Phytophthora ramorum + *P. kernoviae* = International Biosecurity Failure[1]

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Introduction

For a scientist, my title may seem a little sensationalist in tone. This is deliberate - to draw attention to my issue. And here's the issue. About six years ago the previously unknown invasive pathogen *P. ramorum* sp. nov. was found spreading on trees and shrubs in North America and Europe. Almost simultaneously in the U.K. we found another previously unknown invasive *Phytophthora* on trees and shrubs, *P. kernoviae* sp. nov. Shortly before that we found the previously unknown invasive *P. alni* sp. nov. spreading on alders; and a decade before that the invasive *P. ilicis* on holly… Four invasive *Phytophthora* species, each spreading for some years prior to their discovery. And that's just the *Phytophthora* species.… And that's just the invasive *Phytophthora* species we've actually found.

As scientists we usually discuss sudden oak death in the context of our specialist scientific disciplines such as epidemiology, evolutionary biology, fire control, diagnostics and so on. But the bottom line is that the 'sudden' appearance of a *P. ramorum,* a *P. kernoviae,* or a *P. alni* is a symptom of a far bigger underlying problem: the growing threat to our forests and natural ecosystems from invasive forest pathogens (consult Brasier.and others 2006). Or, to put it another way, the low efficacy of current international plant biosecurity protocols. I suggest that this is the *main* scientific issue that we face. *P. ramorum, P. kernoviae, P. alni, P. ilicis* are merely symptoms of the problem.

At the first sudden oak death symposium at Monterey in December 2002 the author presented a scientific health check on the international protocols governing plant movement. I concluded that although the international system was well regulated in many countries, for example North America and the U.K., it could not succeed in protecting our forests and natural ecosystems because of certain fundamental flaws (summarised in Brasier 2005). One proposed flaw was that many invasive pathogens were unknown to science until they escaped from their natural range, in other words, until they became an invasive. There may be many such unknowns (90 percent of all fungi? consult Hawksworth 2001). Such unknown threats cannot be formally regulated against because they do not meet the requirement of being 'listable' as a named threat organism. Before they were discovered causing damaging diseases in the U.S. and Europe both *P. ramorum* and *P. kernoviae* were unknown to science. Obviously, it was by then too late to prevent their initial escape. Both

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Phytophthora species probably arrived on plants imported by the nursery trade or by plant collectors.

As with most new invasives, with the discovery of *P. ramorum* and *P. kernoviae* we have been caught napping and left scrambling for scientific information. In this talk I am going to pick out some of the excellent scientific information now emerging on the behaviour of *P. ramorum* and *P. kernoviae* and consider what it may be telling us about the efficacy of plant biosecurity.

Genetic Structure of *P. ramorum* and *P. kernoviae* Populations: Near Clonal Molecular Polymorphism

Studies by Ivors and others on AFLP or microsatellite polymorphisms in *P. ramorum* populations of Europe and North America (Ivors and others 2006) and by Hughes and colleagues (K. Hughes, personal communication) on *P. kernoviae* populations in the U.K. are revealing remarkable genetic uniformity within each species, consistent with genetic bottlenecks following introductions. Together with evidence for an initially limited geographic distribution of each species followed by spread out of the initial areas, this genetic evidence is consistent with the recent introduction of *P. ramorum* into the U.S. and Europe and of *P. kernoviae* into the U.K. Both species are considered to be introduced from an exotic or unexplored ecosystem in another part of the globe (see Brasier and others 2004).

This re-enforces the point that the escape of scientifically *unknown* pathogens from exotic ecosystems is what we most need to prevent. Yet, as already indicated, our international plant biosecurity protocols are based on lists of known, named organisms. In reality, unknowns may represent a majority of the threats. In my opinion, international plant biosecurity protocols need changing to take account of this reality. Otherwise, as with *P. ramorum* and *P. kernoviae,* we may be will be forever chasing the ripples after the splash, not preventing the splash (Brasier 2005).

Geographical Origins of *P. ramorum*, *P. kernoviae*

The geographic origins of *P. ramorum* and *P. kernoviae* have been suggested to be in places such as Yunnan or the Himalayas (Brasier and others 2004). The 'sudden' appearance of these species in Europe and North America has already spawned two expeditions to search for their origins: a United States Department of Agriculture-Forest Service funded expedition to forests of Yunnan in 2005 involving Ellen Goheen, Everett Hansen and Niklaus Grunwald; and a part FAO/ part self funded expedition to forests of Nepal also in 2005 involving Andrea Vannini, Anna Brown, Anna Maria Vettraino and Clive Brasier. The Yunnan crew isolated *Phytophthora* spp., but unfortunately ran into local difficulties with bringing out their cultures. The Nepal crew was more fortunate. No *P. ramorum* or *P. kernoviae* was found but, in addition to *P. citricola* and a *P. palmivora*-like *Phytophthora,* an apparently previously unknown forest *Phytophthora* species was discovered in the Himalayan sub tropical forest zone.

The probable existence of another 'new' forest *Phytophthora* in Nepal again emphasises that it is the scientifically unknown pathogens that we most need to worry about. Indeed, for a number of reasons we need to foster international cooperation and encourage funding agencies to support more of the above types of expeditionary searches. They represent part of a more proactive approach to providing information for improving international plant biosecurity policy: too much of current research in plant biosecurity is institutionalised and 'box ticking' (Brasier 2005). Such searches can also help identify likely pathways of exotic pathogen arrival. Furthermore, the study of a pathogen in its native habitat can shed light on its natural host range, and ecology and dispersal: and its 'normal' population structure and population dynamics. Indeed the population dynamics of endemic *P. ramorum* may be quite different from invasive *P. ramorum*. Searches in areas where organisms such as *P. ramorum* are indigenous may also lead to discovery of naturally occurring biocontrol agents, and so widen our disease control armoury.

Origins of *P. kernoviae* – Emerging Information

In Cornwall, U.K., *P. kernoviae* is behaving as a recent invasive in terms of its distribution, its spread and its genetic homogeneity. The recent publication of the formal description of *P. kernoviae* (Brasier and others 2005) has led to its being identified from soil at four different field locations in New Zealand (M. Dick and others, New Zealand MAFF/Ensis, personal communications). Circumstantial evidence indicates it has probably been present at one location in New Zealand since at least the 1950s. It is also known that there have been contacts between nurseries and plant collectors in Cornwall and nurseries and plant collectors in New Zealand. However it is still unclear whether *P. kernoviae* is introduced to New Zealand or is native there.

Behavioural Differences Between the EU1 and NA1 Molecular Lineages of *P. ramorum*

Research carried out on both sides of the Atlantic (for example Werres and Kaminski 2005; Huberli and others 2006; Brasier and others 2006) has shown that the two most widespread molecular lineages of *P. ramorum*, one occurring in Europe (termed the EU1 lineage) and the other dominant in North America (the NA1 lineage), differ significantly in continuous phenotypic characters. For example they differ in their colony patterns, in their mean growth rate and in their mean aggressiveness on susceptible hosts; and EU1 isolates are much more stable in culture than NA1 isolates. These differences indicate adaptive differences between the two lineages. In addition the EU1 lineage is of predominantly A2 sexual compatibility type and the NA1 lineage of A1 type.

Since *P. ramorum* is potentially 'heterothallic' in other words, a sexually outcrossing fungus, these differences indicate that genetic recombination between the EU1 (A1) and NA1 (A2) lineages could yield new phenotypes. Even without A1 x A2 sexual mating, genetic recombination might occur via zoospore fusion between the EU1 and NA1 lineages. This emphasises the need to identify and prevent the spread and intermixing of different *genotypes* of invasive pathogens. We cannot concentrate *solely* on preventing the spread of different pathogen species.

Host Range Studies

It is often popularly assumed that the initial pathway of introduction of *P. ramorum* into Europe or North America was via imported *Rhododendron, Camellia,* or *Viburnum* nursery stock; and of *P. kernoviae* via imported *Rhododendron* or *Magnolia* stock. However, experience coming from host records in the field and from laboratory-based host testing could indicate something rather different. The potential host range of both species is clearly very wide: over 100 foliar and tree stem hosts have been recorded in the field in the U.S. and Europe for *P. ramorum*; over 30 in the U.K. for *P. kernoviae*. Laboratory tests reveal an ever widening potential host range for both species. It has also been clear for some time that *Rhododendron* is probably something of a 'universal *Phytophthora* suscept', it apparently being susceptible to most of the *Phytophthora* species that can attack woody hosts. *Rhododendron* may therefore simply be the ideal universal '*Phytophthora* carrier' (consult Brasier and others 2004).

These observations have a range of implications for biosecurity protocols. First, the initial pathway host or a main pathway host in the invaded area, such as rhododendron, may not necessarily be the common host in the pathogen's geographic area of origin. To identify the host(s) in the area of origin the net may therefore have to be thrown very wide. Second, the initial pathway of introduction may not even have involved rhododendron at all but another host altogether, *P. ramorum or P. kernoviae* spreading onto rhododendron subsequently. Third, a *Phytophthora* pathogen listed on quarantine schedules as causing damage on a specific host in country A might be a serious threat to entirely different hosts and ecosystems in country B. The absence of that specific host in country B is therefore insufficient reason to consider the pathogen 'non-threatening'.

Asymptomatic Sporulation on the Host

There have recently been two interesting new developments on the issue of sporulation behaviour. A study by Riedel and colleagues in Germany (Poster, this Proceedings) has shown that *asymptomatic* roots of infected rhododendrons harbour chlamydospores of *P. ramorum*. Similarly studies by Denman, Moralejo and colleagues in U.K. and Spain (this Proceedings) have shown that sporangial production by both *P. ramorum* and *P. kernoviae* occurs on *asymptomatic* infected leaves and fruits of a range of hosts including rhododendron, *Quercus, Rosa, Smilax, Crataegus and Laurus.*

Assessment of the health of planting stock for quarantine certification is normally a visual process. Many shipments of plants for planting are sent bare rooted. The above studies show that visually healthy plants may harbour a sporulating pathogen in the roots or the foliage, in other words, visual inspection alone may be insecure. They also show that shipping stock bare-rooted is not a guarantee of plant biosecurity. Indeed it might provide a false sense of security.

Presence of *P. kernoviae* and *P. ramorum* in Tree Xylem

Historically, tree *Phytophthora* species have not been perceived as inhabitants of secondary xylem but as inhabitants of the phloem and cambium. Studies by Brown and Brasier (2007) in the U.K. and Parke and others (2007) in Oregon have now shown that *P. kernoviae* and *P. ramorum* are significant colonisers of xylem of for example *Fagus, Quercus, Acer* and *Lithocarpus* species; that *P. ramorum* and *P. kernoviae* may spread within xylem and possibly recolonise the phloem from the xylem; and that *P. ramorum* and *P. kernoviae* can remain viable (perennate) within xylem for two or more years.

From a biosecurity perspective, these studies indicate that removal of outer sapwood should be undertaken in protocols aimed at preventing national or international spread of *P. ramorum*, *P. kernoviae* and other tree stem *Phytophthora* species in affected timber: Brown and Brasier (2007) recommend removal of at least 3 cm of outer sapwood with regard to *P. ramorum* and *P. kernoviae*–infected *Fagus* and *Quercus* in the U.K.

Movement of *P. ramorum* and *P. kernoviae* by People

At the first SOD meeting in January 2002, Tjosvold, Davidson and colleagues produced some striking data on the presence of *P. ramorum* inoculum on hikers' boots. *Phytophthora ramorum* was sampled from the soil of hikers' shoes after they walked 2.4 km on a trail at the Sonoma Fairfield Osborn reserve. Soil from shoes yielded 46.7 percent and 33.3 percent positive samples during the two trials in April 2003 (Davidson and others 2005). Recently Webber and Rose (these Proceedings) working at sites in Cornwall, U.K. have collected samples from boots of people leaving *P. kernoviae*-infested woodland, mainly within the official *P. kernoviae* 'Management Zone'. Again, significant levels of infestation in soil adhering to footwear were detected.

Movement of inoculum on feet of people, animals and on wheeled vehicles is clearly a major potential pathway for local and even international spread of *P. ramorum and P. kernoviae*. Yet at most *P. ramorum* infested sites in California and Oregon access by people and vehicles for recreational or employment purposes continues as normal. The same is true for *P. ramorum* and *P. kernoviae* infested sites in Cornwall, U.K. Indeed in Cornwall, some of the infested woodland sites are popular pheasant shooting venues in winter, just when the soil may be at its wettest. As a consequence, movement of infested soil is likely between local shooting estates. Some shooting clients may fly in from as far away as the U.S. What is on their boots when they arrive home?

Movement of *P. ramorum* and *P. kernoviae* inoculum on feet of humans or animals and on wheels of machinery, is probably another of those 'inconvenient truths'. If we are serious about management and control of *P. ramorum* and *P. kernoviae* we may have to severely modify our behaviour in terms of public access to affected sites. Maybe we need to learn from the two already historically well documented situations where inoculum of invasive *Phytophthora* species has been shown to spread on feet and machinery: *P. cinnamomi* in jarrah forests and native vegetation in western Australia (for example Brandis and Batini 1985, Colquhoun and Hardy 2000, Shearer and Tippett 1989) and *P. lateralis* in Port-Orford-cedar areas in the Pacific northwest (for example. Hansen and others 1999; Goheen and others 2006). In these situations strict management regimes have been developed locally including seasonal 'no go' areas and stringent vehicle washing protocols. Similar regimes may be needed for *P. ramorum* and *P. kernoviae* and for other invasive *Phytophthora* species in future.

Concluding Thought: How Many Undescribed *Phytophthora* species?

Hawksworth (2001) estimated that 90 percent of fungi were still unknown to science. About 60 described *Phytophthora* species were known in 2001 (today the figure is about 80 described species). Based on the above admittedly loose estimate, it is possible that there are as many as 540 still undescribed *Phytophthora* species in unexplored ecosystems. More conservatively, it seems reasonable to propose both from the above 90 percent estimate and from the many new species currently being described, that the total number of *Phytophthora* species may lie between 200 and 600. Say 10 percent of these undescribed species were potentially invasive or seriously damaging forest *Phytophthora* species (about 5 to 10, or 8 to 16 percent of the 60 known species in 2001 fall into this category, depending upon how one defines a 'potentially invasive or seriously damaging forest *Phytophthora'*). Then there could be some 10 to 50 unknown potentially damaging forest *Phytophthora* species still out there in their natural habitats, waiting to be introduced into a new evolutionary playground by man. Tomorrow's *P. ramorums*, *P. kernoviaes*, *P. alnis*, *P. cinnamomis*. . . . .

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