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Natural hybrids of resident and introduced *Phytophthora* species proliferating on multiple new hosts

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Abstract

Several atypical *Phytophthora* strains, isolated from a range of horticultural hosts, were tentatively identified as *P. cactorum*. Numerous abortive oospores were observed in these strains and isozyme analysis showed all were heterozygous for the dimeric malic enzyme (MDHP). More detailed comparisons indicated that their MDHP alleles matched those of both *P. cactorum* and *P. hedraiandra*. Cloning and sequencing of the nuclear ribosomal internal transcribed spacer (ITS) regions of the atypical *P. cactorum* strains demonstrated the presence of sequences characteristic for both *P. cactorum* and *P. hedraiandra*. It was concluded that the atypical strains represented hybrids between the resident *P. cactorum* and the apparently recently introduced *P. hedraiandra*. Most strains had the mitochondrially inherited cytochrome oxidase I (Cox I) gene typical of one putative parent *P. hedraiandra*, while one single strain had that of the other putative parent, consistent with the hybrid hypothesis. Our data also suggest that the hybrids are evolving. The hybrids have proliferated on multiple new hosts in the Netherlands.

Introduction

The diploid Oomycete genus *Phytophthora* comprises highly destructive primary plant pathogens, including *Phytophthora ramorum*, causal agent of Sudden Oak Death. Through global trade, phytopathogens are being dispersed around the world, increasing the risk that imported pathogens might hybridize with and modify the virulence of local species (Brasier, 2000). Imported pathogens like *P. ramorum* often have restricted genetic variation (Ivors et al., 2006) consistent with the so-called 'founder effect' (Mayr, 1942), but pathogens generated by hybridization may rapidly develop recombinant genomes (Chamnanpunt et al., 2001), resulting in novel combinations of virulence factors. This process may generate new pathogens against which local plant populations may have no defence.

Traditional identification of *Phytophthora* species is based on morphology. Due to variation in morphological characters and overlap among species, this process is difficult and time-consuming. In recent years it has become clear that species boundaries can be determined unambiguously using techniques such as isozyme analysis (Oudemans and Coffey, 1991), sequence analysis of the nuclear ribosomal internal transcribed spacer (ITS) region (Cooke et al., 2000) and sequence analysis of cytochrome oxidase I (Kroon et al., 2004). Isozyme analysis of dimeric enzymes has the advantage that it can unambiguously distinguish heterogeneous isolate mixtures from crossed

isolates. Only when two different dimeric isozyme alleles are concurrently expressed do products combine to form two homodimeric isozymes and one heterodimeric type, yielding distinctive three-banded patterns in native gel-electrophoresis (Goodwin et al., 1994).

In isolations from diseased plants, we obtained nine homothallic *Phytophthora* strains that had papillate, caducous sporangia, numerous abortive oospores, and paragynous and amphigynous antheridia. These strains were tentatively identified as atypical representatives of *Phytophthora cactorum*. Preliminary experiments studying the dimeric malic enzyme (MDHP), indicated that the atypical strains were heterozygous, whereas it is known that *P. cactorum* is homozygous at the *Mdhp* locus and at least 17 other isozyme loci (Oudemans and Coffey, 1991). It was therefore hypothesized that the atypical strains represented hybrids. The aim of this investigation was to test whether the atypical strains were possibly the result of hybridization of *Phytophthora cactorum* with an, as yet unidentified, other species.

Materials and methods

Fungal isolates and study of morphology

Fungal isolates and their sources are listed in Table 2. Atypical *P. cactorum* -like strains were isolated from leaf spots (*Allium cepa*, *Allium porrum*), from stem-base rot (*Idesia* and *Penstemon*) and from wilting shoots (*Rhododendron*). Cultures were maintained on cornmeal agar slants. Isolates were cultivated on V8 agar at 20 °C in the dark for morphological study. (Gams et al., 1987). The formation of sporangia was stimulated by growing the mycelium, taken from the margin of V8 cultures, in the vicinity of pepper seeds in pond water. The dimensions of oogonia, oospores and sporangia were measured. For all characteristics studied, at least 25 measurements were made for each isolate and the average value was calculated. The maximum temperature for growth was determined by incubating the colonies at different temperatures (28–37 °C) with intervals of 0.5 °C. The highest temperature at which growth occurred was considered to be the maximum growing temperature.

Isozyme analysis

Isolates of all *Phytophthora* spp. were grown in 50 ml of Tryptone Soy Broth medium (TSB) in 250 ml Erlenmeyer flasks on a rotary shaker at 40 rpm in the dark. Each flask had been inoculated with three V8 agar discs (5 mm diam) of mycelium, taken from actively growing margins of three day-old colonies. Cultures were incubated at 23 °C. After 7 days the mycelium of each isolate was collected by sieving, after which the tissue was dried by pressing between layers of filter paper. The mycelium was stored overnight at −80 °C prior to extraction of enzymes. Frozen mycelium was thawed at 4 °C for 3 h before enzyme extraction. Routinely, about 0.5 g of mycelium was ground in a chilled mortar with sand and 70 μl of extraction medium. The extraction medium consisted of 0.1 M Tris-HCl (pH 7.0), 1 mM dithiothreitol, 50 mM Ethylene Diamine Tetra-acetic Acid (EDTA), 10% Poly Vinyl Pyrrolidone (PVP) w/v, 50 μg ml^{-1} soybean trypsin inhibitor, 0.1 mM Phenyl Methyl Sulphonyl Fluoride (PMSF) and 5% glycerol (v/v). All mycelia were ground for 3 min. The homogenate was transferred to Eppendorf Safe-Lock micro test tubes (Eppendorf AG, Hamburg, Germany) and centrifuged for 10 min at 14,000 rpm (4 °C). The supernatant (40–80 μl) was collected and stored at −80 °C before use. Electrophoresis was carried out at 4 °C, using the automated PhastSystem (Amersham Pharmacia Biotech Europe GmbH, Germany). Crude extracts, obtained as described before, were routinely analysed for malic enzyme (MDHP, EC 1.1.1.40) on native, 12.5% homogeneous polyacrylamide gels. The gels were made with 0.11 M Tris-acetate buffer (pH 6.4). The running buffer, contained in 2% agarose gel, consisted of a 0.25 M Tris and 0.88 M L-alanine buffer (pH 8.8). At completion of electrophoresis, gels were immersed in freshly prepared staining solutions in the dark at 37 °C for 20 min.

The reaction ingredients for malic enzyme were as follows: 25 ml 0.2 M Tris-HCl pH 8.0, 440 mg L-malic acid (di-sodium salt), 12.5 mg Nicotinamide Adenine Dinucleotide Phosphate (NADP), 7.5 mg Nitro Blue Tetrazolium (NBT) (N 6876, Sigma Zwijndrecht, The Netherlands), 1 mg Phenazine Metho Sulphate (PMS) (Sigma P 9625).

Alleles were scored alphabetically according to their mobility. Since *Phytophthora* spp. are diploid,

two identical letters were assigned to one band. A three-banded pattern was interpreted as the product of two different alleles encoding for a dimeric enzyme (Richardson et al., 1986). Malic enzyme is known to be a dimeric enzyme in *Phytophthora* spp. (Mosa et al., 1993)

ITS and Cox I sequencing and analysis

For DNA studies isolates were cultivated in pea broth (de Cock et al., 1992). Mycelium from 5–14 day-old cultures was harvested by vacuum filtration and the DNA was extracted following the protocol of Möller et al. (1992). The primers ITS1 and ITS4 (White et al., 1990) were used to prepare the sequencing template by amplifying part of the nuclear rRNA operon comprising the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, and the second ITS region (ITS2). The PCR reaction mixture consisted of 0.75 units Biotaq (Bioline, London, U.K.), PCR buffer, 1.5 mM $MgCl_2$, 0.2 μM of each dNTP, 5 pmol of each primer, approximately 10–30 ng of fungal genomic DNA and was made up to a total volume of 25 μl with sterile water. Reactions were performed on a GeneAmp PCR System 2700 (Applied Biosystems, Foster City, CA) and the cycling conditions consisted of denaturation for 5 min at 96 °C, followed by 30 cycles at 96 °C (30 s), 55 °C (30 s), 72 °C (90 s) and a final 7 min extension step at 72 °C to complete the reaction. Amplification products were purified according to the manufacturer's instructions using a commercial kit (GFX PCR DNA and Gel Band Purification Kit, Amersham Pharmacia Biotech Europe GmbH, Germany). Sequencing reactions were carried out using the PCR primers in ABI PRISM Big Dye Terminator Cycle v 3.0 Sequencing Ready Reaction Kit (Applied Biosystems) following the manufacturer's recommendations. The reactions were analysed on an ABI Prism 3100 Genetic Analyser (Applied Biosystems).

The complete ITS region of two strains (CBS100426 and CBS100427) was amplified with primers ITS4 and ITS5 of White et al. (1990) as described by Goodwin et al. (2001) and purified products were cloned with the TA cloning kit (Invitrogen Corp., Carlsbad, CA). Fourteen clones from each strain were selected at random and the sequencing was done with ABI BigDye v3.1 terminator chemistry and ABI 3730xl DNA Analyzer. Cox I was sequenced using COXF4N and COXR4N primers according to Kroon et al. (2004). Sequences were edited using Seqman 4.05 (DNASTAR, Madison, WI). Mega-Tree was used to calculate bootstrap values, based on 1000 replicates.

Results

Morphology

All atypical *P. cactorum* strains formed papillate sporangia, were homothallic with predominantly paragynous (occasionally amphigynous) antheridia, and had numerous (> 50%) abortive oospores. The morphological characters of two atypical *P. cactorum*-like strains were measured in detail; the same was done for one typical strain of *P. cactorum* and *P. hedraiandra* (Table 1).

Characterization of single-zoospore strain

We established strain CBS 100426 by isolating a single zoospore from CBS 100425. The isozyme pattern, generated by malic enzyme, the ITS sequence and the cytochrome oxidase I sequence of CBS 100426 were exactly identical to those of the CBS 100425 parent (Table 2). This confirmed that CBS 100425 was not simply a mixed culture.

Isozyme analysis

Isozyme analysis of MDHP showed the presence of a single band in *P. cactorum* strains and a single band at a different position in *P. hedraiandra* strains. All nine atypical *P. cactorum*-like strains displayed a three-banded pattern, in which the uppermost band comigrated with the single *P. cactorum* band and the lowermost comigrated with the single *P. hedraiandra* band. The ratio of intensities of the bands of the atypical *P. cactorum* strains was mostly 4:4:1 indicating the presence of two copies of the *P. cactorum* allele and one *P. hedraiandra* allele (Figure. 1a, Table 2). Isozyme analysis of a large collection of 47 *P. cactorum* isolates, including European strains, revealed that there was only one MDHP allele in the population (Oudemans and Coffey, 1991). The same allele was present in 36 *P. cactorum* strains held at the Dutch Plant Protection Service (unpublished results). One

Table 1. Morphological features of *Phytophthora* hybrids, *P. cactorum* and *P. hedraiandra* isolates

Species, isolate number	Oogonia dimensions (μm)		Oospores dimensions (μm)		Sporangia Length/breadth (μm)		L/B ratio	T_{max} (°C)
	Range	Average	Range	Average	Range	Average		
P. cactorum[a]	23–35	27.4	18–27	24.0	24–55 × 19–40	35.7 × 26.8	1.33:1	32.0
P. cactorum P6183	25–30	27.8	22–25	23.6	35–40 × 26–30	36.6 × 28.0	1.31:1	32.0
Hybrid CBS100425	26–40	32.5	22–34	26.7	26–52 × 20–34	40.1 × 28.7	1.39:1	35.0
Hybrid CBS100427	18–38	28.4	16–31	23.8	26–48 × 20–36	35.6 × 26.0	1.36:1	34.5
P. hedraiandra. CBS111725	26–38	32	23–36	29.5	21–53 × 16–34	37.0 × 28.3	1.31:1	30.0

[a]General features according to the discription of Kröber (1985).

Dutch *P. hedraiandra* strain and four Italian strains contained an identical MDHP allele, which differed from that observed in *P. cactorum* (Table 2).

Sequence analysis of the ITS region

Comparison of the ITS regions against data in GenBank confirmed that these atypical strains were most similar in sequence to *P. cactorum* and *P. hedraiandra* in ITS clade 1 (Cooke et al., 2000). *Phytophthora. cactorum* and *P. hedraiandra* sequences differ by four single base pairs and, in all nine atypical strains examined in this study, these matched precisely a series of double bases observed in the electropherograms at four positions 74, 100, 101 and 686 (Figure. 1b, Table 2). Cloning of the ITS regions of CBS100426 and CBS100427 and analysis of 28 cloned inserts revealed twenty clones with the ITS sequence of *P. cactorum* and eight clones with that of *P. hedraiandra*. The ITS sequences deposited in GenBank for 11 *P. cactorum* strains were identical to that of *P. cactorum* as seen in 20 analysed clones. The ITS sequences in GenBank for eight other *P. cactorum* strains differed by just one base pair. The ITS sequence of the type species of *P. hedraiandra* deposited at GenBank (AY707987) was identical to the sequence present in eight analysed clones. The sequences of *P. idaei* and *P. pseudotsugae* in GenBank differed at several positions from those of *P. cactorum* and *P. hedraiandra* (de Cock and Lévesque, 2004).

Sequence analysis of Cox I

Phylogenetic analysis revealed that eight atypical *P. cactorum*-like strains contained the *P. hedraiandra* Cox I sequence, whereas one strain contained the *P. cactorum* sequence (Table 2, Figure. 2). The sequences of *P. idaei* and *P. pseudotsugae* in GenBank differed from those of *P. cactorum* and *P. hedraiandra* (Figure. 2).

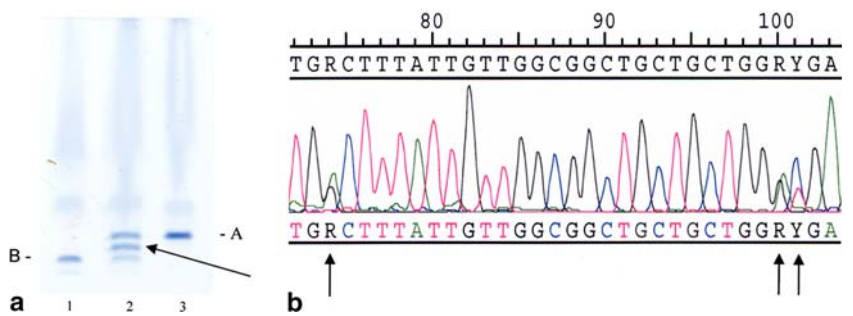

Figure 1a. Isozyme patterns of dimeric malic enzyme (MDHP) generated by *Phytophthora* spp. and visualized by enzymatic staining using the nitroblue-tetrazolium system after native polyacrylamide gel electrophoresis; lane 1: *Phytophthora hedraiandra* CBS 111725*;* lane 2: *Phytophthora* hybrid CBS 100425 (arrow indicates the heterodimeric band); lane 3: *Phytophthora cactorum* P6183. 1b: Electropherogram of part of the internal transcribed spacer sequence of the ribosomal DNA gene repeat (ITS) of *Phytophthora* hybrid strains showing double bases at three positions (see arrows), where the sequences of *P. hedraiandra* and *P. cactorum* differ.

Table 2. Features of *Phytophthora* spp. Variable positions in sequences of the internal transcribed spacer region of the ribosomal DNA gene repeat (ITS), cytochrome oxidase I (Cox I) type and malic enzyme isozyme alleles A and B (*Mdhp*) of *Phytophthora* hybrids and their parents *P. cactorum* and *P. hedraiandra*. (R = A + G, Y = C + T and K = T + G)

Phytophthora species	Strain number	Year of isolation	Host	ITS: variable nucleotide positions				Cox-I type	Isozymealleles *Mdhp*
				74	100	101	686		
Cactorum[b]	P6183[a]	–	*Rubus idaeus*	G	G	T	T	*Cactorum*	AA
Hybrid	CBS 116572[a]	1997	*Allium cepa*	R	R	Y	K	*Hedraiandra*	AAB
Hybrid	CBS 100425	1992	*Rhododendron*	R	R	Y	K	*Hedraiandra*	AAB
Hybrid	CBS 100426-ms[c]	1992	*Rhododendron*	R	R	Y	K	*Hedraiandra*	AAB
Hybrid	CBS 100427	1995	*Idesia*	R	R	Y	K	*Hedraiandra*	AAB
Hybrid	CBS 111731	1998	*Allium porrum*	R	R	Y	K	*Hedraiandra*	AB
Hybrid	CBS 112274	2001	*Penstemon*	R	R	Y	K	*Cactorum*	AAB
Hybrid	CBS 113345	2001	*Rhododendron*	R	R	Y	K	*Hedraiandra*	AAB
Hybrid	CBS 113348	2002	*Rhododendron*	R	R	Y	K	*Hedraiandra*	AAB
Hybrid	CBS 113349	2002	*Rhododendron*	R	R	Y	K	*Hedraiandra*	AAB
Hedraiandra[d]	CBS 111725	2001	*Viburnum*	A	A	C	G	*Hedraiandra*	BB
Hedraiandra[e]	PD01838040[a]	2004	*Viburnum*	A	A	C	G	*Hedraiandra*	BB
Hedraiandra[e]	PD01838059	2004	*Viburnum*	A	A	C	G	*Hedraiandra*	BB
Hedraiandra[e]	PD01838067	2004	*Viburnum*	A	A	C	G	–	BB
Hedraiandra[e]	PD01838075	2004	*Viburnum*	A	A	C	G	–	BB
Hedraiandra[f]	P3842	2005	*Viburnum*	A	A	C	G	*Hedraiandra*	-
Hybrid related	BBA 5/94[a]	1994	Soil[g]	G	G	T	T	*Cactorum*	AB
Hybrid related	CBS 114342	2001	*Rhododendron*	A	A	C	G	*Cactorum*	AA

[a]P: University of California, Riverside, U.S.A.; CBS: Centraal Bureau voor Schimmelcultures, The Netherlands; PD: Plant Protection Service, The Netherlands; BBA: Federal Biological Research Centre for Agriculture and Forestry, Germany.
[b]GenBank accession number AY564167 (Cox I). All GenBank accession numbers of the ITS sequence of *P. cactorum* contained the same nucleotides at those positions mentioned.
[c]ms = monospore strain, GenBank accession number DQ836127 (ITS).
[d]GenBank accession numbers AY707987 (ITS) and AY769115 (Cox I) of type species CBS 111725.
[e]Italian strains.
[f]Spanish strain; GenBank accession numbers AY881005 (ITS) and DQ220015 (Cox I).
[g]Containing dying young trees of *Fagus sylvaticus*, *Acer pseudoplatanus* and *Alnus glutinosa*

Discussion

As a matter of serendipity, we found that nine *P. cactorum* strains, isolated from diseased plants within the last decade, were all atypical in producing numerous abortive oospores and in generating identical three-banded patterns for the dimeric malic enzyme (MDHP, EC 1.1.1.40, Figure. 1a). As mentioned above, the uppermost band co-migrated with the single band characteristic of the consistently homozygous *P. cactorum* (Oudemans and Coffey, 1991). The lowermost co-migrated with the single band characteristic of *P. hedraiandra*. Incidentally, the heterodimeric band should normally be twice as intense as the homodimeric bands; however, instead in most atypical *P. cactorum* strains the ratio of intensities of the bands was 4:4:1 (Figure. 1a, Table 2), indicating that these strains are probably trisomic for the chromosome containing the *Mdhp* locus. (Goodwin et al., 1995). These results strongly suggest that the atypical *P. cactorum*-like strains were hybrids between *P. cactorum* and *P. hedraiandra*, both of which under normal circumstances are inbreeding (homothallic) species. When nuclear ribosomal ITS sequences were examined for the nine atypical strains, the presence of four double peaks (Figure. 1b) in sequence traces signified that two different sequences, with base pairs corresponding to those of *P. cactorum* and *P. hedraiandra*, were present in each strain. Since through concerted evolution ITS sequences are normally homogeneous in *Phytophthora* species, this finding is consistent with hybridization. Cloning of the ITS regions of the atypical *P. cactorum* strains confirmed the presence of both

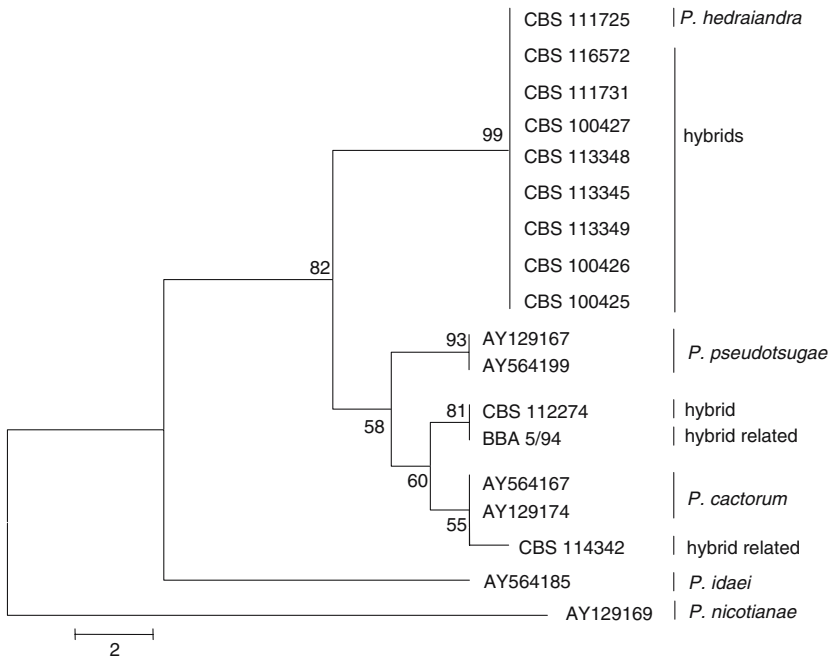

Figure 2. Phylogenetic tree, calculated using bootstrap Maximum Parsimony, based on the cytochrome oxidase I (Cox I) gene of *Phytophthora hedraiandra* × *cactorum* hybrids and related species. The numbers at the branch points indicate the percentage of bootstrap values, based on 1000 replicates. Scale bar indicates 2 nucleotides.

parental ITS sequences in these strains. Incidentally, it may be helpful to note that restriction enzyme analysis of the ITS region cannot distinguish the parental species from one another, or from the hybrids, since no restriction sites are present at the positions where the three taxa differ.

Mitochondrial DNA is maternally inherited, and only one type is thus expected in hybrids. Indeed, eight strains possessed the mitochondrial cytochrome oxidase I gene of *P. hedraiandra*, whereas one strain contained the *P. cactorum* gene, differing at 15 positions from that of *P. hedraiandra* (Table 2, Figure. 2). This confirmed the parenthood of the hybrids and indicated that these strains are not derived from a single clone. We conclude therefore that the atypical *P. cactorum*-like strains represent hybrids of *P. cactorum* (Lebert & Cohn) J. Schröter and *P. hedraiandra* de Cock & Man in 't Veld. Remarkably, two genetically different strains in the current investigation, BBA 5/94 and CBS 114342, contained homogeneous ITS sequences and had alleles consistent in part with those of *P. cactorum* and in part with those of *P. hedraiandra*, but also had an allelic distribution differing from that seen in the hybrids (Table 2). These two hybrid-related strains may originate from intercrossing of hybrids, or from backcrossing or mitotic genome rearrangements (Chamnanpunt et al., 2001). The occurrence of such strains tends to confirm that new genotypes develop rapidly after hybridization as has been reported in another hybrid *Phytophthora* (Brasier et al., 1999). Further work on this new pathogen is needed to reveal more about its ongoing evolution and pathological significance.

According to Arnold (1997), natural hybrid individuals derive from crosses in nature between individuals from two populations, or groups of populations, which are distinguishable on the basis of one or more heritable characters. The strains studied fulfil the requirements for classification as interspecific hybrids; moreover, the hybrids and their parents can easily be identified by examining the loci studied here.

Phytophthora species have been extensively monitored for several decades in northwestern Europe and the recently described *P. hedraiandra* has been found only once in the Netherlands. This record was from an exotic *Viburnum* species sold by a commercial nursery. There are four arguments in

favour of regarding *P. hedraiandra* as an introduced species rather than a rare indigenous species in the Netherlands and nearby parts of northwestern Europe: (1) *Phytophthora hedraiandra* has been discovered on only one occasion in the Netherlands on a nursery, suggesting a link with international plant trade. Most *Viburnum* species grown in nurseries are exotics, e.g. the south European/North African *V. tinus* and the East Asian *V. plicatum*. *P. hedraiandra* has never been isolated from the native *Viburnum* species *V. lantana* and *V. opulus*, despite high attention in the Netherlands to nature conservation and the health of wild plants. Moreover, in a nationwide survey for *P. ramorum*, held in the Netherlands from 2002-until the present, and in which *Viburnum* and *Rhododendron* were deliberately monitored, *P. hedraiandra* has never been discovered. (2) If *P. hedraiandra* was a rare resident species in the Netherlands, the discovery of only one strain during the 40 years in which *Phytophthora* species in the Netherlands have been diligently monitored is, even for a rare species, highly unlikely. (3) *Phytophthora hedraiandra* has been found exclusively on *Viburnum* in Europe, but strains recently obtained in the USA were isolated from *Rhododendron* (Schwingle et al., 2006). Our studies show that *P. hedraiandra* and *P. cactorum* appear to have hybridized in nature. The lack of such hybrids in the *Phytophthora* studies done through most of the later 20th century suggests that these species must originally have been isolated, reproductively or geographically. If *P. hedraiandra* is indeed pathogenic on *Rhododendron*, a known host of *P. cactorum*, this isolation is more likely to have been geographic rather than being based on host specificity since there have been opportunities to hybridize on a common host in nature. (4) There is no plausible explanation as to why the one resident species, *P. cactorum*, suddenly would start to hybridize in the beginning of the 1990s with another resident species, *P. hedraiandra*.

The probability that *P. hedraiandra* is a rare, resident species in the Netherlands is thus extremely low. The fact that the present hybrids were first detected in 1992, while *P. hedraiandra* itself was only detected for the first time in 2001 suggests that *P. hedraiandra* entered the Netherlands before 1992 but that, like many introduced species, it was not able to expand its population into a permissive niche. It is possible that the hybrids themselves formed elsewhere and were subsequently imported to the Netherlands, but this seems less likely since compatible isolates have not been reported or received from any other locations that predate the cases observed in the Netherlands. For several years, *P. hedraiandra* was known only from its type culture and its provenance was a mystery. Recently *P. hedraiandra* has been reported from Italy, Spain (Table 2), USA (Schwingle et al., 2006) and Poland (GenBank DQ403788). This situation in regard to this organism appears to be developing similarly to what was seen during the emergence of *P. ramorum*, which was first discovered in the beginning of 1990s simultaneously in Europe and America. It is generally assumed that *P. ramorum* is an introduced species, but its origin, like that of *P. hedraiandra*, is still not known.

In Dutch monitoring programmes in the past, *P. cactorum* was isolated from several hosts including *Rhododendron* spp. In recent years, however, only the hybrids discussed here have been obtained from *Rhododendron*. Additional hybrid strains have been detected on *Rhododendron* since the present study was completed, indicating that they are still successfully proliferating on this host. Among phytopathogenic Oomycetes, as mentioned above, natural hybrids have been discovered previously (Man in 't Veld et al., 1998, Brasier et al., 1999). Unlike those hybrids, the present hybrids were obtained from multiple new hosts not colonized by either parent (Erwin and Ribeiro, 1996), including monocots (*Allium* spp.) and dicots (*Idesia* and *Penstemon*). As mentioned, prior to 1992 neither the present hybrids nor *P. hedraiandra* were detected in the Netherlands, or are they found in stored isolates from years prior to that time.

Five of the hybrid strains studied were found on fields belonging to nurseries. The remaining four strains were found in parks: collections were made in three Dutch provinces at sites more than 100 km apart including Hoorn in Noord-Holland province, Buitenpost in Groningen, province and Gendringen and Tiel in Gelderland province. This broad distribution strongly suggests that the hybrids have proliferated possibly via human transportation of infected plants, and/or by means of a reproductive structure that enables rapid, broad dissemination, namely, by caducous sporangia. Since the distance between these locations is probably too great to have been traversed by a

single sporangial dissemination event, other, as yet unidentified hosts may have played a role in their dispersal. The hybrids appear to be considerably more able than the parental *P. hedraiandra* to find and occupy permissive local niches, even though both species are equally efficiently disseminated by means of dispersed sporangia, similar to those responsible for rapid dispersal of *Phytophthora infestans* (Zwankhuizen et al., 1998).

It is remarkable that *P. cactorum* apparently hybridizes with *P. nicotianae* (Man in 't Veld et al., 1998) as well as *P. hedraiandra*. *Phytophthora nicotianae* is a longtime resident species in greenhouses in Europe (the first strain from ornamentals in the collection of the Centraalbureau voor Schimmelcultures dates back to 1927), but it was at first isolated from black shank disease of tobacco in Indonesia (van Breda de Haan, 1896) and hence it may be assumed that *Phytophthora nicotianae* was introduced from Indonesia. The two types of hybrids occupy different niches and have different host ranges. *P. nicotianae* × *cactorum* hybrids have only been found in greenhouses in Europe and in the tropical climate of Taiwan (Chern et al., 1998; Man in 't Veld, 2001), but *P. hedraiandra* × *cactorum* hybrids have seemingly only proliferated in the environment of the Netherlands to date. The *P. nicotianae* × *P. cactorum* hybrids do not share any common hosts with *P. hedraiandra* × *P. cactorum* hybrids as yet, and the chance that they might intercross is therefore estimated to be low.

It is striking that most of the present hybrid strains contain mtDNA of the rare introduction, *P. hedraiandra*. Also mitochondrial DNA of three *P. nicotianae* × *P. cactorum* hybrid strains was analysed and contained mtDNA of the introduced *P. nicotianae* (Man in 't Veld et al., 1998). The predominant presence in the hybrids of mtDNA of the introduced species may lend some support to a theory developed by Olson and Stenlid (2001). These researchers established artificial hybrids in the laboratory of the so-called 'S' and 'P' types of *Heterobasidion annosum*. Infection trials resulted in higher mortality rates of pine seedlings by hybrids containing 'P' type mitochondria than those containing 'S' type mitochondria. They concluded that the mitochondrial genome in *H. annosum* encodes factors contributing to virulence. When introduced species hybridize with native species, selection may favour progeny with exotic, mitochondrially-borne virulence factors to which local plant populations are not adapted.

It is now clear that European strains morphologically resembling *P. cactorum* in reality constitute a complex containing multiple taxa, including two categories of hybrid types as well as evolving strains. Characterization of *P. cactorum*- like strains based on morphological criteria alone is insufficient to distinguish the different taxa due to variation and overlap. The only way to establish the true identity of these strains is the genetic analysis of several loci.

Notoriously destructive introduced oomycetes like *P. ramorum* have often been found to be clonal species with the restricted genetic variation (Ivors et al., 2006) typical of 'founder' populations (Mayr, 1942). Hybridization between resident and introduced species may combine the vigour of these introduced pathogens with a new level of adaptability and a capacity for attacking new hosts. This process thus poses a novel threat not just to crops and natural ecosystems, but also, by extension, to states and their economies.

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