Rapid Evolution of Introduced Tree Pathogens Via Episodic Selection and Horizontal Gene Transfer

Clive Brasier[1]

Episodic and Routine Selection

Routine selection is simply defined as "the ecological constraints experienced by an endemic organism that favor a relatively stable but fluctuating population structure over time." Its antithesis is episodic selection, defined as "any sudden ecological disturbance likely to lead to a significant alteration in a species' population structure" (Brasier 1986, 1995). In plant pathogens, examples of episodic selection are common in the context of modern anthropogenically driven disturbance conditions such as sudden exposure to crop monoculture or to a new fungicide, host, or vector; or sudden introduction into a new biogeographic zone; an increasingly frequent event because of increasing international trade in plants.

The introduction of pathogens into new biogeographic zones is the concern of the present paper. Such an introduction is a major episodic selection event for any organism, let alone a plant pathogen. It can result in sudden release from many of the routine selection constraints experienced in the endemic environment; and sudden exposure to new constraints such as new hosts, substrates, competitors, parasites, phylogenetic relatives, vectors, and microclimates (Brasier 1995). For this reason, every introduced pathogen is an uncontrolled, open-ended experiment in evolution (Brasier 2008).

In populations of outcrossing (heterothallic) pathogens, two significant evolutionary processes can occur as a result of introduction-mediated episodic selection. One is the emergence of highly fitted clones, examples being the clones of the Dutch elm disease pathogens *Ophiostoma ulmi* and *O. novo-ulmi* that spread across Eurasia and North America between 1910 to1930 and 1940 to 1990 respectively (Brasier 1996, Brasier and Kirk 2000, Brasier et al. 2004a, Mitchell and Brasier 1994). If the episodic selection conditions are maintained over the longer term, this process could ultimately lead to clonal speciation (Brasier 1995). The second process, and again the particular concern of this paper, is rapid evolution via interspecific hybridization.

Interspecific Hybridization Between Introduced and Resident Pathogens

Novel contact between phylogenetically related introduced and resident pathogens will often result in their direct ecological competition for resources. It also provides an opportunity for rapid evolution via the transfer of genes between the residents and the immigrants (Brasier 2000b). Sympatric fungi, like animals, are likely to exhibit strong genetic or behavioral barriers to inter-specific gene flow as a result of prior co-evolution. Allopatric fungi, arising in geographical isolation, may not have evolved such barriers. Today, many previously allopatric fungal pathogens are being brought into contact by man, especially through inappropriate plant imports (Brasier 2008). Figure 1 shows an increase in major tree disease events in the United Kingdom since the mid-1990s, some 25 years after the arrival of *O. novo-ulmi*. Many of these new events are caused by introduced pathogens.

[1]Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, U.K.
Corresponding author: clive.brasier@forestry.gsi.gov.uk.

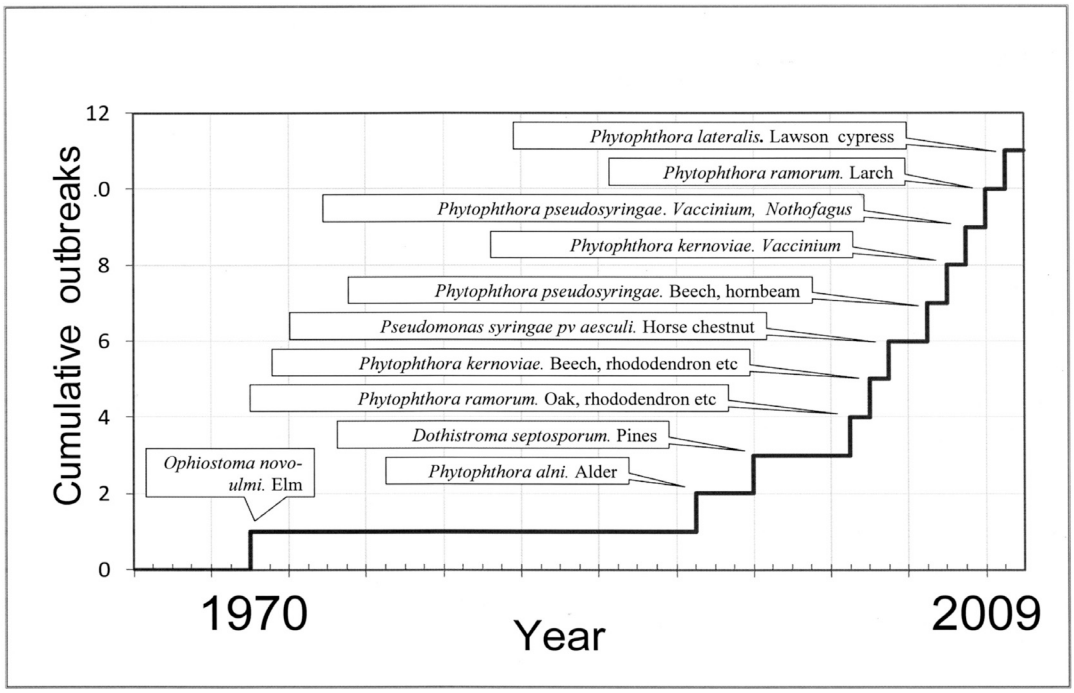

Figure 1—Cumulative major tree disease outbreaks in the United Kingdom since 1970. (Courtesy of Sandra Denman, Forest Research, UK)

A variety of factors will influence whether or not contact between two previously geographically isolated pathogens will result in hybridization (Brasier 1995, 2000b). These include their level of reproductive isolation (both pre-zygotic and post- zygotic); the degree of niche contact between them (fig. 2); and the genetic and ecological conditions that will determine whether any hybrids can survive in the presence of the parent species. Often, hybrids will need to be fitter than one or both parents to become established. Modern conditions of anthropogenically driven environmental disturbance are more likely to favor the successful establishment of hybrids. In horticultural nurseries, which these days can be infested with non-native pathogens, unusual combinations of introduced and resident pathogens may be brought together. The plants themselves may be stressed by the watering and chemical regimes and therefore susceptible to pathogens and hybrids that would not normally colonize them under natural conditions (Brasier 2008). Plantations of non-native trees may also be more susceptible to infection because they are growing ‘off site,’ i.e., under sub-optimal conditions.

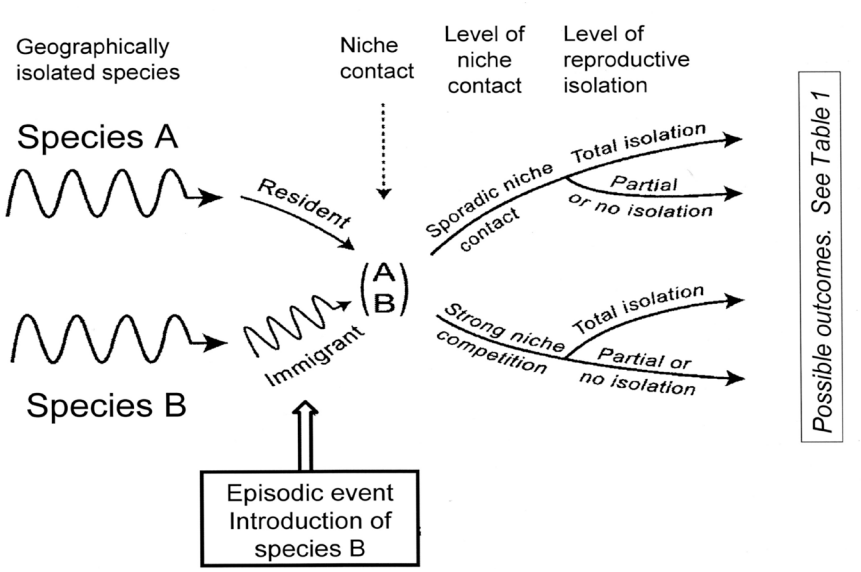

Figure 2—Genetical and environmental constraints on emergence of species hybrids in fungal pathogens. Adapted from Brasier (1995).

There are many possible outcomes to fungal interspecific hybridization, as shown in table 1.These range from the acquisition of a few nuclear genes or cytoplasmic elements to the emergence of a new organism that contains the genomes of both parents. Either way, with fungal pathogens there is a risk that the resulting genetic modification will be damaging to the natural environment. Before 1990, examples of hybridization between fungal pathogens were very rare. Recently, striking examples have occurred in all the major fungal groups, and most especially among tree pathogens. Several of the more prominent examples will now be discussed. A basic knowledge of the biology of the organisms will be assumed.

Table 1—Possible outcomes of contact between two previously geographically isolated pathogens (see fig. 2)[a]

- No change: no ecological displacement and no interspecific gene transfer.
- Introgression: acquisition and fixation of genes (also viruses, plasmids) by one or both parent species. Example: *Ophiostoma ulmi* x *O. novo-ulmi*.
- Extinction of the ecologically 'weaker' species via competition.
- Extinction of the 'weaker' species and genetic modification of the other species via introgression. Example: *Ophiostoma ulmi* x *O. novo-ulmi*.
- Emergence of a hybrid swarm: free recombination between two species resulting in many genotypes. Optimal phenotypes may emerge via natural selection. Examples: the *Ophiostoma novo-ulmi* subspecies hybrids; *Heterobasidion annosum* x *H. irregulare*; *Melampsora occidentalis* x *M. medusae*.
- Emergence of an allodiploid or allotetreploid hybrid species: a hybrid with the chromosome compliments of both parents. May be stable or give rise to further new heteroploid forms via haploidisation or diploidisation. Example: *Phytophthora alni*, subspecies *alni*.

[a] Adapted from Brasier (1995). The examples given are described in the main text.

Emerging Hybrids in the Dutch Elm Disease Pathogens (Ascomycetes)

The intercontinental spread of Dutch elm disease in the last century resulted in two massive pandemics on elms across the Northern Hemisphere (fig. 3). It has also provided classic examples of hybridization operating at two different taxonomic levels with two very different outcomes: introgression and full genetic recombination.

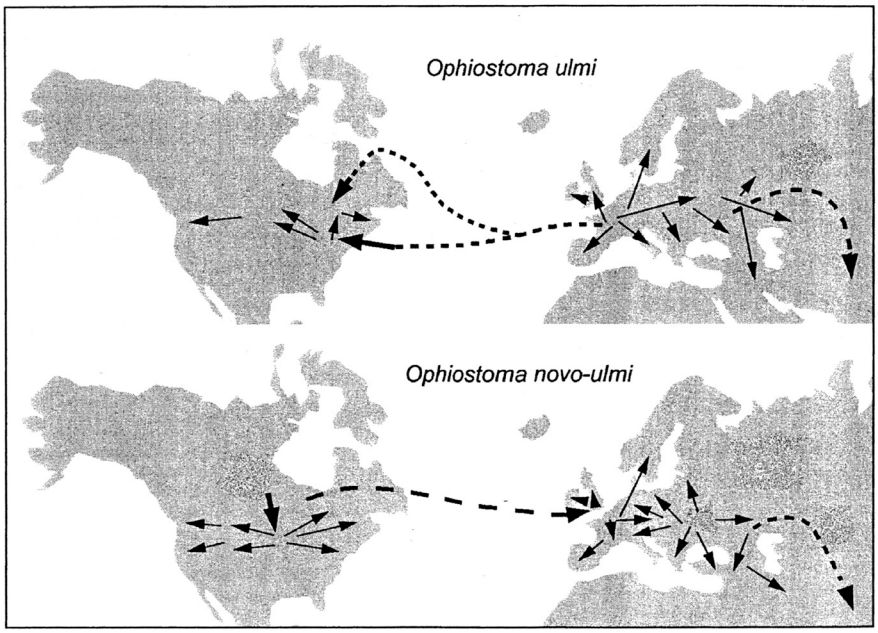

Figure 3—Spread of the pathogens *Ophiostoma ulmi* and *O. novo-ulmi* in the two pandemics of Dutch elm disease. Adapted from Brasier (2001).

At the species level, *Ophiostoma novo-ulmi*, responsible for the current second pandemic of Dutch elm disease beginning in the 1940s, has been displacing *O. ulmi*, responsible for the first pandemic in the 1920s to 40s (fig. 3). The two species are rather differently adapted, *O. novo ulmi* being an aggressive pathogen that grows fastest at ~ 22 °C; *O. ulmi* being a weaker pathogen that grows fastest at ~28 °C. Their molecular profiles indicate they are anciently divergent. The geographic origin of *O. novo-ulmi* remains unknown, but *O. ulmi* may have come from Japan. During the replacement of *O. ulmi* by *O. novo-ulmi* across Eurasia, a series of remarkable evolutionary events has occurred. These can be summarized as follows:

1. When *O. novo-ulmi* arrived in Europe (coming from east and west, fig. 3) the resident *O. ulmi* population was highly variable both phenotypically and genetically. Whereas *O. novo-ulmi* initially spread as frontal clones of a single vegetative compatibility (*vic*) type and only a single sexual mating type: *Mat*-2. Hence these frontal clones could not undergo sexual reproduction.
2. *O. novo-ulmi* and *O. ulmi* occupied the same ecological niche, coming into close physical contact in the breeding galleries of the vector beetles in diseased elm bark.
3. *O. novo-ulmi* and *O. ulmi* are only partially reproductively isolated (at both pre-zygotic and post-zygotic levels), probably due to their having evolved allopatrically. Hybrids formed between them via sexual crosses. These hybrids were highly unfit and transient, but they provided a genetic bridge between *O. ulmi* and *O. novo-ulmi*, probably via backcrosses.
4. Debilitating dsRNA viruses also spread from *O. ulmi* into the *O. novo-ulmi* frontal *vic* clones, again as a result of their close contact in the bark phase. The build-up of these viruses considerably lowered the fitness of *O. novo-ulmi* and therefore 'threatened' its survival.

5. Simultaneously, *O. novo-ulmi* populations acquired the 'missing' *Mat*-1 mating type allele and new *vic* alleles from *O. ulmi* via introgression from the hybrids.
6. As a result the *O. novo-ulmi* population rapidly diversified into many new *vic* types via sexual recombination (crosses between *Mat*-1 and *Mat*-2 types).*O. novo-ulmi* also acquired other *O. ulmi* genes such as pathogenicity and cell surface hydrophobin genes at this time.
7. Virus spread in the *O. novo-ulmi* population was suppressed by a sudden increase in the diversity of its *vic* types.
8. *O. ulmi* rapidly became extinct in any one location, declining at circa 10 percent per annum. It could not compete with the more aggressive, faster growing *O. novo-ulmi*.
9. *O. novo-ulmi* 'retained' the novel *Mat*-1 and *vic* genes by fixation but 'discarded' other *O. ulmi* DNA that rendered it less fit, such as *O. ulmi* pathogenicity genes. Figure 4 shows the strong negative impact of small amounts of *O. ulmi* DNA on the fitness of emerging *O. novo-ulmi* introgressants.
10. Some key references: Brasier 1986, 1988, 1996, 2000a; Brasier and Kirk 2000; Brasieret al. 1998, 2004a; Buck et al.2002; Et Touil et al. 1999; Masuya et al. 2009; Paoletti et al. 2006; Pipe et al. 2000, 2001. For further references see www.forestry.gov.uk/fr/infd-8mraqc.

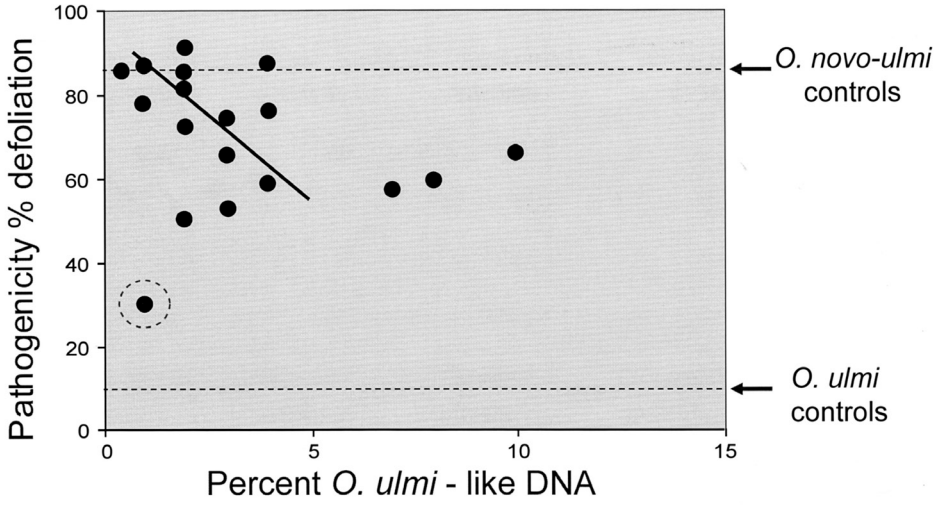

Figure 4—Influence of introgressed *Ophiostoma ulmi* DNA on fitness (pathogenicity) of *O. novo-ulmi* isolates. Note that small amounts (2 to 4 percent) of introgressed DNA have a major effect on fitness. (M.Paolettii, C.M. Brasier, and K.W. Buck, unpublished data)

The *O. novo-ulmi* x *O. ulmi* interaction illustrates most of the main variables that influence the outcome of interspecific hybridization (see fig. 2). It also shows how complex, rapid, and dynamic the underlying evolutionary events can be. *Ophiostoma ulmi* was replaced because it could not compete either with the invading *O. novo-ulmi* or with the ensuing introgressants. Strong selection pressure exerted by the deleterious viruses probably favored the survival of the introgressants over the original *O. novo-ulmi* genotypes. It is somewhat ironic that the viruses as well as the *Mat*-1 and *vic* genes were probably acquired by *O. novo-ulmi* from *O. ulmi* via somatic or sexual fusion—rather like a sexually transmitted disease. Also, that in Eurasia and North America today *O. novo-ulmi* now carries *O. ulmi* genes almost by definition, much as *Homo sapiens* now carries the DNA of *H. neanderthalensis*. *Ophiostoma novo-ulmi* is no longer the same organism that it was when it was first introduced into Eurasia and North America around 70 years ago (fig. 3).

But that is far from the end of the matter. Another remarkable hybridization event is now occurring. *Ophiostoma novo-ulmi* actually spread across the northern hemisphere as two subspecies named *americana* and *novo-ulmi* (fig. 5). These differ in colony development, perithecial (sexual fruit body) morphology, mean pathogenicity, and molecular profiles (Brasier and Kirk 2001). During the

1980s, the distributions of the two subspecies began to overlap along a zone running approximately from southern Sweden to southern Italy, with an outlier in southern Ireland (fig. 6). In this zone they have come into strong niche contact in the galleries of the vector beetles.

There is also only a weak pre-zygotic mating barrier between the two subspecies and their sexual progeny are highly fit. Fully recombinant hybrid swarms have begun to appear, probably involving many further backcrosses and intercrosses (Brasier and Kirk 2010). A recent study has shown that 30 years on the surviving hybrids in the overlap zones are still very genetically heterogeneous, but now exhibit the colony and pathogenicity characteristics of subspecies *americana* and the perithecial characteristics of *O. novo-ulmi* (C.M. Brasier, unpublished). Evidently a new phenotype of *O. novo-ulmi* is emerging from the hybrid swarms under natural selection. *Ophiostoma novo-ulmi* is undergoing yet another evolutionary transformation. If this new phenotype stabilizes and becomes widespread it will need to be formally taxonomically recognized.

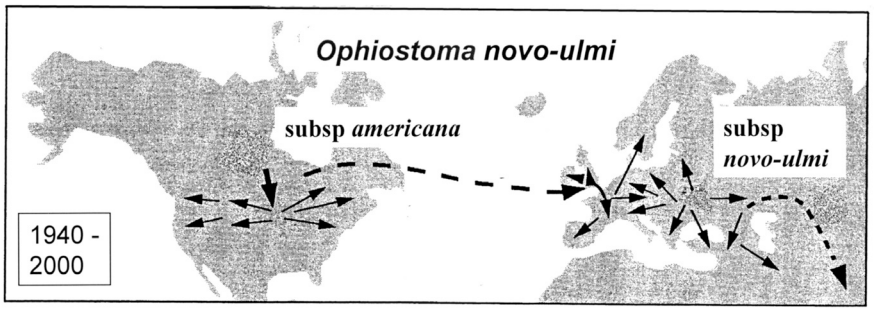

Figure 5—Spread of the two *Ophiostoma novo-ulmi* subspecies. Note their recent overlap in western Europe. Adapted from Brasier (2001).

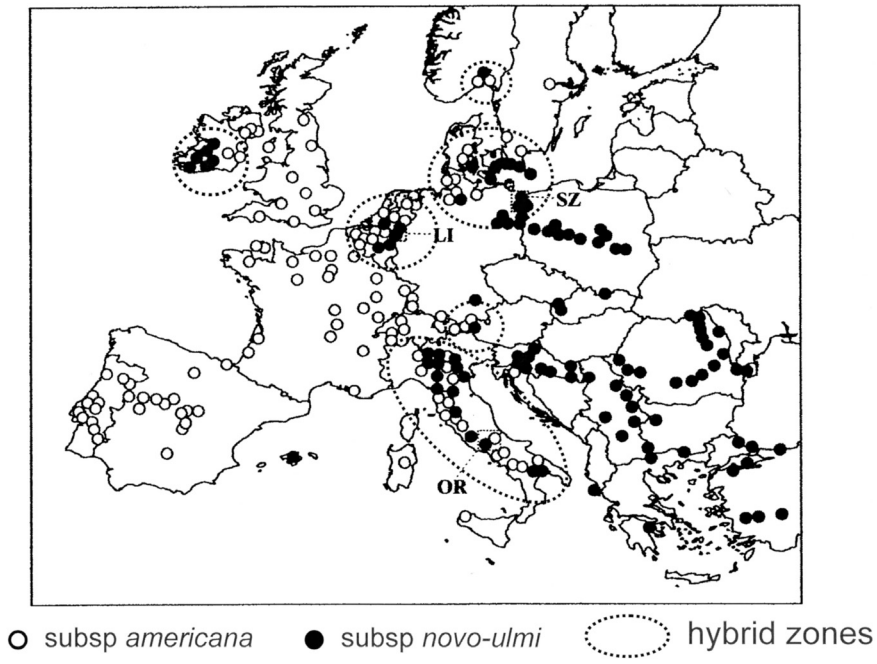

Figure 6—Known distribution of the two *Ophiostoma novo-ulmi* subspecies in 1995 (omitting central Asia), showing potential hybrid zones. Adapted from Brasier and Kirk (2010) and based on >2,500 field samples collected by the author.

Emerging Hybrids in *Melampsora, Heterobasidion,* and *Phytophthora* (Basidiomycetes and Oomycetes)

In the Pacific Northwest of North America, hybrids have recently appeared between the resident Basidiomycete rust pathogen *Melampsora occidentalis* and the introduced *M. medusae*. *Melampsora occidentalis* occurs naturally on the leaves of the local *Populus trichocarpa* in the Columbia Valley. *Melampsora medusae* is endemic on *P. deltoides* in the southeastern United States. A swarm of hybrid rust genotypes and morphotypes ('*M.* x *columbiana'*) has emerged as a result of backcrosses and intercrosses between the two species on *P. trichocarpa* (Newcombe et al.2000, Newcombe et al. 2001). The hybrids show abundant pathogenic variation and commercially produced *P. trichocarpa* x *P. deltoids* clones are vulnerable to this variation. It is noteworthy that screening using host differentials carrying known resistance genes using *M.* x *columbiana* hybrids has led to the discovery of three new genes for rust resistance in poplar. The long-term evolutionary potential of these hybrids is presently unclear.

In the economically important conifer root rot Basidiomycete *Heterobasidion*, a wide range of hybrids and introgressants between the resident *H. annosum* and the introduced North American *H. irregulare* have recently appeared on native pines in the Castel Porziano region of southwest Italy (Gonthier and Garbelotto 2011, Gonthier et al. 2007). Both species specialize in attacking pines. *Heterobasidion irregulare* may have been imported into the area on pine packing material during the Second World War (M. Garbelotto, professor, University of California, Berkeley, personal communication). In this case it is especially noteworthy that the hybridization process has included the evolution of novel alleles via intralocus recombination. Introgression is mostly occurring unilaterally from the resident to the introduced species. Currently it also appears to be occurring randomly—by population expansion rather than natural selection—but again, the long-term evolutionary potential of this development is unclear.

In the well-known Oomycete genus *Phytophthora*, a very aggressive new hybrid species, *P. alni*, has emerged on alder in Europe, possibly as a result of a recent hybridization event in a European nursery. *Phytophthoras* are diploid in the vegetative state but the common hybrid form, *P. alni* subspecies *alni*, is a near allotetreploid (i.e., with two sets of chromosomes, one from each parent). It appears to be sexually sterile (probably due to pairing failures at first meiotic division) and its genome, such as its rDNA ITS region profile, is apparently still evolving (Brasier 2003, Brasier and Kirk 2001, Brasier et al. 1999, Brasier et al. 2004b, Delcan and Brasier 2001). *Phytophthora alni* subspecies *alni* is causing serious damage to native riparian alders, especially *A. glutinosa*, along rivers in France, Germany, and the United Kingdom (Gibbs et al. 2003, Jung and Blashke 2004) and has now spread to at least 15 neighboring countries.

The 'parents' of *P. alni* subspecies *alni* were originally suggested to be taxa close to *P. cambivora* and *P. fragariae* (Brasier et al. 1999). New evidence suggests otherwise. Two additional phenotypically and genetically unique forms of *P. alni* also occur in Europe, but at low frequency: *P. alni* subspecies *uniformis* and *P. alni* subspecies *multiformis* (Brasier et al. 2004b). Both are only weak pathogens on alder (Brasier and Kirk 2000). Since these forms had chromosome compliments closer to diploid they were originally suggested to be genetic breakdown products of the allotetreploid *P. alni* subspecies *alni*. The new evidence, from additional molecular profiling, indicates that these two forms are most probably the parents of *P. alni* subspecies *alni* (Ioos et al. 2006, 2007a, 2007b). This event has therefore resulted in a hybrid that is much more aggressive than either parent. *Phytophthora alni* subspecies *multiformis* may itself be a product of a reticulation event.

Conclusions

The five examples provided illustrate most of the theoretical outcomes of interspecific hybridization between fungal pathogens, ranging from genetically modified pathogens to new pathogen species (table 1). Similar examples are being reported increasingly in the literature. An increase in the

introduction of plant pathogens into new biogeographic areas (by the plant trade and other agencies)—episodic selection events—coupled with increasing environmental disturbance, is leading to an increase in rapid pathogen evolution via interspecific hybridization.

At present we may be recording only the 'tip of the iceberg': hybridization events associated with more overt disease episodes such as epidemics. More subtle events—such as the introgression of one or two loci into organisms currently viewed only as a minor threat—are perhaps less likely to be recorded unless they lead to a significant change in pathogen behavior.

Interspecific hybridization between pathogens, whether overt or subtle, must now be considered a major genetic risk issue, not only for the biosecurity of our forests and natural ecosystems, but also for tree disease resistance breeding programs. It adds another element of uncertainty to disease control policy and to practice. How do you breed against an emerging hybrid swarm or against the risk of future horizontal gene transfer?

Unfortunately present international plant health protocol, formulated by the Food and Agriculture Organization of the United Nations (FAO) and the World Trade Organization, and operated by organizations such as the Animal and Plant Health Inspection System (APHIS) in the United States and the Standing Committee on Plant Health in the European Union, has failed to provide an acceptable level of protection against the export and import of exotic pathogens; and failed to educate the public on the issue (Brasier 2008, Stenlid et al. 2011, Webber 2010). In present circumstances, none of the world's tree populations, whether native or introduced, whether natural or the products of sophisticated tree breeding, can be considered secure.

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