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Species hybrids in the genus *Phytophthora* with emphasis on the alder pathogen *Phytophthora alni*: a review

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Abstract This review provides a summary of recent examples of interspecific hybridisation within the oomycetous genus *Phytophthora*. Species hybrids either created in the laboratory or evolved in natural environments are discussed in association with evolutionary issues and possible threats they may pose to agriculture, horticulture and forestry. It is suggested that sustainable control of such hybrids will depend on the better understanding of temporal and spatial aspects of genetic mechanisms and environmental factors that lead to the hybridisation process and thus the genetic diversity in *Phytophthora* populations.

Keywords Evolution · Interspecific hybridisation · Oomycetes

Introduction

Hybridisation between individuals from two populations has long been known in the plant kingdom, but its occurrence among populations of eukaryotic microorganisms has received delayed recognition. Indeed, eukaryotic microorganisms, such as fungi and oomycetes, possess a variety of reproductive mechanisms whereby they might undergo interspecific genetic exchange. As a consequence of the combination of two distinct genomes via sexual or parasexual processes, new allopolyploid hybrid species may evolve.

The possibility of hybridisation between two closely related species of plant pathogenic fungi or oomycetes has been considered for many years. For instance, seven decades ago Flor (1932) pointed out the potential for hybridisation among fungi based on the appearance of isolates of *Tilletia* with atypical morphological phenotypes. Decades later, Burdon et al. (1981) used isozyme analysis to confirm that a rust virulent on rough wheat grass and barley evolved via somatic hybridisation between rye stem rust and wheat stem rust i.e., between two formae speciales (f.sp.) of *Puccinia graminis*. Conclusive proof of species hybridisation has been provided only recently with the arrival of modern molecular genetic tools. Since the mid 1990s, a limited number of phytopathogenic species hybrids have been detected in the fungal phyla, Ascomycota and Basidiomycota (Brasier 2000; Olson and Stenlid 2002; Schardl and Craven 2003).

Within the Oomycota in the Kingdom Staminipila (Dick 2002), efforts to establish the occurrence of species hybridisation have focused on the genus *Phytophthora*. This genus contains approximately 80

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species, including the infamous late blight pathogen, *P. infestans*. These species are primarily soil-borne pathogens that affect a wide range of crop plants, shrubs and trees throughout the world. Although fungus-like in appearance, phytophthoras differ fundamentally from true fungi in terms of cell wall composition, reproductive biology and genetics (Erwin and Ribeiro 1996). In this review we summarise the evidence for species hybridisation in this important genus. Evidence for hybridisation as a source of genetic variability has derived from both laboratory attempts to create hybrids, and from studies of *Phytophthora* hybrids found in either natural or agro-ecosystems.

Hybrids created in the laboratory

Species hybrid formation in nature is likely to be a rare event and, consequently, it is difficult to detect and study. A tractable approach to the study of the possibility of genetic exchange and evolution derived from species hybridisation would be to create such organisms artificially.

Sexual crosses

An early report of this approach was by Boccas (1981), who induced sexual crosses among isolates of numerous heterothallic (outcrossing) *Phytophthora* species. This first effort was minimally successful and produced only one putative species hybrid among 220 progeny derived from several species crosses. Subsequent attempts to induce and confirm species hybridisation in other laboratories were more successful. For instance, Goodwin and Fry (1994) induced sexual crosses of the sympatric, heterothallic species, *P. mirabilis* and *P. infestans*. They confirmed that 79 out of 86 progeny were species hybrids based on DNA fingerprinting and isozyme analyses. Notably, mitochondrial DNA was uniparentally inherited, predominantly from the *P. infestans* parental isolates. Interestingly, most of these hybrids lost their ability to attack hosts of either parental species, including *Mirabilis jalapa*, the host for *P. mirabilis*, and potato or tomato, common hosts for *P. infestans*. May et al. (2003) more recently induced sexual crosses between the homothallic (self-fertile) species, *P. sojae* and *P. vignae*. They confirmed the hybrid nature of offspring by RAPD and AFLP analyses. They also noted that both of the tested F1 hybrids were pathogenic to soybean, the host for *P. sojae*, and cowpea, the host for *P. vignae*. However, the aggressiveness of these hybrids was reduced and was substantially more variable when compared to the parental isolates on their respective hosts.

Somatic hybridisations

Somatic fusion has also been suggested as a mechanism for hybridisation in nature among *Phytophthora* species that are heterothallic and that temporarily or spatially lack compatible mating types (Brasier 1992; Érsek et al. 1995). Although protoplast fusion between strains of a *Phytophthora* species was relatively easy to induce, the same method appeared to be insufficient for the creation of interspecific hybrids between *P. sojae* (syn.: *P. megasperma* f.sp. *glycinea*) and *P. medicaginis* (syn.: *P. megasperma* f. sp. *medicaginis*) (Layton and Kuhn 1988) or between *P. nicotianae* (syn.: *P. parasitica*) and *P. capsici* (Gu and Ko 2000). The first evidence of the formation of such hybrids was obtained by the induced fusion of uninucleate zoospores (Fig. 1) derived from non-compatible mating-type isolates of the closely related heterothallic species, *P. capsici* and *P. nicotianae* (Érsek et al. 1995). In these laboratory experiments, the morphologies of the four resultant hybrid isolates resembled *P. capsici* more closely than *P. nicotianae.* All of the hybrids were pathogenic to tomato, a plant

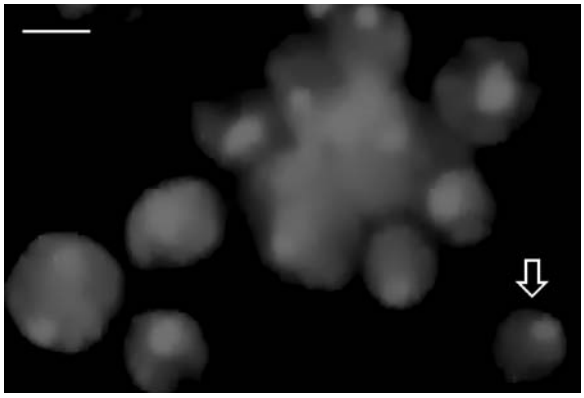

Fig. 1 Nuclear status of regenerating cells stained with DAPI following induced fusion of zoospores of *Phytophthora capsici* and *P. nicotianae*, as viewed by epifluorescence microscopy. Note enlarged cells and/or nuclei and multiple nuclei as compared to a uninucleate cell of normal size (*arrow*). Bar: 10 μm

susceptible to both parental species. However, two of the hybrid isolates exhibited an expanded host range that included both radish and lemon, hosts that are susceptible to *P. capsici* or *P. nicotianae*, respectively. The hybrid nature of the fusion offspring was confirmed by detection of DNA sequences specific to each parental species. In these hybrids repetitive DNA of *P. capsici* was detected readily by hybridisation with a species-specific DNA probe, whereas *P. nicotianae*-specific DNA was revealed after PCR amplification of DNA from hybrids using *P. nicotianae*-specific primers or random primers (Érsek et al. 1995; English et al. 1999). Bakonyi et al. (2002) also induced zoospore fusion to create hybrids from the morphologically distinct species, *P. nicotianae* and *P. infestans*. Resultant fusion offspring were more similar to *P. nicotianae* than to *P. infestans* on the basis of morphological and molecular evidence. Again, these hybrids expressed modified pathogenicity traits compared to parental species.

As a final example of the tractability of somatic fusion, Érsek et al. (1997) created tri-parental hybrids derived from *P. capsici*, *P. nicotiane* and *P. citrophthora.* In these studies, zoospore fusion offspring contained DNA from each parental species; however, all offspring failed to express pathogenicity to any of the hosts susceptible to the parental species. Notwithstanding the failure of protoplast fusion to create hybrids between *P. nicotianae* and *P. capsici*, Gu and Ko (2000) successfully generated hybrids by transfer of isolated nuclei from one species to the other. Analysis of zoospore progeny of these nuclear hybrids suggested the completion of events leading to a parasexual cycle.

Studies on interspecific zoospore fusion and nuclear transfer support the suggestion of Brasier (1992) that non-sexual genetic exchange might generate variability in pathogenicity or virulence within pathogen populations, particularly when complementary mating types needed for sexual reproduction are lacking. Artificially induced hybrids also suggest that it may be difficult to predict the effects of hybridisation on pathogen survival and dominance among populations in nature. With the development of molecular and biochemical markers, however, there have recently been noteworthy findings of naturally occurring *Phytophthora* species hybrids that may provide further insight into the mechanism of interspecific genetic exchange.

Naturally formed hybrids

Phytophthora alni

The potential for hybrid formation among *Phytophthora* species that has been established in laboratory studies has, in recent years, been confirmed by the detection of true or putative hybrids in natural and agro-ecosystems. One of the best-studied examples of *Phytophthora* species hybridisation in nature is that of *P. alni*, a newly recognised pathogen of alder (*Alnus* spp.). This pathogen was first discovered on dying alders in southern Britain in the beginning of the 1990s, and it has since been found throughout Europe, including Hungary (Brasier et al. 1995; Szabó et al. 2000; Streito 2003). *Phytophthora alni* killed approximately 10% of the alders in southern Britain within a few years of its initial discovery (Brasier et al. 1995; Gibbs et al. 1999). Recent surveys indicate that the disease is even more severe in riparian ecosystems in north-eastern France (Streito et al. 2002) and in Bavaria (Jung and Blaschke 2004).

Initial studies showed that certain isolates of this new pathogen of alder resembled *P. cambivora*. The similarity to *P. cambivora* was notable through the morphology of the gametangia (Brasier et al. 1995). However, the new pathogen differed from *P. cambivora* in several other traits, for instance, in being homothallic rather than heterothallic and in exhibiting an extremely high level of zygotic abortion. These properties, in addition to assessments of internal transcribed spacer (ITS) sequences and genomic polymorphisms, ultimately suggested that the alder pathogen might be a hybrid of two species, the heterothallic *P. cambivora* and a homothallic *P. fragariae*-like species (Brasier et al. 1999). Neither of these organisms is a known pathogen of alder. Ultimately, the alder *Phytophthora* was formally designated by Brasier et al. (2004) as a new species, *P. alni* Brasier & S.A. Kirk.

Because the newly defined species comprises a range of phenotypically diverse allopolyploid genotypes, *P. alni* was split into three subspecies, *P. alni* ssp. *alni (Paa), P. alni* ssp. *uniformis (Pau)* and *P. alni* ssp. *multiformis (Pam)* (Brasier et al. 2004). In addition to the three subspecies, a series of emerging variant types of *P. alni* have recently been recovered (Brasier et al. 2004; Jung and Blaschke 2004). Prior to their designation as subspecies, isolates of *Paa* and

Pau were termed standard types and Swedish variants, respectively, whereas *Pam*, including divergent hybrid types, were considered to be Dutch, German and UK variants of the pathogen (Brasier et al. 1999).

Paa occurs more commonly across much of Europe, and isolates of this form are generally more aggressive than those of the other two subspecies that are also present in several countries (Brasier and Kirk 2001). Furthermore, *Paa* produces *P. cambivora*-like ornamented oogonia and elongated two-celled antheridia. In contrast, the other two subspecies exhibit unique reproductive structures. Isolates of *Pam* produce oogonia that are typically ornamented, but antheridia and gametangial fusions may vary in morphology. *Pau* uniformly forms oogonia with a smooth surface under ordinary conditions but develops *Paa*-like ornamented female organs when grown at sub-optimal temperatures, i.e. ≤15°C, thus indicating that morphology-based differentiation of the two subspecies might fail under variable conditions (Fig. 2).

As opposed to typical *Phytophthora* species, which are diploid (2n) organisms, the standard type isolate of *Paa* was determined by acetoorcein staining to be an approximate tetraploid (~4n), having a chromosome number of ca. 18–22 at the first metaphase division. This isolate, however, is unable to complete meiosis (Brasier et al. 1999). In contrast, ploidy levels of *Pau* and *Pam* are intermediate between diploid and tetraploid and range from 2n+2 to 2n+4–7, respectively.

In addition to differences in ploidy, subspecies of *P. alni* differ in details of molecular features. For instance, *Paa* has dimorphic sites in the ITS region of its rDNA genes, in which DNA sequences are representative of two species, *P. cambivora* and a *P. fragariae*-like species. In contrast with *Paa*, ITS sequences in both *Pam* and *Pau* are homogeneous and resemble the ITS sequences of either *P. fragariae* or *P. cambivora*, respectively (Brasier et al. 1999, 2004). Subspecies of *P. alni* also differ on the basis of AFLP profiles (Brasier et al. 1999), RAPD or isozyme patterns (Nagy et al. 2003; Brasier et al. 2004) and diagnostic *P. alni*-specific PCR primer sets (Table 1). A firm correspondence, as shown in Table 2, has also been established between PCR markers and expression patterns for glucose-phosphate isomerase (Gpi) and malate dehydrogenase (Mdh) (Bakonyi et al. 2007). Since both isozymes are known to be nuclear-encoded, this suggests that the above-mentioned PCR-targeted DNAs that differentiate the subspecies are likely to be of nuclear origin.

Mitochondrial (mt) genomic variability within *P. alni* has been examined to only a limited extent. In studies by Nagy et al. (2003), RFLP analyses of mtDNA showed several bands that co-migrated between *P. alni* isolates and either *P. cambivora* or *P. fragariae*. However, it was not clear whether the appearance of such bands refers to the presence of biparental mtDNA fragments in hybrid isolates or whether it reflects intraspecific variation in the mt genome of either parental species. Based on sexual crosses within individual *Phytophthora* species, it has been suggested that the mitochondrial genome is transmitted uniparentally through the maternal line, whereas the nuclear genome is inherited from both the maternal and paternal lines (Förster and Coffey 1991; Whittaker et al. 1994).

The comparatively meagre knowledge about the mt genome of *P. alni* has been broadened recently. Ioos et al. (2006) performed phylogenetic analysis of the mt genes, *cox*1 and *nadh*1, that revealed that mtDNA sequences from either *P. cambivora* or *P. fragariae*

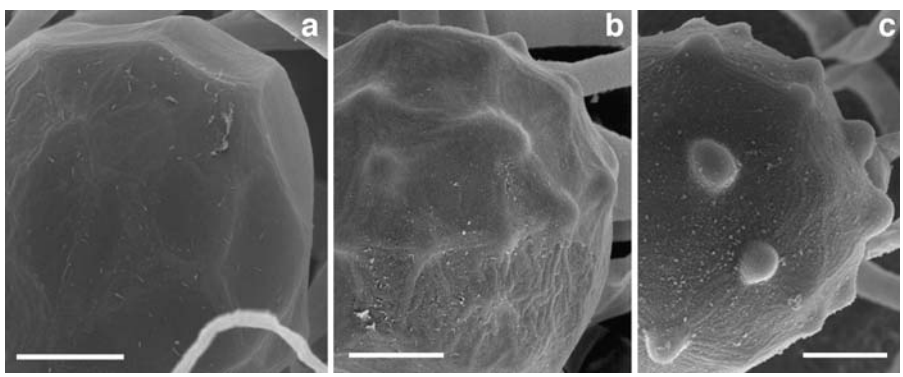

Fig. 2 Scanning electron micrographs of the surface of oogonia of *Phytophthora alni* subsp. *uniformis* grown at optimal temperature (**a**), sub-optimal temperature (**b**) and of *P. alni* subsp. *alni* (**c**). Note the environment-dependent change in morphology of *P. alni* subsp. *uniformis*. Bars: 20 µm

Table 1 PCR primer pairs developed for specific detection of *Phytophthora alni*

Primer name	Sequence (5′–3′)	Amplicon size (bo)	Specificity[a]	Reference
SAP1	GGC ACT GAG GGT TCC TC	930	*Paa*, *Pam*	Bakonyi et al. (2006)
SAP2	GGC ACT GAG GTC TAG ATT			
SWAP1	TGG CCC TCA CAT TAA AAC TGC TGC	1130	*Pau*, *Paa*	
SWAP2	GGC CCT CAC CAA ATG CGA AAT GA			
PA-F	GGT GAT CAG GGG AAT ATG TG	450	*Paa*, *Pau*, *Pam*	Ioos et al. (2005)
PA-R	ATG TCG GAG TGT TTC CCA AG			
PAM-F	CTG ACC AGC CCC TTA TTG GC	590	*Paa*, *Pam*	
PAM-R	CTG ACC AGC CAT CCC ACA TG			
PAU-F	GAG GAT CCC TAA CAC TGA ATG G	750	*Pau*, *Paa*	
PAU-R	GAT CCC TGG TTG AAG CTG AG			
D16F	AGG GCG TAA GGG TGC GAA ATA	366	*Paa*, *Pau*	De Merlier et al. (2005)
D16R	AGG GCG TAA GCC TGG ACC G			

[a] *Paa*, *Pau* and *Pam* are for *Phytophthora alni* subsp. *alni* subsp. *uniformis* subsp. *multiformis*, respectively.

did not cluster with those from the hybrid isolates, likely to be the result of uniparental inheritance of the mt genome. Furthermore, mtDNA sequences of *P. alni* isolates from the three subspecies clustered into only two groups, one that included *Paa* and *Pam*, and the other, *Pau*. Surprisingly, the mtDNA profiles of certain isolates that had been identified as *Paa* using morphological and nuclear markers were identical to those of *Pau* isolates (Ioos et al. 2006; Bakonyi et al. 2007). Such isolates may represent additional hybrid forms that encompass the nuclear type of *Paa* and a mitotype represented by *Pau* (Table 2).

The complexity of the nuclear and mitochondrial genomes of *Paa*, *Pau*, and *Pam* suggests that *P. alni* may be a species in a state of continuing evolution. As compared to its putative parental species, *P. alni* has exploited a new host (Brasier and Kirk 2001). The source of the parental species is not clear, since *P. cambivora* and *P. fragariare* are believed to be exotic to Europe. The evolutionary mechanism leading to the formation of subspecies of *P. alni* is also uncertain. Based on cytological and molecular analyses, Brasier et al. (1999) favoured the view that *Paa* could have arisen via somatic fusion followed by further segregation, rather than via a sexual cross between *P. cambivora* and a *P. fragariae*-like species. These authors further suggested that *Pau* and *Pam* might have then evolved through subsequent recombination events and chromosome losses in *Paa* that led to reversions towards the *P. cambivora*-like or *P. fragariae*-like parental genotypes.

Recently, Ioos et al. (2006) proposed an alternative evolutionary model by which *Paa* might have arisen via hybridisation of *Pam* and *Pau*. They suggested

Table 2 Patterns of nuclear and mithochondrial traits in subspecies of *Phytophthora alni* according to the results of Bakonyi et al. (2006, 2007)

Marker type	*Pam* (M)[a]	*Pau* (U)	*Paa* (A)
PCR with primer set SAP1/SAP2	+[b]	–	+
PCR with primer set SWAP1/SWAP2	–	+	+
RAPDs with primer OPG-02 or OPG-05	M	U	M + U
Isozyme locus *Mdh-1*	91/100	83/83	83/91/100
Isozyme locus *Mdh-2*	94/94	100/100	94/100
Isozyme locus *Gpi*	85/100	93/93	85/93/100
mtDNA-RFLP with *Msp*I or *Hae*III	M (=A)	U	A (=M) or U

[a] *Pam* (M) *Pau* (U) *Paa* (A) are for *Phytophthora alni* subsp. *multiformis*, subsp. *uniformis* and subsp. *alni*, respectively.

[b] Specific amplicon is produced (+) or not produced (−).

that *Pau* might have evolved from *P. cambivora*, whereas *Pam* might have either been generated itself by an ancient reticulation or by autopolyploidisation. Their hypotheses were based on analyses of a large European-wide collection of *P. alni* isolates showing that *Paa* possessed three alleles for each of four nuclear genes studied, two of which were also present in *Pam*, and a third one that matched a single allele in *Pau*. Furthermore, the *Paa* isolates displayed a mtDNA RFLP pattern identical to isolates of either *Pam* or *Pau*, implying uniparental inheritance of the mt genome in the suspected hybridisation process. These results are supported by data of Bakonyi et al. (2007) who found that the studied nuclear-encoded traits expressed in *Paa* included combined expression profiles of *Pam* and *Pau*, whereas mtDNA restriction profiles of *Paa* matched that of either *Pam* or *Pau* (Table 2).

Isolates of *Pam* and *Pau* have been recovered from alder lesions far less frequently than have isolates of *Paa*, and they have also proven to be significantly less aggressive in colonising alder bark (Brasier and Kirk 2001). On the basis of these observations, Bakonyi et al. (2007) suggested that the emergence of atypical *Paa* isolates with a *Pau* mitotype might have occurred in bark tissue co-colonised by *Paa* and *Pau*. In this niche, *Paa* and *Pau* isolates might have hybridised by either somatic or gametangial interaction. In this scenario, Bakonyi et al. (2007) also suggested the possibility that these atypical *Paa* isolates may have arisen through the introgression of mitochondria from *Pau* into the nuclear background of *Paa*. Interactions like these must be very rare in nature, and indeed, there has been only one such report, in association with the causal agents of Dutch elm disease. According to Bates et al. (1993), certain *Ophiostoma novo-ulmi* isolates exhibited typical *O. novo-ulmi* nuclear DNA profiles, but they also exhibited *O. ulmi*-like mtDNA patterns. They attributed these patterns to somatic fusion between the two related species.

The hybridisation event that led to the emergence of *P. alni* is believed to be recent, and it may have occurred in a European nursery, perhaps on raspberry or another host that is common to the putative parental species (Brasier et al. 1999; Brasier and Jung 2003). It is assumed that *P. alni* arrived in Britain, the country of first record, as a result of commercial trade of colonised plant material. Its subsequent spread over long distances is likely to have occurred via distribution and planting of infested nursery stock (Brasier et al. 1999).

Local spread from points of *P. alni* introduction is not likely to be related to the movement of oospores, since these structures have poor survival ability in soil (Delcan and Brasier 2001). More likely, zoospores and plant debris containing mycelium contribute to pathogen movement at this scale. Alders are key trees in wetlands and riparian environments, where they stabilise river and stream banks. In these habitats, the presence of saturated or flooded soils, and water movement, would enhance spread of zoospores and debris. This scenario is supported by observations of higher disease incidence among alders growing near rivers than those some distance away (Gibbs et al. 1999).

Phytophthora cactorum × *P. nicotianae*

Species hybridisation has also been reported in hydroponic greenhouse systems in The Netherlands. Under such circumstances novel *Phytophthora* diseases have appeared on diverse ornamental species. For instance, Man in't Veld et al. (1998) reported the isolation of *Phytophthora* that differed morphologically from known pathogenic species from *Spathiphyllum* and *Primula* plants. Isozyme and RAPD analyses revealed that the unusual isolates represented hybrids of *P. nicotianae* and *P. cactorum*. In addition, mtDNA restriction patterns of the hybrid isolates were identical to those of *P. nicotianae* (Man in't Veld et al. 1998). *Phytophthora nicotianae* is an introduced species in The Netherlands, and it can infect both *Spathiphyllum* and *Primula*. In contrast, *P. cactorum* is a resident species, but it does not cause disease on these host plants. Additional hybrid isolates were obtained from a *Cyclamen* sp., which is not known to be a host of either of the parental species (Bonants et al. 2000). Subsequent analysis of the ITS region of rDNA and AFLP analyses provided further evidence of the biparental origin of the recovered isolates.

Similar hybrids have been characterised recently from loquat trees (*Eriobotrya japonica*) grown in orchards in central Taiwan (Man in't Veld 2001). The unlikely movement of hybrid isolates between such distinctly different and separated agricultural and horticultural systems suggests a potential for hybridisation between *P. cactorum* and *P. nicotianae* when both species occupy the same habitat.

Phytophthora cactorum × *P. hedraiandra*

Man in't Veld et al. (2007) recently reported the involvement of *P. cactorum* in the formation of yet another hybrid species after hybridisation with *P. hedraiandra*. These new hybrids were shown to be heterozygous at the dimeric malic enzyme (*Mdhp*) locus, possessing the MDHP alleles of the two parental species. They also contained dimorphic sites in the ITS region, exactly at those positions where the parental sequences differ. Consistent with the hybrid hypothesis, most hybrid isolates contained the mitochondrial-encoded cytochrome oxidase I (Cox I) gene sequences that were identical to those of *P. hedraiandra*, and one isolate had the gene sequences of the other putative parent.

Phytophthora cactorum has been isolated from numerous hosts, including *Rhododendron* spp. During the past decade, however, only these novel hybrids have been found on *Rhododendron* in the Netherlands, suggesting that they are replacing the resident *P. cactorum* population on this host. While *P. cactorum* is an indigenous species in Europe, *P. hedraiandra* is believed to be a recent introduction from North America, where it infects *Rhododendron* spp. In comparison with the parental species, the hybrid isolates exhibit expanded host ranges, including monocots (*Allium* spp.) as well as dicots (*Idesia* and *Penstemon* spp.). These isolates are known to be proliferating in the environment in the Netherlands and in Germany (Man in't Veld et al. 2007).

Concluding remarks

Laboratory and field studies suggest that interspecific hybridisation in *Phytophthora* populations occurs rarely. However, such rare events may prove to be an important source of genetic diversity, in addition to the more commonly recognised processes of mutation and sexual or parasexual reproduction within individual species. Studies summarised in this review suggest that *Phytophthora* species hybridisation may produce unique offspring capable of exploiting an expanded range of host plants. In addition, hybrid offspring with increased aggressiveness may be selected to such an extent that they become a dominant component of *Phytophthora* populations within a region.

The studies summarised here also suggest that species hybridisation occurs readily between allopatric species that have not co-evolved in the same location. To date, known *Phytophthora* species hybrids represent the offspring of a native and an exotic or two exotic species that occupy the same habitat and niche. No reports have described similar hybridisation among indigenous, sympatric *Phytophthora* species populations, even though such hybrids can be generated in the laboratory. The reason for this limitation is uncertain, although it is believed that strong genetic barriers have evolved to restrict hybridisation among sympatric oomycete and fungal species (Brasier 2000; Olson and Stenlid 2002; Schardl and Craven 2003).

The mechanisms of species hybridisation in *Phytophthora* populations are not known, but studies of several cases have provided evidence for hybrid populations in various states of genomic evolution. The role of diverse ploidy levels and genomic reorganisation in determining host range, aggressiveness, and population dynamics bears further investigation. In addition, it is noteworthy that most *Phytophthora* species hybrids have acquired the mitochondrial genome of the exotic, introduced parental species (Man in't Veld et al. 2007). Since mitochondrial control of virulence was reported for artificially made hybrids of the basidiomycete fungus *Heterobasidion annosum* (Olson and Stenlid 2001), further research is needed to examine the influence of the acquired mitochondrial genome on host selection by species hybrids of *Phytophthora*.

Interspecific hybridisation among *Phytophthora* species is likely to increase with expanding world trade that introduces plants and associated pathogens into new regions with uniquely different environmental conditions (Brasier 2000). Opportunities for interactions between *Phytophthora* species are also enhanced as plants are managed under hydroponic and other non-traditional agricultural conditions (Man in't Veld et al. 1998, 2007; Bonants et al. 2000). Finally, over longer periods of time, human disturbance factors such as pollution, climate change, and land use may accentuate the emergence of newly adapted hybrids with unique pathogenicity attributes. Limited evidence for these possibilities was provided by Gibbs et al. (1999), who showed that pollution of water with oxidised nitrogen can sensitise alder trees to *P. alni* infection. Consequently, interspecific

hybrids appear to be the products of recent evolutionary events. However, some of them might have existed for a long period of time without being identified as hybrids, due to the lack of appropriate tools. Natural hybridisation has been suspected but never proven with *P. meadii* (Sansome et al. 1991). As for fungi, a particular poplar rust identified recently as a hybrid of *Melampsora medusae* and *M. occidentalis* is represented in specimens from nearly a century ago (Newcombe et al. 2000).

Although species hybridisation as a source of new epidemic outbreaks should not be exaggerated, it is of interest to regulatory officials to monitor the emergence of new hybrid genotypes. Unfortunately, emerging hybrid populations are unlikely to be detectable by conventional, morphology-based approaches. Consequently, population sampling methods and molecular techniques for characterising pathogen genetic structure require further development for effective detection and management.

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