Rapid Evolution of Introduced Plant Pathogens via Interspecific Hybridization

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Plant disease epidemics resulting from introduction of exotic fungal pathogens are a well-known phenomenon. Limited resistance in the host and excessive aggressiveness in the pathogen (reflecting their lack of prior coevolution) can result in an explosive outbreak of disease. Introduction events also present a window of evolutionary opportunity for the pathogen. In its endemic location, a plant pathogen tends to be subject to routine selection constraints, favoring maintenance of a relatively stable, if fluctuating, population structure over time. When introduced into a new environment, it will often be subject to novel or episodic selection, reflecting sudden exposure to new biotic and abiotic influences, such as a new host population, new vectors, new competitors, or a different climate. These influences provide the potential for rapid evolution (Brasier 1995).

Falling within the category of episodic selection is the sudden contact that can occur between closely related but previously geographically isolated pathogens as a result of introductions. Theoretically, this process presents an opportunity for rapid emergence of new or modified pathogens via interspecific gene flow (Brasier 1995). Until recently, this phenomenon has been little studied. Now Dutch elm disease, a major ecological accident of the 20th century (Heybroek 1993), is providing remarkable insights into rapid evolution of a plant pathogen outside its endemic environment. This article describes the migratory events and unusual genetic events that have occurred in this fungus and other new examples of rapid pathogen evolution via interspecific gene flow, and discusses some of the wider environmental, evolutionary, and quarantine implications.

The Dutch elm disease pathogens

Elm trees (*Ulmus*) are confined mainly to the temperate regions of the Northern Hemisphere. China and Japan have a total of about 25 elm species, while Eurasia, North America, and the Himalayas each have about five or six species.

HYBRIDIZATION IS LEADING TO RAPID EVOLUTION OF DUTCH ELM DISEASE AND OTHER FUNGAL PLANT PATHOGENS

Dutch elm disease (so called because the early seminal research was in The Netherlands [Holmes and Heybroek 1990]) is the elm's main enemy. It is a wilt disease, caused by ascomycete fungi of the genus *Ophiostoma*, that spreads within the tree's vascular system. The pathogens are transmitted from diseased to healthy elms by elm bark beetles of the genus *Scolytus* (Fransen 1935, Webber and Brasier 1984).

Dutch elm disease was unknown in Europe and North America before 1900, but there have been two enormously destructive pandemics of disease across the northern hemisphere in this century. These two pandemics were caused by the spread of two different species of fungal pathogen, *Ophiostoma ulmi* and *O. novo-ulmi*, respectively (Figure 1). The geographic origins of the pathogens remain unknown, despite a number of expeditionary searches, but they are believed to have come from Asia (Brasier 1990, Brasier and Mehrotra 1995).

O. ulmi and *O. novo-ulmi* differ markedly in many of their behavioral and genetical properties, such as their optimum temperatures for growth, colony morphologies (Figure 2), molecular fingerprints (Figure 3), and pathogenicity to elms (Brasier 1991). From their behavioral differences, it appears that they probably have rather different ecological strategies in their original, or endemic, locations. For example, the optimum temperature for growth of *O. novo-ulmi* is approximately 22°C and that of *O. ulmi* 28°C, suggesting

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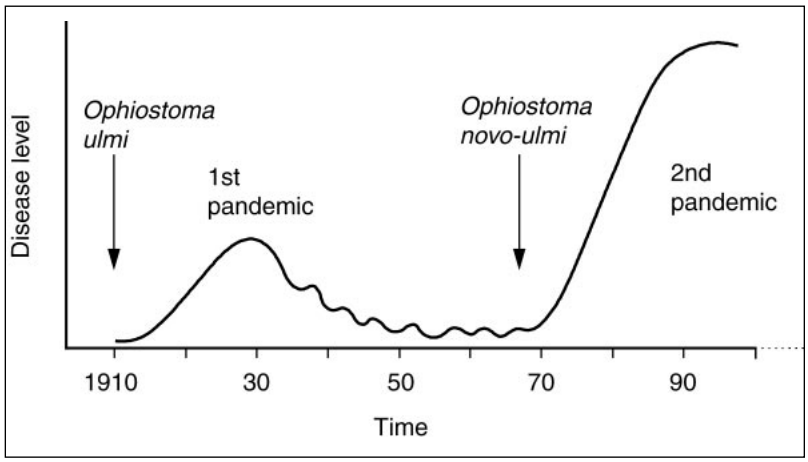

Figure 1. Approximate chronology and impact of the first and second pandemics of Dutch elm disease in Britain and northwest Europe. Note the decline in the first pandemic in the 1940s. The chronology in North America is believed to be very similar but without the decline in the first pandemic, owing to the greater susceptibility of American elm. From Brasier (1996).

that the former may be naturally adapted to a temperate and the latter to a subtropical environment (Brasier and Mehrotra 1995). Another difference is that *O. ulmi* is a moderate and *O. novo-ulmi* a highly aggressive pathogen of European elms. American elms, however, are generally more susceptible to Dutch elm disease than European elms; therefore, *O. ulmi* causes significantly greater damage to American elms than it does to European elms (Gibbs et al. 1975). *O. ulmi* and *O. novo-ulmi* are genetically divergent based on molecular fingerprint studies, although studies on DNA sequences of their cerato-ulmin genes (cerato-ulmin is a glycoprotein implicated as a wilt toxin) indicate that they are also anciently divergent taxa (Bates et al. 1993, Pipe et al. 1997).

Ophiostoma novo-ulmi is not a single entity but exists as two distinct forms, called the Eurasian, or EAN, and the North American, or NAN, races (Brasier 1979). In practice, these races are equivalent to subspecies (and will shortly be formally named as such; Brasier and Kirk 2001). On average, the EAN race is slightly less pathogenic then the NAN, but both are very aggressive pathogens of European and North American elms (Brasier 1991). The EAN and NAN races also differ in a number of other properties, such as their colony morphologies (Figure 2), their perithecial sizes, and their molecular fingerprints (Figure 3).

It is important to appreciate that *O. ulmi* and *O. novo-ulmi* are not totally reproductively isolated. Both species are obligatorily outcrossing, with two sexual compatibility types. Crosses within each species are highly fertile and "breed" true. In crosses between them, however, *O. novo-ulmi* strongly rejects *O. ulmi* as a fertilizing (male) sexual partner, although *O. ulmi* can be fertilized by *O. novo-ulmi*. The resulting ascospore progeny show a remarkable range of nonparental phenotypes, including female sterility. Many are of low vigor and fitness, and most are weaker pathogens even than the *O. ulmi* parent (Brasier 1977, Kile and Brasier 1990). *O. ulmi* and *O. novo-ulmi* are therefore strongly but not totally reproductively isolated at both prezygotic and postzygotic levels. This reproductive isolation has been interpreted as evidence that they were previously geographically separated as well as differently adapted species (Brasier 1977, 1986, Kile and Brasier 1990).

Intercontinental spread of Dutch elm disease

The Dutch elm disease pandemics of the 20th century involved major migratory episodes for the causal pathogens. These events are summarized below.

***First pandemic:* Ophiostoma ulmi.** The first recorded pandemic of Dutch elm disease, caused by Ophiostoma ulmi, began in northwest Europe around 1910 (Figure 4a). Thereafter the disease spread rapidly eastward on a series of epidemic fronts across Europe and into southwest Asia. It was also introduced to the United Kingdom and North America in about 1927 and into central Asia in the late 1930s as a result of a series of importations of infested elm timber (Peace 1960, Brasier 1990). Initially, the spread of O. ulmi resulted in an intense epidemic in Europe, but during the 1940s this first epidemic unexpectedly declined after losses of 10% to 40% of the elms in most European countries (Figure 1;

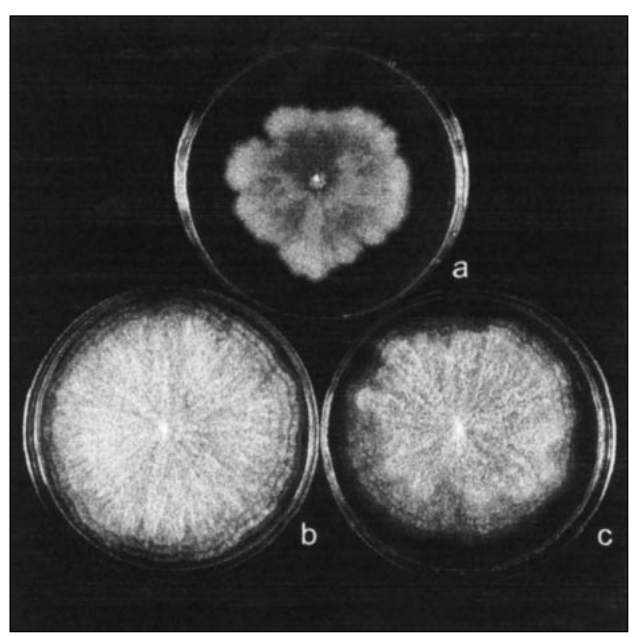

Figure 2. Characteristic colonies of the Dutch elm disease fungi: (a)* Ophiostoma ulmi*, cause of the first pandemic; (b), (c) NAN and EAN races of* O. novo-ulmi*, cause of the current pandemic.

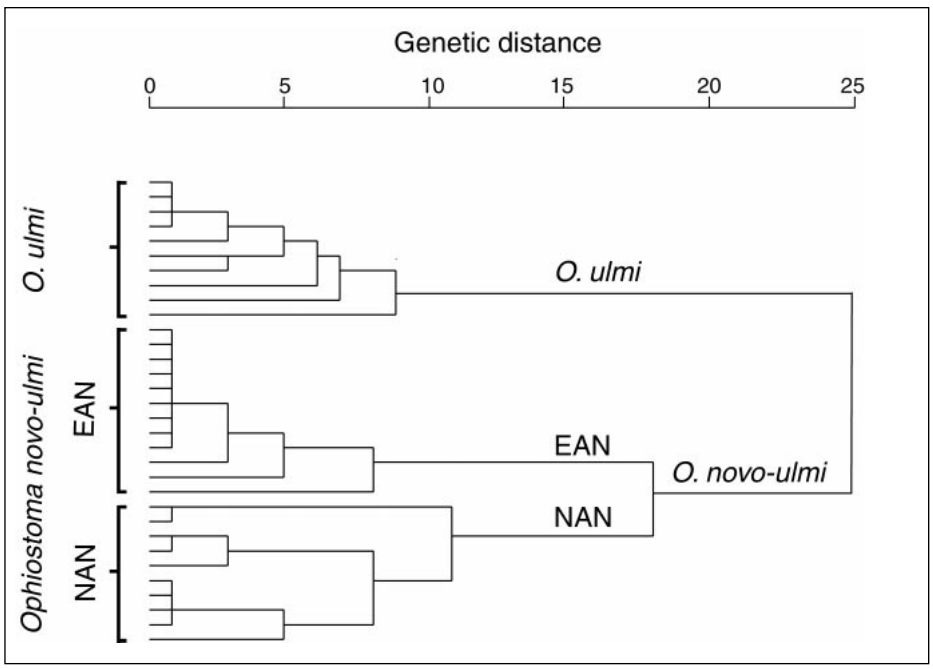

Figure 3. The phylogenetic relationship between isolates of Ophiostoma ulmi and the EAN and NAN forms of* O. novo-ulmi*, based on their molecular fingerprints (RFLPs of mitochondrial DNA; in this case, a PvuII digest). Adapted from Bates et al. (1993).

Peace 1960). This decline is now thought to have involved the spread of deleterious viruses in the O. ulmi population (Mitchell and Brasier 1994). In North America, however, no such decline occurred.

***Second pandemic:* Ophiostoma novo-ulmi.** In the early 1970s, a severe new Dutch elm disease outbreak occurred in Britain and neighboring parts of Europe, caused by the previously unknown *O. novo-ulmi*. Later, sample surveys across much of the Northern Hemisphere indicated that a second pandemic of Dutch elm disease, caused by O. novo-ulmi, had actually begun in the 1940s at two very different locations: the Moldova–Ukraine region in eastern Europe (EAN form, Figure 4a) and the southern Great Lakes area in North America (NAN form, Figure 4b) (Brasier 1990, 1996). Thereafter, the EAN form migrated steadily westward across Europe, reaching the Netherlands by the mid-1970s, and eastward to Southwest Asia. In the 1970s, it arrived some distance away in central Asia, probably as a result of an importation jump. Likewise, NAN *O. novo-ulmi* spread steadily outward across the North American continent, reaching both the east and west coasts by the 1970s and 1980s (Figure 4b). In another importation jump, it was introduced from Canada into Britain on diseased elm logs during the 1960s (Brasier and Gibbs 1973). It quickly spread to the Netherlands, France, Spain and many other countries of western Europe. The geographical ranges of EAN and NAN *O. novo-ulmi* now overlap in several parts of Europe (Figure 4b; see also Figure 1).

The international spread of Dutch elm disease has been very much a story of sequential introductions of these pathogens on infested elm timber. This mode of introduction highlights the risk posed by inappropriate timber importations between or across continents (Gibbs and Wainhouse 1986, Goheen 1993). Unfortunately, in the case of the second pandemic, the existence of a second species of Dutch elm disease pathogen (*O. novo-ulmi*) was not recognized until it was too late for many countries to take appropriate quarantine measures. The risk presented by unrecognized pathogens has since been further highlighted by the recent discovery of yet another previously unknown Dutch elm disease pathogen, *O. himal-ulmi*.

The spread of *O. novo-ulmi* has resulted in a catastrophic epidemic in which most mature European elms have died, with some 30 million elms killed in the United Kingdom alone. In North America, the destructive impact of *O. novo-ulmi* has been even greater: Losses have run into hundreds of millions of elms. Across North America, Europe, and southwest Asia, recurrent cycles of elm recovery via emerging young seedlings and root suckers, followed by further attacks by *O. novo-ulmi*, are predicted to occur well into the future (Brasier 1986).

Discovery of O. himal-ulmi

A major objective of the author's international surveys of *O. ulmi* and *O. novo-ulmi* (Figure 4) has been to locate the pathogens' geographic origins. China was long considered a likely origin for Dutch elm disease, but a 1986 survey across central China and Xinjiang Province revealed no evidence of the pathogens (Brasier 1990). They are also absent from Japan. However, a survey in the western Himalayas led to the discovery (Brasier and Mehrotra 1995) of an entirely new, endemic species of Dutch elm disease pathogen, now named *Ophiostoma himal-ulmi*. *O. himal-ulmi* is another Dutch elm disease fungus that is very aggressive to European elms. It also shares many other physiological similarities to *O. novo-ulmi* (Brasier and Mehrotra 1995), but it is apparently in natural balance with the native Himalayan elms and elm bark beetles.

Despite this new discovery, the geographic origins of *O. ulmi* and *O. novo-ulmi*, and indeed the origins of the EAN and NAN races of *O. novo-ulmi* (which also may have separate geographical origins), still remain to be identified. Suggested areas for further surveys are the eastern Himalayas, Burma, other parts of southeast Asia bordering China, and the floristically unique Yunnan Province in China itself (Brasier and Mehrotra 1995).

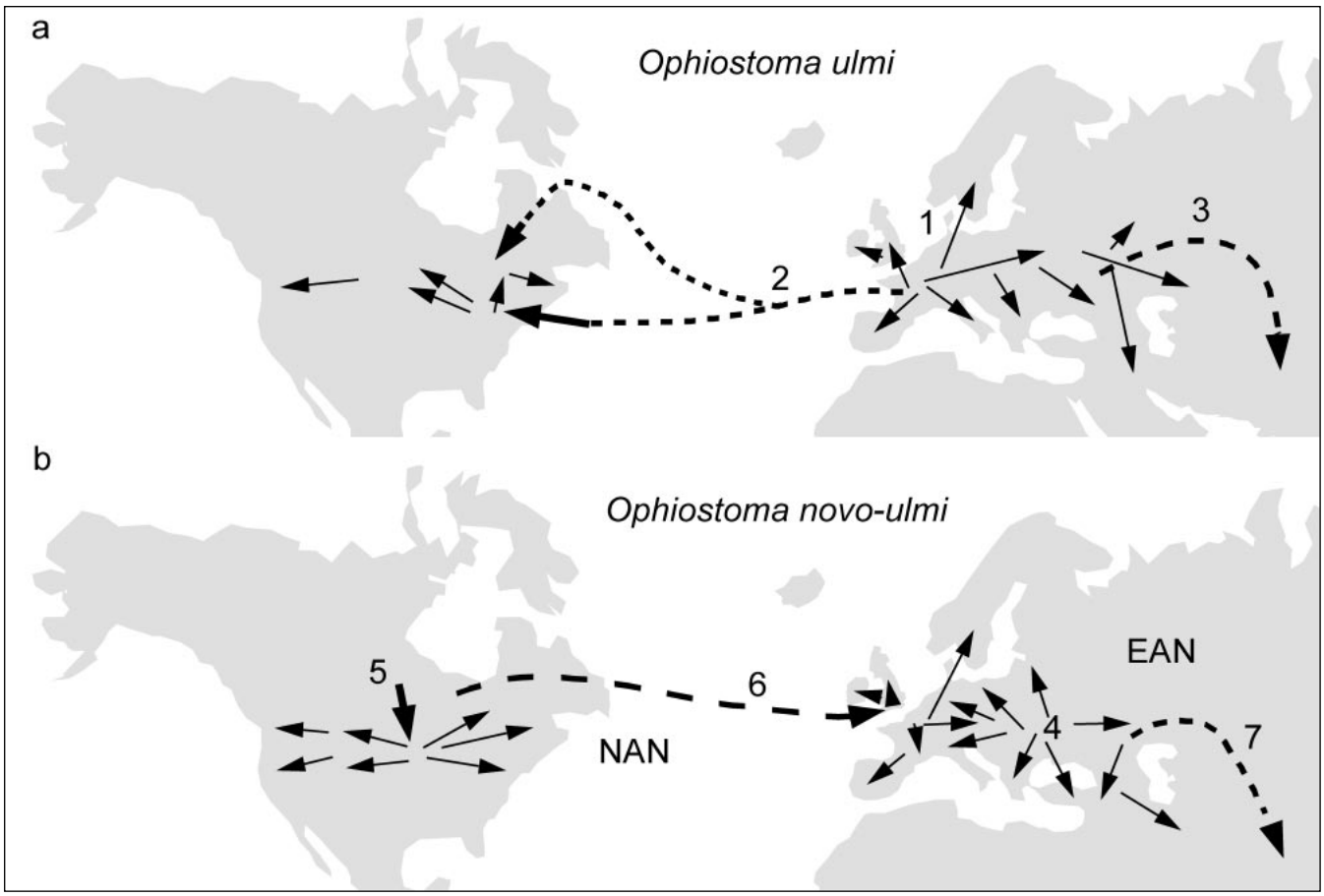

Figure 4. Intercontinental spread of* Ophiostoma ulmi *and O. novo-ulmi in the first and second pandemics of Dutch elm disease. Solid arrows show natural migrations from probable sites of initial introduction; dashed arrows, subsequent spread via additional importation events. (a) Spread of* O. ulmi*: 1, its appearance in northwest Europe around 1910; 2, introduction to North America in the 1920s; 3, introduction from Krasnodar to Tashkent, late 1930s. (b) Spread of EAN and NAN races of* O. novo-ulmi*; 4, 5, original centers of appearance of the EAN and NAN in the Romania–Moldova and southern Great Lakes regions, respectively; 6, introduction of NAN* O. novo-ulmi *from Toronto area to Britain, c. 1960; 7, introduction of EAN* O. novo-ulmi *to Tashkent area, 1970s. Adapted from Brasier (1990).

Replacement of O. ulmi *by* O. novo-ulmi *and the potential for genetic exchange*

Commonly, when *O. novo-ulmi* arrives at a "new" location, *O. ulmi* is already present. *O. novo-ulmi* rapidly replaces *O. ulmi*, and the latter declines at about 10% of the total pathogen population per annum (Figure 5; Brasier 1986). This phenomenon appears to be a classic example of a fitter species replacing a less fit species. The fitness advantage exhibited by *O. novo-ulmi* probably has several components. First, there appears to be direct competitive antagonism of *O. ulmi by O. novo-ulmi* when the two species come into close physical contact in the bark around the breeding galleries of the elm bark beetles (Figure 5; Mitchell 1988). Second, by virtue of its greater pathogenic ability, *O. novo-ulmi* captures more of the host resource than *O. ulmi* (resource meaning here the internal sapstream or xylem of the tree and the highly nutritious inner bark around the beetle breeding galleries). Third, *O. novo-ulmi* may be better adapted to the temperate, elm-inhabiting regions of Europe and North America, and *O. ulmi* may be disadvantaged through being a tropically or subtropically adapted organism (Brasier and Mehrotra 1995).

During this replacement process, the close proximity of *O. ulmi* and *O. novo-ulmi* in the bark around the beetle galleries (Figure 6) provides the physical opportunity for interspecific genetic exchange. In addition, as outlined above, *O. ulmi* and *O. novo-ulmi* are not fully reproductively isolated. Limited sexual hybridization between them is possible. Until recently, because of the low fitness of their progeny, it was considered that any hybrids produced would not survive in nature (Brasier 1977, Kile and Brasier 1990). However, new research has shown that rare *O. ulmi–O. novo-ulmi* hybrids do occur in nature but are transient, quickly disappearing in competition with the parent species (Brasier et al. 1998). Despite being transient, the hybrids could act as "genetic bridges," allowing unilateral gene flow from one species to the other.

Evidence for gene transfer from O. ulmi *to* O. novo-ulmi

Several lines of evidence indicate that introgressive gene flow has occurred between *O. ulmi* and *O. novo-ulmi* during the latter's migration.

Introgression of* O. ulmi *DNA, including a pathogenicity gene. One clue that gene flow was occurring between O. ulmi and O. novo-ulmi came from RFLP-based DNA fingerprinting studies. In a search for cloned DNA fragments that unambiguously discriminated O. ulmi isolates from O. novo-ulmi isolates when used as hybridization probes, some EAN O. novo-ulmi isolates were found to exhibit rare O. ulmi-like DNA polymorphisms, suggesting that they had acquired O. ulmi genes via introgression (Figure 7). The DNA of 50 EAN isolates from across Europe was therefore digested with two restriction enzymes and probed with a range of cloned DNA fragments that discriminated O. ulmi from O. novo-ulmi. Fifteen EAN isolates were found to carry O. ulmi-like polymorphisms (Bates 1990). These isolates were concentrated around the Romania–Black Sea area (Figure 8), suggesting that the introgressed O. ulmi genes were being lost or "discarded" as EAN O. novo-ulmi migrated away from its initial center of appearance.

Of the 15 EAN isolates with *O. ulmi*-like DNA, only one exhibited a non-*O. novo-ulmi*-like array of phenotypes. This isolate also had an unusually low level of pathogenic aggressiveness on elm. In a subsequent cross, the isolate's low aggressiveness was shown to be controlled by a single gene. An analysis based on cosegregation of AFLP markers has now shown that the gene involved is an *O. ulmi* pathogenicity gene that has been acquired by *O. novo-ulmi* via interspecific gene transfer (Abdelali et al, 1999).

Rapid changes in O. novo-ulmi population structure. Another clue to the existence of gene flow between *O. ulmi* and *O. novo-ulmi* has come from ultra-rapid

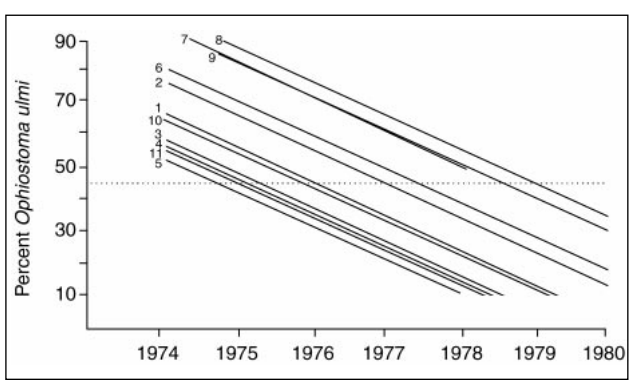

Figure 5. The replacement of* Ophiostoma ulmi *by* O. novo-ulmi. *The figure shows the steady decline in the percentage of O. ulmi, following the arrival of* O. novo-ulmi. *The data are for 11 provinces of the Netherlands between 1974 and 1980. The dashed line is the 50% level (transformed). Redrawn from Brasier (1983).

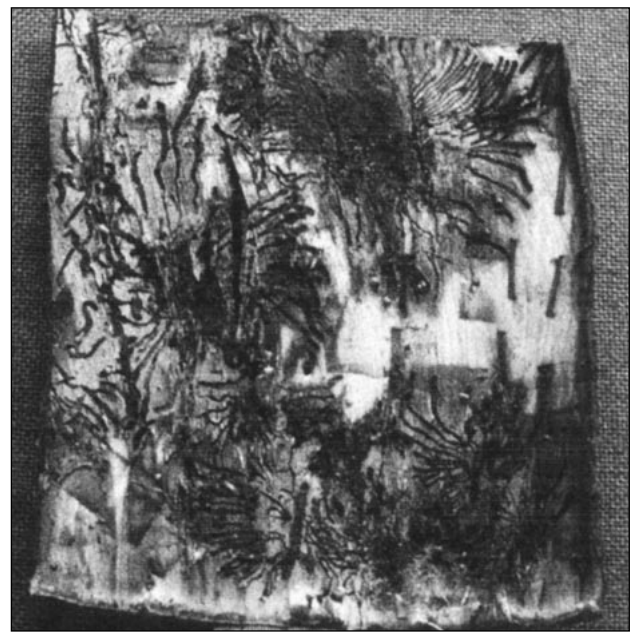

Figure 6. Elm bark beetle breeding galleries in a piece of dying elm bark. The pathogens,* Ophiostoma ulmi *and* O. novo-ulmi, *live in and around the galleries.

changes in the genetic structure of local *O. novo-ulmi* populations. These changes involve, in particular, a sudden increase in the frequency of so-called vegetative compatibility (vc) types. Fungal vegetative incompatibility systems are analogous to tissue incompatibility systems in animals. They are controlled by multiple genes with multiple alleles; thus, many different vc types potentially can exist in a population. One function of vc systems is probably to restrict the spread of deleterious intrahyphally transmitted viruses (Figure 9; Caten 1972). If adjacent colonies of *O. novo-ulmi* are of a different vc type—that is, vegetatively incompatible—viruses cannot pass readily from one colony to the other via hyphal fusions because the fusion cells die. However, when adjacent colonies are of the same vc type—that is, vegetatively compatible—viruses can spread readily between them because the fusion cells are functional (Brasier 1986). The more vc types in a population, therefore, the more the spread of viruses should be restricted.

Each time *O. novo-ulmi* has arrived at a "new" location in Europe, it has usually spread as a clone of a single vc type. These clones are also of a uniform colony morphology and of a single sexual mating type (Brasier 1988). Deleterious viruses (known as d-factors) tend to spread abundantly in the expanding vc clones. However, within only a few years, the clonal population diversifies into multiple new vc types (Figure 10). This change is accompanied by a sudden increase in diversity in colony patterns and other characteristics, and by the appearance of the "other" sexual mating type. Furthermore, as the new vc types appear, the frequency of deleterious viruses in the population falls rapidly.

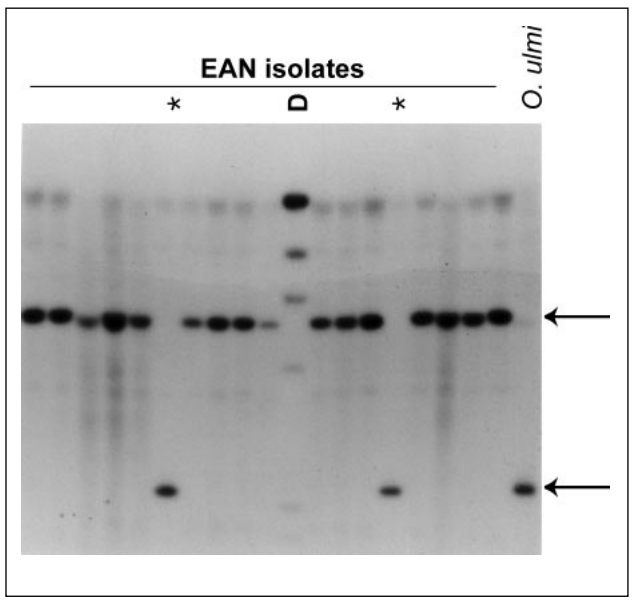

Figure 7. Presence of* O. ulmi *DNA in EAN* O. novo-ulmi *isolates. Nuclear DNA of 15 EAN* O. novo-ulmi *and one* O. ulmi *isolate probed with a DNA clone from* O. ulmi. *Arrows show the presence of* O. ulmi*-like bands in two EAN isolates. D, DNA ladder. From Bates (1990).

Comparable studies on migrating *O. novo-ulmi* populations in North America show that a similar change from a clonal population to multiple vc types has occurred, but that the rate of change has been much slower and so far only partial (Figure 10b). In addition, virus pressure on the clones in North America has remained low (Brasier 1996, Milgroom and Brasier 1997). In New Zealand (where *O. novo-ulmi* arrived in the late 1980s), an immigrant vc clone has continued to persist, apparently unchanged, for over a decade, and no viruses have been detected in the clone (Figure 10c; Brasier and Gadgil 1992, Brasier 2000). Moreover, in the New Zealand case, *O. ulmi* was not present prior to the arrival of *O. novo-ulmi*.

Two inferences may be drawn from the different outcomes in the European, American, and New Zealand situations. First, *O. novo-ulmi* vc clones tend to diversify into new vc types only where *O. ulmi* was originally present, as in Europe and North America. Second, only where virus activity in the vc clones is very high, as in Europe, do the clones diversify both rapidly and extensively. Those inferences suggest, in turn, that the novel vc genes are acquired by *O. novo-ulmi* from *O. ulmi*, and that the selection pressure exerted by the viruses favors the survival of novel vc types over the original vc clones. Results of a molecular study initiated to test the hypothesis that the novel vc genes come from *O. ulmi* are consistent with the hypothesis. Segments of *O. ulmi* DNA have been found flanking the novel vc genes in *O. novo-ulmi*. The results also indicate that the "missing" mating type gene may also be acquired by *O. novo-ulmi* vc clones from *O. ulmi*.

Transfer of viruses from O. ulmi *to* O. novo-ulmi?

There exists a further, equally biologically significant possibility: that the deleterious viruses that spread in the *O. novo-ulmi* vc clones are also acquired from *O. ulmi*. A preliminary comparison of viruses in *O. ulmi* and *O. novo-ulmi* isolates obtained from the same epidemic front site in Europe indicates very close similarity in their RNA sequences.

Together, these possibilities suggest that a remarkable series of events has occurred. *O. novo-ulmi* has competitively eliminated *O. ulmi* across much of the Northern Hemisphere, causing *O. ulmi* to become extinct. At the same time, *O. novo-ulmi* may have "caught" debilitating virus infections from *O. ulmi*. These viruses would have brought about its demise, but for the fact that it simultaneously acquired vc genes from *O. ulmi* that allowed it to escape the consequences of the viruses. It also seems that "unuseful" genes, such as *O. ulmi* pathogenicity genes, may have been acquired by *O. novo-ulmi*, but these genes appear to be eliminated by selection (Abdelali et al. 1999).

Yet to be tested is another fascinating possibility concerning *O. ulmi* and unusual gene transfers. When *O. ulmi* originally spread across Europe, it probably did so as a single vc clone, which also subsequently became highly variable in vc types (Mitchell and Brasier 1994). *Ophiostoma ulmi* may therefore have acquired its novel vc genes, and perhaps also its viruses (which were abundant in some of its populations), from *Ophiostoma quercus* (previously called *O. piceae*), a saprotrophic fungus believed to have been the resident associate of the elm bark beetles before the arrival of *O. ulmi* (Brasier 1990).

Unrestricted EAN–NAN hybridization: Emergence of a new form of O. novo-ulmi?

Unlike *O. ulmi* and *O. novo-ulmi*, the EAN and NAN forms of *O. novo-ulmi* hybridize freely in the laboratory and in nature. Locations in Europe where the EAN and NAN are known to overlap are shown in Figure 11. A preliminary study at two such locations, Limburg, in the Netherlands, and Orvieto, in Italy, has shown that hybrids with phenotypes intermediate between EAN and NAN—and with recombinant vc and molecular (RAPD) genotypes—but no less aggressive to elm, are emerging and replacing the pure EAN and NAN forms (Brasier and Kirk 2001). In the future, therefore, swarms of EAN/NAN hybrids are likely to emerge at such overlap sites (Figure 11). Initially, the hybrids are likely to intercross with other hybrids and to backcross with the surviving pure EAN and NAN forms.

From such a melange of recombinants, natural selection may in the future favor a particular set of genotypes that will be neither NAN nor EAN, but a new race or subspecies of the pathogen. *Ophiostoma novo-ulmi* is therefore undergoing another phase of evolutionary development in Europe. Outside Europe—in North America and in central and Southwest Asia, for example (Figure 4b)—the pure NAN and EAN forms may remain geographically isolated from these

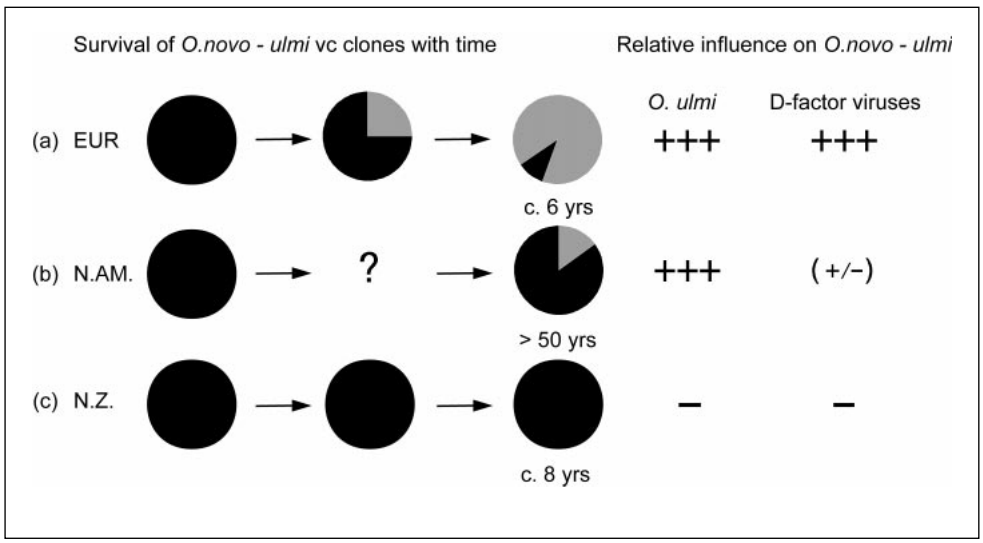

Figure 8. Sample positions of 50 EAN* O. novo-ulmi *isolates from across Europe and central Asia, showing the distribution of isolates exhibiting* O. ulmi*-like DNA polymorphisms. Compiled from Bates (1990).

events for a while, and so survive into the future. Gradually, however, they are likely to be overtaken by hybrid genotypes as these migrate into the outlying areas or are introduced into them. The present situation in Europe thus provides an outstanding and unique opportunity to investigate the evolutionary development of a plant pathogen undergoing a full-scale hybridization process.

Potential for rapid evolution of introduced fungal pathogens

This glimpse of the migratory history of *O. novo-ulmi* from the 1940s to the 1990s demonstrates clearly how the "escape" of a fungal pathogen beyond the routine selection constraints of its endemic environment presents it with new evolutionary opportunities. It also shows that the traditional focus of concern about the risk posed by introduced exotic plant pathogens—namely, disease impact—must be extended to include the risk of accelerated pathogen evolution and the emergence of new or altered pathogens. Gene flow between fungal species is, however, a neglected area of mycology and plant pathology. Reports of species hybrids in fungi are surprisingly rare, with only about six clear examples prior to 1990 (Burnett 1983, Brasier 1995).

The example of the Dutch elm disease pathogens led me to a theoretical assessment of the potential for horizontal gene transfer when related fungal species come into contact suddenly, as through immigration or geographic transposition (Brasier 1995). Among the main variables likely to be involved are the level of niche contact and the degree of reproductive isolation between the immigrant and resident species (Figure 12) and the potential for survival of any resulting hybrids or introgressants—for example, whether or not any hybrids exhibit greater fitness than the parent species and whether new substrates or hosts are available for their exploitation. The possible evolutionary outcomes range from relatively small (if highly significant) genetic modifications, such as the acquisition by one parent species of a virus or of a single gene for a new host specificity, to the emergence of an entirely new fungal taxon combining the genomes of both parents.

Hybridization is perhaps most likely when related but previously allopatric (geographically isolated) fungal taxa, lacking strong genetic barriers to hybridization, come into regular contact in the same niche. This is almost certainly the case in the current hybridization events between the Dutch elm disease pathogens; indeed, these events encompass several of the outcomes shown in Figure 12.

New examples of hybridization between pathogens

Recently, several new examples of pathogen hybrids have come to light, all involving important plant pathogens. In the Netherlands, a new *Phytophthora* pathogen on *Primula* and *Spathiphyllum* has been shown to be a hybrid between *P. cactorum*, which is probably an endemic resident in the Netherlands, and *P. nicotianae*, an introduced species (Man in't Veldt et al. 1998). Also, in Europe, a new, aggressive *Phytophthora* pathogen of alder has been found spreading along river systems and in crop shelterbelts in several countries. This appears to be a recent allopolyploid interspecific hybrid between the introduced *P. cambivora*, which is a

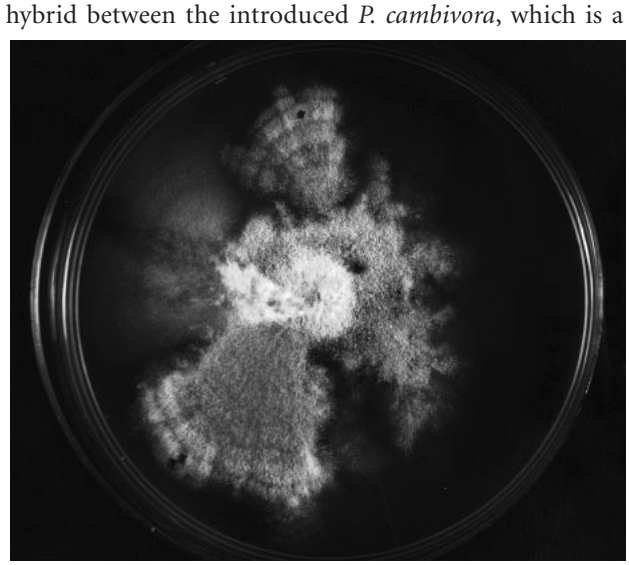

Figure 9. A virus (d-infected) culture of* Ophiostoma novo-ulmi*, showing a typical irregular, unstable growth pattern associated with severe virus infection.

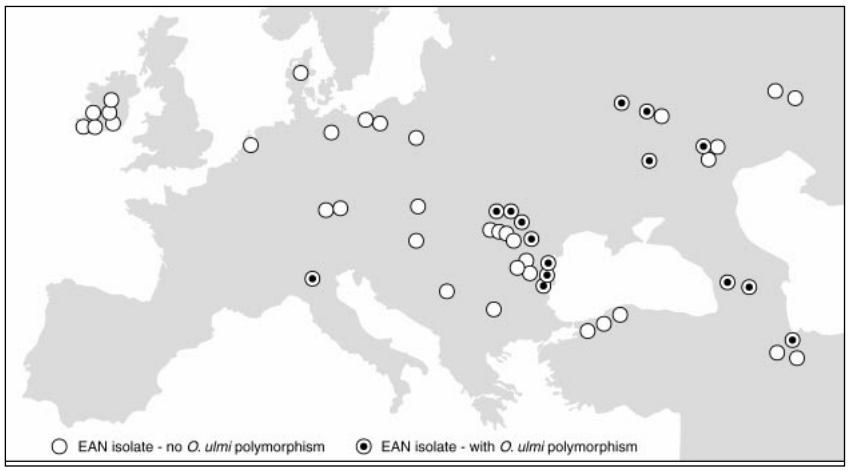

Figure 10. Changes in the structure of the* Ophiostoma novo-ulmi *population from dominant single vc type clone(s) (black) to many new vc types (gray) since the beginning of the current Dutch elm disease epidemic; (a) Europe, (b) North America, (c) New Zealand. The level of involvement of two other variables in the survival of the clone is indicated: the presence (or absence) of* O. ulmi *before the arrival of* O. novo-ulmi *and the level of virus infection in the dominant vc clone. +++, strong influence; +/- weak or no influence; –, absent.

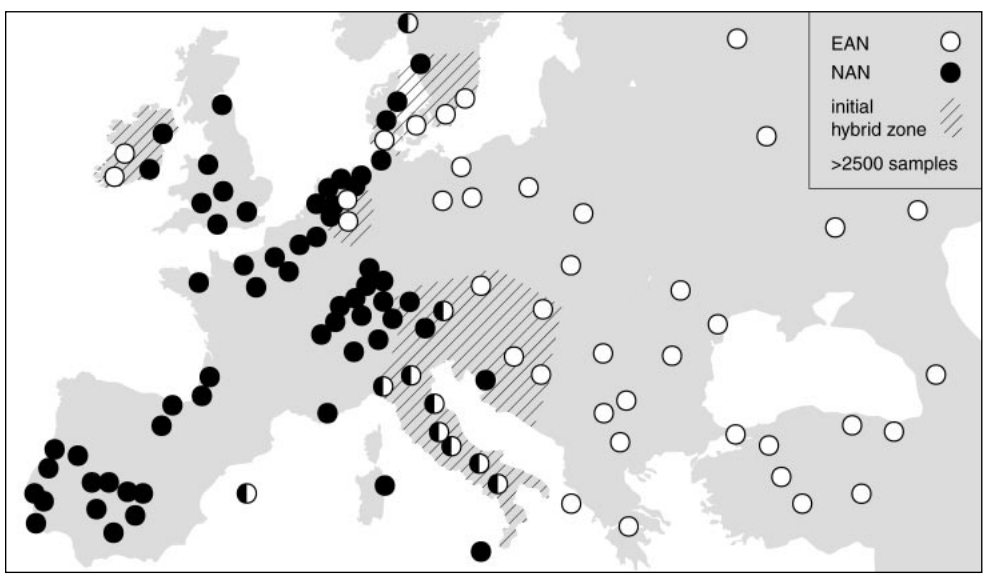

Figure 11. Distribution of EAN and NAN races of* O. novo-ulmi *across Europe in 1990 (representative sample points only). EAN and NAN overlap locations occur in Ireland, the Netherlands, Scandinavia, Germany, and Italy. Hatched areas show known and probable zones of emergence of EAN–NAN hybrids. Based on more than 2500 samples.

well-known pathogen of hardwood trees but not a pathogen of *Alnus*, and another *Phytophthora* species close to *P. fragariae*, which is a pathogen specialized on raspberry and strawberry (Brasier et al. 1999). In New Zealand and North America, a range of newly evolved interspecific hybrids have been found between different introduced *Melampsora* rust species on poplar trees (Spiers and Hopcroft 1994, Frey et al. 1999, Newcombe et al. 2000). While in the forests of northeastern California, hybrids have been identified between the host-specialized "S" and "P" taxa of the conifer root pathogen *Heterobasidion* (Garbelotto et al. 1996).

The latest reports indicate that many cases of hybrid pathogens remain to be discovered. They also raise additional environmental and evolutionary issues. The new hybrid alder Phytophthoras in Europe attacks a host (alder) that the presumptive parent species are unable to attack (Brasier et al. 1999). Such a possibility was recently demonstrated experimentally in *Phytophthora*: Progenies with novel host specificities have been recovered among *Phytophthora* interspecific hybrids generated in the laboratory (Ersek et al. 1995). On the other hand, the new *Melampsora* rust hybrids on poplar in the American Pacific Northwest (and also the *Melampsora* hybrids in New Zealand; Frey et al. 1999) combine the host ranges of both parent rust species. The hybrids can therefore act as a genetic bridge, transferring pathogenicity traits from one rust species to the other (Newcombe et al. 2000). The alder *Phytophthora* and *Melampsora* examples therefore demonstrate a potential for evolution of new organisms with new host ranges.

Previously, this risk that pathogens with novel host specificities might arise through hybridization has been discussed theoretically in terms of the potential for emergence of new "superpathogens" (Brasier 1995). For example, *Ophiostoma quercus*, a common oak saprotroph and oak bark beetle associate in Europe, is closely related to the recently immigrant elm pathogen, *O. novo-ulmi*. It has also been found on elm (see above), leading to the suggestion that it might become a potential wilt pathogen of oak if it acquired a toxin gene or a pathogenicity gene from *O. novo-ulmi* (Brasier 1990, 1995). This potential has now been partially confirmed. When the *O. novo-ulmi* cerato-ulmin gene is artificially transferred into *O. quercus, O. quercus* becomes a vascular wilt pathogen of elm (Del Sorbo et al. 2000). Furthermore, in a simple laboratory test, it has been shown that perithecia (sexual fruiting structures) develop in laboratory crosses between *O. novo-ulmi* and *O. quercus*, but to date these have been infertile (Brasier 1993).

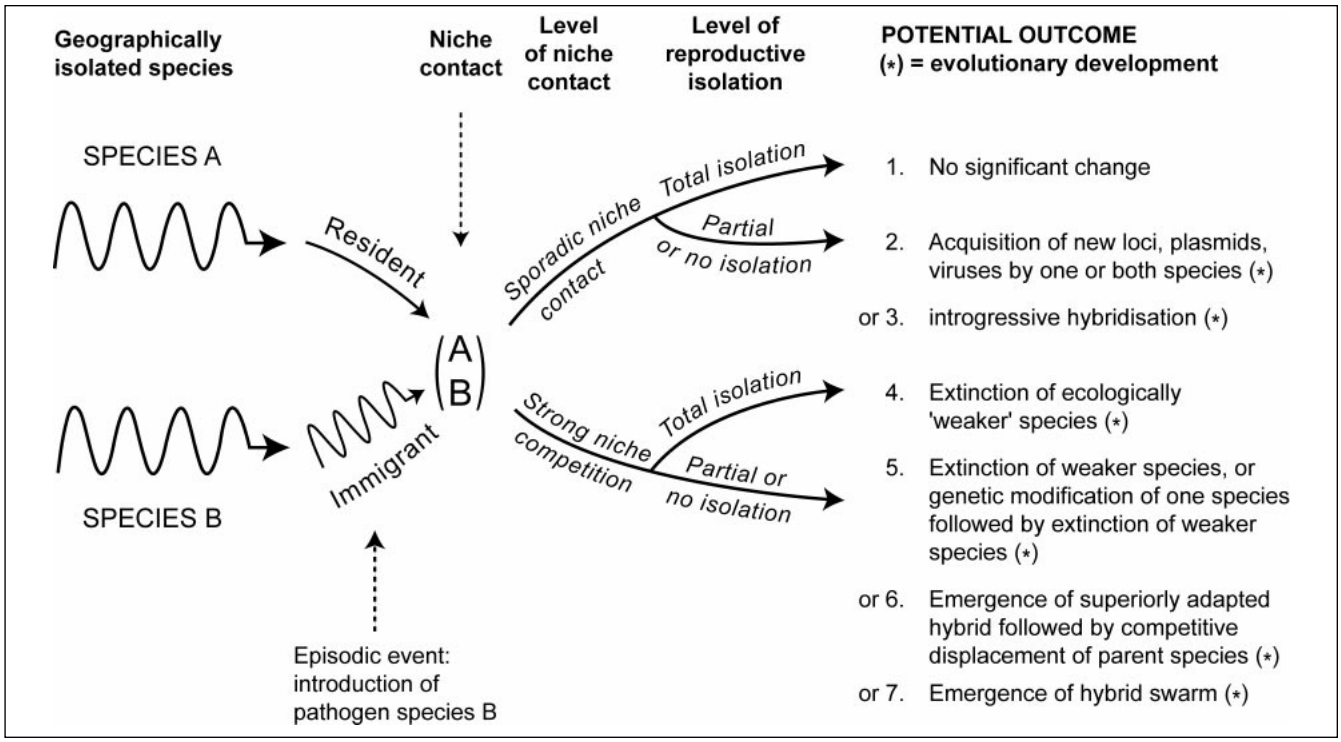

Figure 12. Potential evolutionary outcomes when closely related but previously geographically isolated pathogen species come into contact. The outcome will depend upon many factors, including the frequency of niche contact, the nature of any genetic barriers to hybridization, the degree to which the genomes of the two species can recombine, and the ability of any resulting hybrids to compete with the "parent" species. Redrawn from Brasier (1995).

The new alder *Phytophthora* is not a single entity but comprises a swarm of sterile to fertile heteroploid genotypes. Some of these have unique, unstable, and often highly unusual phenotypes, and appear to be breakdown products of the full allotetraploid hybrid, the most frequent hybrid type found. Hence the evolution of the alder *Phytophthora* pathogens appears to be continuing, a conclusion that is further supported by evidence of homogenization and continuing recombination in the ITS (internal transcribed spacer) regions of the rDNA genes in some isolates. In addition, several of the isolates of *P. cambivora* examined for comparison were shown to have variable ITS sequences, indicating that they have been involved in other interspecific hybridization events (Brasier et al. 1999).

The new *P. cactorum–P. nicotianae* hybrid in the Netherlands is so far confined mainly to nurseries and greenhouses. Interestingly, like *P. nicotianae*, a number of other exotic introduced *Phytophthora*s are now being found in hydroponics facilities in Europe (Man in't Veldt et al. 1998) and in intensive nurseries. Such facilities, especially continuous hydroponics systems, can allow multiple *Phytophthora* species access to a wide variety of hosts. They may therefore provide an ideal environment for evolutionary "experimentation" in this major pathogen genus (Man in't Veldt et al. 1998). Since the flagellate, swimming zoospores of *Phytophthora* lack cell walls, they may behave more like fungal protoplasts when it comes to cell fusion events. Fusion of zoospores, as opposed to fusion of mycelia or gametangia, could represent a fast track to interspecific hybridization in hydroponics facilities and in the field.

The opportunity for hybridization between previously allopatric fungal pathogens is likely to increase as world trade in plants intensifies and more plants and their associated pathogens are introduced into new biogeographic environments. Increasing stress levels on plants due to global environmental disturbance effects such as climate change and pollution, together with the planting of more host species geographically off-site, are likely to increase the availability of hosts suitable for colonization by novel genotypes (Brasier 1995). Particularly vulnerable may be the slower-reproducing components of natural ecosystems such as trees and shrubs in forests and wildlands. Among the higher-risk pathogen types may be those with limited dispersal ranges, such as the soil-borne Phytophthoras. Some Phytophthoras may also have greater interspecific hybridization potential because they already have broad host ranges. Another high-risk pathogen type may be those dispersed by insect vectors, as exemplified by Dutch elm disease and oak wilt. By "host jumping," the vectors may bring different introduced, or introduced and resident, pathogens into contact (Brasier 1993). The occurrence of two clear *Melampsora* examples to date suggests that another higher-risk group may be the tree rusts.

Quarantine issues

Another major issue raised by these developments is that of the regulation, detection, and diagnosis of hybrids or introgressants. From the early 1900s, plant quarantine regulations were introduced by many countries, aimed at excluding well-known "dangerous" exotic pathogens. However, those regulations, together with methods of pathogen diagnosis currently used in international quarantine, tend to be founded on traditional, usually morphology-based fungal species concepts (Brasier 1997). They are therefore unlikely to readily encompass or detect the international movement of hybrids—especially where a genetic alteration is confined to a few genes, which, although pathologically significant, have little effect on the morphology of fruiting structures. For example, the new alder *Phytophthora* has already been incorrectly classified as *P. cambivora* on purely morphological grounds in some culture collections. Hence there is a need for quarantine regulations to be targeted to particular genotypes, as well as to particular species.

To identify hybrids and to control and detect their movements, molecularly based protocols will probably be needed. Even where these are applied, as in PCR diagnostics based on ITS sequences of rDNA, a hybrid may not be detected if its ITS sequence is identical to that of one of the parent species (Brasier et al. 1999). Complementary molecular criteria, such as fingerprinting of "total" DNA, may therefore be needed. In addition, how to nomenclaturally designate hybrids or hybrid swarms, such as the EAN–NAN *O. novo-ulmi* hybrids or the hybrid alder *Phytophthoras*, in a way that meets the needs of scientific communication and the legal requirements of international quarantine legislation has yet to be seriously addressed in mycology and plant pathology. Here again, molecular criteria are likely to be important.

Although current quarantine schedules are targeted mainly at traditional high-risk species, the emergence of new hybrid pathogens indicates a need to consider whether unscheduled, apparently low-risk organisms can hybridize to produce high-risk organisms. This possibility in turn implies a need for environmental risk assessments similar to those used prior to the release of a genetically modified organism. This form of risk assessment would require the development of a knowledge base founded on an understanding of fungal breeding systems, including the strength of barriers to gene flow between species, the likely genetic outcome of hybridization events, and the likely impact of any hybrids under field conditions. From the recent examples of hybrid pathogens, it is already clear that further research is needed on the genetic stability and potential for further evolution of emerging hybrids; on the potential for nascent hybrids, such as the alder *Phytophthoras*, to hybridize with other species in the ecosystem; and on the potential for evolution of novel host specificities in hybrids.

In today's world of rapid transport and communication, there is also a need for international quarantine procedures to become more proactive and rapid responsive. In particular, international agreements may be needed under which newly identified pathogens or hybrids are quickly assessed, in their place of initial identification, for the risks they pose to potentially susceptible host plants on other continents or in other biogeographic environments. More proactive initiatives are also needed with regard to previously identified quarantine threats. For example, cooperative research has recently demonstrated, contrary to the expectation of some, that European oaks are highly susceptible to North American oak wilt, *Ceratocystis fagacearum* (Macdonald et al. 1998). And although *C. fagacearum* lacks an efficient vector in North America, if it were ever to become "linked up" with the European oak bark beetle *S. intricatus*, which has an elm bark beetle-like lifestyle (Yates 1984), the effects could be catastrophic to oak forests across the Northern Hemisphere (Gibbs et al. 1984). Strategic research should be considered to identify potential control measures, such as the deployment of natural or modified viruses against *C. fagacearum* (Brasier 1998), based on knowledge of the pathogen's ecology, its breeding systems, and its likely evolutionary development following a link with a much more efficient vector (Brasier 1986, 1995).

The examples of Dutch elm disease, the new alder *Phytophthoras*, and oak wilt, among others, are a reminder that not only food crops but entire ecosystems are at risk from exotic pathogens, newly evolving pathogens, and inappropriate plant and timber importations (Goheen 1993). On the short human time scale of evolution, the impact of these events can be just as damaging to our environment and to our economic well-being as many social problems with a higher public profile. However, the international spread of a plant disease is often insidious; hence, its impact is more difficult to assess. The social and environmental costs even of major environmental catastrophes such as Dutch elm disease are rarely accounted for in national or corporate economic balance sheets. Consequently, despite the fact that the long-term costs can be enormous and open-ended, market forces tend not to operate in favor of strong quarantine protection.

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