Phytophthora spp. a new threat to tree seedlings and trees

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Abstract

At least 60–80 *Phytophthora* species has been described and most of them are soil-borne pathogens causing damping off, root rot, collar and stem rot and foliar blight on different woody plant species. These microbes are sometimes difficult to isolate and even more difficult to identify. A general review of isolation, detection and some newly identified species, including *Phytophthora alni* complex and *P. ramorum*, is presented in this article. The disease symptoms, host species and geographical range are also shortly described.

Phytophthora

Phytophthora and other oomycetous micro-organisms were long included within the fungi, but today, because of evolutionary phylogeny and structure of biflagellate zoospores, they are grouped in the kingdom Chromista, which includes e.g. brown algae (Erwin & Ribeiro 1996, Baldauff *et al.* 2000). *Phytophthora* is a genus that is mainly parasitic on plants including trees and tree seedlings. Tsao (1990) has presented that most crown diseases of woody plants can be attributed to *Phytophthora* although in most cases proper techniques have not been used to reveal these pathogens behind the symptoms.

Phytophythora spp. produce mainly diploid hyphae, oospores and chlamydospores within plant tissue. Although oospores can survive in organic part of soil for a long time the asexual chlamydospores are the main resting stage of oomycetes. The asexual, biflagellate, swimming zoospores, produced in vessels called sporangia, are responsible for plant infection under wet conditions. Some homothallic species are self-fertile and they produce oospores after fusion of oogonium and antheridium. In heterothallic species, oospore production needs a presence of two mating types called A1 and A2. Sexual recombination or somatic fusion might create new races having higher pathogenic ability than the parents. Typical for *Phytophthora* are also hybrids, a new combination produced by parents representing two different *Phytophthora* species as in the case of *P. alni*-complex (Brasier *et al.* 1999, 2004a).

Identification

At least 60–80 *Phytophthora* species have been described and most of them are soil-borne causing damping off, root rot, collar and stem rot and foliar blight on different woody plant species (Erwin & Ribeiro 1996). The traditional identification of *Phytophthora* spp. is based on the morphology of sporangia, oogonia and antheridia, presence or absence of chlamydospores, and the growth and colony characters of cultures on special agars (Waterhouse 1963, Stamps *et al.* 1990). Morphological grouping segregated the species into six main groups based on 1) the structure of the sporangium apex and the width of the exit pore, 2) the caducity of sporangia and the length of pedicel and 3) the antheridial attachment. [A sporangium may be papillate, semi-papillate or non-papillate, caduous sporangia shed at maturity and an antheridial attachment may be paragynous, amphigynous (Fig. 1) or both]. However, these morphological keys are not distinct and stable and might differ within a species or be similar between species. In addition the traditional taxonomic grouping does not reflect true phylogenetic relations (Kroon *et al.* 2004).

Fig. 1. Amphigynous antheridium on oospore.

Many molecular techniques such as protein electrophoresis, isozymes and PCR-based methods such as DNA fingerprinting and direct sequencing have been investigated in the search for more effective and rapid identification of the species within the genus *Phytophthora*. (eg. Bielenin *et al.* 1988, Oudemans & Coffey 1991, Cooke *et al.* 2000). Today, the internal transcribed spacer (ITS) sequence of most *Phytopthora* species is available in the GenBank, and thus this information can be used to determine the identity of unknown isolates.

Detection

Most *Phytophthora* spp. cannot be isolated directly from diseased plants, soil or water as easily as many other pathogens. The affected material should be in a stage of active infection since the ability of *Phytophthora* to compete with other microbes is restricted (Erwin & Ribeiro 1996, Martin

et al. 2004). A common reason for the failure of isolation procedure is also a dry season or too dry samples (Kox *et al.* 2002, Garbelotto 2003).

The main idea of baiting is the activation of the pathogen. The generally used baits are highly susceptible hosts such as unripe fruits (apples, pears etc.) or seedlings (lupine, alder etc.). Small cores are made in fruits and they are stuffed with soil or small fragments of wood tissue taken from a necrotic lesion on roots or bark. After incubation a *Phytophthora* 'rot' will develop on the host's exterior (Fig. 2) and isolation by e.g. plating on agar medium (with or without selective chemicals) can be done from this 'fresh', active infection (Jeffers & Martin 1986). Another option is to add water to the samples and use suitable living plant tissue floated on the surface or fruits in the water as baits (Streito *et al.* 2002, Themann *et al.* 2002).

Thus the need for more reliable approaches has created new methods. For example PCR- techniques used in studies on many *Phytophthora* spp. take advantage of the sequence in the ITS region of the ribosomal DNA or are based on the sequences for nuclear genes such as beta-tubulin or mitochondrial genes such as cytochrome oxidase subunits coxI and coxII and NADH dehydrogenase subunit 5 nad5 (Schubert *et al.* 1999, Nechwatal *et al.* 2001, Grote *et al.* 2000, 2002, Ivors & Garbelotto 2002, Kox *et al.* 2002, Garbelotto 2003, Martin *et al.* 2004).

Fig. 2. Phytophthora *'rot' in apple baits after incubation. Before inoculation small cores were made in raw, green fruits and they were stuffed with tissue taken from a necrotic lesion on diseased plants.*

Alder *Phytophthora*

Symptoms and distribution

During 1993 and 1994 an unusual *Phytophthora* was consistently isolated from bark lesions at the stem bases of dying *Alnus glutinosa* along riverbanks, in orchard shelter belts and in woodland plantations in southern Britain (Brasier *et al.* 1995, Gibbs 1995). Typical for affected trees were abnormally small, yellow and sparse leaves and the presence of tarry or rusty colored exudations on stem lesions. In the following years, the disease was also found on *A. incana* and *A. cordata*, and it has been reported to be present in many countries in Europe: Austria, Belgium, France, Estonia, Germany, Hungary, Italy, Lithuania, Netherlands and Sweden (Gibbs *et al.* 2003). Field studies showed that it might be locally very damaging and an easily spreading disease.

Origin and variants

The microbe behind the disease is a group of heteroploid hybrids. Nucleotide sequence of the ITS-region and amplified fragment length polymorphism (AFLP)-analysis of total DNA have shown that the parents of these hybrids are probably *P. cambivora* and *P. fragariae* (Brasier *et al.* 1999). The hybrid variants (standard, Swedish, German, Dutch and UK) differ in their chromosome numbers (n=11–22), oogonial and antheridial morphology, oospore viability and colony characters. The origin of different variants may be the breakdown products of the first isolated standard hybrid or products of subsequent back-crosses or inter-crosses (Brasier *et al.* 1999, 2004a). However all variants seem to be relatively host specific pathogens of alders (Gibbs *et al.* 2003). The most aggressive are the standard- and Dutch-type variants. Recently the standard-type was described as *P. alni* subsp. *alni* and the Swedish variant as *P. alni* subsp. *uniformis*. Although the German, Dutch and UK variants have shown phenotypic diversity, they have identical ITS-profiles and thus they have been grouped together as *P. alni* subsp. *multiformis* (Gibbs *et al.* 2003, Brasier *et al.* 2004a).

Phytophthora ramorum

Morphology and distribution

In 2001 *Phytophthora ramorum* associated with twig blight disease in *Rhododendron* and *Viburnum* in Germany and Netherlands was described as a new species (Werres *et al.* 2001). This heterothallic *Phytophthora* was first characterized by abundant production of chlamydospores and elongate, ellipsoid, deciduous sporangia. Oogonia with amphigynous antheridia were produced by parings with *P. chryptogea* representing mating type A2 (Werres *et al.* 2001). Later the same pathogen was found to be responsible for the Sudden Oak Death disease (SOD) of *Quercus* and *Lithocarpus* spp. in California (Rizzo *et al.* 2002). The disease was first discovered on *Lithocarpus* spp. near Mill Valley in 1995. Since that time, it has spread throughout coast counties around the San Franscisco Bay area and numbers of *L. densiflorus*, *Q. agrifolia*, and *Q. kelloggii* have died (Rizzo *et al.* 2002, Davidson *et al.* 2002, 2005). Later the pathogen has been found in Oregon, Washington, and British Columbia (Anon 2003, Davidson *et al.* 2005, Hansen *et al.* 2003a). Recent findings of *P. ramorum* in North American nurseries and in trees in Europe have shown that the pathogen is a real threat to forests in both continents (Anon 2004a,b, 2005).

In the course of time *P. ramorum* has been found in many European countries: Germany, Netherlands, Belgium, Denmark, Ireland, Italy, France, Norway, Slovenia, Spain, Sweden, Switzerland, the UK and Poland (Werres

et al. 2001, Delatour *et al.* 2002, Moralejo & Werres 2002, Orlikowski & Szkuta 2002, De Merlier *et al.* 2003, Heiniger *et al.* 2004, Zerjav *et al.* 2004). In 2004 the Finnish Food Safety Authority, Evira found *P. ramorum* on *Rhododendron* in one Finnish nursery producing horticultural plants. It was detected by species-specific PCR and identified morphologically (Fig. 3).

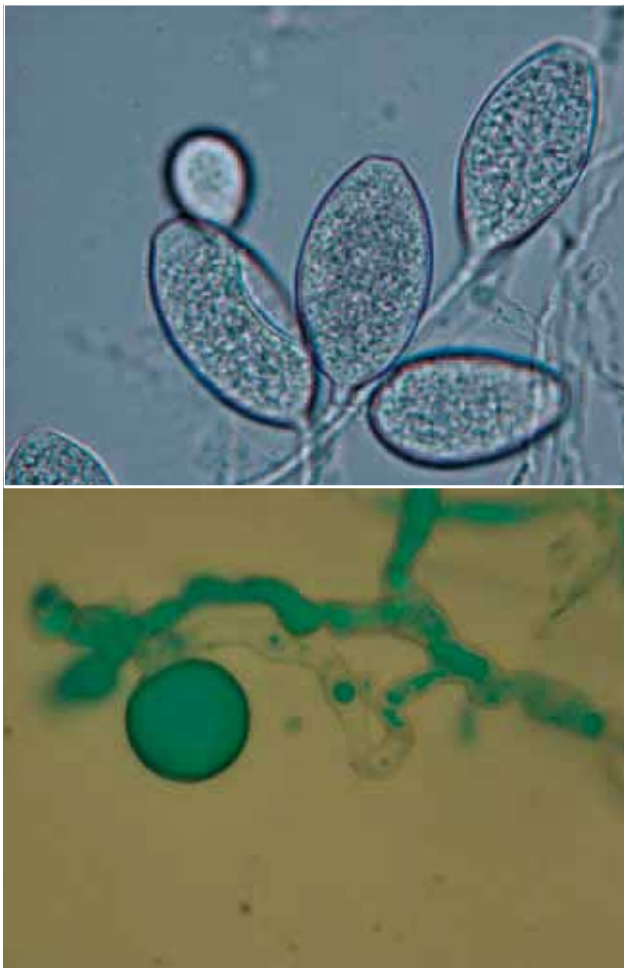

Fig. 3. Sporangia (a), chlamydospores and coralloid hyphae (b) typical for Phytophthora ramorum.

Symptoms and hosts

P. ramorum invades susceptible trees through the bark on which cankers with tarry or rusty colored exudations are developed. Later the leaves of infected trees may turn to brown over a short period (Garbelotto *et al.* 2001). Non-lethal foliar infections on woody shrubs or other hosts in understorey serve as a source of inoculum for trees (Davidson *et al.* 2005). Today over 40 plant genera have been found to be susceptible for *P. ramorum* (Rizzo *et al.* 2005). These include in North America besides *L. densiflorus*, *Q. agrifolia*, *Q. kellogii* and *Q. parvula* var. *shrevei* species such as *Q. chrysolepis*, *Umbellularia californica*, *Sequoia sempervirens, Pseudostuga menziesii, Acer macrophyllus* and *Aesculus californica* . The pathogen was also found on *Vaccinium ovatum, Arbutus menziesii, Arctostaphylos manzanita, Heteromeles arbutifolia, Lonicera hispidula, Maianthemum racemosum, Rhamnus californica, Rosa gymnocarpa, Toxicodendron diversilobatum, Rubus spectabilis, Rhamnus purshiana, Corylus cornuta, Pittosporum undulatum, Trientalis latifolia* (Davidson *et al.* 2002, Goheen *et al.* 2002, Rizzo *et al.* 2002, Knight 2002, Hong 2003, Hüberli *et al.* 2004, 2005, Murphy & Rizzo 2003, Maloney *et al.* 2005). In Europe, *P. ramorum* was first found on *Rhododendron* and *Viburnum*, but later it has also been isolated e.g. from *Arbutus*, *Camellia, Hamamelis, Kalmia, Leucothoe, Pieris* and *Syringa* (Werres & De Merlier 2003, Beales *et al.* 2004a,b). In 2003 the pathogen was found on *Quercus falcata* in the UK, and shortly after on *Fagus sylvatica*, *Quercus ilex*, *Q. cerris*, *Castanea sativa, Taxus baccata* and *Aesculus hippocastanum* (Anon 2004a, Brasier *et al.* 2004b, Lane *et al.* 2004). In the Netherlands infection has also been identified on *Q. rubra* near diseased *Rhododendron*s (Anon 2004b).

Mating type and origin

At first it was believed that the reason why we have not had a same kind of epidemic in Europe than in North America was that different mating types were found in Europe (A1) and in North America (A2). However, in 2003 the occurrence of isolates of *P. ramorum* belonging to A1 and A2 mating types was respectively reported in North America and Europe (Hansen *et al.* 2003a, Werres & De Merlier 2003). The AFLP-fingerprinting clustered European and American isolates separately within individual clades according the mating type (Ivors *et al.* 2004). Also the morphological characters separated the mating types in most cases so that the European isolates were much more homogenous than the North American isolates (Werres & Kamiski 2005). However, the genetic diversity among European isolates was greater than among *P. ramorum* isolates from North America (Brasier 2003, Werres & Zielke 2003, Brasier & Kirk 2004, Ivors *et al.* 2004). The A1 isolates grew faster, had larger chlamydospores and did not produce gametangia with *P. cambivora* (Werres & Kaminski 2005). This might prove that the pathogen was separately introduced into North America and Europe from a third area, which remains unknown, but probably locates in Asia.

Other *Phytophthora* spp.

A new *Phytophthora* species, described few years ago, is *P. inundata*, which infects *Salix* in riparian ecosystems (Brasier *et al.* 2003). It has also other woody hosts as *Aesculus, Olea* and *Prunus*, and might be highly pathogenic after flooding or waterlogging (Brasier *et al.* 2003). The extensive study on oak decline has revealed *P. quercina, P. psychrophila, P. europaea, P. uliginosa* and *P. pseudosyringae* (Jung *et al.* 1999, 2002, 2003). The latter *Phytophthora* was also found in necrotic fine roots and in stem lesions of *F. sylvatica* and *A. glutinosa* (Jung *et al.* 2003). *P. quercina* was the most frequently recovered species from rhizosphere soil near declining oaks in Sweden (Jönsson *et al.* 2003). There was also a correlation between

the presence of the pathogen and the vitality of oak stands (Jönsson *et al.* 2005). *P. nemorosa* is also a newly described species, which was found during an intensive survey on sudden oak death and *P. ramorum* in California and Oregon (Hansen *et al.* 2003b). A similar survey in the UK found *P. kernoviae*, which was isolated most frequently from *F. sylvatica*, but it has also been present on necrotic lesions of *Q. robur* and *Liriodendron tulipifera* (Brasier *et al.* 2005).

In Finland, a new homothallic *Phytophthora* sp. from *Rhododendron* was found to be highly pathogenic to many woody hosts including Norway spruce (Fig. 4).

Fig. 4. Norway spruce seedlings inoculated with a homothallic, unidentified Phytophthora *sp.*

Conclusion

The past decade has shown, that many new *Phytophthora* species are associated with diseased trees. Most of them are not native in the area where they are a serious problem: e.g. *P. ramorum*, the cause of sudden oak death, was introduced separately to North America and Europe. Even old, native species might create through sexual recombination or somatic fusion new combinations with higher pathogenic ability than their parents have. Typical for *Phytophthora* are also hybrids, a new combination produced by parents representing two different *Phytophthora* species, as was in the case of *P. alni*-complex, which has caused changes in riparian ecosystems all around the Europe. The fact that *P. ramorum* is present in large forest area in Oregon shows that the assumption that *Phytophthora* spp. cannot adapt to weather conditions in Nordic countries is not true. Thus we must be ready to prevent the spread of these introduced pathogens. The movement of infected plants should be avoided by strict quarantine regulations and control of all suspicious ornamentals and seedlings.

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