**Phytophthora kernoviae** sp. nov., an invasive pathogen causing bleeding stem lesions on forest trees and foliar necrosis of ornamentals in the UK

Clive M. BRASIER1, Paul A. BEALES2, Susan A. KIRK1, Sandra DENMAN1 and Joan ROSE1

1Forest Research Agency, Farnham, Surrey GU10 4LH, UK.
2Central Science Laboratory, Sand Hutton, York YO41 1LZ, UK.
E-mail: clive.brasier@forestry.gsi.gov.uk

Received 25 February 2005; accepted 13 May 2005.

A new *Phytophthora* pathogen of trees and shrubs, previously informally designated *Phytophthora* taxon C, is formally named here as *P. kernoviae*. *P. kernoviae* was discovered in late 2003 during surveys of woodlands in Cornwall, south-west England, for the presence of another invasive pathogen, *P. ramorum*. *P. kernoviae* is self-fertile (homothallic), having plerotic oogonia, often with distinctly tapered stalks and amphigynous antheridia. It produces papillate sporangia, sometimes markedly asymmetric with medium length pedicels. Its optimum temperature for growth is $ca$ $18^\circ C$ and upper limit $ca$ $26^\circ C$. Currently, *P. kernoviae* is especially noted for causing bleeding stem lesions on mature *Fagus sylvatica* and foliar and stem necrosis of *Rhododendron ponticum*. *P. kernoviae* is the latest of several invasive tree *Phytophthoras* recently identified in the UK. Its geographical origins and the possible plant health risk it poses are discussed.

**INTRODUCTION**

In the early 1990s various developments led to rising concern about the threat posed by invasive *Phytophthora* pathogens to European forests (Brasier 1999, 2000a, Brasier & Jung 2003). These included the association of *P. cinnamomi* with cork oak mortality in Iberia, the spread of the new hybrid, *P. alni* subspecies on alder (Brasier et al 2004a) and evidence that several Phytophthoras, including the newly recognized *P. quercina*, were associated with deciduous oak declines across northern and central Europe (Jung, Blaschke & Oßwald 2000). This concern was heightened when another new and invasive pathogen, *P. ramorum*, the new *Phytophthora* was causing widespread foliar necrosis and shoot dieback of the often dense understory rhododendrons. Also like *P. ramorum*, the new *Phytophthora* had caducous sporangia and was probably aerially or splash dispersed. It was subsequently found to be infecting other trees and shrubs in the locality, including stems of *Quercus robur* and foliage of *Magnolia* and *Pieris*. The new *Phytophthora* was informally designated *Phytophthora* taxon C and referred to as such in preliminary publications (e.g. Brasier et al. 2004b, Sansford, Brasier & Inman 2004).

*P. taxon C* exhibits a unique combination of behavioural and morphological properties including its breeding system, gametangial morphology, sporangial morphology, growth-temperature relationships and colony patterns. Its (ITS) rDNA sequence is also unique, and unrelated to that of *P. ramorum* (David E. L Cooke & Kelvin J. D. Hughes pers. Comm.). Information on phenotypic variation in *P. taxon C* is necessarily limited, since available isolates come mainly from a very small area of Cornwall. However, *P. taxon C* may present a threat to forests and natural...
**MATERIALS AND METHODS**

SMA + MRP medium was prepared as for the *Phytophthora* minimal medium (SMA medium) of Elliott *et al.* (1966) and then amended before autoclaving with 0.5 ml of a 4% MBC (benomyl hydrochloride) solution. The pH was adjusted to 6.5 with 1 M NaOH. After autoclaving at 121 °C for 15 min the agar was cooled then further amended with 0.4 ml of a 2.5% suspension of Pimaricin and 3 ml of a 1% w/v solution of Rifamycin SV. Carrot agar (CA) was prepared as described by (Brasier 1967, Erwin & Ribeiro 1996).

*P. kernoviae* (P. taxon C) was isolated from necrotic inner bark or leaf lesions onto plates of SMA + MRB. For each sample, 20 pieces of tissue, 10 per 9 cm Petri dish, were incubated at 20 °C in darkness. Resulting colonies were subcultured initially to a fresh SMA + MRB plate and from there onto CA. Origins and collection accession numbers of the principal isolates studied are shown in Table 1. Stock cultures for experimental purposes were maintained on CA plates at 20 °C in darkness and subcultured at 4 wk intervals.

Growth rate and colony morphology tests were carried out in 9 cm Petri dishes containing 20 ml of CA. A 5 mm diam inoculum plug from the edge of a 2–3 d culture was placed in the centre of the plate and two colony diameters were measured at right angles after 3 or 7 d. There were either two or three replicate plates per isolate. Colony morphology was assessed after 10 d in the dark at 20 °C.

Gametangia were measured from colonies on CA 10 or more days old. Small pieces of colony ca 3 × 3 mm were removed to a glass slide, a drop of lactic acid cotton blue and a coverslip added and the slide gently warmed until the agar was soft. The coverslip was then pressed firmly down to remove excess agar and 20 mature, well formed gametangia were measured for each isolate.

To produce sporangia, 1 cm diam plugs from the edge of an actively growing colony on CA were transferred to unsterile pondwater and incubated at 20 °C in darkness. After 15–18 h the length, breadth and pedicel length of 20 mature sporangia were measured for each isolate.

Pathogenicity to beech, *Fagus sylvatica*, was initially tested in a quarantine chamber by wound inoculating 50 cm × 16 cm diam fresh cut stems; a second test was performed on larger (1.2 m × 18 cm diam) logs. In both tests 10 mm plugs of *P. taxon C* colonies grown on CA and similar plugs of plain CA as controls were used according to the method of Brasier & Kirk (2001). In each case the stems were incubated for 3 wk at 20 °C before being destructively sampled to assess the extent of developing lesions in the inner bark. Pathogenicity to foliage of *R. ponticum* was tested by dipping the apical end of detached unwounded leaves into a...
zoospore suspension of \textit{P.} taxon C. Lesion formation was assessed and confirmed by re-isolation after 8 d.

**TAXONOMY**

\textbf{Phytophthora kernoviae} Brasier, Beales & S. A. Kirk, \textit{sp. nov.}

\textit{Etym.}: from ‘Kernow’, the Cornish noun for Cornwall.

\textit{Phytophthora kernoviae} differt ab aliis speciebus papillatis per suam combinationem characterum; systema sexus homothallica; oogonia saepe cum base attenuata, diam. in medio ca 21–28 \textmu m; antheridia semper amphigynosa, in medio ca 10–14\times 9–12 \textmu m; sporangia papillata, saepe cum vacuola conspicua, decidua cum pedicellulibus brevibus (in medio ca 5–19 \textmu m); longitudo et altitudo sporangiorum ca 34–52\times 19–31 \textmu m; temperaturae crescentiae in agaro ‘carrot agar’, optima ca 18 °C (incrementum radiale ca 3.8–4.6 mm per diem) et maxima ca 26 °C. Growth rate at 20 °C in darkness ca 3.8–4.6 mm d\textsuperscript{-1} (mean 4.2 mm d\textsuperscript{-1}).

\textit{Homothallus, gametangia} usually frequent to abundant after 10 d on CA (Figs 9–17). \textit{Oogonia}, diam range of means (4 isolates, Table 1) 23.5–25.5 \textmu m, common range ca 21–28 \textmu m; often with tapered stalks (Figs 10–14, 16). \textit{Antheridia} amphigynous. Antheridial length \times width range of means ca 11.5–12.5\times 10–10.5 \textmu m, common range ca 10–14\times 9–12 \textmu m. \textit{Oospores} plerotic, diam range of means ca 21.1–22.5 \textmu m, common range ca 19–25 \textmu m; wall thickness average ca 3.5 \textmu m, common range 3.5–5 \textmu m.

\textit{Sporangia} occasional on CA in the light. Produced abundantly on CA plugs immersed in unsterile pond water or soil leachate; with sympodial sporangio-phores. Papillate, caducous, from regular ovoid or limoniform (Figs 18–22) to distinctly asymmetrical or ‘mouse-shaped’ with one rounded and one flatter side (Figs 23–26). Most have a conspicuous vacuole. Sporangial length \times width range of means (4 isolates, Table 1) ca 38.5–45.5\times 22.5–27 \textmu m, common range ca 34–52\times 19–31 \textmu m. Length:width ratio average ca 1.5 \textmu m. Sporangial pedicels range of means ca 8.6–14.1 \textmu m, common range ca 5–19 \textmu m. Hyphae sometimes denticulate or tuberculate. No chlamydospores observed.

\textit{Other representative cultures examined}: IMI 393171, IMI 393172, IMI 393173, and IMI 1393176 (details in Table 1).


*P. kernoviae* can be distinguished from other homothallic *Phytophthora* species with caducous papillate sporangia and medium length pedicels as follows: from *P. botryosa* and *P. heveae* by its much lower optimum and maximum temperatures for growth (cfr Erwin & Riberio 1996); and from *P. nemorosa* (Hansen et al. 2003) by its higher optimum temperature for growth. It can also be distinguished from *P. meadii*, *P. botryosa* and *P. nemorosa* by its often tapered oogonial stalks; *P. meadii*, *P. megakarya* and *P. nemorosa* by its often asymmetric sporangia; and from *P. boehmeriae* (its possible nearest relative) by its much longer sporangial pedicels.

Currently, in parts of Cornwall, south-west England, *P. kernoviae* often occurs at the same locations and on similar hosts to the semi-papillate caducous species *P. ramorum*. It is easily distinguished from *P. ramorum* by its self fertility (homothallism), longer sporangial pedicels, and lack of chlamydospores. It can also be distinguished readily from another semi-papillate, caducous species on beech in Europe, *P. pseudosyringae* (Jung et al. 2003), by its predominantly amphigynous antheridia and longer sporangial pedicels.

**PATHOGENICITY, DISTRIBUTION, ECOLOGY AND PHYLOGENY**

**Pathogenicity**

Initially, isolate P1553, the first isolate of *Phytophthora kernoviae* to be obtained from bark of a beech tree in the field, was wound inoculated into 50 cm × 16 cm diam fresh cut stems of *Fagus sylvatica*. Following 3 wk incubation at 20 °C, the outer bark was removed and the inner bark examined for lesions. No lesions developed in the control inoculations. Eight wound inoculations with P1553 resulted in necrotic bark lesions of average area 23 ± 3.7 (s.e.) cm². *P. kernoviae* was successfully re-isolated from the margins of the lesions onto SMA + MRB plates.

In subsequent tests, a wider range of isolates were inoculated into larger ca 1.2 m × 18 cm diam stems of *F. sylvatica* and incubated for 3 wk at 20 °C (Brasier & Kirk 2001). In all cases, substantial lesions (ca 34–100 cm²) developed, and the pathogen was successfully reisolated onto selective medium. In pathogenicity tests on detached, unwounded leaves of *Rhododendron catawbiense* cv. ‘Cunningham White’ dipped in zoospore suspensions of isolates P1560 and P1576 (from rhododendron and beech respectively, Table 1), lesions developed within 3–6 d. The pathogen was re-isolated on selective medium.

---

**Figs 9–17.** Representative oogonia, antheridia and thick walled plerotic oospores of *Phytophthora kernoviae*. Compare oogonia with tapered bases (Figs 10–14, 16) with those without this feature (Figs 9, 15, 17). Bar = 10 μm.
Phytophthora kernoviae is especially associated with bark necrosis and bleeding stem lesions above ground level (‘aerial stem lesions’) on European beech, Fagus sylvatica. It has also been isolated from similar lesions on Quercus robur and Liriodendron tulipifera. The lesions often develop into sunken or erumpent bark cankers. *P. kernoviae* is also especially associated with shoot dieback, foliar necroses and wilting of rhododendron, notably *Rhododendron ponticum*. Dieback is often observed on both lower and upper stems. Leaves may abscise rapidly, leading to defoliation. In particularly severe infections, the shrub is killed. *P. kernoviae* also causes foliar necroses of *Magnolia* spp., *Pieris formosa*, *Gevuina avellana*, *Camellia* spp. and *Michelia doltsopa*, and leaf and shoot dieback of *Q. ilex*.

The present known distribution is: local in woodlands in Cornwall, UK, mainly at multiple sites between Redruth and Falmouth, at a site to the south west of St Austell and at another north of Truro. Also at single outlier locations in gardens/nurseries at Swansea (south Wales) and a single nursery in Cheshire (England). Geographical origin unknown.

**Distribution and ecology**

**Phylogeny**

In a study of the ITS rDNA sequence of several *Phytophthora kernoviae* isolates, the closest known sequence match was to *P. boehmeriae* (David E. L. Cooke & Kelvin J. D. Hughes pers comm.). GenBank accession nos. are shown in Table 1. *P. kernoviae* is distinct from *P. boehmeriae*, differing from it by over 50 bp. *P. kernoviae* and *P. boehmeriae* are more closely related to each other and to members of ITS clades 9 and 10, including *P. macrochlamydospora, P. insolita* and *P. richardiae* than to members of the main cluster of Phytophthoras in ITS clades 1–8 (Cooke et al. 2000). *P. kernoviae* is not related to *P. ramorum* which, on the basis of its ITS sequence data, groups with the main *Phytophthora ITS cluster* as a sister taxon to *P. lateralis* and *P. hibernalis* (Martin & Tooley 2003, Ivors et al. 2004).

**DISCUSSION**

Having papillate, caducous sporangia, *Phytophthora kernoviae* appears to be a typical ‘aerial’ *Phytophthora* adapted more for splash or wind dispersal. It is readily self-fertile or ‘homothallic’ in culture, and is therefore adapted more for splash or wind dispersal. It is readily keeping out what may be the many scientifically unknown or ‘unescape’ pathogens (Brasier 2000b, 2001). The potential for the latter is under investigation.

**ACKNOWLEDGEMENTS**

We thank David Cooke and Kelvin Hughes for carrying out ITS sequence analyses of *Phytophthora kernoviae* isolates; Thomas Jung for preparing the Latin diagnosis; and David Slawson for suggesting the epithet ‘kernoviae’.

**Phytophthora kernoviae** sp. nov., an invasive pathogen 858

As with most invasive *Phytophthoras*, the geographical origins of *P. kernoviae*, are unclear. Its temperature – growth relationships, such as its optimum temperature close to 18 ° and upper limit of 26 °, suggest it is adapted to a temperate climate. Other circumstantial evidence, such as its main current host range in the UK of *Eriaceae*, *Fagaceae*, *Magnoliaceae* and its phylogenetic affinity to *P. boehmeriae*, which itself has geographical affinities with China and the western Pacific (cfr Erwin & Ribeiro 1996), could indicate a possible origin in temperate forests of the eastern Himalaya, China or Taiwan (Brasier et al. 2004b). Favoured origins for *P. kernoviae* (and for *P. ramorum* and *P. ilicis*, two other possibly invasive *Phytophthoras* that occur alongside *P. kernoviae* in Cornwall) are Yunnan in south-west China and the Himalayas because of these regions’ historical popularity with plant collectors (Brasier et al. 2004b).

Another possible origin for *P. kernoviae*, because of its association with the proteaceous Chilean hazelnut, *Gevuina avellana*, is Patagonia. These possibilities must, however, be viewed as speculative until more evidence, positive or negative, can be obtained from expeditionary searches in the relevant areas.

Currently, *P. kernoviae* is largely confined to woodlands in a small area of Cornwall, south-west England. There it is causing locally intensive disease on understorey rhododendron, and from light to severe bleeding lesions on stems of mature *F. sylvatica, Q. robur* and *L. tulipifera*. Other isolated occurrences of the pathogen in south Wales and Cheshire, UK, may reflect its further spread from the Cornish sites, via the plant trade. Current plant health control policy is to attempt to eradicate the fungus by destroying understorey rhododendrons in disease management zones, and affected plants in nurseries (Sansford, Inman & Brasier 2004). Should this policy prove unsuccessful, there is a risk that the pathogen could spread to nurseries or forest ecosystems in other parts of Europe, the Americas, southern Africa or Australasia (Brasier et al. 2004b). As with all invasives, the evolutionary potential of *P. kernoviae* outside its natural habitat and probably without its natural enemies, is unknown and open-ended. Possible developments include its intrinsic adaptation to the new hosts and new environments to which it is exposed, or its adaptation through lateral transfer of genes from other *Phytophthoras* (cfr Brasier 2000b, 2001). The potential for the latter is under investigation.

**ACKNOWLEDGEMENTS**

We thank David Cooke and Kelvin Hughes for carrying out ITS sequence analyses of *Phytophthora kernoviae* isolates; Thomas Jung for preparing the Latin diagnosis; and David Slawson for suggesting the epithet ‘kernoviae’.