimated under a Bayesian multispecies coalescent model of internal transcribed spacer and cytochrome oxidase 2 data. Numbers on nodes indicate Bayesian posterior probabilities. The root of the topology was arbitrarily placed at the midpoint.

Table 6. Intraspecific variation in the isolate collection of *Phytophthora pisi* based on nuclear (ITS) and mitochondrial (cox2) sequences

Isolate name	Isolate number	ITS genotype/allele	cox2 genotype/allele
2150	1	1	1
5582A2	2	2	1
3370 E	3	3	1
3127 B	4	1	1
MW	5	2	2
5213A	6	2	2
5536C	7	1	1
5526C	8	2	2
3353X	9	2	2
6127H	10	2	1
FA	11	4	2
Vara	12	4	1
97603 (type isolate)	"SI"	4	2

21. Persson, L., Bødker, L., and Larsson-Wikström, M. 1997. Prevalence and pathogenicity of foot and root rot pathogens of pea in southern Scandinavia. *Plant Dis.* 81:171-174.
22. Persson, L., and Olsson, S. 2000. Abiotic characteristics of soils suppressive to *Aphanomyces* root rot. *Soil. Biol. Biochem.* 32:1141-1150.
23. Posada, D., and Crandall, K. A. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14:817-818.
24. Rambaut A., and Drummond A. J. 2010. TreeAnnotator version 1.6. <http://beast.bio.ed.ac.uk/TreeAnnotator>
25. Rasband, W. S. 1997-2012. ImageJ. U.S. National Institutes of Health, Bethesda, MD. <http://imagej.nih.gov/ij/>
26. Riethmüller, A., Voglmayr, H., Goker, M., Weiss, M., and Oberwinkler, F. 2002. Phylogenetic relationships of the downy mildews (Peronosporales) and related groups based on nuclear large subunit ribosomal DNA sequences. *Mycologia* 94:834-849.
27. Ronquist, F., and Huelsenbeck, J. P. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572-1574.
28. Schmitthenner, A. F. 1959. The effect of media concentration on sporangia production in *Phytophthora*. (Abstr.) *Phytopathology* 49:550.
29. Walker, A. K., and Schmitthenner, A. F. 1984. Comparison of field and greenhouse evaluations for tolerance to *Phytophthora* rot in soybean. *Crop Sci.* 24:487-489.
30. Waterhouse, G. M. 1963. Key to species of *Phytophthora* de Bary. *Commonw. Mycol. Inst. Mycol. Pap.* 92:1-22.
31. White, T. J., Bruns, T., Lee, S., and Taylor, J. W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 in: *PCR Protocols: A Guide to Methods and Applications*. M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, eds. Academic Press, Inc., New York.
32. Wojciechowski, M. F., Lavin, M., and Sanderson, M. J. 2004. A phylogeny of legumes (Leguminosae) based on analyses of the plastid *matK* gene resolves many well-supported subclades within the family. *Am. J. Bot.* 91:1846-1862.
33. Zwickl, D. J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. dissertation, The University of Texas at Austin.