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Comparative aggressiveness of standard and variant hybrid alder phytophthoras, *Phytophthora cambivora* and other *Phytophthora* species on bark of *Alnus*, *Quercus* and other woody hosts

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Pathogenicity tests were carried out on the bark of *Alnus glutinosa* with 19 isolates of the standard (near-tetraploid) hybrid alder phytophthora, nine isolates representing its known heteroploid variants and 11 isolates of *P. cambivora*, a probable parent species of the hybrid. Over a 4-year period, 12 experiments were conducted on living alder logs incubated at 20°C. Most isolates of the standard hybrid and those of the 'Dutch variant' were highly aggressive to alder bark. Isolates of the 'Swedish', 'UK' and 'German variants', and of *P. cambivora*, were only weakly pathogenic. Also, isolates of *P. fragariae*, *P. cinnamomi*, *P.* sp. 'O-group', *P. cryptogea*, *P. megasperma*, *P. gonapodyides* and *P. citricola* were either weakly or nonpathogenic. Rates of lesion development were greatest on logs cut during July–October, slower on logs cut between November and March and zero on logs cut during April, indicating a strong seasonal effect. Other evidence indicated that lesion development was subject to critical thresholds of host resistance. The standard hybrid was nonpathogenic to the bark of four other hardwood and two conifer species, indicating that it is relatively host specific. In contrast, *P. cambivora* was an aggressive pathogen on live bark of *Quercus* and *Castanea*. The significance of these results is discussed.

Keywords: aggressiveness, *Alnus*, bark lesions, *Castanea*, *Phytophthora*, *Quercus*, seasonal variation

Introduction

During 1993–95, an unusual *Phytophthora*, morphologically similar to *P. cambivora*, was isolated from dead and dying alder (*Alnus*) in Britain. On the basis of its self-fertility and developmental instability, including a high level of zygotic abortion, it was postulated that this *Phytophthora* might be a species hybrid involving *P. cambivora* (Brasier *et al*., 1995). A study of chromosome numbers, nuclear behaviour and the sequence of the internal transcribed spacer (ITS) region of the rDNA was carried out on similar *Phytophthora* isolates from Britain and continental Europe. This confirmed that the common, 'standard' form of the alder phytophthora is a near-tetraploid species hybrid, and that it is probably of recent origin, with *P. cambivora* and a *Phytophthora* closely related to *P. fragariae* as parents (Brasier *et al*., 1999). *P. cambivora* is a common pathogen of *Castanea*, *Fagus* and other hardwoods in Europe (Peace, 1962), whilst *P. fragariae* is a major pathogen of strawberry and raspberry (Wilcox *et al*., 1993).

The standard alder phytophthora type is widely distributed across Britain, France, Germany and Austria. In addition, a range of unstable variant types, showing unique combinations of morphological and behavioural characters, chromosome numbers intermediate between diploid and tetraploid, different ITS profiles and variable amplified fragment length polymorphism (AFLP) of genomic DNA, were identified amongst isolates from the UK, The Netherlands, Germany and Sweden. These natural variants may be segregants from the standard alder phytophthora resulting from chromosome loss and homogenization of the ITS arrays. Even standard isolates are probably still evolving, since their ITS arrays show evidence of continuing recombination (Brasier *et al*., 1999).

These newly recognized phytophthoras can cause a rapid necrosis of the inner bark of the collar and stems and roots of alder trees (Brasier *et al*., 1995), the lesions sometimes reaching 2 m above soil level. Since April 1995, representative standard and variant alder phytophthora isolates and a wide range of *P. cambivora* isolates have been tested, at different times, for their

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comparative ability to cause lesions on living alder logs. Also tested have been isolates of *P. fragariae* var. *rubi*; *P. cinnamomi*, a common tree pathogen in Europe related to *P. cambivora* and *P. fragariae* (Cooke & Duncan, 1997); *P. gonapodyides*, *Phytophthora* sp. 'O-group' (Brasier *et al.*, 1993a), *P. citricola* and *P. megasperma*. These last four *Phytophthora* morphospecies are frequently found in ponds, rivers or wet soil on riverbanks in Europe, and therefore often inhabit the same riparian ecosystems as the alder phytophthoras. More limited pathogenicity tests were also carried out on logs of other tree species. In all, 12 pathogenicity experiments were carried out over 4 years, spread over different times of the year. The results of these experiments are reported here.

Materials and methods

The origins of the *Phytophthora* isolates studied are given in Table 1. For experimental work, isolates were maintained on carrot agar (CA) (Brasier, 1969) at 20°C and subcultured at 3-week intervals. For long-term storage, cultures were grown for 4 days on oatmeal agar slopes in universal bottles and submerged in sterile paraffin oil. Synthetic mucor agar (SMA) + rifamycin selective medium comprised SMA agar (Elliott *et al.*, 1966) containing a 4% solution of methyl benzimidazol-2-ylcarbamate (MBC) (4 g MBC heated in 47·2 mL H_2O + 2·8 mL HCl, then made up to 100 mL with H_2O), 0·1 g L^{-1} pimaricin powder and 3·0 mL L^{-1} of a 1% w/v aqueous solution of rifamycin.

For inoculation tests, 1·2 m long × 20–30 cm diameter billets were cut from stems of living *Alnus glutinosa* trees 24–48 h before the experiment and the cut ends sealed with Isoflex (bitumen) (Ronseal Ltd, Sheffield, UK). A modification of the elm inoculation protocol of Webber & Hedger (1986) was used. A 5 mm diameter hole was punched through the bark to the wood surface with a cork borer. A 5 mm agar plug from the margin of an actively growing colony on CA was then inserted and the bark plug replaced. Moist cottonwool was placed over the wound, and covered with a 5 × 5 cm piece of aluminium foil secured by adhesive PVC tape. Inoculation points were staggered as shown in Fig. 1. There were eight replicates per isolate (10 in the first experiment), inoculated so as to ensure that the different *Phytophthora* species were evenly distributed over the logs. Control inoculations (one per log) were as above, but of plain CA. Inoculated logs were stood upright, covered individually in loose polythene sleeves (sealed at both ends) and incubated at 18–22°C (setting 20°C) in an air-conditioned laboratory for 26–55 days (see Table 4 for details of duration).

The logs were destructively sampled by removing the periderm with a drawknife to expose the phloem. Any lesion or stained necrotic area around an inoculation point was quickly outlined with a marker pen prior to the oxidative staining of the surrounding live bark tissue. Lesion outlines were then traced onto tracing paper. Maximum length and average breadth measurements were made from photocopies of the traced lesions. Areas were calculated by cutting and weighing the tracings. The mean lesion length, width and area for each isolate, together with the SE, were then calculated. Bark shavings removed during assessment were bagged and autoclaved. The logs were air dried and burnt.

Between April 1995 and November 1998, a total of 19 standard alder phytophthora isolates from different countries and nine isolates representing the UK, Dutch, German and Swedish variant types were tested, plus isolate P818v, a morphologically unique sector from a colony of standard isolate P818. In addition, 11 isolates of *P. cambivora* were tested, together with 21 isolates of other species. The experiments in which each isolate was used are indicated in Table 1. Some alder isolates (e.g. standard type P772 and Dutch variant P770) were used repeatedly to maintain a level of continuity between experiments. However, a balance also had to be struck between repeating the same isolates, the need to test freshly collected alder phytophthora isolates or isolates of other *Phytophthora* species, and the practical limits on the size of the experiments.

The different experiments and isolates resulted in very different extents of lesion development. To allow a direct visual comparison to be made of the results between experiments (see Figs 2 and 3), the mean lesion areas of all the isolates and the controls within each experiment are shown as a percentage of the largest mean lesion area caused by any isolate. In consequence, SE cannot be shown in Figs 2 and 3. Statistical analyses (either analysis of variance (ANOVA) or general linear models) of lesion sizes, however, were performed on the raw data sets, with lesion areas transformed to square roots. Because of considerable replicate variation within isolates, skewed distributions of the data sets and the unequal group sizes, suitable error and link functions were applied as appropriate.

In Experiment II, re-isolation of all the inoculated fungi onto SMA + rifamycin selective medium was attempted from the lesion margins.

Results

Pathogenicity tests on *Alnus glutinosa* logs

Twelve pathogenicity experiments were conducted between 1995 and 1998. Experiment I was conducted in April 1995 (Table 4), shortly after the local alder trees had commenced flushing. Although a wide range of *Phytophthora* species and isolates were used, the experiment resulted in little or no lesion development. It was repeated in October 1995, as Experiment II, when it was markedly more successful. In most subsequent experiments, conducted in various months of the year, some degree of lesion development usually occurred. However, Experiment VI which, like Experiment I, was set up in April also resulted in little or no

lesion development (Table 4). No further details of Experiments I and VI are presented.

As a representative data set, the mean lesion lengths, widths and areas caused by each isolate in Experiment II are shown in Table 2. Two standard alder phytophthora isolates P772 and P773, and Dutch variant isolate P770, produced substantial lesions approximately 350–500 mm long and 45–100 mm wide. As can be seen from the respective SEs, there was considerable variation between replicates. A standard isolate, P668, and one of three *P. cambivora* isolates, P199, produced moderately long lesions (78–86 mm). However, these lesions were extremely narrow (approximately 13–20 mm mean width) and resulted in only a small lesion area. Two *P. cambivora* isolates, two *P. cinnamomi* isolates, two *P. gonapodyides* isolates and one *P. cryptogea* isolate caused only nil to very slight necrosis compared to the controls. All inoculated isolates were re-isolated onto SMA + rifamycin selective medium from the lesion margins or from the zone of necrosis around the inoculum plug.

Nine further experiments produced broadly similar results. The results of all 10 positive experiments are summarized in Fig. 2 in terms of mean lesion area per isolate.

Standard and variant alder phytophthoras

The largest mean lesion area produced by any isolate from experiment to experiment varied greatly (discussed below). However, with the exception of isolate P818v in Experiment X, the isolates producing the largest lesions in each experiment were always standard alder isolates or, in the case of P818v (shown as d2 in Fig. 2), a sector derived from a standard isolate. Indeed, the standard alder isolates were, in general, considerably more aggressive than the other *Phytophthora* isolates and species tested, apart from the German and Dutch variants. This is well illustrated by Experiments VII, XI and XII (Fig. 2). However, a few standard isolates exhibited only low levels of aggressiveness. Notable amongst these were isolates P668 and P669 (Experiments II, III, V), which were also the first standard alder phytophthora isolates collected in the UK (Table 1; Brasier *et al*., 1995).

The Dutch variant (isolates P770 and P972) was tested in eight of the 10 experiments represented in Fig. 2. This was consistently the most aggressive of the natural variants. Indeed, in some experiments, lesion sizes produced by the Dutch variant and the standard isolates could not be distinguished. Thus, in Experiment VII (Fig. 2), both the standard isolates and the Dutch variant P770 produced significantly larger mean lesion areas than the *P. cambivora* isolates ($P < 0{\cdot}001$), and the mean lesion area of P770 was not significantly different from the mean of the four standard alder isolates. Generally, however, the Dutch variant produced more moderate-sized lesions. These averaged 45% of the mean area of the most active standard isolates in these eight experiments, and 68% of the average lesion area of all the standard isolates in these experiments: Dutch variant, average lesion area $111{\cdot}5 \pm 51{\cdot}5$ cm^2; standard isolates, average lesion area $164{\cdot}4 \pm 53{\cdot}4$ cm^2 (not significantly different). It should also be noted that, in Experiment XII (Table 3), Dutch variant isolate P972 was significantly more aggressive than isolate P770.

The German, UK and Swedish variants exhibited consistently lower levels of pathogenicity than the standard isolates (see Fig. 2, Experiments IX, X and XII). The results of Experiment XII are shown in more detail in Table 3. In this experiment, the mean lesion area of the three standard isolates was $99{\cdot}1$ cm^2, that of the two Dutch variant isolates $50{\cdot}4$ cm^2, that of the five German, UK and Swedish variant isolates combined $15{\cdot}8$ cm^2, and that of the two *P. cambivora* isolates $7{\cdot}4$ cm^2. The means of the standard isolate and Dutch variant groups were significantly different from each other, from the means of the combined German, UK and Swedish variant group, and from *P. cambivora* (all at $P < 0{\cdot}05$). The mean of the combined German, UK and Swedish variant group, however, was not significantly different from that of *P. cambivora*. There was also considerable variation in lesion sizes of replicates within isolates ($P < 0{\cdot}001$), as well as between isolates within the above groups. Overall, however, an approximate order of aggressiveness was as follows: standard isolates > Dutch variant >> German variant > Swedish variant, UK variant and *P. cambivora*.

The pathogenicity of P818v, a unique sector type derived from standard isolate P818, was compared with its 'parent' culture P818 in Experiment X. P818v produced large lesions, the largest recorded in the test, but was not significantly different from P818.

Other *Phytophthora* species

Phytophthora cambivora is a probable parent of the hybrid alder phytophthoras. Eleven isolates of *P. cambivora* were tested over the 12 experiments, at least one being included in each. These generally produced only a limited lesion area, usually around 5% of the area of the most active standard isolate in the test. Isolates P199 and P819 appeared to be the most aggressive of the *P. cambivora* isolates. In experiments in which lesions produced by the standard alder isolates were more restricted (see below), such as Experiments V, IX and X (Fig. 2), the lesions produced by P199 and P819 were approximately 20–30% of the area of those of the standard isolates. However, these lesions were still only about 3–4 times the area of the necrotic zone produced in the noninoculated controls. In experiments in which the standard isolates were very active (e.g. Experiments II, VII or VIII), the *P. cambivora* isolates, including P199 and P819, showed little more aggressiveness than other *Phytophthora* species tested (e.g. Table 3). *P. fragariae* var. *rubi*, a possible parent of the

Table 1 Key *Phytophthora* isolates studied and experiments in which they were used

Phytophthora species	Isolate	Location, country	Sampled/isolated by	Sampled from	Sampling or receipt date	Experiment[a] I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Alder phytophthoras																	
Standard form	P668	Worcestershire, UK	J. Rose	abl[b]	1994	●	●			●							
	P669	Worcestershire, UK	J. Rose	as[c]	1994			●		●							
	P670	Gwent, UK	J. Rose	abl	1994			●	●	●							
	P677	West Sussex, UK	J. Rose	abl	1994	●											
	P765	West Sussex, UK	J. Rose	abl	1994					●							
	P766	Oxfordshire, UK	J. Rose	abl	1994			●		●							
	P768	Essex, UK	J. Rose	abl	1994	●											
	P772	South Yorkshire, UK	G. MacAskill	abl	1994	●	●	●	●	●	●	●	●			●	●
	P773	Dyfed, UK	J. Rose	abl	1994		●			●							
	P785	Norfolk, UK	J. Rose	abl	1995			●	●	●							
	P806	Berkshire, UK	D. Kennedy	abl	1995						●						
	P807	Hampshire, UK	M. Lipscombe	abl	1995			●	●	●	●	●	●				
	P817	North Germany	G. Hartmann	abl	1994			●		●							
	P818	North Germany	G. Hartmann	abl	1995			●	●		●	●	●		●	●	
	P834	Léon, France	C. Delatour	abl	1996							●	●			●	●
	P844	Upper Austria	T. Cech	abl	1996									●			
	P891	North Yorkshire, UK	G. MacAskill	abl	1997										●		
	P938	Hampshire, UK	J. Delcan	abl	1997										●	●	●
	P976	Grampian, UK	J.N. Gibbs	abl	1998											●	
Sector variant	P818v[d]	n/a	n/a	Cultural variant of P818	1997										●		
Dutch variant	P770	Wageningen, Netherlands	H. Van Kesteren	abl	1994	●	●	●	●	●	●	●	●				●
	P972	De Wieden, Netherlands	C. Van Dyck	abl	1998											●	●
UK variant	P841	North Yorkshire, UK	S.C. Gregory	abl	1996										●		●
Swedish variant	P875, P876	Gothenburg, Sweden	C. Olsson	abl	1997									●	●		
	P887	Save River, Sweden	C. Olsson	abl	1997												●
	P888	Alingsas, Sweden	C Olsson	abl	1997												●
German variant	P889, P890	Freising, Germany	T. Jung	abl	1997										●		●
P. cambivora	P28	Cheshire, UK	R.G. Strouts	*Chamaecyparis* root/soil	1975		●										
	P199	Unknown, UK	M.G. Griffin	*Fagus* sp.	1969		●	●	●	●	●	●	●	●			
	P203	Surrey, UK	R. Reeves	*Castanea sativa*	1969	●		●									
	P219	Hampshire, UK	R.G. Strouts	*Acer* soil	1971	●											
	P238	Unknown, UK	R. Reeves	*Fagus* sp.	1971	●											
	P239	Surrey, UK	R.G. Strouts	*Fagus* sp.	1972	●	●	●									

Table 1. *continued*

Phytophthora species	Isolate	Location, country	Sampled/isolated by	Sampled from	Sampling or receipt date	Experiment[a] I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
	P281	Hampshire, UK	C. Gulliver	*Fagus* soil	1975			●									
	P315	Bristol, UK	C. Gulliver	*Platanus* soil	1976			●	●	●	●	●	●				
	P819	Perthshire, UK	D. Cooke	*Rubus* sp.	1985			●	●	●	●	●	●	●	●	●	●
	P820	Somerset, UK	D. Cooke	*Rubus* sp.	1995			●	●		●	●	●				
	P821	Unknown, Italy	T. Turchetti	*Castanea sativa*	1980					●	●	●	●			●	●
P. cinnamomi	P382	Surrey, UK	R.G. Strouts	*Nothofagus procera* soil	1980				●		●	●	●	●			
	P402	Unknown, Canary Islands	L. Gallo	*Persea* sp.	1976		●										
	P404	Unknown, Malaysia	L.B. Siew	*Syzygium* sp.	1990	●											
	P592	Yorkshire, UK	R.G. Strouts	*Crataegus* roots	1991				●								
	P596	Badajoz, Spain	C.M. Brasier	*Quercus ilex* roots/soil	1992	●	●		●		●	●	●				
	P612	Western Cape, South Africa	S. von Broembsen	*Leucospermum comosum*	1994	●											
	P724	Lagos, Portugal	E. Sanchez	*Quercus suber* soil	1995				●			●	●				
P. citricola	P812	Oregon, USA	C.M. Brasier and E.M. Hansen	*Alnus* roots	1995				●		●	●	●				
	P813	Oregon, USA	C.M. Brasier and E.M. Hansen	River water	1995				●								
P. cryptogea	P187	Surrey, UK	M.G. Griffin	*Hebe* sp. roots/stems	1972	●											
	P241	Kent, UK	R.G. Strouts	*Prunus* sp. stems	1972	●	●	●									
	P242	Kent, UK	R.G. Strouts	*Viburnum* sp. roots	1972	●											
P. fragariae var. *rubi*	P823	Scotland, UK	D. Cooke	*Rubus* sp.	1971				●	●	●						
P. gonapodyides	P236	Gloucestershire, UK	C.M. Brasier and R.G. Strouts	*Prunus* sp. roots	1971	●											
	P245	Kent, UK	C.M. Brasier and R.G. Strouts	*Salix* sp. roots	1972	●	●										
	P501	Surrey, UK	T. Reffold	*Ilex* sp.	1995	●	●										
	P786	Norfolk, UK	J. Rose	abl	1995												●
	P878	Unknown, Denmark	K. Thingaard	Alder debris in pond	1995												●
P. megasperma	P426	Unknown, UK	C. Brasier	*Populus* sp.	1984			●									
	P922	Hampshire, UK	J. Delcan	as	1997												●

Table 1. *continued*

Phytophthora species	Isolate	Location, country	Sampled/isolated by	Sampled from	Sampling or receipt date	Experiment[a] I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
P. sp. 'O'-group	P210	Buckinghamshire, UK	C.M. Brasier and R.G. Strouts	*Aesculus hippocastanum*	1970												●
	P246b	Kent, UK	C.M. Brasier and R.G. Strouts	*Salix* sp. roots	1970												●
	P845	Nancy, France	J.C. Streito	*Alnus* sp.	1996												●
	P877	Unknown, Denmark	K. Thingaard	*Alnus* debris in pond	1995												●

[a]●, Isolate used in the experiment indicated.
[b]abl, alder bark lesion.
[c]as, alder soil.
[d]See Delcan & Brasier (2001).

hybrid alder phytophthora, was tested in Experiments IV and V. It caused very little necrosis.

Isolates of *P. cinnamomi* (Fig. 2, Experiments II, IV, VII, VIII and IX), *P. gonapodyides* (Experiments II and XII), *P.* sp. 'O-group' (Experiment XII), *P. citricola* (Experiments IV, VII and VIII), *P. cryptogea* (Experiments II and III) and *P. megasperma* (Experiments III and XII) also produced very limited necrosis, usually little more than the noninoculated controls. The two *P. citricola* isolates appeared to be the most aggressive isolates of this group, producing lesions of about the same size as or slightly larger than those of the more active *P. cambivora* isolates.

Seasonal variation in lesion area and critical threshold effects

The average lesion areas and lesion extension rates of the standard alder isolates in each of the 12 experiments on alder are shown in Table 4. The mean lesion areas produced in logs cut between July and October, at approximately 240–408 cm^2, were considerably larger than those produced in logs cut between November and March, at approximately 12–26 cm^2. In the two experiments involving logs cut in April, virtually no lesion development occurred. Because of variations in the number of days between inoculation and assessment, a statistical analysis was performed on the mean daily longitudinal extension rates rather than on the lesion areas (Table 4). Lesion extension rates in logs cut in July–October were significantly greater, at range 3·1–5·8 mm day^{-1}, average 4·65 mm day^{-1}, than those cut in November–March at range 0·8–3·2 mm day^{-1}, average 1·75 mm day^{-1} ($P < 0·001$). This indicates that lesion development is strongly suppressed in April, moderately restricted during November–March, but unrestricted during July–October.

There is also an indication, particularly with the March to November experiments, that critical threshold effects were operating on host susceptibility. Thus, in Experiment V, a 'higher' group of four standard isolates produced considerably larger lesions than a 'lower' group of five standard isolates. The mean lesion areas of the 'higher' and 'lower' groups were significantly different ($P < 0·01$). Moreover, the mean lesion area of the 'lower' group did not differ significantly from that of the four *P. cambivora* isolates in the experiment.

Further evidence for a critical threshold effect can be seen in the behaviour of standard isolate P772, which was used in eight of the 10 positive experiments (see * in Fig. 2). In Experiment II, P772 produced the largest lesion area of any isolate (Table 2). In Experiment V, however, it fell within the 'lower' group of isolates, i.e. it resembled *P. cambivora* (cf. also Experiment IV). Yet, in Experiment XI, it again produced large lesions, over 20 times the area of those produced by the *P. cambivora* control.

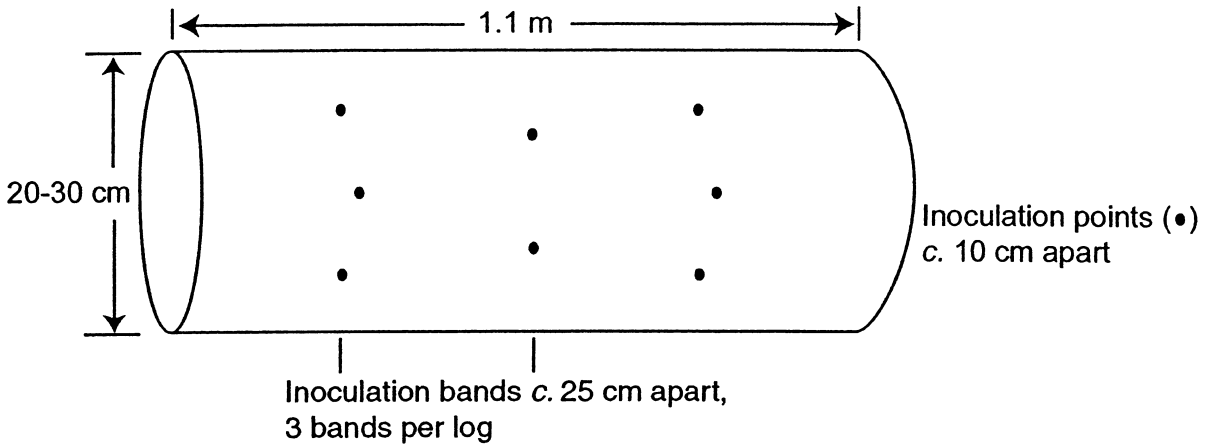

Figure 1 Position of inoculation points in the log inoculation method.

Pathogenicity tests on other tree species

Experiment IV included a concurrent pathogenicity test on comparably sized logs of *Castanea sativa* and *Quercus robur*; and Experiment VII included logs of *Fagus sylvatica* and *Acer pennsylvannicum*. Under natural conditions, mature trees of these four genera are susceptible to root or collar infections caused by *P. cambivora*, *P. cinnamomi* or both of these species. Experiment VIII also included logs of *Chamaecyparis lawsoniana*, which is susceptible to a wide range of phytophthoras in the field and in nurseries, and *Taxus baccata*, which is susceptible to *P. cinnamomi* as a mature tree under forest conditions and in horticulture (cf. Brasier, 2000). The results of these additional tests are summarized in Fig. 3. Mean lesion areas are again expressed as a percentage of the largest mean lesion area caused by any isolate on that host in the same experiment.

On *Castanea*, *Quercus*, *Fagus* or *Acer*, none of the five standard alder phytophthora isolates tested, nor Dutch variant P770, caused any lesion development compared with the noninoculated control; yet substantial lesions were produced by some of these isolates on *Alnus* in the same experiment (cf. Fig. 2). There was considerable variation in the behaviour of different *P. cambivora* or *P. cinnamomi* isolates on these hosts. Most of the *P. cambivora* isolates produced substantial lesions on both *Castanea and Quercus* (3/4 and 4/4 isolates, respectively), a majority (3/5 isolates) produced moderate lesions on *Fagus*, but none (0/5) produced significant lesions on *Acer*. *P. cinnamomi* isolate P382 produced substantial lesions on *Castanea*, *Fagus* and *Quercus* and limited lesion development on *Acer*. *P. cinnamomi* isolate P596 produced moderate-sized lesions on *Castanea* only. Two other *P. cinnamomi* isolates, P724 and P592, failed to produce significant lesions on any of these hosts.

Phytophthora citricola isolate P812 was by far the most aggressive of all the phytophthoras tested on *Acer*. It also produced substantial lesions on *Castanea* and *Fagus*, but only limited lesions on *Quercus*. Its lesions on *Acer and Fagus* tended to be broad and 'diamond-shaped', whereas those produced by the other *Phytophthora* species were usually narrow and lenticular. This suggests that *P. citricola* P812 has a greater ability to colonize phloem tissues laterally. *P. fragariae* var. *rubi* failed to produce lesions on *Castanea* or *Quercus*.

On *Chamaecyparis and Taxus*, four standard alder phytophthoras again caused no lesion development, despite their being very active on *Alnus* in the same experiment (cf. Fig. 2). *P. cinnamomi* P382 produced substantial and two other *P. cinnamomi* isolates moderate lesions on *Taxus*, whereas the five *P. cambivora* isolates tested produced no lesions on *Taxus*. *P. cinnamomi* P382 was the most aggressive isolate on *Chamaecyparis*, producing moderate lesions, as also did *P. citricola* P812. Two other *P. cinnamomi* isolates and five *P. cambivora* isolates caused nil or very small lesions on *Chamaecyparis*.

Discussion

The heteroploid alder phytophthoras are believed to be hybrids between *P. cambivora* and a taxon closely related to *P. fragariae* (Brasier *et al.*, 1999). *P. cambivora* and *P. fragariae* are related through ecologically distinct taxa, the former being a pathogen of various hardwood trees, the latter a specialized pathogen of strawberry and raspberry. In the present study, isolates of the standard alder phytophthora from several European countries were shown to be potentially highly aggressive pathogens of alder bark. However, they were non-pathogenic to the bark of six other tree genera, including tree species such as *Castanea sativa* and *Chamaecyparis lawsoniana* that are otherwise susceptible to a range of phytophthoras including *P. cambivora*. The standard alder phytophthora therefore appears to be relatively specific to alder. In contrast, 11 isolates of *P. cambivora* caused very limited lesion development on alder, whereas selected *P. cambivora* isolates caused substantial lesions on other tree genera,

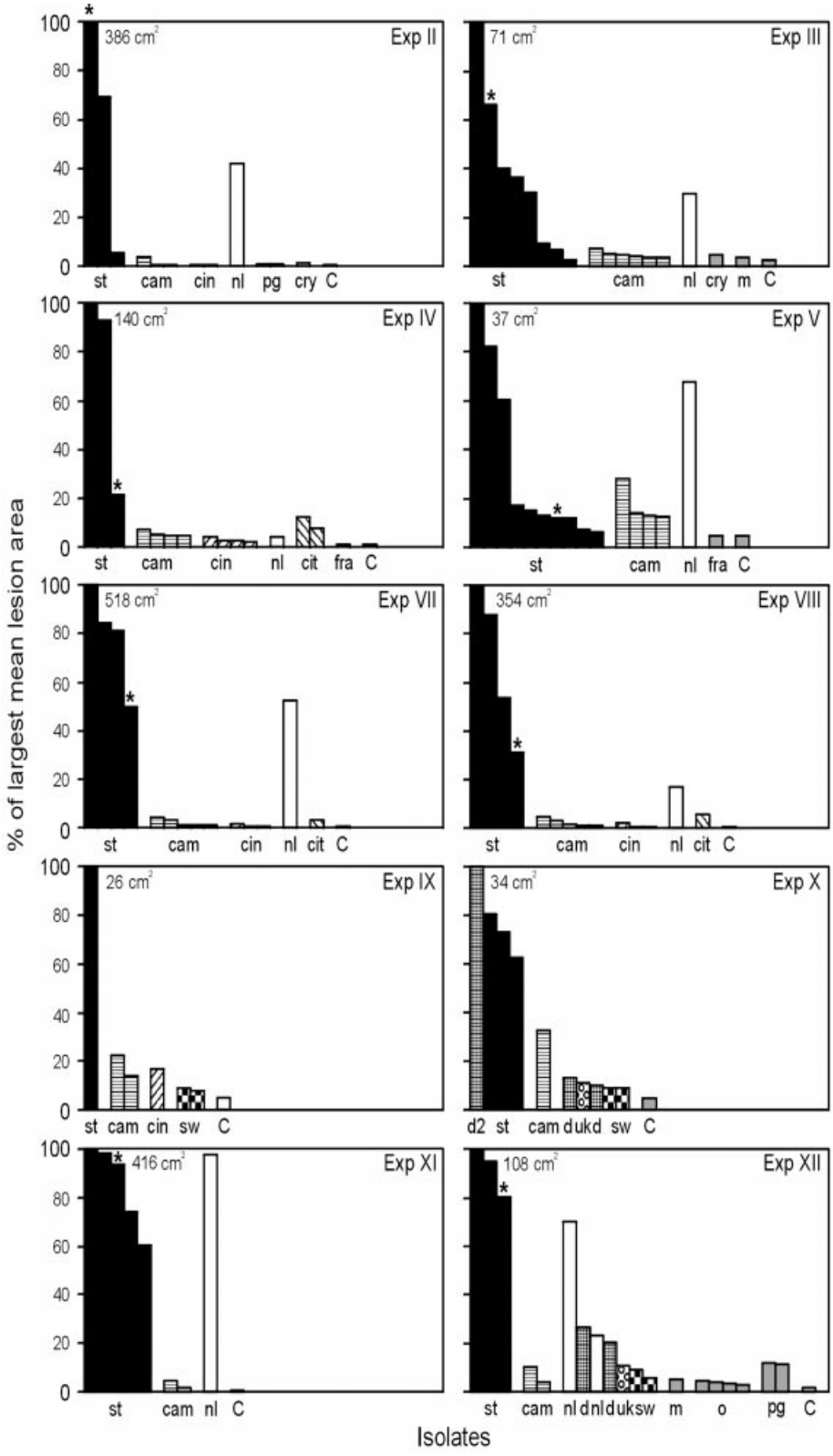

Figure 2 Lesion sizes caused by *Phytophthora* isolates on *Alnus glutinosa*. Area in cm^2 is the largest mean lesion area of any isolate in that experiment. st, isolates of standard, near-tetraploid alder phytophthora; *, isolate P772; nl, d, uk, sw, isolates of the Dutch, German, UK and Swedish variants; d2, cultural variant of standard German isolate P818; cam, *P. cambivora*; cin, *P. cinnamomi*; cry, *P. cryptogea*; fra, *P. fragariae* var. *rubi*; cit, *P. citricola*; o, *Phytophthora* sp. 'O'-group; pg, *P. gonapodyides*; m, *P. megasperma*; C, control.

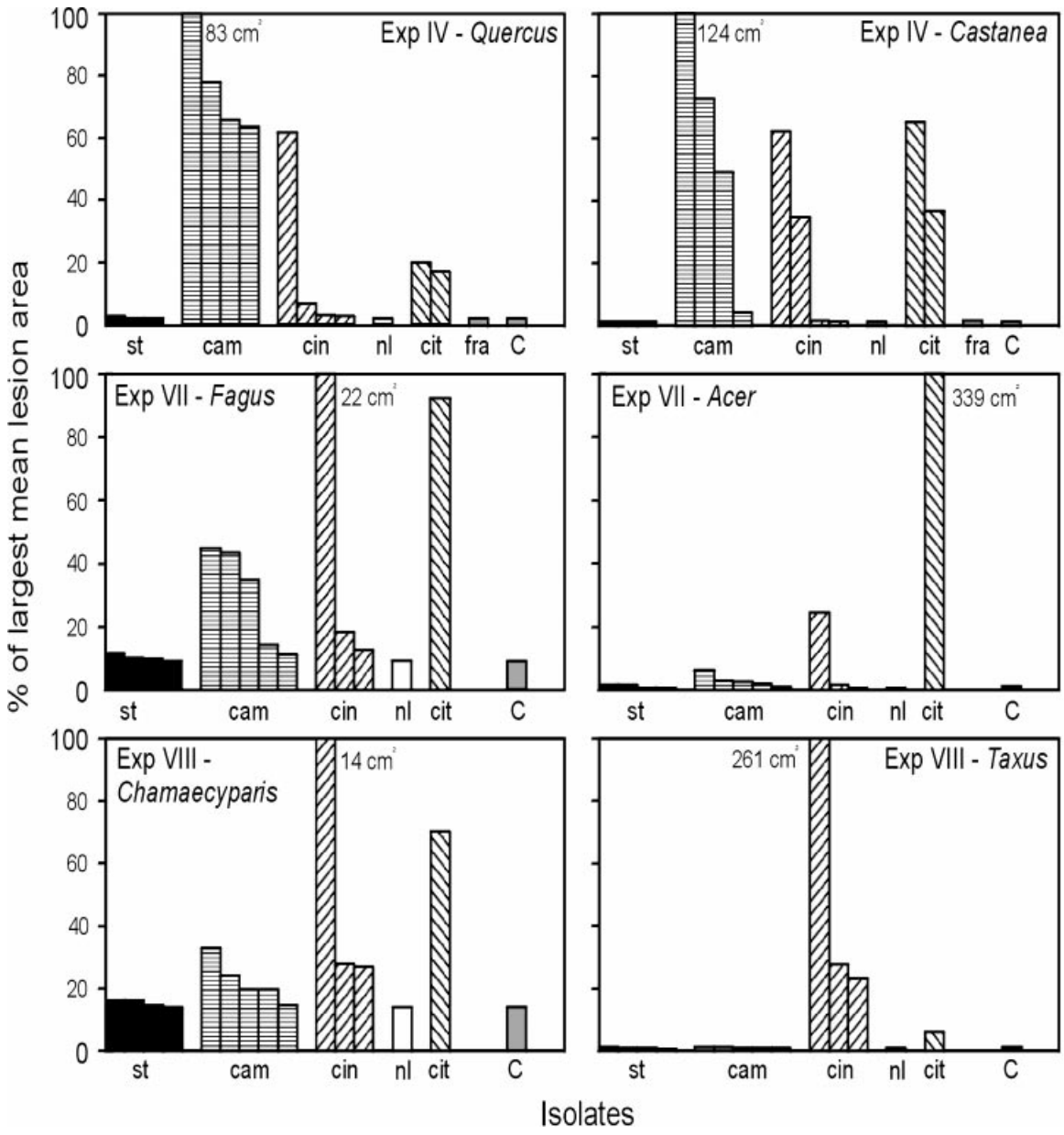

Figure 3 Lesion sizes caused by *Phytophthora* isolates on *Quercus*, *Castanea*, *Fagus*, *Acer*, *Chamaecyparis* and *Taxus*. Key as in Fig. 2.

such as *Castanea* and *Quercus*, under the same conditions. An isolate of *P. fragariae* var. *rubi* caused almost no lesion development on alder, *Quercus* or *Castanea*.

The genetically and phenotypically unique Swedish, Dutch, German and UK natural variants are suggested to be either genetic breakdown products or backcross products of the standard type (Brasier *et al*., 1999). Only one of these, the Dutch variant, was also a strong pathogen of alder bark. The others were comparatively weak pathogens. If the variant types are breakdown products of the standard type, three of them appear to have lost the high level of aggressiveness to alder bark in the process. All the natural variants, however, were originally isolated either from alder bark or from a root lesion on alder. They must therefore have some ability to attack alder in the field. Nonetheless, the present results suggest that the disease is likely to be more aggressive in regions where the standard type is predominant, such as the UK, France and Austria.

Only the Dutch variant was tested on hosts other than alder. It was found, like the standard type, to be nonpathogenic on these hosts. The host ranges of the other variants remain to be tested. Of particular interest is the Swedish variant, which in several other respects shows the greatest similarity of all the variants to *P. cambivora* (Brasier *et al*., 1999). It might therefore have a more *P. cambivora*-like host range.

Table 2 Lesion sizes in Experiment II on *Alnus glutinosa*, October 1995

Phytophthora type or species and isolate no.	Mean lesion length(mm) and SE	Mean lesion width (mm) and SE	Mean lesion area (cm^2) and SE[a]
Standard alder P772	492·0 ± 39·3	100·4 ± 15·0	385·7 ± 82·8 a
Standard alder P773	450·2 ± 66·9	70·5 ± 13·3	267·8 ± 72·8 ab
Standard alder P668	78·4 ± 33·3	19·8 ± 4·1	21·2 ± 12·2 c
Dutch variant P770	375·0 ± 46·1	46·0 ± 13·0	161·8 ± 52·6 b
P. cambivora P28	18·3 ± 0·9	12·6 ± 0·6	1·9 ± 0·2 e
P. cambivora P199	85·7 ± 15·7	17·3 ± 3·2	14·7 ± 5·3 c
P. cambivora P239	18·1 ± 1·1	12·9 ± 0·6	1·9 ± 0·1 e
P. cinnamomi P596	22·6 ± 3·6	12·3 ± 0·7	2·3 ± 0·3 de
P. cinnamomi P402	16·1 ± 0·4	12·4 ± 0·6	1·8 ± 0·1 e
P. cryptogea P241	49·9 ± 5·9	13·3 ± 0·6	5·1 ± 0·7 d
P. gonapodyides P245	30·8 ± 3·1	12·7 ± 0·6	3·3 ± 0·3 de
P. gonapodyides P501	17·9 ± 1·6	12·6 ± 0·8	3·0 ± 0·9 de
Control	12·3 ± 1·0	13·3 ± 0·6	1·5 ± 0·2 e

[a]Lesion areas followed by a different letter are significantly different at the 95% level ($P < 0·05$).

Isolate P818v was derived from a unique sector that arose in a colony of German standard isolate, P818. It differs from P818 in its densely aerial colony type, in producing many single-celled (as opposed to two-celled) antheridia, and in having the same ITS pattern as the UK, German and Dutch natural variants (D.L. Cooke and C.M. Brasier, unpublished results; Delcan & Brasier, 2001). P818v therefore shows phenotypic abnormalities comparable to the natural variants, and could have originated through the type of genetic rearrangement event in a standard isolate that has given rise to the variants in nature. In the present tests, P818v was found to have retained its high level of pathogenicity to alder.

Table 3 Lesion areas of alder phytophthora isolates and *P. cambivora* isolates on alder bark in Experiment XII (October 1998)

Isolate no.	Isolate type or species	Mean lesion area (cm^2) and SE[a]
Isolates		
P938	Standard	108·0 ± 11·9 a
P834	Standard	102·6 ± 8·5 a
P772	Standard	86·6 ± 13·1 ab
P972	Dutch variant	75·7 ± 11·7 b
P890	German variant	28·9 ± 3·7 c
P770	Dutch variant	25·1 ± 4·5 c
P889	German variant	22·3 ± 6·4 cd
P841	UK variant	11·9 ± 2·2 de
P819	*P. cambivora*	10·8 ± 3·4 de
P887	Swedish variant	9·7 ± 1·3 de
P888	Swedish variant	6·3 ± 1·2 e
P821	*P. cambivora*	4·0 ± 0·9 e
Control		1·7 ± 0·1 e
Isolate groups[b]		
	Standard isolates (3)	99·1 ± 6·4 x
	Dutch variant (2)	50·4 ± 25·3 y
	German + Swedish + UK variant (5)	15·8 ± 4·2 z
	P. cambivora (2)	7·4 ± 3·4 z

[a]Lesion areas followed by a different letter are significantly different at the 95% level ($P < 0·05$).
[b]Number of isolates in parentheses.

Of the other *Phytophthora* species tested, none was a significant pathogen of alder bark. These included *P. cinnamomi*, an aggressive pathogen of certain tree species in warm, temperate ecosystems, and *P. gonapodyides*, *Phytophthora* sp. 'O-group', *P. megasperma* and *P. citricola*, four taxa commonly isolated from river water and wet soil in riparian ecosystems, including alder habitats, in Britain and Iberia (Brasier *et al*., 1993b; C.M. Brasier, J. Delcan and E.M. Sánchez-Hernandez, unpublished observations). They are therefore phytophthoras to which European *Alnus* spp. are likely to have been exposed prior to the appearance of the hybrid alder phytophthoras.

Evidence was obtained for marked seasonal variation in the susceptibility of alder logs to the standard alder phytophthora. If repeated in the field, development of lesions in the bark, postinfection, is likely to be most rapid between July and October, i.e. the period of leaf retention, restricted from November to March, after leaf fall, and strongly if not totally suppressed during April, the approximate time of leaf flushing. Sometimes, therefore, lesion development might cease during the winter, allowing an opportunity for a tree to recover. The physiological basis of these seasonal differences in host susceptibility is unknown. Low bark water content has been shown to restrict the development of *Phytophthora* pathogens (e.g. Tippett *et al*., 1987; Marcais *et al*., 1993). Hence, one factor involved (but unfortunately not measured in these tests) might be a lower bark water content during the autumn and winter months. Another factor is likely to be the bark's ability to mobilize stored photosynthates or antifungal metabolites for defence (cf. Cahill & McComb, 1992).

There was also evidence, within some experiments, for 'higher' and 'lower' levels of lesion development amongst the standard isolates and the Dutch variant. This was further indicated by the variable position of standard isolate P772 (used as a control) in relation to other standard isolates. These observations suggest that critical thresholds of host resistance were operating. In the field, such threshold effects could mean a difference between chronic, suppressed disease and acute, potentially lethal disease. Internal (host) and external influences, such as temperature or microbial competition, could be additional interactive variables in such a threshold phenomenon. *P. cambivora*, the UK, German and Swedish variants and the other *Phytophthora* species tested appeared unable to cross the threshold at any time.

In tests on other tree species, *P. cambivora* was highly and moderately aggressive to the bark of *Castanea* and *Fagus*, respectively. This is consistent with the well-known susceptibility of these taxa to *P. cambivora*

Table 4 Seasonal influence on lesion sizes produced by standard alder phytophthora isolates[a]

Experiment	Date of inoculation	Length of experiment (days)	No. of isolates tested	Mean longitudinal lesion extension rate (mm day^{-1}) and SE	Mean lesion area (cm^2) and SE	Largest mean lesion area of any isolate (cm^2)
XI	14 July 1998	42	5	5·8 ± 0·2	354 ± 32	415·6
VII	8 October 1996	42	4	5·5 ± 0·3	408 ± 55	517·5
XII	21 October 1998	26	3	5·0 ± 0·1	99 ± 6	108·0
VIII	22 October 1996	42	4	4·3 ± 0·3	240 ± 56	353·4
IV	12 February 1996	42	3	3·2 ± 0·7	100 ± 35	139·6
II	4 October 1995	55	3	3·1 ± 1·2	225 ± 107	385·7
IX	19 December 1996	35	1	2·0	26	26·0
X	2 February 1998	35	3	1·9 ± 0·1	25 ± 2	27·3
III	13 December 1995	43	8	1·6 ± 0·4	26 ± 8	71·0
V	4 March 1996	45	10	0·8 ± 0·2	12 ± 4	36·7
I	17 April 1995	42	4	Nil/trace	Nil/trace	(1·0) nil/trace
VI	15 April 1996	43	4	Nil/trace	Nil/trace	(1·0) nil/trace

[a]Experiments are shown in descending rank according to mean longitudinal extension rate.

under forest conditions (e.g. Day, 1938, 1939; Peace, 1962). *P. cambivora* was also shown here to be an aggressive pathogen of *Quercus* bark (cf. also Jung & Blaschke, 1996). Other recent research shows that *P. cambivora* can be isolated from small roots of *Q. robur* in the field, that it is a frequent associate of *Q. robur* on heavy soils in Germany and the UK and that it can cause significant fine root loss in inoculated oak seedlings (Jung *et al.*, 1996, 1999; C.M. Brasier and J. Rose, unpublished results). These combined observations suggest that, under suitable conditions, *P. cambivora* is potentially a serious primary pathogen of *Quercus*.

Phytophthora cinnamomi is associated with root rot and mortality of *Q. suber*, *Q. ilex* and other *Quercus* spp. in Mediterranean Europe, and with stem canker of *Q. rubra* in France (e.g. Brasier *et al.*, 1993b; Marcais *et al.*, 1993). Australian and South African *P. cinnamomi* isolates showed considerable variation in aggressiveness when inoculated into *Eucalyptus* spp. (Dudzinski *et al.*, 1993; Linde *et al.*, 1999). In addition, Robin & Desprez-Loustau (1998) observed a wide range in aggressiveness of *P. cinnamomi* isolates on excised bark of *Q. rubra*. In the present tests, isolate P382 of *P. cinnamomi*, from *Nothofagus* soil in the UK, was a particularly aggressive bark pathogen on all hosts tested except *Acer and Alnus*. Two other *P. cinnamomi* isolates (P596 and P724), from *Quercus* roots and associated soil in Iberia, were considerably less aggressive than P382 on the susceptible hosts, including *Q. robur*. This again demonstrates marked variation in the aggressiveness of individual isolates of this species. *P. cinnamomi* was the only *Phytophthora* tested that was an aggressive bark pathogen of *Taxus*. This is consistent with the known susceptibility of *Taxus* to *P. cinnamomi* in the field (cf. Brasier, 2000).

P. *citricola* isolate P812 was obtained from soil around roots of red alder, *Alnus rubra*, in Oregon, USA during a preliminary (negative) search for the new alder phytophthoras in the Pacific North-west. Similar *P. citricola* isolates were obtained by baiting from the water of adjacent alder-lined rivers (C.M. Brasier and E.M. Hansen, unpublished results). Isolate P812 was only a weak pathogen of alder bark in the present test, but proved to be an extremely aggressive pathogen of *Acer* bark, producing broad, diamond-shaped lesions. Along with another *P. citricola* isolate, it was also a significant pathogen of bark of *Castanea*, *Fagus* (cf. also Jung & Blaschke, 1996) and *Chamaecyparis*. In the UK, *P. citricola* tends to be associated with wet soils, including soil around oak trees (Brasier, 2000; J. Rose and C.M. Brasier, unpublished results), and, as in Oregon, with rivers (J. Delcan and C.M. Brasier, unpublished results). It is also found in flooded alder carr sites in the Netherlands (C. van Dyck, personal communication). It may therefore be well adapted to attacking tree roots on wet soils or in flooded situations.

The present pathogenicity tests were conducted at approximately 20°C, close to the temperature growth optimum for the standard alder phytophthora of approximately 22°C (Brasier *et al.*, 1995). *P. cinnamomi* is reported to be most active as a pathogen at 25–30°C (Shearer & Tippett, 1989), and is therefore unlikely to have been at its maximum aggressiveness in these tests. This might also apply to *P. cambivora*, which has an optimum temperature for growth of approximately 27–30°C (Brasier *et al.*, 1995). It should also be emphasized that the log inoculation method used here tests an isolate's capacity for spread in the host, not for infection. The common mode of entry of phytophthoras into the host is via the fine root tips, which could involve a different set of pathogenicity and resistance attributes. The comparative ability of the standard and variant alder phytophthoras to colonize such roots therefore needs to be investigated.

If, as proposed, the standard alder phytophthora is a recently evolved hybrid involving *P. cambivora* and a *P. fragariae*-like species as parents (Brasier *et al.*, 1999), it appears to have become pathogenic to alder in the process, whilst the wide host range of the *P. cambivora*

parent has apparently been lost. That *Phytophthora* interspecific hybrids can acquire host specificities that are not expressed in the parent species has recently been demonstrated via laboratory crosses (Ersek *et al*., 1995). However, little is known about the genetic basis of host specificity in *Phytophthora* pathogens of trees, or about their natural variability in aggressiveness. These aspects are in need of study: firstly, so that the risk of damage by existing or newly evolving *Phytophthora* tree pathogens can be better assessed; secondly, to provide information of use in breeding trees for resistance. The log inoculation method used here provides a simple assay that could be used in assessing pathogenic variation amongst progeny of *Phytophthora* crosses.

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