

## Pathogen profile

***Phytophthora ramorum*: a pathogen with a remarkably wide host range causing sudden oak death on oaks and ramorum blight on woody ornamentals**

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*Phytophthora ramorum* is an oomycete plant pathogen classified in the kingdom Stramenopila. *P. ramorum* is the causal agent of sudden oak death on coast live oak and tanoak as well as ramorum blight on woody ornamental and forest understorey plants. It causes stem cankers on trees, and leaf blight or stem dieback on ornamentals and understorey forest species. This pathogen is managed in the USA and Europe by eradication where feasible, by containment elsewhere and by quarantine in many parts of the world. Genomic resources provide information on genes of interest to disease management and have improved tremendously since sequencing the genome in 2004. This review provides a current overview of the pathogenicity, population genetics, evolution and genomics of *P. ramorum*.

**Taxonomy:** *Phytophthora ramorum* (Werres, De Cock & Man in't Veld): kingdom Stramenopila; phylum Oomycota; class Peronosporomycetidae; order Pythiales; family Pythiaceae; genus *Phytophthora*.

**Host range:** The host range is very large and the list of known hosts continues to expand at the time of writing. Coast live oak and tanoak are ecologically, economically and culturally important forest hosts in the USA. *Rhododendron*, *Viburnum*, *Pieris*, *Syringa* and *Camellia* are key ornamental hosts on which *P. ramorum* has been found repeatedly, some of which have been involved in moving the pathogen via nursery shipments.

**Disease symptoms:** *P. ramorum* causes two different diseases with differing symptoms: sudden oak death (bleeding lesions, stem cankers) on oaks and ramorum blight (twig dieback and/or foliar lesions) on tree and woody ornamental hosts.

**Useful websites:** <http://nature.berkeley.edu/comtf/>, <http://rapra.csl.gov.uk/>, [http://www.aphis.usda.gov/plant\\_health/plant\\_pest\\_info/pram/index.shtml](http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/index.shtml), [http://genome.jgi-psf.org/Phyra1\\_1/](http://genome.jgi-psf.org/Phyra1_1/)

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[Phyra1\\_1.home.html](http://Phyra1_1.home.html), <http://pamgo.vbi.vt.edu/>, <http://pmgn.vbi.vt.edu/>, <http://vmd.vbi.vt.edu/>, <http://web.science.oregonstate.edu/bpp/labs/grunwald/resources.htm>, <http://www.defra.gov.uk/plant/pramorom.htm>, <http://www.invasive.org/browse/subject.cfm?sub=4603>, <http://www.forestry.gov.uk/forestry/WCAS-4Z5JLL>

**INTRODUCTION**

*Phytophthora ramorum* (Werres, De Cock & Man in't Veld) is an exotic pathogen of tanoak (*Lithocarpus densiflorus*), coast live oak (*Quercus agrifolia*) and other tree species as well as a number of woody and herbaceous perennials (Frankel, 2008; Rizzo *et al.*, 2002, 2005; Werres *et al.*, 2001). Broadly speaking, this pathogen causes two kinds of disease although distinctions are not absolute: sudden oak death on oaks in native forests in the USA and ramorum blight on trees and woody ornamentals in forest, nursery and garden environments in Europe and North America (Table 1). The pathogen causes sudden mortality in tanoak and coast live oak in the USA and has been responsible for the rapid decline of forest populations of these species in the California coastal forests and parts of coastal southern Oregon (Rizzo *et al.*, 2002, 2005) (Fig. 1A). This pathogen is also of concern with respect to native and ornamental trees such as southern red oak (*Q. falcata*), American red oak (*Q. rubra*) and European beech (*Fagus sylvatica*) in urban gardens in Europe and heathland plants in the UK (Brasier *et al.*, 2004a,b). In the USA, *P. ramorum* causes bleeding cankers and mortality of coast live oak, Shreve oak (*Q. parvula*) and tanoak and tip blighting and die-back on woody and herbaceous perennials including *Rhododendron* and *Viburnum* (Davidson *et al.*, 2003).

The rapid onset and death of infected tanoaks in Marin County, California, in the early 1990s raised alarm among residents and researchers and led to the term sudden oak death. The cause of the outbreak was not known until 2000 when the pathogen, a

**Table 1** Broadly speaking, two distinct disease symptoms can be observed for infections by *P. ramorum*. Sudden oak death is mainly observed in oak and tanoak forests in coastal forests of California and Oregon, but can also be observed on specimen trees in European or American gardens. Ramorum blight is observed in forest, nursery and garden environments in both North America and Europe on trees, ornamentals and woody shrubs. Sudden oak death is the ecologically and economically important disease in North America.

Disease	Symptoms	Host categories	Typical hosts*	Geography and environment
Sudden oak death	Stem cankers; bleeding cankers	Forest trees; garden trees	Coast live oak, tanoak, European beech	North American forests; European gardens
Ramorum blight	Foliar and twig blight; tip and shoot dieback; leaf blight	Ornamental trees and woody shrubs; forest understorey plants	Viburnum, rhododendron, pieris, lilac; coast redwood, Douglas fir, huckleberry, madrone, yew, tanoak, California bay laurel	European nurseries and gardens; North American nurseries and forest

\*Only a small selection of typical hosts is presented. For a complete list of hosts refer to section on host range for appropriate references.



**Fig. 1** (A) Tanoak mortality near Bolinas Ridge, Mt. Tamalpais, Marin County, California. Photo courtesy: Janet Klein, Marin Municipal Open Space District. (B) Eradication of sudden oak death in Curry County, Oregon, through slash and burn. Photo courtesy: Oregon Department of Forestry.

then unknown *Phytophthora* sp., was isolated from cankers on dying trees (Frankel, 2008; Rizzo *et al.*, 2002). Simultaneously, it was recognized to be the same pathogen causing tip dieback, leaf blight and stem cankers on *Rhododendron* and *Viburnum* in European greenhouse settings (Frankel, 2008; Werres *et al.*, 2001). This discovery prompted an examination of the nursery

stock in the infested areas in California and soon thereafter infected *Rhododendron* were found (Frankel, 2008). This pathogen is currently managed by containment in woodlands in California, the UK and the Netherlands, by eradication in US nurseries and Oregon forests (Fig. 1B), and by quarantine in many other parts of the world. In the USA, the economic impact of losses due to *P. ramorum* is estimated to be in the tens of millions of dollars due to the direct loss of nursery and ornamental crops, the decrease of property values due to dead/dying trees, the cost of monitoring, tracking and eradicating the disease, the societal impact through loss of recreational value and cultural value, and the ecological impact through loss of food resources for native fauna (Cave *et al.*, 2005; Dart and Chastagner, 2007; Frankel, 2008).

## TAXONOMY

### Morphology

*P. ramorum* can be readily identified based on morphology. It is different from other *Phytophthora* species in that it forms large and abundant chlamydospores (Werres and Kaminsky, 2005; Werres *et al.*, 2001). Sporangia are elongate, ellipsoid, semi-papillate and deciduous with a short pedicel, thus resembling *P. palmivora*. However, *P. palmivora* has smaller chlamydospores, papillate sporangia and higher temperature requirements for growth (Werres *et al.*, 2001). Optimal growth for *P. ramorum* occurs at 20 °C over a range of 2–26 °C. *P. ramorum* is heterothallic requiring a compatible response between opposite mating types to form oospores (Brasier and Kirk, 2004; Werres *et al.*, 2001), but oospores are not readily produced in culture and there has been no evidence of oospore formation reported in nursery settings where both mating types of the pathogen have been found (Grünwald *et al.*, 2008a).

### Phylogenetic context

Although *Phytophthora* species resemble fungi morphologically, based on their hyphal growth patterns and nutritional requirements, they differ from true fungi in being diploid through much

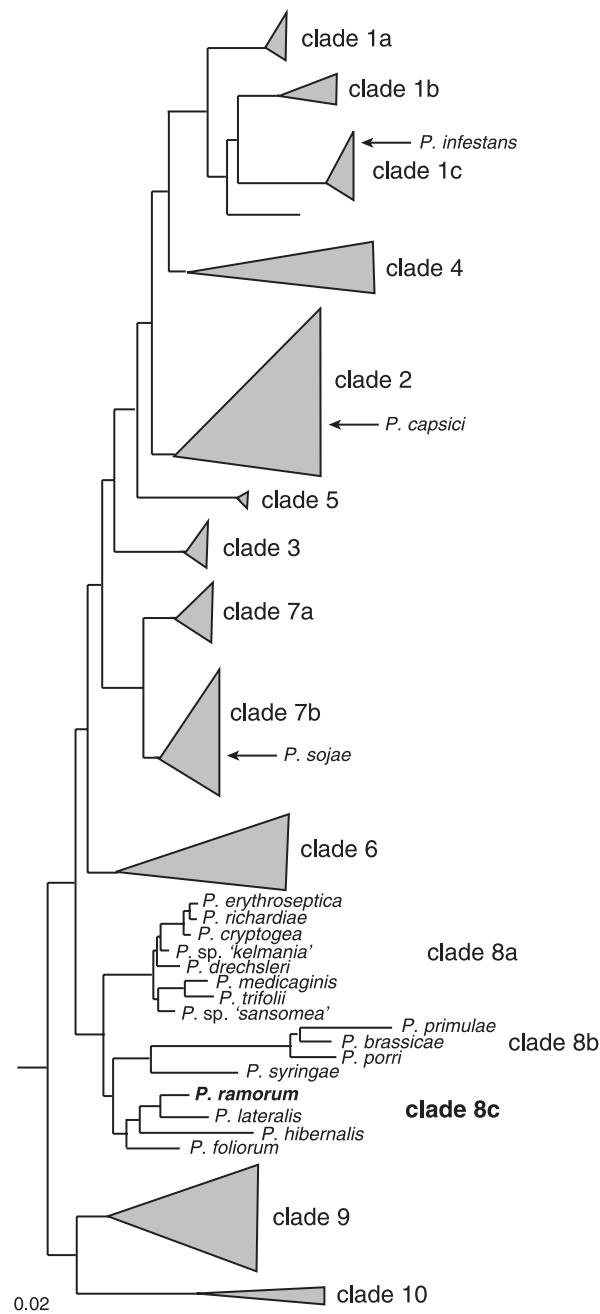
of their life cycle and in producing swimming zoospores. Rather, they are taxonomically placed within the kingdom Stramenopila (Baldauf, 2003; Förster *et al.*, 1990), a distinct eukaryotic branch that includes golden-brown algae, diatoms and brown algae. Furthermore, analyses of the *P. ramorum* and *P. sojae* genome sequences revealed genes of apparent plant origin, which suggests that the ancestor of oomycetes and other Stramenopiles was photosynthetic (Tyler *et al.*, 2006).

Within the genus *Phytophthora*, *P. ramorum* falls into clade 8c (Blair *et al.*, 2008; Cooke *et al.*, 2000; Martin and Tooley, 2003). Clade 8 is the second most basal *Phytophthora* group (Fig. 2) (Blair *et al.*, 2008). *P. ramorum* shares clade 8c with *P. lateralis*, *P. hibernalis* and *P. foliorum*; all except *P. hibernalis* are thought to be exotic in their known ranges (Donahoo *et al.*, 2006; Erwin and Ribeiro, 1996; Ivors *et al.*, 2004). Given that these four species are fairly genetically distant from each other, that their centres of origin are unknown and that new *Phytophthora* species are continually being discovered, it is likely that the closest relative of *P. ramorum* is unknown to science. *P. lateralis* is fairly well supported by molecular phylogenetics as the closest known relative to *P. ramorum*, yet it remains unclear whether *P. hibernalis* or *P. foliorum* is the sister taxon of the *P. ramorum*/*P. lateralis* lineage (Blair *et al.*, 2008; Ivors *et al.*, 2004; Kroon *et al.*, 2004; Martin and Tooley, 2003).

## LIFE HISTORY AND DISEASE BIOLOGY

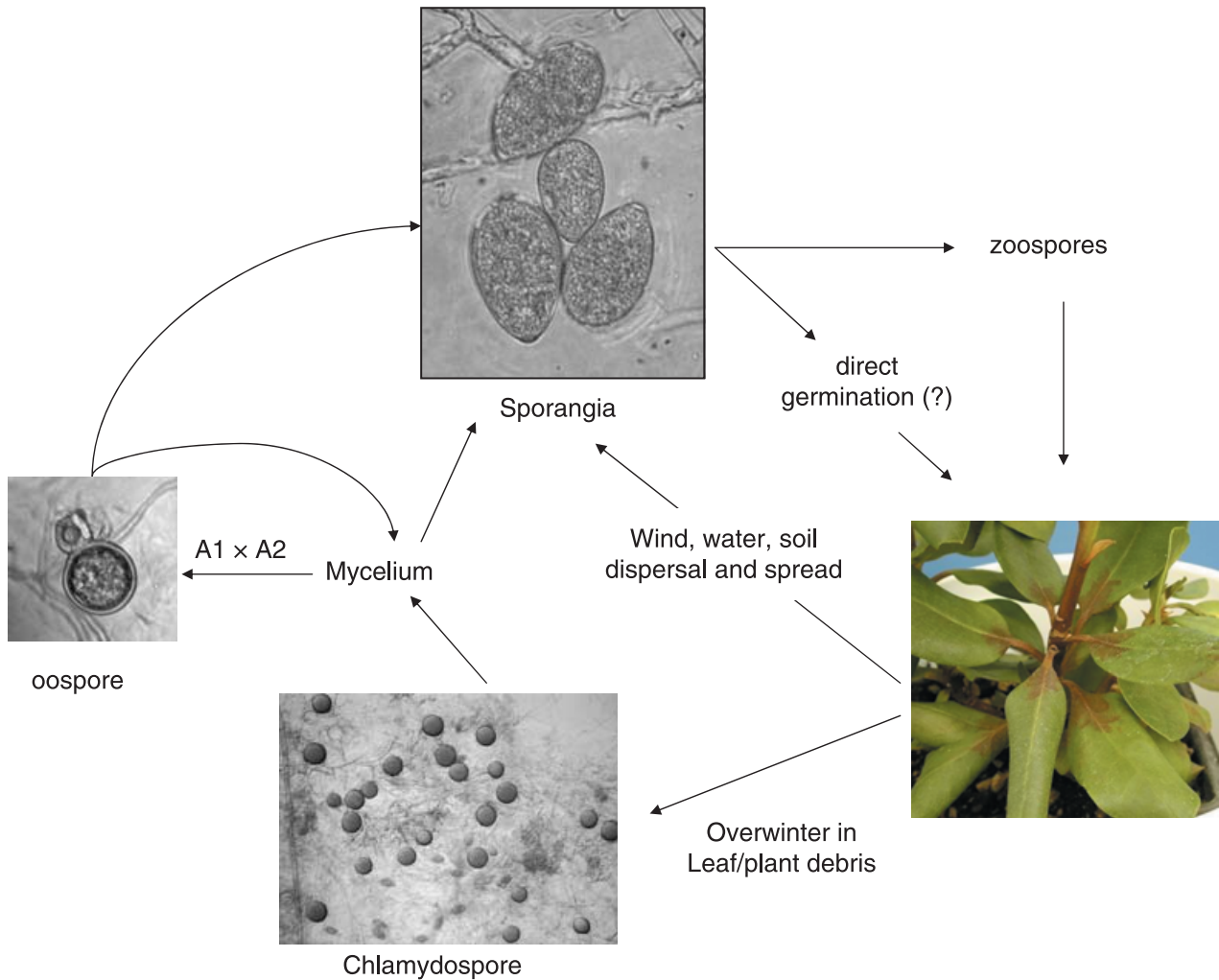
### Natural history

The life cycle of *P. ramorum* is similar to that of other *Phytophthora* species and is broadly outlined in Fig. 3. The *P. ramorum* life and disease cycles are not well understood and are thus a major focus of research, both in natural and in nursery settings. *P. ramorum* produces sporangia on the surfaces of infected leaves and twigs of foliar hosts (Davidson *et al.*, 2005). These sporangia can be splash-dispersed to neighbouring hosts or driven longer distances by wind and rain (Davidson *et al.*, 2005). *P. ramorum* has also been baited from rivers and streams downstream of infested areas (Davidson *et al.*, 2005; Frankel, 2008). Inoculum can additionally be carried on soil or debris attached to boots of walkers, tyres, etc. (Davidson *et al.*, 2005). At the same time, the distribution of *P. ramorum* within infested areas is patchy both in California (Rizzo *et al.*, 2005) and in the UK (Denman *et al.*, 2006a), suggesting some limitations in its ability to colonize new areas. Upon contact with a suitable host environment, it is thought that the sporangia germinate to produce zoospores that encyst, penetrate the host and initiate a new infection (Fig. 3). Direct germination of sporangia has not been documented in *P. ramorum*, but occurs in other *Phytophthora* species (Erwin and Ribeiro, 1996). Although *P. ramorum* is primarily a foliar pathogen, it appears to be able to survive in soil (Fichtner *et al.*,



**Fig. 2** Phylogeny of the genus *Phytophthora* highlighting clade 8. Clade locations of other *Phytophthora* species with published or forthcoming whole genome sequences are also indicated (modified from Blair *et al.*, 2008).

2007; Shishkoff, 2007), and has been shown to infect the roots of *Rhododendron*, colonize the vascular tissue and spread to the stem (Parke and Lewis, 2007). Chlamydospores are readily produced in infected plant material and can serve as resting structures, allowing the pathogen to survive adverse conditions, and may be particularly important for survival in soil (Shishkoff, 2007; Tooley *et al.*, 2008). Oospores are currently missing in the



**Fig. 3** Generic life cycle of *Phytophthora ramorum*, broadly outlining the major life stages. Note that the sexual cycle to date has only been described under controlled conditions in the laboratory and has not been documented in nature (Brasier and Kirk, 2004; Werres and Kaminsky, 2005).

life cycle but have been produced under controlled conditions in the laboratory, although very few and largely unviable oospores are formed in these pairings (Brasier and Kirk, 2004; Werres *et al.*, 2001). Nevertheless, the presence of both mating types in US (Grünwald *et al.*, 2008a) and Belgian nurseries (Werres and De Merlier, 2003) presents a potential for sexual recombination: establishment of sexual reproduction after migration of individuals of opposite mating type from a centre of sexual reproduction has been well documented for *P. infestans* (Drenth *et al.*, 1995; Fry *et al.*, 1993; Grünwald and Flier, 2005).

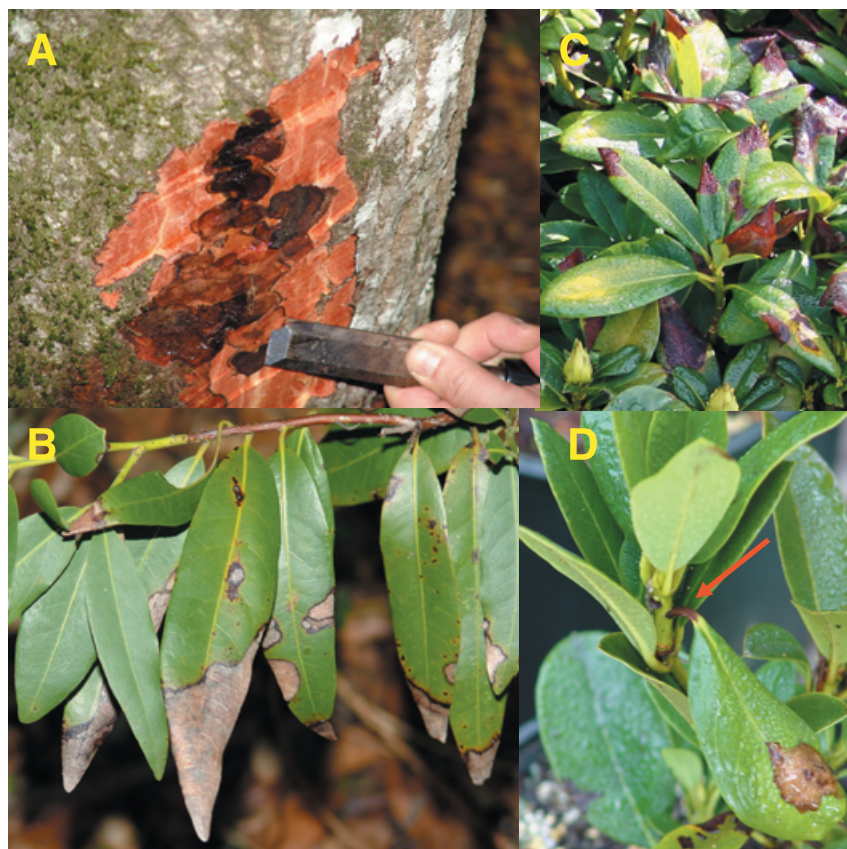
### Disease symptoms

*P. ramorum* causes two symptom classes consisting of the foliar phase (ramorum blight) or the bole canker phase (sudden oak death) that are considered to be separate and distinct (Table 1).

On oaks, tanoaks and European beech, as well as poison oak, *P. ramorum* causes bleeding cankers on the stem. The cankers themselves may only be visible under the cambium after removal of the bark (Fig. 4A). It is these cankers that cause 'sudden oak death' through killing of the inner bark (phloem) and girdling of the tree, resulting in the canopy often rapidly and completely wilting and trees dying even though the plant might have been infected for several years before the apparent 'sudden death'.

*P. ramorum* also causes ramorum blight on foliar and twig hosts. This form of the disease can be evident as a shoot tip dieback or leaf spots (Fig. 4B–D). In forests in the USA, hosts that experience dieback include California bay laurel (*Umbellularia californica*), coast redwood (*Sequoia sempervirens*), Douglas fir (*Pseudotsuga menziesii*), evergreen huckleberry (*Vaccinium ovatum*), Pacific madrone (*Arbutus menziesii*), Pacific yew (*Taxus brevifolia*), Pacific rhododendron (*Rhododendron macrophyllum*) and tanoak.





**Fig. 4** Typical symptoms observed on different hosts of *Phytophthora ramorum*. (A) Bleeding canker of tanoak in the exposed inner bark (phloem) after removal of the outer bark; note typical lesions with clearly differentiated margins; (B) leaf tip necrosis or leaf spots on leaf margins of California bay laurel; (C) rhododendron—arrow marks necrosis of leaf petiole; (D) leaf necrosis on nursery ornamental rhododendron.

Affected shoots may develop discoloration or wilting. Many species have been documented as exhibiting leaf blight in response to *P. ramorum* infection, the most prominent being California bay laurel, tanoak, rhododendron and other Ericaceae species. Lesions on California bay laurel are distinctive as they are often found on the downward pointing tips of leaves where water puddles (Fig. 4B). Leaf infections may also become evident as water-soaked lesions on leaf margins, petioles or tips, rapidly become necrotic, and spread to cover the entire leaf and the shoot. At least two research groups have observed sporulation of *P. ramorum* on asymptomatic leaves (Denman *et al.*, 2006b; Frankel, 2008; Vettraino *et al.*, 2007) and rhododendron roots can be infected from infested potting media while remaining asymptomatic (Parke and Lewis, 2007).

#### Host range and susceptibility

The known host range for *P. ramorum* continues to expand and currently includes over 109 plant species (Denman *et al.*, 2005; Hansen *et al.*, 2005; Tooley and Kyde, 2007; Tooley *et al.*, 2004). An up-to-date list of hosts is maintained by the US Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) ([http://www.aphis.usda.gov/plant\\_health/plant\\_pest\\_info/pram/index.shtml](http://www.aphis.usda.gov/plant_health/plant_pest_info/pram/index.shtml)). Although many nursery hosts are regulated at

the genus level for interstate shipping, as is the case for *Rhododendron* or *Viburnum*, it has now become clear that plants can exhibit a considerable range of susceptibility to *P. ramorum* by species and even by cultivar within species. For example, most *Viburnum* cultivars evaluated for resistance to *P. ramorum* were not susceptible or developed only small lesions (Grünwald *et al.*, 2008b).

Oaks in the red oak group (section *Lobatae*) are taking the brunt of the coastal California epidemic, yet *Quercus* species appear to be a dead-end host for *P. ramorum*. They do not support foliar infections and sporulation has not been observed on stem cankers (Davidson *et al.*, 2005; Garbelotto *et al.*, 2003). Coast live oak (*Quercus agrifolia*) exhibits up to an eight-fold difference in susceptibility among individuals within populations, which may spare some of these trees in coastal populations from *P. ramorum*-induced mortality (Dodd *et al.*, 2005). Tanoaks (*Lithocarpus densiflorus*), by contrast, develop lethal stem cankers and also support sporulation on leaves and twigs. Tanoaks appear to be more susceptible to *P. ramorum* than true oaks (Davidson *et al.*, 2003). *P. ramorum* colonizes the xylem of tanoaks and reduces stem water transport (Parke *et al.*, 2007). In fact, *P. ramorum*, *P. kernoviae* and other *Phytophthora* species have been repeatedly isolated from discoloured xylem of oaks, beech and maples in the UK (Brown and Brasier, 2007), so colonization

of the xylem may be more common than previously thought. In California forests, *P. ramorum* epidemics are driven by California bay laurel, on which *P. ramorum* sporulates prolifically (Davidson *et al.*, 2005). These trees exhibit genetic variation in susceptibility, but this variation is overwhelmed in the field by local environmental conditions, such as temperature and humidity, which ultimately determine disease levels (Anacker *et al.*, 2008). In south-west Oregon, bay laurel is not the key foliar host and producer of inoculum, and instead tanoak itself is a major source of sporangia (Rizzo *et al.*, 2005). In Europe, heavily infected wild *Rhododendron ponticum* in the understorey appears to be responsible for producing inoculum that infects neighbouring tree trunks (Brasier *et al.*, 2004a).

### Management

*P. ramorum* is managed differently in nursery versus forest systems. In Europe, nurseries are under EU directives to prevent the spread of *P. ramorum* within the European Community by eradicating outbreaks, using good phytosanitary practices and employing plant passports for trade in host species. Currently, a passport is required both for movements within and between member states, and additional requirements apply for movements into and within EC Protected Zones. A passport is issued by the authorized and registered grower of the plant material that is also subject to official, annual inspections of this material. Due to market demands it is highly unlikely that the nursery industry will limit its trade to resistant cultivars or species, and therefore the emphasis in managing the pathogen in nurseries is on best management practices and vigilance. In the USA, nurseries in Oregon, Washington, and California that ship host plants out of state are subject to a federal quarantine. When *P. ramorum* is found during routine inspections, a government-regulated protocol must be followed to attempt to eradicate the pathogen.

Sudden oak death in California is managed largely through containment, with a focus on limiting the spread of the pathogen to the north. Individual high-value oaks can be treated with a phosphite fungicide preventively or very early in the course of infection to slow the progress of the disease (Garbelotto *et al.*, 2007). In Oregon, the infested area is geographically limited and a policy of eradication has been pursued, which includes cutting

and burning all hosts within an infected area and surrounding buffer zone and has thus far succeeded in limiting the extent of the epidemic (Frankel, 2008) (Fig. 1B).

## GENETICS AND GENOMICS

### Population genetics

Currently *P. ramorum* is known only in Europe and North America. Populations on both continents are clonal and belong to three distinct lineages (Table 2). Population genetic analyses have shown them to be genetically distinct and asexually reproducing yet clearly conspecific (Ivors *et al.*, 2004, 2006; Prospero *et al.*, 2007). In 2006 there was an informal agreement within the *P. ramorum* research community on designated names for these clonal lineages: EU1, NA1 and NA2. The EU1 clonal lineage was first identified on *Rhododendron* and *Viburnum* in European nurseries (Werres *et al.*, 2001). It is the only lineage found in Europe to date, but it is now regularly found in nurseries on the west coast of the USA (Grünwald *et al.*, 2008a; Hansen *et al.*, 2003; Ivors *et al.*, 2006). Clonal lineage NA1 is responsible for the natural infestations in California and Oregon and many of the nursery infections in North America (Ivors *et al.*, 2006; Prospero *et al.*, 2007). The third clonal lineage, NA2, has a limited distribution. It has only been isolated in a few instances from nurseries in North America (Ivors *et al.*, 2006). All NA1 and NA2 isolates that have been tested are of mating type A2 (Table 2). Interestingly, EU1 is predominately mating type A1 (Brasier and Kirk, 2004; Werres and Kaminsky, 2005), yet rare A2 EU1 isolates have been identified in Belgium (Werres and De Merlier, 2003). It is unclear how the mating type switch occurred in the EU1 lineage as the genetics of mating type are complex and poorly understood. In the case of *P. infestans*, the A1 mating type behaves as a heterozygote and the A2 as a homozygote (Judelson and Blanco, 2005). It is thus possible that the A2 genotypes observed in the EU1 lineage are the product of a mutation to the homozygous state. As already discussed, there is currently no evidence for sexual reproduction in the nurseries in which both mating types are present, yet the levels of heterozygosity observed in *P. ramorum* are consistent with an outcrossing species (Tyler *et al.*, 2006). In addition, sequencing both alleles of several nuclear genes in each

Clonal lineage	Current distribution	Habitat	Mating type	Colony growth	Colony stability	Aggressiveness
EU1	EU, North America	Nurseries	A1 (A2)†	fast	Stable	Higher
NA1	North America	Forests, nurseries	A2	slow	Unstable	Lower
NA2	USA	Nurseries	A2	fast	Stable	ND*

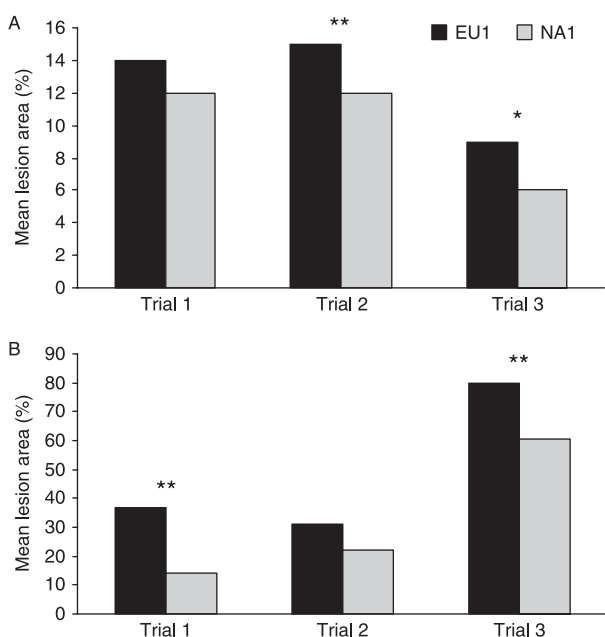
\*ND = not determined.

†EU1 is predominately mating type A1 (Brasier and Kirk, 2004; Werres and Kaminsky, 2005), yet several A2 EU1 isolates have been identified in Belgium (Werres and De Merlier, 2003).

**Table 2** Currently recognized nomenclature and behavioural characteristics of *Phytophthora ramorum* clonal lineages (adapted from Ivors *et al.*, 2006).

clonal lineage reveals that the alleles for each lineage do not cluster together and, in fact, the genealogical relationships among the lineages change with each gene (E. M. Goss and N. J. Grünwald, unpublished data). This suggests that the clonal lineages are descendants of sexually recombining populations.

The three clonal lineages also show phenotypic differences (Table 2). The most notable difference is relative homogeneity among EU1 isolates as compared with phenotypic variation and intrinsic instability in NA1. Instability in NA1 has been suggested by variation in colony morphology and vegetative growth rates among subcultures (Brasier *et al.*, 2006) as well as the observation of abnormally shaped sporangia in single colony segments and between subcultures (Werres and Kaminsky, 2005). On average, EU1 isolates have shown faster growth rates in culture and larger chlamydo-spore size, although the growth rate of NA1 isolates can equal that of EU1 isolates (Brasier, 2003; Brasier *et al.*, 2006; Werres and Kaminsky, 2005). NA2 exhibited a relatively rapid growth rate in culture, similar to EU1 isolates (Ivors *et al.*, 2006). EU1 isolates have been found to produce larger lesions than NA1 isolates on cut stems of *Quercus rubra* (Brasier *et al.*, 2006) and detached *Rhododendron* leaves (Fig. 5), but differences in other measures of fitness were not consistent across experiments. No



**Fig. 5** Difference in aggressiveness of *P. ramorum* clonal lineages EU1 and NA1. (A) Leaf lesion area 10 days post inoculation on two *Rhododendron* cultivars as a function of clonal lineage. The difference between EU1 and NA1 was significant in trials 2 and 3 ( $P < 0.005$  and  $P < 0.05$ , respectively) (V. T. McDonald and N. J. Grünwald, unpublished data). (B) Mean lesion area in wound inoculated lower stems of *Quercus rubra* (data from Brasier *et al.*, 2006). The differences between EU1 and NA1 were significant in trials 1 and 3 ( $P < 0.001$  and  $P < 0.0012$ , respectively).

differences in aggressiveness between the two major lineages were observed on a variety of other host species (Denman *et al.*, 2005; Tooley *et al.*, 2004). Variation in aggressiveness among isolates within both NA1 and EU1 lineages has been documented on coast live oak seedlings, detached bay laurel leaves and *Q. rubra* cut stems (Brasier *et al.*, 2006; Huberli *et al.*, 2006). None of these studies has observed genotype-specific (gene-for-gene-like) variation in virulence.

Amplified fragment length polymorphism (AFLP) and fast-evolving microsatellites (simple sequence repeats) have revealed genetic variation within the lineages, which has allowed for studies of population structure and change. Given the asexual reproduction of these populations, this variation is hypothesized to be the result of mutation or mitotic recombination (Goodwin, 1997). The first AFLP and microsatellite studies found the European population to be more variable than the NA1 population (Ivors *et al.*, 2004, 2006). Conversely, Prospero *et al.* (2007) found microsatellites that are variable within NA1 but not EU1. Yet these loci may be evolving so quickly in NA1 as to be considered unstable, providing an interesting parallel to the phenotypic data. These markers significantly differentiated the nursery and forest populations in Oregon and showed that the genotypic composition of nursery populations had changed from 2003 to 2004 (Prospero *et al.*, 2007). Microsatellite variation has also proven valuable for rapid and accurate diagnosis of clonal lineages and is being used to monitor nursery finds in the USA. There are numerous simple sequence repeats identified in the *P. ramorum* genome sequence that have not been screened for variation and may yet provide useful markers (Garnica *et al.*, 2006; Tyler *et al.*, 2006) (<http://web.science.oregonstate.edu/bpp/labs/grunwald/resources.htm>).

### Genome sequence

A 7X draft whole-genome sequence for *P. ramorum* (an NA1 isolate from California) was published in 2006 along with that of the soybean pathogen *P. sojae* (Tyler *et al.*, 2006), and the genome sequences of *P. infestans* and *P. capsici* are forthcoming (Broad Institute and Joint Genome Institute, respectively). The complete mitochondrial genome of *P. ramorum* has also been sequenced (Martin *et al.*, 2007) and there are several other oomycete mitochondrial genomes to which it can be compared (Avila-Adame *et al.*, 2006; Grayburn *et al.*, 2004; Martin *et al.*, 2007; Paquin *et al.*, 1997). The mitochondrial genomes are largely conserved between *Phytophthora* species, although there are a number of more rapidly evolving genes, some of which may be specific to *Phytophthora*, as well as apparent hotspots for gene rearrangement. *P. ramorum*, in particular, contains a short inverted repeat, which among *Phytophthora* has previously been observed in only one other species, as inversions in other Stramenopile mitochondrial genomes typically represent much larger proportions of the genome (Martin *et al.*, 2007).

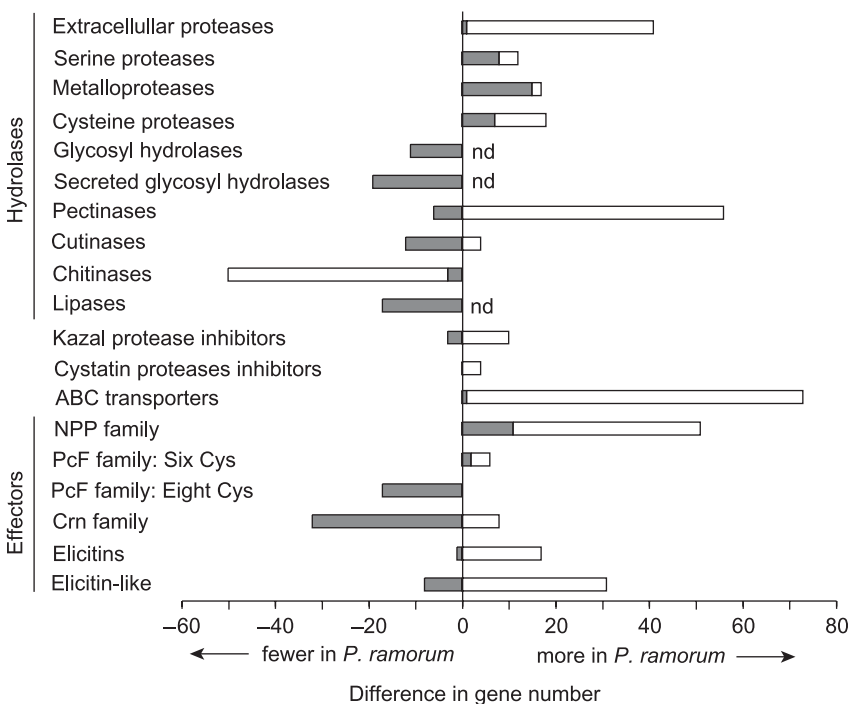
Since the first release of the *P. ramorum* and *P. sojae* whole genome sequences, there has been rapid progress in the area of *Phytophthora* genomics, especially as pertains to genes involved in pathogenicity (Kamoun, 2006; Lamour *et al.*, 2007; Morgan and Kamoun, 2007). The follow-up functional studies are mostly being conducted in the established model systems *P. infestans* and *P. sojae*, which are both fairly host-specific pathogens and may rely on different strategies for infection than the generalist *P. ramorum*. As such, it is interesting to note how the *P. ramorum* genome differs from these other *Phytophthora* genomes.

The *P. ramorum* genome size is 65 Mb, smaller than *P. sojae* (95 Mb) and *P. infestans* (240 Mb), but approximately the same size as *P. capsici* (Lamour *et al.*, 2007). Proteins that are secreted from the pathogen are thought to have functions specific to the host–pathogen interaction, and thus the predicted secretomes are of particular interest to the plant pathology community. Comparison of the *P. ramorum* and *P. sojae* genomes showed these genes to be evolving faster than the rest of the proteome (Tyler *et al.*, 2006). In addition, there has been family-specific expansion of pathogenicity-related genes. Figure 6 shows the families that are expanded in *P. ramorum* as compared with *P. sojae* and the autotrophic diatom *Thalassiosira pseudonana*. *P. ramorum* has more protease genes than *P. sojae*, but fewer of the other groups of hydrolytic enzymes, proteins that may be important to the necrotrophic stage of infection. Among effectors, the necrosis and ethylene-inducing protein (NPP) family shows an expansion in *P. ramorum*, while *P. sojae* contains relatively more of the

necrosis-inducing PcF and Crn families, which are also large and diverse families in *P. infestans* (Kamoun, 2006).

The avirulence genes cloned from oomycetes to date have all contained the conserved amino acid motif RXLR (Arg, any amino acid, Leu, Arg), which has recently been shown to be required for translocation of the protein into the host cell (Dou *et al.*, 2008b; Whisson *et al.*, 2007). Following the RXLR is an additional motif, dEER, which takes on more variable functional forms (Allen *et al.*, 2004; Jiang *et al.*, 2008; Shan *et al.*, 2004) but is also required for translocation (Dou *et al.*, 2008b; Whisson *et al.*, 2007). Fascinatingly, the RXLR motif shares similarity with the motif in the malaria parasite *Plasmodium falciparum* (RXLX followed by E, D or Q) that serves the equivalent function of exporting pathogen proteins to the host erythrocyte (Bhattacharjee *et al.*, 2006).

When homologues of these genes were examined in the *P. ramorum* and *P. sojae* draft genome sequences, a large and diverse family of potential effectors was found (Jiang *et al.*, 2008; Tyler *et al.*, 2006). The number of ‘true’ RXLR-class effectors (i.e. avirulence gene homologues) in the *Phytophthora* genomes is under debate, as the vast majority of them have not been functionally validated; certainly none has been validated in *P. ramorum*. As a result, different numbers exist based on the bioinformatic method used to mine them (Table 3). These genes are so variable between *Phytophthora* genomes that orthology is difficult to determine. Of the nearly 400 genes identified in the *P. ramorum* and *P. sojae* genomes by Jiang *et al.* (2008), only 34 syntenic orthologues could be definitively assigned as such. Most of these genes have



**Fig. 6** Relative number of genes potentially involved in infection in *P. ramorum* compared with *P. sojae* (grey bars) and the diatom *Thalassiosira pseudonana* (white bars). For three gene families, the number of genes in *T. pseudonana* was not determined (nd). Data are from Tyler *et al.* (2006: table 1).



**Table 3** Estimates of numbers of RXLR-class effectors in three *Phytophthora* genomes.

Method	<i>P. ramorum</i>	<i>P. sojae</i>	<i>P. infestans</i>
Jiang <i>et al.</i> (2008)	374	396	–
Win <i>et al.</i> (2007)*	531	672	716‡
Whisson <i>et al.</i> (2007)†	314	400	425

\*Upper limit established by combining results of two methods.

†Upper limit (signal peptide plus RXLR between amino acids 30 and 60).

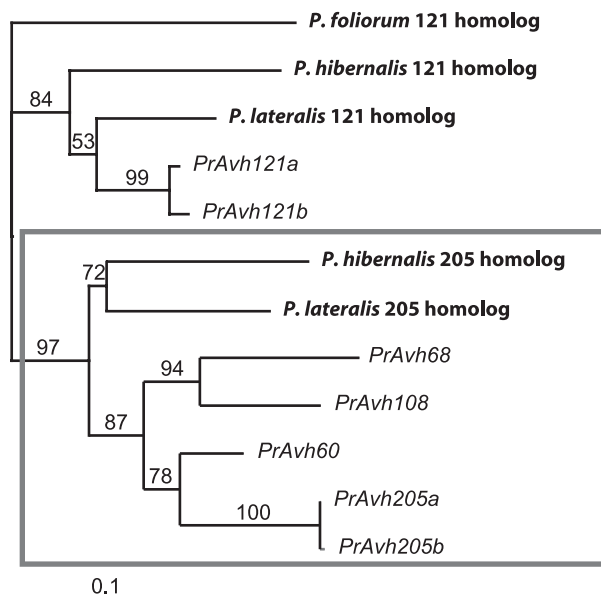
‡From Lamour *et al.* (2007).

only distant homologues based on weak similarity between inferred amino acid sequences.

RXLR-type effectors have a modular structure: the RXLR and dEER motifs are localized to the N-terminus, while the C-terminal domain is thought to be responsible for activity within the host plant cell (Dou *et al.*, 2008a; Jiang *et al.*, 2008; Kamoun, 2007; Whisson *et al.*, 2007). The majority of likely RXLR-class effectors in both *P. ramorum* and *P. sojae* contain a conserved C-terminal motif, called the W motif, which has recently been shown in *P. sojae* to suppress programmed cell death and thus increase virulence in the initial biotrophic phase of infection (Dou *et al.*, 2008a; Jiang *et al.*, 2008). The W motif is often followed by two other conserved motifs (Y and L) forming a W–Y–L module that may be repeated up to eight times (Jiang *et al.*, 2008). As the function of these and other motifs are characterized in model organisms, they may elucidate the function of effectors with these motifs in *P. ramorum*.

### Effector evolution

Within genomes, recently duplicated genes (paralogues) can be identified based on sequence similarity. Analysis of paralogous RXLR-class effectors in *P. ramorum* showed many to be under detectable positive selection, often localized to the C-terminal region and within the W and Y motifs (Jiang *et al.*, 2008; Win *et al.*, 2007). Of 59 groups of closely related paralogues examined by Win *et al.* (2007), 41 showed evidence of positive selection. Of those 41 groups, 21 had two to four members clustered in the genome (within 100 kb). The *P. sojae* genome contained a comparable number of putative RXLR-type effectors, but only 28 groups of closely related paralogues were identified, of which 18 had experienced positive selection and only five of these were clustered. These data indicate that *P. ramorum* may have experienced a more recent expansion of RXLR-class effectors than *P. sojae*. We examined a group of four paralogues in *P. ramorum* more closely and found only a single homologue in *P. lateralis* and *P. hibernalis* (Fig. 7; E. M. Goss and N. J. Grünwald, unpublished), suggesting that this group is a result of gene duplication since the divergence of *P. ramorum* and *P. lateralis*. Win *et al.* (2007) found that for those *P. ramorum* RXLR-type effectors that have



**Fig. 7** Maximum likelihood genealogy of a subfamily of RXLR-class effector genes (avr gene homologues, Avh) in *Phytophthora* clade 8c. *PrAvh* gene sequences are from *P. ramorum* isolate Pr102. For heterozygous genes, the two alleles are distinguished by the suffixes 'a' and 'b'. Homologous sequences are in bold typeface. The *P. lateralis* and *P. hibernalis* homologues to *PrAvh60*, *PrAvh68*, *PrAvh108* and *PrAvh205* (genes in box) appear to be syntenic to *PrAvh205* based on flanking sequence similarity. Bootstrap support is shown for all branches (E. M. Goss and N. J. Grünwald, unpublished data).

paralogues, an estimated 67–75% had between 70 and 99% amino acid identity to their closest paralogue. The most divergent of the four genes in Fig. 7 have 72% amino acid identity (21% divergence at synonymous sites in the whole genome sequence isolate Pr102) and thus fall in the lower end of the above range. Therefore, many of the expansions may indeed have occurred since divergence from *P. lateralis*, although one would expect rates of evolution to vary widely among effector genes.

### PROSPECTS

Several features make *P. ramorum* a particularly interesting candidate for further genomic and genetic analysis. *P. ramorum* is unique among the sequenced oomycete pathogens in that it has a very wide host range. Thus, genes involved in host–pathogen interactions must have been subject to very different evolutionary dynamics. Similarly, in contrast to *P. infestans*, *P. sojae* and *P. capsici*, *P. ramorum* can infect mature trees and is able to penetrate bark and colonize the xylem (Brown and Brasier, 2007; Parke *et al.*, 2007). It is thus expected that a unique set of biochemical pathways and novel chemical functions will have evolved to enable these distinct infection pathways. Yet, these characteristics of *P. ramorum* also make it a challenging organism for molecular genetics because its host plants are

primarily woody perennials that exhibit poorly characterized multigene resistance.

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