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Testing variability in pathogenicity of *Phytophthora cinnamomi*

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Abstract

Forty eight isolates of *Phytophthora cinnamomi* from various host plants in France (35 isolates) and in other countries were tested for pathogenicity. Seedlings of chestnut, northern red oak, pine and eucalyptus were infected by soil contamination. Taproots, stems and bark strips of plants of chestnut and different oak species were inoculated with mycelium agar disks. Results of the different experiments were in good agreement. All isolates appeared pathogenic to all the different test species but with variable levels of virulence. Isolates with consistent low or high level of virulence, which could be used as standards in further studies, were identified. Interaction between *P. cinnamomi* isolates and host plant species was significant in terms of lesion lengths. These interactions could not be related to host from which *P. cinnamomi* was isolated. Consistent with this, in *Quercus rubra*, the isolate-provenance interaction was not significant. This feature is encouraging for provenance screening for resistance to *P. cinnamomi* in this species. The variation in virulence was not related to other isolate characteristics (mating type, electrophoretic type, age).

Introduction

Phytophthora cinnamomi Rands is responsible for severe losses in a number of plantations and serious damage to native ecosystems. In its suspected original region (New Guinea-Celebes-Malaysia), it has been introduced to southern Australia, Hawai, central Africa, southern and northern America and Europe, where it is now widespread (Zentmyer, 1980). Isozyme variability of *P. cinnamomi* populations is low (Old et al., 1983; Old et al., 1988; Oudemans and Coffey, 1991; Linde et al., 1997), suggesting that the worldwide dissemination of the pathogen has involved a few clones of the A2 mating-type (Brasier, 1992). In Europe, it might have been introduced as early as the 18th century, and only the A2 mating type has been reported. In France, *P. cinnamomi* is a frequent soil pathogen in forest and ornamental nurseries (Vegh and Bourgeois, 1975). It is also responsible for the ink disease of chestnuts (*Castanea sativa* Miller) and northern red oaks (*Quercus rubra* L.) (Moreau and Moreau, 1952). Root infection by *P. cinnamomi* is followed by die-back and collar lesions in chestnut and by development of trunk cortical lesions in red oak (Robin et al., 1992). Breeding programs have been initiated for these two species, for which improvement of resistance to *P. cinnamomi* is one of the main objectives. For chestnut, this is attempted by interspecific hybridizations and clonal selection (Salesses and Chapa, 1993). For red oak, provenance and progeny tests are in progress.

Durability of resistance may depend on *P. cinnamomi* variability and on isolate-host plant interaction. Screening clones or provenances for resistance also requires an accurate knowledge of the pathogenic variation inside *P. cinnamomi* species and the use of well-characterized isolates. Moreover, because chestnut and red oak extension areas overlap in France, it is necessary to assess the level of host specialization of *P. cinnamomi*. Concepts of pathogenicity and virulence have evolved in plant pathology and confusion may exist if definitions are not clearly stated. In the following text, we will use the terms of pathogenicity, virulence and specific pathogenicity, as defined

by Shaner et al. (1992). Pathogenicity encompasses two components: the virulence (synonym of aggressiveness, measured by the amount of disease) and the parasitic fitness, which is the result of the reproductive fitness and the specific pathogenicity, that describes the behavior of an isolate on specific hosts. Early studies on intraspecific variability of *P. cinnamomi* showed differences of host range, leading some authors to suggest the existence of 'strains' or 'races' differing in their capacity to induce symptoms on different hosts (White, 1937; Torgesson, 1954; Zentmyer and Guillemet, 1981). Based on the observation that walnut (*Juglans nigra*) was infected only by walnut isolates, Crandall et al. (1945) hypothesized the existence of 'slightly different physiologic strains of *P. cinnamomi*'. Manning and Crossan (1966 a) showed variation in specific pathogenicity of 13 isolates of *P. cinnamomi* on six cultivars of *Taxus.* In a following experiment, they reported the existence of 'biotypes', based on 'differential pathogenic effects to the same and different host plants' (1966 b). However, the seven isolates they studied all induced disease to the different coniferous and deciduous tree species. In more recent investigations, variation in virulence, measured by the amount of disease caused by the isolates, is reported in *P. cinnamomi* (Vegh and Bourgeois, 1975, Podger, 1989, Dudzinski et al., 1993). As pointed out by Zentmyer (1980) and given the potential for damage in European forest (Brasier et al., 1993; Brasier, 1996), further studies on the variability of this pathogen are required. The objectives of this work were to assess the variation in specific pathogenicity and virulence for a large set of isolates of *P. cinnamomi*, from various geographic origins and host plants in order to investigate the possible host specialization and plant-isolate interactions. Several tests were used and their respective merits will be discussed.

Materials and methods

Isolates

Table 1 lists the origins of the isolates of *P. cinnamomi* employed in the study. Most came from various woody hosts in France, but in this study we also included some reference cultures coming from around the world and mono-zoospore isolates characterized with RFLP markers in a previous study (Robin, 1991). These mono-zoospore cultures, referred to as MZ9, MZ16, MZ23, MZ64, MZ214, MZ242, MZ243 and MZ245, had been obtained from the mass isolates 9, 16, 23, 64, 214, 242 and 245, with the method described by Byrt and Grant (1979) and were cultured twice on apple to check their pathogenicity. Table 1 also indicates the inoculation experiment in which the isolates were used, and the electrophoretic types (ET) of isolates, when available, as defined by Oudemans and Coffey (1991).

Isolates were stored in sterile water at room temperature and grown routinely in V8 agar. Mycelial agar disk inocula of *P. cinnamomi* were removed in the edge of a 8- to 10-day-old culture with a cork borer (5 mm-diameter). For soil contamination, inoculum of *P. cinnamomi* was produced on a vermiculite medium which consisted in a mixture (1/1/1 in volume) of vermiculite, bran and malt water suspension (20 $g.l^{-1}$), autoclaved three times at 24 h intervals. *P. cinnamomi* was grown on this medium (ten disks on 100 cm^3 of medium), for eight days at 25 °C in the dark.

Potting-mix inoculation

The differences in pathogenicity of the mono-zoospore isolates were evaluated in *Q. rubra* (from Doat, France), *C. sativa* (Dordogne, France), *Pinus pinaster* Ait (Landes, France) and *Eucalyptus gunnii* Hook. f. (Australia). All plants were produced from seed, which were surface-sterilized with sodium hypochlorite (4% w/v for 20 mn) and germinated in a moist chamber. Plants were grown in steamed peat for one month (oaks, chestnuts and pines) or three months (eucalyptus), in a glasshouse, in small pots (125 cm^3). At the time of inoculation, plants were gently removed from their pots, and transferred into 300 cm^3 of peat, which was infested by adding and mixing 0.25% (v/v) of *Phytophthora* culture on vermiculite medium. To avoid cross contamination, the 15 replicates of each species-isolate combination were gathered in one plastic tray (30 × 20 × 13 cm). Trays were randomly placed in a growth cabinet (25 °C, 300 $\mu mol.m^{-2}.s^{-1}$, 16h-8h day-night photoperiod). Plants were allowed to drain freely and were regularly watered to maintain a constant moisture in the potting-mix. Two weeks after inoculation, roots were washed and the taproot was plated on selective medium (PARB: pimaricin 10 $mg.l^{-1}$, ampicillin (sodium salt) 250 $mg.l^{-1}$, rifampicin 10 $mg.l^{-1}$, benomyl 15 $mg.l^{-1}$, agar 20 $g.l^{-1}$, malt 15 $g.l^{-1}$). The length of infected taproot from which *P. cinnamomi* grew out was measured and expressed as a percentage of total root length (PIT). The experiment was performed four times. Due to technical constraints and difficulties in obtaining a suf-

Table 1. Isolates of *Phytophthora cinnamomi*

Isolate	Mating type	Location	Year of isolation	Host	Reference	Electrophoretic type [Oudemans and Coffey 1991]	Pathogenicity test:				
							Potting mix	taproot	stem	bark (1)	bark (2)
7	A2	France	1988	*Quercus rubra*							y
8	A2	France	1988	*Liquidamber styraciflua*						y	
9	A2	France	1988	*Q. rubra*			y		y	y	y
13	A2	France	1987	*Q. rubra*						y	y
16	A2	France	1988	*Pinus pinaster*			y	y	y	y	y
23	A2	France	1974	*Rhododendron* sp.			y		y	y	y
44	A2	France	1988	*Q. robur*						y	
47	A2	France	1988	*Q. rubra*				y		y	y
48	A2	France	1988	*Q. rubra*						y	
49	A2	France	1988	*Q. robur*						y	
50	A2	France	1988	*Q. rubra*						y	
57	A2	France	1982	*Q. robur*						y	y
59	A2	France	1983	*Q. rubra*						y	y
60	A2	France	1983	*Q. rubra*						y	
61	A2	France	1984	*Q. rubra*							y
62	A2	France	1984	*Eucalyptus gunnii*						y	y
63	A2	France	1984	*E. gunnii*						y	y
64	A2	France	1982	*Castanea sativa*			y		y	y	y
66	A2	France	1989	*Q. robur*							y
205	A2	France	1989	*Q. rubra*						y	
208	A2	France	1989	*Q. rubra*							y
214	A2	N. Carolina	1979	*Rhododendron* sp.	ATCC 46292		y		y	y	
242	A2	Australia		*Eucalyptus* sp.	P 3565	CINN 4	y		y	y	y
243	A1	Australia		*Eucalyptus* sp.	P 3664	CINN 2	y		y	y	y
244	A2	California		*Persea americana*	P 2410	CINN 4				y	y
245	A2	California		*Juglans* sp.	P 2411	CINN 4				y	
246	A2	Papua New Guinea		*Nothofagus* sp.	P 3656	CINN 7					y
248	A1	South Africa		*Vitis viniferea*	P 2159	CINN 3					y
249	A2	China		*Camellia japonica*	P 3233	CINN 1					y
250	A1	Madagascar		*P. americana*	P 2121	CINN 2				y	y
251	A1	Papua New Guinea		*P. americana*	P 3663	CINN 6					y
252	A2	France	1989	*Q. ilex*						y	y
298	A2	France	1989	*C. sativa*							y
302	A2	France	1989	*Q. rubra*						y	
305	A2	France	1989	*Q. rubra*				y		y	y
306	A2	France	1989	*Q. rubra*						y	
307	A2	France	1989	*Q. rubra*						y	
308	A2	France	1989	*Q. rubra*				y			y
309	A2	France	1989	*Q. cerris*					y	y	
312	A1	Australia		soil	P 3471		y			y	
320	A2	Taiwan		soil	P 6377	CINN 5		y		y	
327	A2	France	1982	*C. sativa*						y	
340	A2	France	1990	*Q. palustris*				y		y	y
344	A2	France	1992	*C. sativa*						y	
348	A2	California		*Q. ilex*					y	y	
353	A2	France	1992	*Q. rubra* (soil)			y			y	
400	A2	France	1992	*Q. rubra* (soil)			y			y	
410	A2	France	1992	*Q. rubra* (soil)			y			y	

ficient number of plants, the different species were not tested each time. Trial 1 involved eucalyptus, pines and oaks, trial 2 chestnuts and pines, and trials 3 and 4 chestnuts, pines and eucalyptus.

Taproot inoculation

Seedlings of *Q. rubra* (from Doat, France) and *Q. palustris* (Saint-Sever, France) were grown in mini-rhizotrons which consisted in plastic bags (15 cm × 5 cm) filled with vermiculite, with the inner face covered with filter paper (Desprez-Loustau and Dupuis 1994). Germinating acorns were placed at the top of the mini-rhizotrons which were installed in trays, stacked with their long axis at an angle of 60 ° to vertical, so that the root system grew between the filter paper and the plastic bag. Taproots were inoculated by applying an inoculum disk onto the tip. Three plants of *Q. rubra* and two plants of *Q. palustris* were used for each isolate. Seedlings were incubated at 25 °C, with a 12h-12h day-night photoperiod. After six days, lesion length was measured and PIT calculated.

Stem inoculation

Stem inoculations were performed on 2-year old plants of four French provenances of *Q. rubra* (Ainhoa, Doat, Azereix, La Houve), one of *Q. robur* (Orleix, France) and one of *Q. palustris* (Saint-Sever, France). Plants were grown in a greenhouse, regularly watered and fertilized. Inoculation was carried out in June, before the plants started a second shoot growth cycle. Plugs of *P. cinnamomi* cultures were applied on a light scar made with a scalpel, 10 cm above the collar. Fifteen plants were inoculated per isolate-provenance combination. The visible lesion length was measured 1, 2, 3, 5 and 9 weeks after inoculation. The final lesion length was measured, 16 weeks after inoculation, on the cambial surface after removing the bark.

Bark test

The bark test method previously described for the study of red oak resistance (Robin et al., 1994) was used in this study to compare virulence of *P. cinnamomi* isolates. Selected trees (20-year-old, 20 cm-diameter) were felled in May and their boles cut into three parts, which were brought back to the laboratory. The following day, pieces of cortical tissues (3 × 9 cm) were removed from trees and a disk of *P. cinnamomi* culture (5 mm-diameter) applied to the inner face, at the top end of each strip. Three strips were inoculated with each isolate tested and all the pieces were incubated in a moist chamber (25 °C, in the dark). Lesion lengths were measured after five days.

Statistical analyses

Data were subjected to an analysis of variance (ANOVA) when appropriate. Type III sums of squares were calculated by the SAS General Linear Model procedure (1989). When there was a significant isolate by inoculated species (or provenance) interaction, the interaction value between the isolate i and the species s was calculated as:

$$I_{is} = X_{is} - X_{i.} - X_{.s} + X_{..}$$

where X is the variable used as a measurement of virulence (lesion length or PIT), X_{is} the mean value for the isolate i on trees of the species s, $X_{i.}$ the mean value for the isolate i, $X_{.s}$ the mean value for the species s and $X_{..}$ the general mean value. The interaction I_{is} was called 'homologous' when the species s was the host plant from which the isolate i, had been obtained or, otherwise, 'heterologous'. The null hypothesis (no specialization of isolates, and then no difference between homologous and heterologous interaction values) was tested by a t-test.

Results

Test of pathogenicity and assessment of virulence by potting-mix inoculations

Two weeks after inoculation, the decay of fine roots and necrosis of taproots were observed in all the species. Depending on the isolates, the necrosis was restricted or extended and PIT varied from 0 (no lesion in the taproot, but infection of fine roots) to 1 (total colonization of the taproot). A PIT 1 was sometimes associated with plant wilting. Figure 1 shows the mean PIT calculated for each isolate-species combination for the four trials. ANOVA were performed per trial. Effects of the species (trials 2 and 3), of the monozoospore isolate (trials 1, 3 and 4) and of the isolate-species interaction (all trials), were significant on PIT (with $P < 0.05$). Different rankings of the isolates according to their virulence were observed per species. However, the isolate MZ64 exhibited the lowest aggressiveness in three trials. The mean of homologous

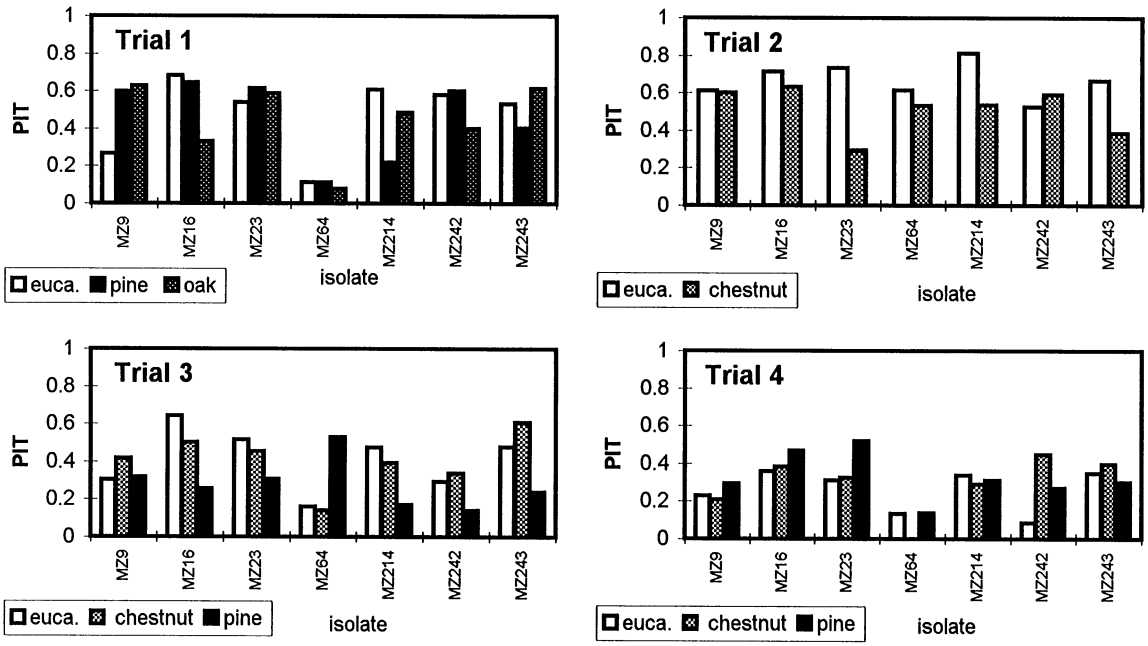

Figure 1. Mean percentage of infected taproot (PIT) for each *Phytophthora cinnamomi* isolate-species interaction (15 replicates) studied by potting-mix inoculation.

Table 2. Mean percentages of infected taproot (PIT) for each *Phytophthora cinnamomi* isolates inoculated on *Quercus rubra* (three replicates) and *Q. palustris* taproots (two replicates)

Isolate	PIT		General mean	SNK test (a)
	Q. rubra	*Q. palustris*		
400	0.99	0.86	0.94	A
305	0.70	0.72	0.71	AB
308	0.71	0.67	0.70	AB
16	0.75	0.36	0.65	BC
47	0.66	0.60	0.64	BC
410	0.61	0.63	0.62	BC
353	0.67	0.37	0.55	BC
340	0.58	0.42	0.54	BC
312	0.56	0.31	0.50	BC
320	0.43	0.63	0.35	C

(a) Student-Newman-Keuls test for general mean PIT calculated per isolate: means with the same letter are not significantly different.

interaction values (-0.03) was not significantly different to the mean of heterologous interaction values (0.015).

Assessment of virulence by taproot inoculation

The day following the inoculation lesions were observed at the tip of taproots and six days after inoculation, PIT varied between 31% and 99% depending on the isolate and on the *Quercus* species which was inoculated (Table 2). The ANOVA showed that the isolate and species effects were significant (at P = 0.0001), but not the isolate-species interaction. The isolate 400, the most virulent one on *Q. rubra* and *Q. palustris*, was significantly more virulent than isolates which had been obtained from an infected red oak (# 47) or from soil (# 410 and 353) in the same oak stand (Table 1). The general means of PIT per species were 67% and 50% for *Q. rubra* and *Q. palustris*, respectively.

Assessment of virulence by oak stem inoculations

One week after inoculation, bark necrosis was observed on stems. For the first two weeks longitudinal lesion development was quite rapid (3 to 6 mm per day), then slowed down (less than 0.5 mm per day) and finally stopped. Most extensive lesions girdled the stem, causing wilting of the crown. By the end of the experiment (16 weeks), rolls of callus had formed and some lesions had healed.

Two isolates (MZ64 and MZ242) caused small lesions on all the hosts (Figure 2). Red oaks appeared on

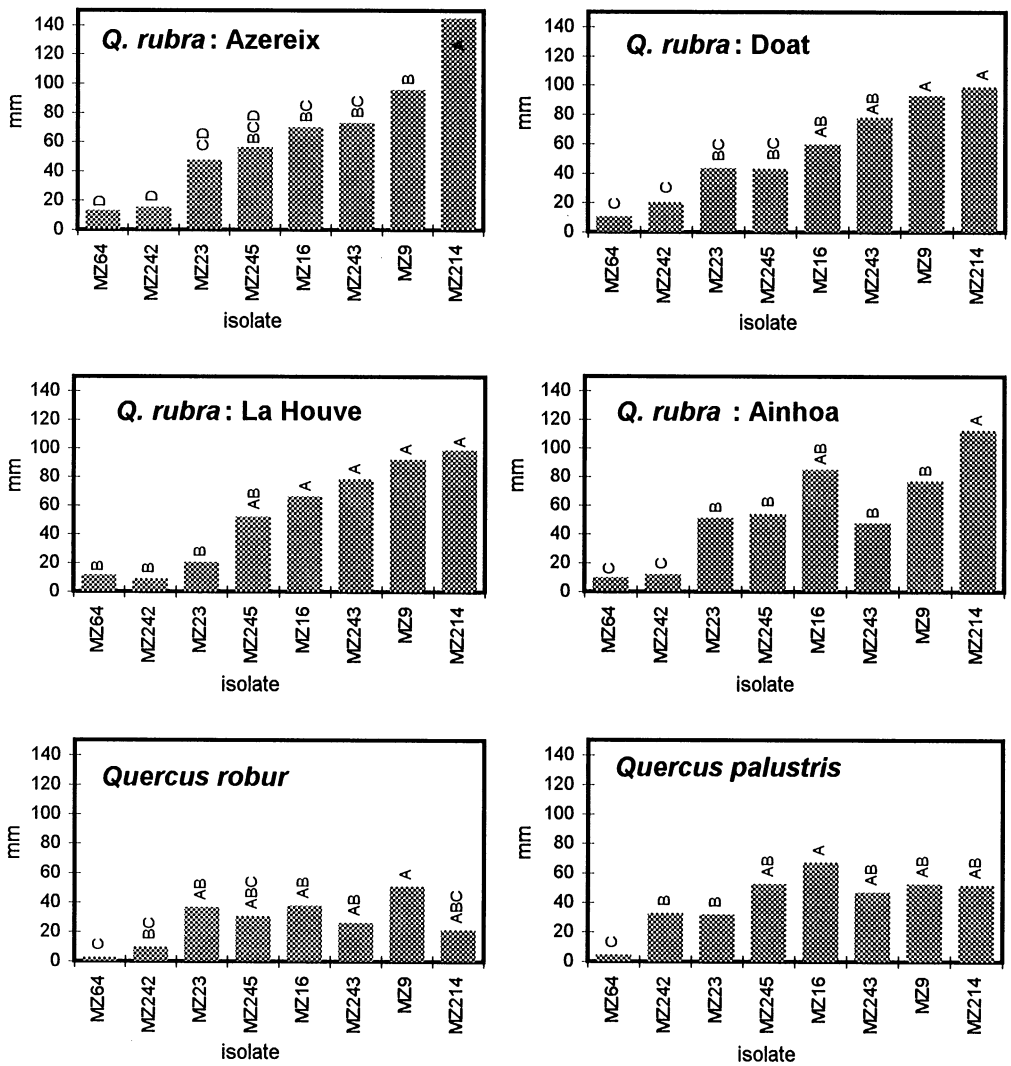

Figure 2. Mean lesion lengths caused by 7 mono-zoospore isolates of *Phytophthora cinnamomi* inoculated on stems of different oak provenances (15 replicates). Values with the same letter, for a given provenance, are not significantly different according to Student-Newman-Keuls multiple range test.

average more susceptible to *P. cinnamomi* than pin and pedunculate oaks. An analysis of variance was performed on the final lesion length. There was an isolate (7 df, F = 43.46, P = 0.0001) and a provenance effect (5 df, F = 13.51, P = 0.0001) on the lesion length. A significant strain-provenance interaction was detected (35 df, F = 2.75, P = 0.0001) for the whole experiment. This was principally due to isolate MZ214 which was the most pathogenic on *Q. rubra* but one of the least pathogenic on *Q. robur*. But within *Q. rubra*, no differences between provenances were observed and no isolate-provenance effect was demonstrated; only the isolate effect was significant on the lesion length (at P = 0.0001).

Assessment of pathogenicity with bark tests

Two separate trials were performed. In the first one, one red oak was used and 33 isolates were screened. Three bark strips excised at three different heights (0, 0.5 and 1 m, three strips) were inoculated per isolate. Lesion lengths varied significantly (P= 0.0187) from 3.5 to 6.3 cm depending on the isolates (Figure 3). Isolates 57, 64 and 246 caused the smallest lesions (inferior to 4 cm), whereas 12 isolates induced lesions larger to 6 cm, the general mean being 5.5 cm. Isolates

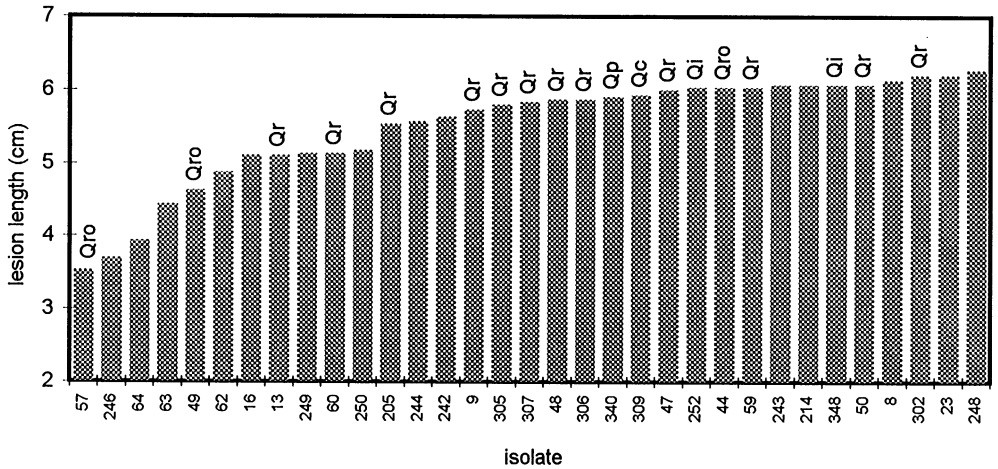

Figure 3. First bark test: lesion length measured on bark strips removed from one red oak (*Quercus rubra).* Means of three replicates. Qro: isolates from *Quercus robur*, Qr from *Q. rubra*, QP from *Q. palustris*, Qc from *Q. cerris*, Qi from *Q. ilex.*

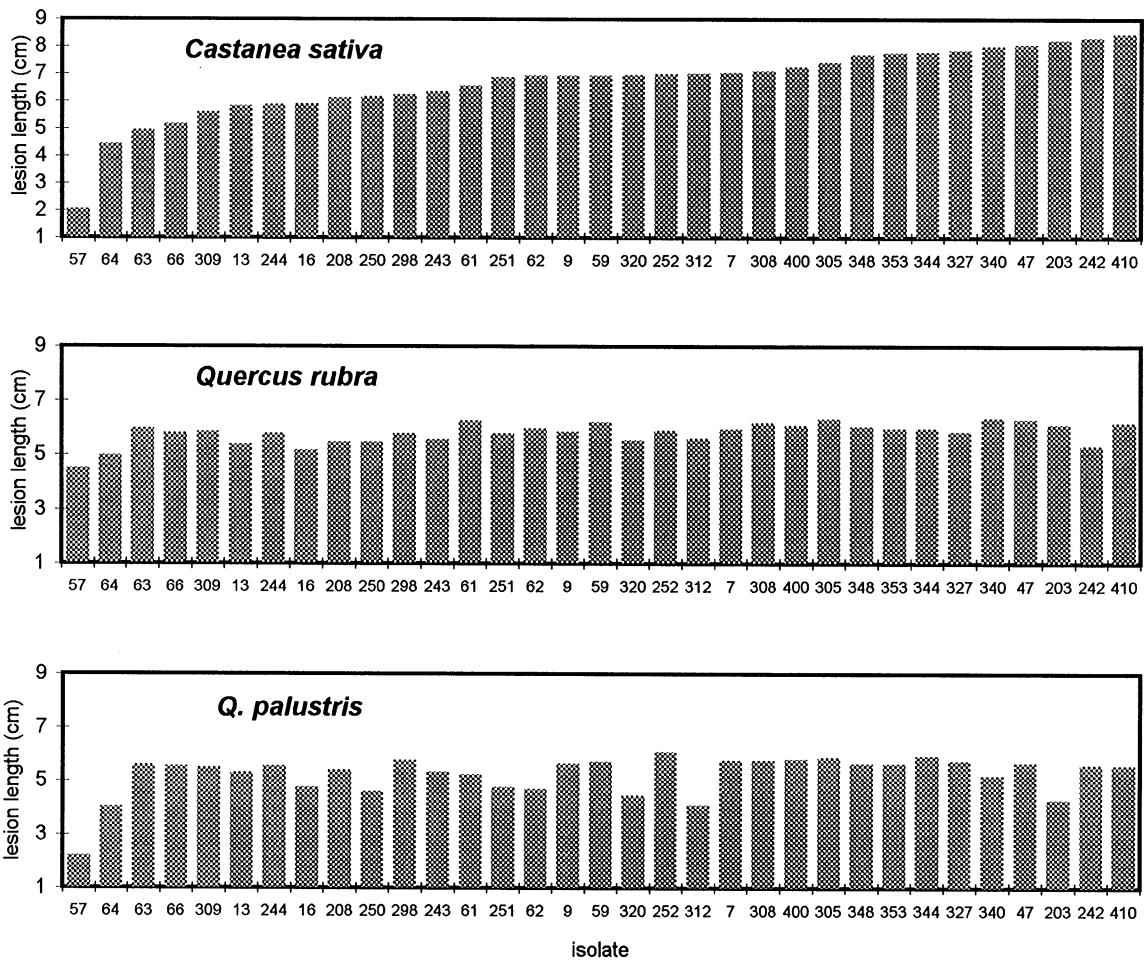

Figure 4. Second bark test: lesion lengths caused by *Phytophthora cinnamomi* isolates inoculated on a chestnut (*Castanea sativa),* a red oak (*Quercus rubra)* and a pin oak (*Q. palustris).* Means of three replicates.

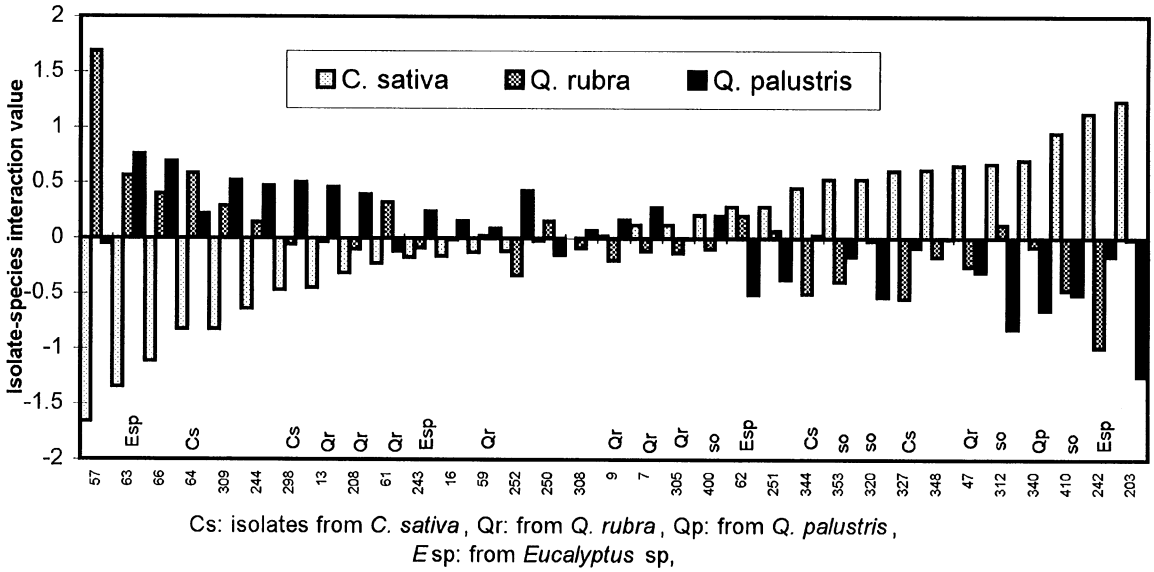

Figure 5. Interaction values calculated for the lesion lenth caused by each *Phytophthora cinnamomi* isolate used in the second bark test on *Quercus rubra*, *Quercus palustris* and *Castanea sativa.*

of the A1 mating type showed moderate (isolate 250) or high aggressiveness (isolates 243 and 248). Isolates from the same electrophoretic type caused lesions of either similar (isolates 242 and 244, ET CINN4) or different size (isolates 250 and 243, ET CINN2). Isolates which host plant was an oak species did not show specific virulence on red oak bark. The mean lesion lengths caused by the 12 isolates from red oak or by the, 19 isolates from oak species were 5.8 and 5.6 cm, respectively.

In the second trial, a different batch of 33 isolates (18 isolates present in the first trial, Table 1) was inoculated on bark strips excised from one chestnut, one red oak and one pin oak. *C.sativa* was significantly more susceptible than *Q. rubra* and *Q. palustris:* mean lesion lengths were 6.6, 5.8 and 5.2 cm, respectively (P=0.0001). *C.sativa* was also the tree which allowed the best discrimination of isolates. Length of the lesions developed on chestnut, red oak and pin oak bark varied between 2 and 8.5 cm, 4.5 and 6.3 cm and 2.2 and 6.5 cm, respectively (Figure 4). Differences among isolates were significant (P=0.0001), but there was a significant isolate-tree interaction (at P = 0.0001) and ranking of isolates, according to their virulence, varied with the tree species. Interaction values were calculated for each isolate-species combination (Figure 5). Fifteen isolates, among them isolates 57 and 242, contributed heavily to the interaction (I_{is} > 0.5 or I_{is} < -0.5). The t-test calculated on the interaction values did not differentiate homologous versus heterologous interactions (P = 0.41), indicating isolates from red oak, pin oak and chestnut caused similar lesions on their respective host plant and on other plants. No consistent ranking of isolates was detected according to mating type or to electrophoretic type.

The correlation coefficient calculated between the mean lesion lengths measured on red oak, at the first and second trial, was highly significant ($R_{Spearman}$ = 0.71, 16 df, P< 0.01). Correlation between lesion lengths measured on bark strips (second trial) and on taproot (taproot inoculation test) was significant on *Q. palustris* ($R_{Spearman}$= 0.81, 8 df, P< 0.01) but not on *Q. rubra*.

Discussion

To assess the pathogenicity of *P. cinnamomi* isolates, several methods are required in order to study different parts of the pathogenic process. Zoospores released in free water by sporocysts are the principal infectious propagules of *P. cinnamomi.* They encyst and colonize fine roots of a wide range of susceptible and tolerant hosts (Shearer and Tippett, 1989). Such natural process of infection is mimicked during the potting-mix inoculations, in which sporulation is presumed to occur in the potting-mix from the imported inoculum. This inoculation technique may contribute some useful information on the isolate capacity to infect plant

host and to use root tissue as a food source. Because seedlings were used in our experiments, the period during which infection and root colonization could occur was limited to two weeks. This short inoculation period, and the different colonization of the vermiculite medium by *P. cinnamomi* isolates may explain some discrepancies between the different trials.

Direct inoculations onto taproot, stem or excised bark only allow assessment of the ability of the isolates to develop lesion once they are inside the host plant. Although *P. cinnamomi* diseases are initiated by root infections, variability in host susceptibility to this pathogen has been demonstrated in several species with trunk (Marks et al., 1991; Robin, 1990) and stem inoculations (Dixon et al., 1984). Stem inoculations may also be useful to predict and test oak susceptibility to *P. cinnamomi* since significant differences were observed among oak provenances in our study. Moreover, assessment of isolates by the stem inoculation was in agreement with results of the potting-mix inoculations and development of lesions caused by isolates were consistent whatever the plant organ used. Similarly, Dixon et al. (1984) and Dudzinsky et al. (1993) showed that stem and root lesions were highly correlated in *Banksia* spp. and *Eucalyptus* spp., respectively. A limitation of stem and bark inoculations with *Phytophthora* spp. as screening tests for host resistance may be the seasonal variability of susceptibility of deciduous host plants (Matheron and Matejka, 1989; Robin et al., 1994). However, these two techniques appeared reliable to assess virulence of many isolates, as they allowed stable and consistent discrimination of isolates and were more convenient to carry out and to analyze than soil inoculations.

All the experiments conducted in this work provided some evidence of variability in virulence within *P. cinnamomi* species. Although all the isolates appeared pathogenic to all the different host species used, there were significant variations in quantity of colonized or diseased tissue. Some of the isolates studied (64, MZ64 and 57) always ranked among the least virulent isolates whatever the test. Other isolates (7, 9 and 305) always showed high or moderate level of virulence. Such isolates can be useful as reference material in any subsequent pathogenicity studies.

The isolate by plant interactions were significant in the potting-mix inoculation and bark strip tests. However, homologous interaction values did not differ from heterologous ones which indicates that these interactions were not related to host specialization. Our results are in good agreement with many studies of *P. cinnamomi* pathogenicity which could not provide any evidence of host specialization. Chee and Newhook (1965) who tested 17 isolates, Podger (1989) who studied 14 Australian isolates from subtropical, Mediterranean, uniform and temperate areas, and Dudzinski et al. (1993) who focused on collecting and screening 42 Australian isolates, were unable to distinguish races or biotypes in *P. cinnamomi*. Variability in virulence rather than in specific pathogenicity is consistent with the general occurrence of *Phytophthora* pathogenicity attributes: in this genus biotrophy is characteristically linked with host specialization and necrotrophy with variability of virulence and a broad host-range, as observed in *P. cinnamomi* (Brasier, 1992). Given the absence of host specialization in *P. cinnamomi*, it seems essential to prevent cross contamination between different host species, with appropriate prophylactic measures, especially in nurseries or in plantations.

In the stem inoculation trial, some isolates showing a moderate or low virulence on *Q. rubra*, caused relatively large lesions on *Q. palustris*, a field resistant oak. These isolates (MZ242 and MZ23) did not discriminate oak species for resistance to *P. cinnamomi* but more virulent isolates did. However, within *Q rubra*, the interaction between provenances and isolates was not significant. This is an encouraging result for the screening of red oak provenances for *Phytophthora* resistance. For breeding programs aiming at the clonal selection and resistance to *P. cinnamomi*, like that developed in chestnut, interactions with *P. cinnamomi* isolates remained to be studied at the clonal level. Only a few reports have been published on such interactions in other species. For example, in *Eucalyptus marginata*, three clones inoculated with six *P. cinnamomi* isolates showed consistent ranking whatever the isolate (Dudzinski et al., 1993).

French isolates showed the same extent of variation in virulence as the isolates coming from countries close to the presumed *P. cinnamomi* centers of origin or to other areas of introduction (United States, South Africa). Variation was detected among isolates collected from the same site (cf. taproot inoculation experiment), in agreement with a previous study (Desprez-Loustau and Dupuis, 1994). In our set of isolates, which was principally built up with A2 European isolates, variation in virulence did not appear related to the mating type, as it has been reported by Weste (1975), neither to the age of the isolates. 'Newer' (obtained during and after, 1988, the year we started to collect *P. cinnamomi* isolates) and 'older' (obtained

before 1988) isolates from France were not significantly different. A similar pathogenic variation unrelated to isolates characteristics has been reported by Podger (1989) and Dudzinski et al. (1993). Moreover, these latter reported variation inside the CINN4 electrophoretic group. Ninety percent of the A2 isolates characterized by Oudemans and Coffey (1991), and among them the single French isolate (from chestnut) of this study, belonged to this group.

To our knowledge this is the first study of *P. cinnamomi* involving such a large number of European isolates. Based on our results, pathogenic variability appeared significant among these isolates. However, in introduced populations of a heterothallic plant pathogen exhibiting no host specialization and only one mating-type, a low degree of diversity is expected. A low diversity has been reported in populations from Australia and South-Africa, where both the mating-types occurred but where there was no evidence of sexual reproduction in the field (Old et al., 1983; 1988; Linde et al., 1997). In a previous work total DNA from the mono-zoospore isolate MZ9, digested by the enzyme Sau 3AI and ligated to a plasmid of *Escherichia coli*, was used as genomic probes to detect Restriction Fragment Length Polymorphism among the eight mono-zoospore isolates of *P. cinnamomi*, used in this study, and of *P. cambivora* and *P. megasperma* (Robin, 1991). Hybridizations of 15 genomic probes and an heterelogous probe with total DNA digested by two enzymes, showed that intraspecific variability existed within P. *cinnamomi*, and especially between A1 and A2 isolates, but also among isolates from France. Such powerful DNA markers are required to study the population structure of *P. cinnamomi* in Europe and to confirm or refute the hypothesis of their clonal character (Brasier, 1992).

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