

# Recent developments in *Phytophthora* diseases of trees and natural ecosystems in Europe

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## Introduction

*Phytophthora* diseases of trees is a fast moving field: considerable research progress and further unexpected disease developments have occurred since our last European review of 2001 (see Brasier and Jung, 2003). These developments include accumulating evidence that *Phytophthoras* are spreading from infested nursery stock into forests; advances in the taxonomy and phylogeny of European forest *Phytophthoras*; an upsurge in *Phytophthora* activity on European beech and sycamore; surveys of *Phytophthora* populations on chestnut and oaks; and elucidation of a possible mechanism of pathogenesis of *P. cinnamomi*. Developments on the 'sudden oak death' front include the spread of *P. ramorum* onto trees; progress in assessing the potential host range of *P. ramorum* on European tree species; characterisation of EU and US populations of *P. ramorum* for phenotypes and isozymes; further elucidation of the *P. ramorum* breeding system; and the discovery of another new 'aerial' invasive, *P. kernoviae*, in the UK. Here we review these developments and consider some of their implications.

## Growing biosecurity threat posed by invasive *Phytophthoras*: the spread from nurseries into forests

A growing threat to European forests – and forests around the world – is the introduction and dissemination of non-native *Phytophthoras* by the international plant trade and their spread from nurseries into forests, natural ecosystems or parks and gardens on outplanted nursery and semi-mature tree stock. This problem is exacerbated by only limited commitment from trade and plant health regulators, such as the World Trade Organisation (WTO), the European Community and government agencies, to address a fundamental flaw in current international plant health protocols. Namely that the protocols are based on lists of named organisms most of which have already 'escaped' from their centres of origin, and fail to cover an estimated 80% of unknown or unescaped threat organisms (Brasier, 2005). The result has been trade loopholes that allow the movement and introduction of previously unknown pathogens, including *Phytophthoras*. The present international situation could reasonably be

considered the institutional promotion of biosecurity risk through lack of adequate recognition of the problem and, therefore, neglect. The problem is further exacerbated by the rapidly expanding international trade in plants, and by a lack of awareness of the risks within the forest and horticultural industries.

The link between *Phytophthora* infested nursery stock and damage to forests in Europe was discussed in our last review (Brasier and Jung, 2003). Since then, further evidence has emerged. Retrospectively it is difficult – indeed often impossible – to provide direct evidence of a link to a nursery or an introduction long after the event. The first 'proof' that an epidemic in a natural ecosystem has been caused by distribution of infested nursery stock has come from an investigation of riparian and forest alder ecosystems (Jung and Blaschke, 2004; and Chapter 14). Other direct evidence has come from studies of disease situations in alder, beech and oak forests in Germany. These examples are covered by the different studies of Schumacher *et al.*, Jung and Hartmann *et al.* in this publication (Chapters 13, 30 and 31).

Circumstantial evidence for the link is provided by (1) the recent spread across Europe via the nursery trade of *P. ramorum* (see below); (2) the apparent spread of *P. ramorum* and *P. kernoviae* sp. nov., presumably from outplanted rhododendrons or other nursery stock, on to trees in Cornwall, UK and in the Netherlands; (3) ubiquitous infestation of beech seedlings grown in nursery fields in Germany with *P. cambivora*, *P. citricola*, *P. cactorum* and *P. gonapodyides* (Jung *et al.*, 2005; Jung, 2005 and Chapter 30); (4) a sudden increase in *Phytophthora* diseases of tree nursery stock in the 'new' EU countries such as Poland since these countries were exposed to the European Community nursery trade (L.B Orlikowski, personal communication; Orlikowski *et al.*, Chapter 26); (5) the recent appearance of *P. lateralis* on bare rooted *Chamaecyparis* nursery stock in the Netherlands following the first European records of this pathogen in France in the late 1990s.

The accumulated evidence for the process is, therefore, overwhelming. The threat it poses to our forests, natural ecosystems and public spaces is unpredictable and open ended: once an invasive *Phytophthora* is introduced into a new ecosystem it may never be eradicated. A consequence may be evolution of new

diseases and, via interspecies gene exchange, emergence of new or modified pathogens such as the new hybrid alder *Phytophthora*, *P. alni* (cf. Brasier, 2001; Brasier *et al.*, 1999, 2004a). These risks are likely to be further exacerbated by climate change (cf. Brasier, 2000; Brasier and Scott, 1994). The spread of *Phytophthoras* by the international nursery trade therefore needs to be addressed as a matter of urgency by European and other international plant health agencies and by the forest nursery and plant trade itself.

## Taxonomy and phylogeny of *Phytophthora* pathogens of trees

In our last review (Brasier and Jung, 2003) many new *Phytophthora* taxa on trees, including undescribed taxa, were reported for Europe. Table 1 shows the current taxonomic and ecological status of each taxon. Some have now been formally named and described. Others have been further characterised and given an informal designation, e.g. *P.* taxon Pgchlamydo. One, *P. pseudosyringae* sp. nov., a rare species in Europe, has now turned up more widely on tanoak (*Lithocarpus*) trees in California and Oregon.

**Table 1** New *Phytophthora* taxa in Europe listed at Albany in 2001: status in 2004.

Taxon and status in 2001	Host, ecology, distribution, identified by and date	Status in 2004
<b>Alder <i>Phytophthoras</i></b>	Alder, swarm of species hybrids, Europe. FR, UK 1990s.	Now formally named as <i>P. alni</i> subsp. <i>alni</i> , subsp. <i>uniformis</i> and subsp. <i>multiformis</i> (Brasier <i>et al.</i> , 2004a). Spreading. Threat to North America, elsewhere?
<b><i>P.</i> taxon Pgchlamydo</b>	<i>Prunus</i> , Douglas-fir, wet site, Europe, North America. FR, UK 1970s.	Now informally designated (Brasier <i>et al.</i> , 2003). Reported from Argentina (cedar decline – A.G. Greslebin, personal communication) and Germany (nursery alder plants – Jung and Blaschke, 2004; mature beech – Jung, Chapter 30).
<b><i>P.</i> taxon Riversoil</b>	Forest/riverbank soil UK. FR, UK 1990s.	Now informally designated (Brasier <i>et al.</i> , 2003). No new records.
<b><i>P.</i> sp. O-group</b>	Trees/shrubs ( <i>Aesculus</i> , <i>Salix</i> , <i>Olea</i> ), after flooding Europe, South America. FR, UK 1970s.	Now formally named as <i>P. inundata</i> sp. nov. (Brasier <i>et al.</i> , 2003). No new records.
<b><i>P. quercina</i> sp. nov.</b>	Oak fine roots, ubiquitous, central northern Europe. IFB, Freising 1990s.	Further observations in Sweden (Jönsson <i>et al.</i> , 2003, 2005) and Serbia (T. Jung and M. Glavendekić, unpublished).
<b><i>P. uliginosa</i> sp. nov.</b> (then in press)	Oak soil, rare, central Europe. INRA France/IFB, Freising 1990s.	Now formally named (Jung <i>et al.</i> , 2002). No new records.
<b><i>P. psychrophila</i> sp. nov.</b> (then in press)	Oak soil rare, central Europe. IFB, Freising 1990s.	Now formally named (Jung <i>et al.</i> , 2002). New records from beech in Germany.
<b><i>P. europaea</i> sp. nov.</b> (then in press)	Forest soils localized, France, Germany. INRA/IFB Freising 1990s.	Now formally named (Jung <i>et al.</i> , 2002). Since recorded aeriually on <i>Lithocarpus</i> in California and oak soil in eastern USA.
<b><i>P. ramorum</i> sp. nov.</b> (then in press)	'Sudden Oak Death <i>Phytophthora</i> ' <i>Rhododendron</i> and <i>Viburnum</i> , Germany, Netherlands, Poland. BBA, Braunschweig 1990s.	Now formally named (Werres <i>et al.</i> , 2001). Many new developments.
<b><i>P.</i> taxon Oaksoil</b>	Oak soil, rare, France. INRA, Nancy 1990s.	Now informally designated (Brasier <i>et al.</i> , 2003). No new records.
<b><i>P.</i> taxon Forestsoil</b>	Forest soil (e.g. oak, <i>Carpinus</i> ), rare, France. INRA, Nancy 1990s.	Now informally designated (Brasier <i>et al.</i> , 2003). No new records.
<b><i>P.</i> taxon Pseudosyringae</b>	Oak roots, Europe, North America. IFB, Freising 1990s.	Now formally named <i>P. pseudosyringae</i> sp. nov. (Jung <i>et al.</i> , 2003). Recently found on stems of <i>Lithocarpus</i> in California and Oregon; and causing root rot and bleeding lesions on beech in Italy and Germany (Motta <i>et al.</i> , 2003; Diana <i>et al.</i> , Chapter 33; Hartmann <i>et al.</i> , Chapter 31; Jung <i>et al.</i> , 2005; Jung, Chapter 30).
<b><i>P. italica</i> sp. nov.</b>	Myrtle, Italy. IPV, Palermo 1990s.	No new records? (Cacciola <i>et al.</i> , 1996).

FR, UK: Forest Research, Farnham, UK; IFB: Institute of Forest Botany, Freising, Germany; INRA: INRA Nancy, France; BBA: Braunschweig, Germany; IPV: Instituto di Patalogia Vegetale, Palermo, Italy.

Also, since the 2003 review, three more new taxa have been recognised (Table 2): *P. taxon Salix soil*, *P. kernoviae* sp. nov. and *P. taxon G* (*'P. gallica'*). The potential significance of *P. kernoviae* is discussed later in this chapter. Additional information on the distribution of *P. taxon Salixsoil* is presented by Nechwatal and Mendgen (Poster 27).

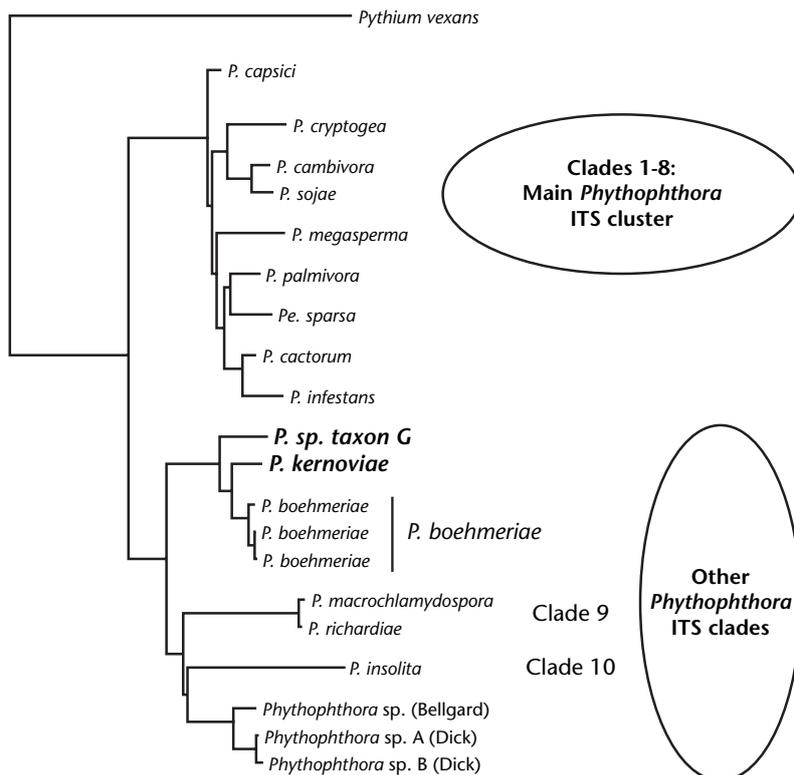
Based on their ITS sequences *P. kernoviae* sp. nov. and *P. taxon G* fall outside the main *Phytophthora* ITS cluster described by Cooke *et al.* (2000). Along with *Phytophthora* ITS clades 9 and 10 they comprise part of a distinct and apparently more ancient set of lineages (Figure 1). *P. kernoviae*'s probable nearest relative, with which it also shares some morphological similarities, is *P. boehmeriae*. *P. taxon G*, which is morphologically highly dissimilar to *P. kernoviae* and *P. boehmeriae*,

appears to be ancestral to these two species (D. Cooke, C. M. Brasier and T. Jung, unpublished).

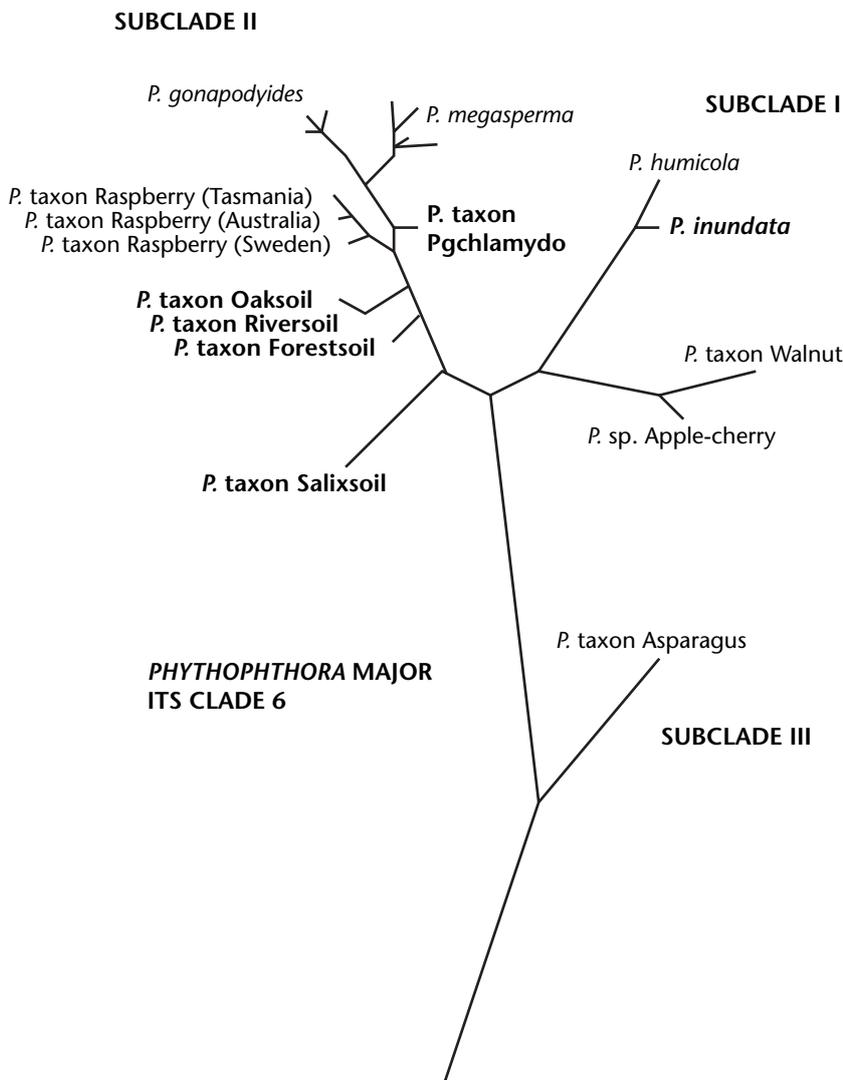
A study of the phenotypes and ITS sequences of a range of unattributable isolates falling within *Phytophthora* ITS Clade 6, which includes *P. gonapodyides* and *P. megasperma*, has led to discrimination of at least seven new taxa within the Clade (Figure 2). Several of these new taxa, including *P. taxon Salixsoil* and *P. taxon Pgchlamydo*, are virtually morphologically indistinguishable from *P. gonapodyides*. Many of these taxa are associated with trees or with riparian ecosystems and many tolerate high temperatures. Species such as *P. gonapodyides*, *P. taxon Pgchlamydo* and *P. taxon Salixsoil* are suggested to be both weak pathogens and litter breakdown and detritus feeders (Brasier *et al.*, 2003).

**Table 2** New *Phytophthora* taxa identified on trees and shrubs in Europe since 2001.

Taxon	Identified by/date	Host, ecology, distribution
<b><i>P. taxon Salixsoil</i></b>	FR, UK 1970s.	Soil around roots of <i>Salix</i> , <i>Viburnum</i> after flooding (UK). <i>Alnus debris</i> (Denmark). Informally designated by Brasier <i>et al.</i> (2003a). Since found associated with reed beds (Nechwatal and Mendgen, 2006) and irrigation streams of nurseries (Jung, unpublished) in Germany.
<b><i>P. kernoviae</i> sp. nov.</b> ( <i>P. taxon C</i> )	FR/CSL, UK 2003.	Papillate aerial, invasive, associated especially with heavy canopy dieback of rhododendron and bleeding lesions on beech. Very local but spreading in Cornwall, southwest England.
<b><i>P. taxon G</i></b> <i>'P. gallica'</i>	LWF, Germany 2003.	Soil around oak roots, northeast France and soil in reed beds of Lake Constance in Germany (T. Jung and J. Nechwatal, unpublished). Very rare?



**Figure 1** Phylogenetic relationship of *P. kernoviae* sp. nov. and *P. taxon G* to other *Phytophthora* species based on ITS sequence (D. L. Cooke, C. M. Brasier and T. Jung, unpublished). Only representative species are shown for major clades 1–8 within the main *Phytophthora* ITS cluster (for further details see Cooke *et al.*, 2000).



**Figure 2** Phylogenetic relationships of six novel taxa in *Phytophthora* major ITS Clade 6 based on ITS sequences (adapted from Brasier *et al.*, 2003 and Cooke *et al.*, 2000). Novel taxa in bold print. See also Tables 1 and 2.

### Upsurge of *Phytophthora* infections on European beech

A collar and root rot of beech in the UK was described by W. R. Day as being caused by *P. cambivora* and *P. syringae* (Day, 1938). Between the 1930s and 1995 there were few additional records – not even during a marked episode of *Phytophthora* activity on trees in the UK in the 1970s. Since 2000 a major upsurge of bark necrosis of beech in Germany, the UK, Italy, Austria and Sweden has occurred (Jung, 2005 and Chapter 30; Jung *et al.*, 2005; Hartmann *et al.*, 2005 and Chapter 31; Motta *et al.*, 2003; Diana *et al.*, Chapter 33; T. Jung, A. M. Vettraino and A. Vannini, unpublished; Cech and Jung, 2005; C. M. Brasier, A. V. Brown and J. Rose, unpublished; T. Jung, U. Jönsson and B. Nihlgard, unpublished). Symptoms include both collar rots and aerial bleeding lesions. *Phytophthora* spp. involved include *P. cambivora*, *P. citricola*, *P. cactorum*, *P. pseudosyringae* sp. nov. (Table 1) and *P. gonapodyides*; plus the newly invasive *P. ramorum* and *P. kernoviae* (see below). It now seems likely that the early records of *P. syringae* on beech in the UK (Day, 1938) were of the morphologically similar species *P. pseudosyringae* sp. nov.

An unexpected finding in both southern England and Bavaria is that the non-caducous soil and root inhabitants *P. cambivora*, *P. citricola* and *P. gonapodyides* can cause aerial bark lesions on beech. *P. cambivora* and *P. citricola* can form extensive aerial lesions over several metres in length. Those caused by *P. gonapodyides* are usually small and discrete. How inoculum of these soil pathogens becomes ‘aerial’ is unknown. Two possible explanations are proposed. One is the transport of inoculum via snails, suggested by Jung (2005 and Chapter 30). This hypothesis is supported by observation of oospores in fresh exudates from *P. citricola* lesions on beech; and by the observation that various species of snail feed on the exudates. Brown *et al.* (see Chapter 32, and Brown and Brasier, 2007) additionally propose that, in the case of *P. cambivora* and *P. citricola*, vertical spread might involve movement of inoculum via embolisms in infected xylem, resulting in linear arrays of island lesions in the bark. For *P. gonapodyides*, they suggest the pathogen may inhabit temporary ponds in major branch forks, and that zoospores in water runoff may infect the bark via epicormic shoots or small wounds.

## Stem bleeding lesions on European sycamore

An outbreak of aerial bleeding lesions on European sycamore, *Acer pseudoplatanus*, is currently occurring in the UK, at least across the southern part of the country. *P. citricola* has been isolated from the bark of almost all the 30 affected trees examined to date (C. M. Brasier, A. V. Brown and J. Rose, unpublished). This appears to be a new European host-pathogen record. It also provides another example of a soil-borne *Phytophthora* with non-caducous sporangia, causing aerial bark lesions. In addition, *P. cambivora* has recently been found causing a collar rot in young plantations of European sycamore in northern Germany (Hartmann *et al.*, 2005).

## Stem bleeding lesions on European horse chestnut

The past few years have also seen a huge upsurge in cases of bleeding lesions on stems of horse chestnut (*Aesculus hippocastanum*) (see Brasier and Strouts, 1976) in the Netherlands, Germany, Austria, Belgium and the UK. In both the Netherlands and UK the disease is epidemic: tens of thousands of trees have died. In Germany, Austria and the UK a small proportion of these trees have been confirmed to be infected by *P. cactorum*, *P. citricola* or *P. cambivora* (Werres *et al.*, 1995; Jung and Blaschke, 1996; Jung, unpublished; T. Cech and Y. Balci, unpublished; Webber, Chapter 27). However in the Netherlands and the UK, many isolation attempts as well as Elisa and PCR tests failed to detect *Phytophthora* in necrotic phloem tissue. Further studies in both countries indicate that a bacterium is involved in the majority of recent deaths (H. deGruyter, J. Webber, personal communications; Webber, Chapter 27) probably spreading as a new and invasive organism. The history and status of this disease is discussed in detail by Webber in Chapter 27. In the Netherlands, baiting tests have revealed the presence of *P. citricola*, *P. syringae*, *P. cambivora* and *P. hibernalis* in the rhizosphere of some affected trees, indicating that fine root damage may sometimes be involved in the decline process (T. Jung, unpublished).

## Ink disease of chestnut in southern Europe

Ink disease of sweet chestnut, *Castanea sativa*, caused by the invasive *P. cinnamomi* and *P. cambivora*, brought about devastating epidemics in southern Europe in the 19th and 20th centuries (reviewed by Robin *et al.*, 2006). The disease remains a serious threat to chestnut forests and chestnut cultivation. As part of the EU-funded research project CASCADE II (1999–2006) the *Phytophthora* species associated with the so-called 'ink disease' (collar and root disease) of *Castanea sativa* were investigated by surveying across 35 sites in Italy, France, Greece, Spain and the UK. In addition to *P. cambivora*

and *P. cinnamomi*, these surveys demonstrated the occurrence of five other *Phytophthora* species in the soils of declining chestnut stands, including *P. cactorum*, *P. citricola* and for the first time *P. megasperma*, *P. cryptogea* and *P. syringae* (Vettriano *et al.*, 2001 and 2005). The role of these *Phytophthoras* in the chestnut ecosystems has yet to be understood. Interestingly, *P. cinnamomi* was found only at sites where no soil frosting occurred, consistent with the predictions of the CLIMEX modelling study of *P. cinnamomi* activity in Europe by Brasier and Scott (1994).

Also within CASCADE II, genetic variation of susceptibility to *P. cambivora* in 23 populations of *C. sativa* was investigated in the same five EU countries (Robin *et al.*, 2006). Susceptibility was measured as lesion length in inoculated excised shoots of mature trees and by root inoculation of seedlings from the same material. Extensive variation in susceptibility was found within and between the different adult tree populations. The authors concluded there was a large amount of genetic variation in resistance. One or more resistant trees were found in 15 of the 25 populations. Lesion lengths in parent trees and percent infection of seedling taproots was not highly correlated. Similar results were found for *Alnus glutinosa* and *P. alni* ssp. *alni* using excised shoot inoculation tests (Jung and Blaschke, Chapter 14).

## Deciduous oak decline across Europe

Several *Phytophthora* species are associated with the current and widespread decline of the deciduous oaks *Q. robur* and *Q. petraea* across central and northern Europe and a number of studies have shown that decline is accelerated or exacerbated by their presence. The biology and distribution of the associated *Phytophthoras* was outlined in our last review (Brasier and Jung, 2003; and see Jung *et al.*, 1999, 2000, 2002, 2003). These studies have since been expanded to Serbia, Turkey, Sweden, Austria and east Germany. Most of the new survey records demonstrate an association of *P. quercina* with oak decline sites (T. Jung and M. Glavendecic, unpublished; Jönsson *et al.*, 2005; Balci and Halmschlager, 2003a,b; Leonhard, 2004). Studies in Sweden by Jönsson and colleagues (Jönsson, 2004; Jönsson *et al.*, 2003, 2005) confirm that *P. quercina* can cause extensive fine root damage of oaks on acidic soils with a mesic water regime (i.e. in the absence of flooding) and in the presence of competitive soil micro-organisms.

## Elucidation of a mechanism of pathogenesis in *P. cinnamomi*

Studies have continued in Portugal and Spain on the association of *P. cinnamomi* with the locally named 'sudden death' and decline of cork oak, *Q. suber*, that began in the early 1980s. An elegant investigation into

the mechanism of pathogenesis of *P. cinnamomi* on *Q. suber* has been carried out by Coelho and colleagues (see Chapter 19). This indicates that a pathogen-produced toxin and a host anti-host toxin gene are involved. The precise nature of the toxin remains unclear (and is under investigation) but the putative host anti-toxin gene probably produces a cinnamyl alcohol dehydrogenase. If this mechanism were confirmed, it would be a major breakthrough in understanding the mechanisms of pathogenesis in tree Phytophthoras, with potentially wide implications for disease control via resistance breeding or via pathogen genetic manipulation.

In a parallel and also potentially significant study, Horta *et al.* (Poster 16) show that the molecular silencing of the beta-cinnamomin elicitor gene does not affect pathogenicity of *P. cinnamomi* to *Q. suber*. In which case, it is possible that this elicitor gene is not directly involved in pathogenesis.

### Spread of *Phytophthora ramorum* (sudden oak death pathogen) and *P. kernoviae*

Since 2001 there has been a growing concern in Europe about the possible impact of the invasive sudden oak death (SOD) pathogen, *P. ramorum* (Rizzo *et al.*, 2002; Werres *et al.*, 2001). A simple chronology of events in Europe is outlined in Table 3. During 2001, regulatory surveys of rhododendron and viburnum nursery stock were initiated in the UK and in The Netherlands. By 2003 similar surveys had been initiated across the EU. By the end of 2004 at least 12 European countries were known to have infested nurseries or to have received

batches of infested stock. It was also clear that >300 nurseries were infested with *P. ramorum* in the UK alone. Procedures were initiated both in the UK and in The Netherlands for the eradication of nursery infestations.

The first evidence that *P. ramorum* was spreading onto trees from diseased rhododendrons outside nurseries came in October 2003, when bleeding lesions caused by *P. ramorum* were found on stems of American red oaks (*Q. falcata* and *Q. rubra*) in the UK and The Netherlands (Brasier *et al.*, 2004c). This was followed by increasing findings of *P. ramorum* on *Fagus*, *Quercus*, and other genera at several woodland sites in Cornwall, southwest England. Both stem bleeding lesions and foliar infections were found. It is now apparent that *P. ramorum* has been spreading to trees from foliar infections on naturalized and semi-naturalized understorey *Rhododendron ponticum*, a native of Iberia and itself an invasive plant in the UK. By mid-2004 about ten *P. ramorum*-infected *Q. rubra* had been found in The Netherlands (M. Steeghs, personal communication). In the UK, *P. ramorum* has caused bleeding lesions on stems of *F. sylvatica*, *Q. falcata*, *Q. cerris*, *Nothofagus obliqua*, *A. pseudoplatanus* and *Aesculus hippocastanum* (Brasier *et al.*, 2004b; Brown *et al.*, Chapter 28). Foliage of *Q. ilex*, *C. sativa* and *Fraxinus excelsior* is also infected in the field (Denman *et al.*, Chapter 16).

Surveys for *P. ramorum* on trees in Cornwall in November 2003 led to the discovery of another new and invasive *Phytophthora* spreading locally on rhododendrons, and from there on to stems of beeches and oaks. The *Phytophthora* was initially labelled as *Phytophthora* taxon C and is now known as *P. kernoviae*

**Table 3** Chronology of *P. ramorum* in Europe 1993–2004.

- **1993** An unknown *Phytophthora* found on rhododendron nursery stock in Germany (D) and The Netherlands (NL).
- **July 2000** An unknown *Phytophthora* found to be the cause of SOD by scientists in California.
- **September 2000** UK Forestry Commission prepares summary PRA on US SOD *Phytophthora*.
- **November 2000** Link proposed between SOD and the new *Phytophthora* on rhododendron.
- **April 2001** Regulatory surveys of nurseries begin in UK and NL.
- **2001** Werres *et al.* describe new *Phytophthora* found in D and NL nurseries as *P. ramorum* sp. nov.
- **April 2002** First finding of *P. ramorum* on nursery stock in UK – on *Viburnum*; followed by records on *Rhododendron*, *Pieris*, *Camellia* etc.
- **June 2003** Nursery surveys initiated across the EU.
- **June 2004** More than 300 nurseries found with *P. ramorum* in UK.
- **June 2004** Nursery or imported plant infections found in at least 11 other EU countries.
- **October 2003** First finding of *P. ramorum* on *Quercus* spp. (red oaks) in UK and NL, associated with infected rhododendron.
- **November 2003–June 2004** Increasing findings of *P. ramorum* on *Fagus*, *Quercus* and other genera in rhododendron infected woodlands in Cornwall, south-west England.
- **November 2003** Discovery of another new invasive aerial *Phytophthora* spreading on trees at the Cornwall sites.
- **October–December 2004** Trees in Cornwall shown to have both stem bleeding lesions and foliar infections. Most but not all affected trees associated with adjacent infected rhododendron.

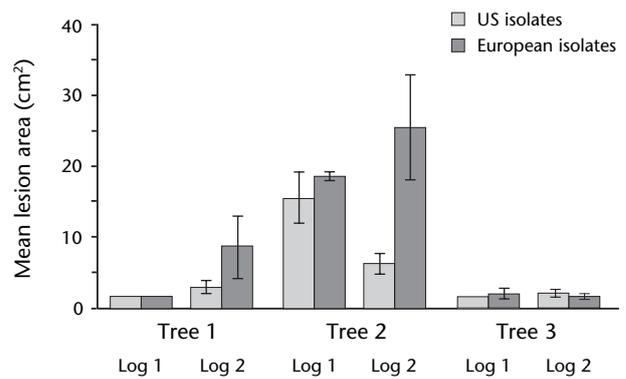
sp. nov. (Brasier *et al.*, 2005). The significance of this development is discussed separately below.

The spread of *P. ramorum* around Europe via the nursery trade has led to increased research activity on the pathogen. Aspects of this research will now be outlined.

### Screening European trees for the risk from *P. ramorum*

Screening of the bark and foliage of European tree species for susceptibility to *P. ramorum* has been carried out in the UK, Spain and The Netherlands, initially with national support and more recently with support from the part-funded EU 'RAPRA' project (2004–06), which covers issues specifically related to pest risk analysis.

In the UK, more than 30 tree hosts have been tested for susceptibility of their phloem or inner bark to *P. ramorum* using the log inoculation method (Brasier and Kirk, 2001) and both European and American isolates of *P. ramorum* (Brasier *et al.*, 2006b). The method involves wound inoculation of 15–20 cm diameter stems with agar culture plugs. The results are summarized in Table 4. Among a number of tree species shown to be potentially at risk (if the fungus can penetrate the bark) were *F. sylvatica*, *Q. rubra* and *Q. cerris*. This is consistent with the finding that these species are becoming infected under field conditions. Pedunculate oak, *Q. robur*, has not so far been found



**Figure 3** Susceptibility of three *Q. robur* trees to *P. ramorum* (two logs per tree). Tree 2 is more susceptible than trees 1 and 3.

infected with *P. ramorum* in the field. *Q. robur* usually falls into a 'less susceptible' category in these tests. However, comparisons of individual *Q. robur* trees (Figure 3) show that some *Q. robur* genotypes fall into the 'more susceptible' category, i.e. there may be significant genetic variation in susceptibility. Parallel studies using zoospore inoculum on unwounded stems showed that zoospores can penetrate intact bark and cause lesions on *F. sylvatica*, *C. sativa*, *Q. rubra* and *Pseudotsuga pseudotsugae*. Zoospores also penetrated the bark of *Q. robur*. No lesions were observed, but the fungus could be reisolated from the phloem below the inoculum zones (C.M.Brasier and S.A.Kirk, unpublished).

**Table 4** Susceptibility of tree stems to *P. ramorum*: summary of UK results up to 2004.

More susceptible <sup>a</sup>	Less susceptible	Resistant
<i>Q. cerris</i> (d, w)	<i>Q. robur</i> (d, w)	<i>Tilia cordata</i>
<i>Q. ilex</i> (e, r)	<i>Q. petraea</i> (d, w)	<i>Carpinus betula</i>
( <i>Q. robur</i> ) (d, w)	<i>Q. suber</i> (e, r)	<i>Populus tremula</i>
<i>Quercus rubra</i> <sup>b</sup> (d, r)	<i>Castanea sativa</i>	<i>Ulmus procera</i>
<i>Fagus sylvatica</i>	<i>Aesculus hippocastanum</i>	<i>Fraxinus excelsior</i>
<i>Rhododendron ponticum</i>	<i>Acer platanoides</i>	<i>Pinus nigra</i>
<i>Eucalyptus</i> sp.	<i>Alnus glutinosa</i>	
<i>Pseudotsuga menziesii</i> <sup>b</sup>	<i>Betula pendula</i>	
<i>Tsuga heterophylla</i> <sup>b</sup>	<i>Prunus laurocerasus</i>	
<i>Picea sitchensis</i> <sup>b</sup>	<i>Taxus baccata</i>	
<i>Chamaecyparis lawsoniana</i>	<i>Pinus sylvestris</i>	
	<i>Picea abies</i>	
	<i>Sequoia sempervirens</i> <sup>b</sup>	
	<i>Abies grandis</i> <sup>b</sup>	

<sup>a</sup> Mean lesion areas for more susceptible, less susceptible and resistant categories c. 20–100 cm<sup>2</sup>, 3–20 cm<sup>2</sup> and < 3 cm<sup>2</sup> respectively after 5 weeks.

<sup>b</sup> North American species.

d: deciduous oak; e: evergreen oak; r: red oak group; w: white oak group.

Tests on susceptibility of tree foliage carried out in the UK indicate that leaves of *Fraxinus excelsior*, *Q. ilex* and *C. sativa* are highly susceptible. Leaves of *F. excelsior* and *C. sativa* may also support significant sporulation by *P. ramorum*, i.e. may potentially spread the pathogen in the field (Denman *et al.*, 2005; Denman *et al.*, Chapter 16). Foliage and stems often show differential susceptibility. For example, bark of *F. sylvatica* and *Q. rubra* is susceptible to *P. ramorum* in the tests, but the foliage of these species is not; whereas foliage of *F. excelsior* is susceptible but its bark is not. A clear distinction therefore needs to be made, when talking about a host, as to whether it is a stem host or a foliar host.

Similar studies have been conducted by E. Moralejo in Spain on the potential stem hosts among Mediterranean tree species using the log inoculation method (summarized in Table 5). In these tests the deciduous oaks *Q. pubescens* and *Q. pyrenaica* were shown to be highly susceptible, *Q. canariensis*, *Q. ilex* and *Pinus halepensis* were rated as susceptible and *Arbutus unedo*, *Q. ilex*, *Q. suber* and *Q. faginea* as less susceptible. Moralejo noted that some species, such as *Q. ilex*, were less susceptible in tests conducted in winter and more susceptible in summer. There is clearly a potential for *P. ramorum* to be damaging to trees in native Iberian vegetation systems, especially perhaps in areas with susceptible understorey shrubs present such as *R. ponticum* or *Viburnum* spp. and a conducive climate.

### Analysis of naturally infected trees at UK field sites

Surveys of aerial stem lesions on trees within *P. ramorum*- and *P. kernoviae*-infested woodlands in Cornwall show that these two invasives are the predominant species present, but other Phytophthoras are also present on the sites. Among these is *P. ilicis*, another aerial *Phytophthora* also believed to be a recent invasive in the UK. *P. ilicis* is causing foliar and stem lesions on several species of holly (*Ilex* spp.). The others are *P. citricola*, *P. cambivora* and *P. gonapodyides*. These three species are established root and collar pathogens

of trees in the UK. All three are usually considered to be soil inhabitants, but, as already mentioned, they have been found causing aerial stem lesions of trees at these survey sites. The presence of at least seven *Phytophthora* species on these sites, with several species sometimes occurring even on the same tree, raises the possibility of hybridization and interspecies gene transfer between them. Further details of these surveys are discussed by Brown *et al.* (Chapter 28).

A wide range of lesion forms and distribution patterns occurs on beech trees infected with *P. kernoviae* or *P. ramorum*. Most infections are associated with the close proximity of diseased rhododendrons, but some infections appear to be associated with water run-off from branch forks, and others can only be explained by inoculum dispersal distances of over 50 m. Isolated single lesions may occur on stems but there is also evidence of massive multiple infection episodes. Lesions may occur from just below ground level to > 11 m up the stem of a tree. Some spread of both pathogens appears to occur within the xylem, resulting in further lesion development above or below the initial bark infection. Some lesions are quickly walled off by the hosts. These may be particularly linked to late season infections. Inner bark lesions produced by *P. kernoviae* appear to 'pump' water from the xylem. Rapid replacement of both Phytophthoras by bark-invading ascomycetes and basidiomycetes may occur (Brown *et al.*, Chapter 32; Brown and Brasier, 2007).

### Enigmatic breeding system of *P. ramorum*

*P. ramorum* is putatively heterothallic (Werres *et al.*, 2001). Thus, recent studies in Europe using A1 and A2 mating type (sexual compatibility type) testers of *P. cambivora*, *P. drechsleri* or *P. cryptogea* *in vitro* have shown European isolates to be almost exclusively of A1 and US isolates to be all of A2 type (excluding European type A1 isolates from Oregon nurseries; Werres and Kaminski, 2005; Werres and Zielke, 2003; Brasier and Kirk, 2004). This difference in the predominant

**Table 5** Susceptibility of tree stems to *P. ramorum* – summary of Spanish results up to 2004 (data courtesy E. Moralejo).

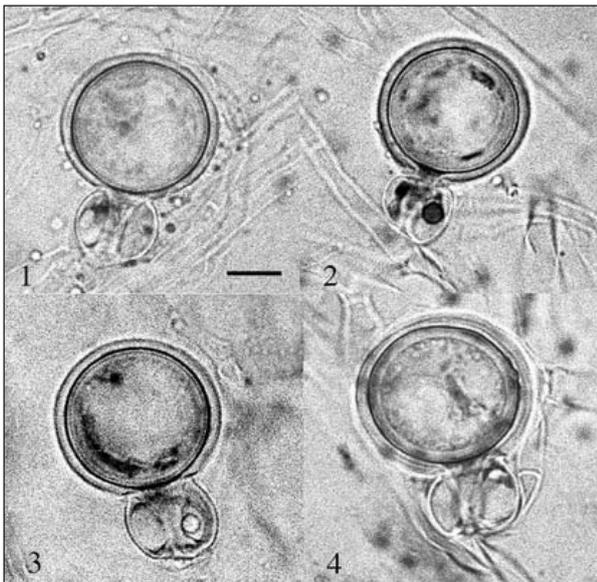
Highly susceptible <sup>a</sup>	Susceptible	Less susceptible	Resistant	Immune
<i>Q. pubescens</i> (d, w)	<i>Q. canariensis</i> (d, w)	<i>Arbutus unedo</i>	<i>P. nigra</i>	<i>Olea europaea</i>
<i>Q. pyrenaica</i> (d, w)	<i>Q. ilex</i> (e, r)	<i>Q. ilex</i> (e, r)	<i>P. pinaster</i>	<i>Fraxinus angustifolia</i>
	<i>Pinus halepensis</i>	<i>Q. suber</i> (e, r)	<i>P. sylvestris</i>	<i>Cupressus sempervirens</i>
		<i>Q. faginea</i> (d, w)	<i>Ilex aquifolium</i>	
			<i>Corylus avellana</i>	
			<i>Acer monspessulanum</i>	

<sup>a</sup> Mean lesion areas: highly susceptible > 100 cm<sup>2</sup>; susceptible 20–50 cm<sup>2</sup>; less susceptible 10–20 cm<sup>2</sup>; resistant < 10 cm<sup>2</sup>; immune, necrotic area not different from control.

d: deciduous oak; e: evergreen oak; r: red oak group; w: white oak group.

compatibility type in Europe and the US also equates to differences in molecular profiles and to differences in behaviour of European and American isolates (see below and Brasier *et al.*, Chapter 39).

However, the production of gametangia in these interspecific pairings is remarkably slow (taking c. 40 days instead of the normal 1–2 days) and their numbers are unusually sparse. Furthermore, pairings of A1 and A2 *P. ramorum* isolates under the same conditions have remained infertile. Recently, a mycelial mixing protocol has been developed which does result in gametangial production between A1 and A2 isolates of *P. ramorum in vitro*; i.e. 'true' gametangia of *P. ramorum* can now be obtained rather than potential interspecific hybrids with other *Phytophthora* species (Figure 4). Their development is considerably faster (7–10 days) but is still sparse and unpredictable (Brasier and Kirk, 2004). Overall, it remains unclear whether *P. ramorum* is truly a regularly A1/A2 outcrossing species or indeed whether its sexual breeding system is functional.



**Figure 4** Four representative gametangia and oospores of *P. ramorum* produced *in vitro* by mixing juvenile mycelia of A1 and A2 isolates (from Brasier and Kirk, 2004).

### Differences between European and American populations of *P. ramorum*

Other research in Europe has revealed behavioural and molecular differences between European (EU) and American (US) isolates of *P. ramorum*. Studies carried out in The Netherlands and the USA by Ivors *et al.* (2004) and Bonants *et al.* (2007, in press) on molecular polymorphism of neutral DNA – both via AFLPs and specific polymorphisms – have revealed small differences between samples of EU and US isolates. These indicate limited but consistent underlying phylogenetic differences, i.e. there is evidence for a low level of phylogenetic divergence of the EU and US populations.

Studies of isozyme profiles in the Netherlands by Man in'T Veld (Poster 33), on the other hand, have not revealed any differences between samples of EU and US isolates, i.e. they provide no evidence of divergence. At the same time, this study has revealed complex and variable banding patterns at the lactate dehydrogenase Ldh-2, Ldh-3 loci that could be an indicator of aneuploidy (Ldh-1) or of non-homologous duplicate genes (Ldh-3). One of several possible interpretations of this phenomenon is that *P. ramorum* has been involved in a reticulation (hybridization) event (W. Man in'T Veld, personal communication).

In a series of gene x environment tests 30 EU and 30 US isolates were compared for variation continuous characters (Brasier, 2003; Brasier *et al.*, 2002, 2006a; and see Chapter 39). EU isolates and US isolates showed similar growth temperature curves across a range of temperatures, indicating conspecificity, but otherwise they showed striking behavioural differences. On average EU isolates grew significantly faster than US isolates although the latter were more variable. EU isolates were also of a uniform wild-type colony type; whereas US isolates included both wild types and a range of morphologically variable non-wild-type colony types that were also developmentally unstable. In pathogenicity tests on the susceptible American red oak *Q. rubra* (log inoculation method; see Brasier and Kirk, 2001), EU isolates were on average significantly more aggressive than US isolates, though the ranges of 10 samples usually overlapped considerably. Werres and Kaminski (2005) have recently reported similar differences in growth rates and colony types between EU and US isolates and greater aggressiveness of EU isolates inoculated onto rhododendron stems.

Taking all the above results into account, Brasier *et al.* (2006a) concluded that the EU and US types should be regarded as adaptively different populations. Also, that if sexual or somatic recombination were to occur between them, further additive allelic variation was likely to be generated. See Chapter 38 for further discussion of this issue.

### Metalaxyl tolerance in *P. ramorum*

Metalaxyl is one of a range of compounds used by nurseries to 'control' *Phytophthora* pathogens. Being fungistatic rather than fungicidal, its main effect is to reduce pathogen development and spread rather than to eliminate the organism. Some nurseries may use it to temporarily suppress visible symptoms on stock. S. Wagner, K. Kaminski and S. Werres (personal communication), working in Germany, have assessed tolerance to metalaxyl-M among 76 *P. ramorum* isolates. Seventy-one of the isolates come from seven different European countries and five isolates from the US. Eighteen (24%) of the isolates were shown to be metalaxyl tolerant. All eighteen were from Europe.

One possible interpretation is that the use of metalaxyl in nurseries has already led to a widespread occurrence of metalaxyl tolerance by *P. ramorum* in Europe. Certainly such a high level of tolerance could reduce the effectiveness of metalaxyl as a 'control' agent. Whether continued use of metalaxyl could result in even higher levels of tolerance is one aspect in need of investigation. Another issue is whether metalaxyl resistant *P. ramorum* isolates are more sensitive to cold winter temperatures and, therefore, their proportion in the population decreases during winter time as reported for *P. infestans* (Kadish and Cohen, 1992; Williams and Gisi, 1992).

### ***Phytophthora kernoviae* sp. nov.: another new invasive pathogen**

In October 2003, during sample surveys for *P. ramorum* on trees in Cornwall, southwest England another previously unknown *Phytophthora*, informally designated *Phytophthora* taxon C, was isolated from a large (> 1 m<sup>2</sup>) aerial bleeding lesion on a mature European beech, *Fagus sylvatica* (Brasier *et al.*, 2006). It has since been formally described as *P. kernoviae* sp. nov (Brasier *et al.*, 2005). Like *P. ramorum*, the new *Phytophthora* has caducous sporangia and is probably aeri ally or splash dispersed. Unlike *P. ramorum*, *P. kernoviae* is inbreeding (homothallic) and has a lower optimum temperature for growth. It is unrelated to *P. ramorum*, falling in an assemblage of *Phytophthora* lineages outside the main *Phytophthora* ITS cluster (see Figure 2).

*P. kernoviae* is currently causing extensive wilting, dieback and mortality of understorey rhododendrons (notably *R. ponticum*) in parts of Cornwall. Indeed, locally it appears to cause a rather heavier dieback in the area than does *P. ramorum*. From its rhododendron base, *P. kernoviae* has spread onto stems and foliage of nearby trees (see Brown *et al.*, Chapter 32). It is especially associated with bark necrosis and bleeding stem lesions on European beech, the lesions usually developing into sunken or erumpent bark cankers. It has also been isolated from similar stem lesions on *Q. robur* and *Liriodendron tulipifera*; and causes foliar necroses of *Magnolia* spp., *Pieris formosa*, *Gevuina avellana*, *Drymis winterii*, *Michelia doltsopa* and leaf and shoot dieback of *Q. ilex* (S. Denman, personal communication). *P. kernoviae* appears to be yet another recent invasive, probably introduced via the specialist plant trade. Suggested geographical origins for *P. kernoviae* include Taiwan, Yunnan in southwest China and the Himalayas (Brasier *et al.*, 2004b).

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