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SCIENCE DIRECT
Forest Ecology and Management 226 (2006) 199–207
Forest Ecology and Management
www.elsevier.com/locate/foreco

Genetic variation in susceptibility to *Phytophthora Cambivora* in European chestnut (*Castanea sativa*)

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Received 28 April 2005; received in revised form 19 January 2006; accepted 30 January 2006

Abstract

Genetic variation in 23 populations of European chestnut (*Castanea sativa* L) to Ink Disease caused by *Phytophthora cambivora* (Petri) Buisman was studied in three labs. The populations represented three domestication levels (naturalized populations, coppice forests and orchards) and were sampled in 10 locations in five countries (Italy, France, Greece, Spain, and the UK). Adult chestnut susceptibility to Ink Disease was assessed by measuring lesion length following inoculation of excised shoots with *P. cambivora*. Half-sib families were harvested in most of these populations and the seedlings were root-inoculated. Provenance and family variance components were estimated. Significant variation in the extent of shoot colonization by *P. cambivora* was observed within and among adult tree populations, suggesting a large amount of genetic variation in resistance. No consistent ranking of the domestication levels for susceptibility was observed. Some results indicate selective pressure exerted by *P. cambivora* on local populations. Lesion measurements in a set of 48 trees inoculated in 2001 and 2002 were correlated ($r = 0.58$). One or more resistant trees (lesion length <10 mm) were identified in 15 populations. Root inoculation of seedlings showed that only three families had a mean value for percentage of infected taproot = 15% or comparable to seedlings of the resistant control 'Marigoule'. Lesion lengths in parent trees and percentage of infection of the taproot of their seedlings were not highly correlated.

Keywords: *Phytophthora cinnamomi*; *Castanea sativa*; Ink Disease; Disease susceptibility; Population differentiation

1. Introduction

European chestnut (*Castanea sativa* Mill.) is a multipurpose species cultivated for its wood and nuts in all Mediterranean and Central regions of Europe (Bounous, 2002). Chestnut forests are estimated to cover 2.5 million ha, principally in France and Italy (60% of the chestnut forest area) as coppice stands (Bourgeois et al., 2004). Besides its rural economic importance, chestnut has an agro-ecological role (protection against fire and erosion, excellent habitat for biodiversity, recreation, etc.), resulting in a general interest in chestnut cultivation and a general concern about the conservation of genetic resources.

European chestnut populations subjected to different environmental and cultural conditions might exhibit genetic differentiation and variation in adaptive traits. A great deal of intraspecific genetic variation, analysed with neutral marker loci, was detected among western and eastern natural populations in Turkey which is one of the assumed refuge zones during the Würm glaciation (Villani and Pigliucci, 1991). The domestication and the widespread use of clonal varieties resulted in a loss of genetic diversity as chestnut migrated to western and Mediterranean Europe during the post-glacial period (Frascaria et al., 1993; Fineschi et al., 1994, 2000; Machon et al., 1996; Pereira-Lorenzo et al., 1996; Villani et al., 1991, 1994). Significant genetic among-population variations in growth traits, drought tolerance and phenology have been reported (Pliura and Eriksson, 2002; Lauteri et al., 2004; Fernandez-Lopez et al., 2005). Estimates of heritability and of the coefficient of genetic variation led the authors to conclude that *C. sativa* has a high potential to respond to environmental changes. However, despite the economic importance of fungal pathogens which represent a key component of the chestnut environment, genetic variation for disease resistance remains poorly understood.

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doi:10.1016/j.foreco.2006.01.035

One of the major chestnut diseases which are widely distributed in Europe is Ink Disease, caused by *Phytophthora cambivora* and *Phytophthora cinnamomi* (Crandall et al., 1945; Peace, 1962; Grente, 1961). These soil-borne Oomycetes infect the root system, causing the wilting and death of chestnut trees. They also may colonize bark and cambial tissue reaching up the stem for several centimetres. Ink Disease is thought to have been present in Europe since the 1700's (Crandall et al., 1945; Grente, 1961; Peace, 1962). Devastating epidemics occurred in the 19th and 20th Centuries (Del Cañizo, 1942; Pimentel, 1949; Moreau and Moreau, 1952). According to Grente (1961), every chestnut growing area in France had been infected by 1960. Recently, a dramatic resurgence of the disease, causing a high mortality rate, has been reported in different areas of Europe, especially Portugal, Italy and France (Vannini and Vettraino, 2001; Fleisch, 2002; Robin et al., 1994; Vettraino et al., 2001). Nowadays *P. cinnamomi* and *P. cambivora* are present from Greece to Great Britain (Vannini and Vettraino, 2001; Vettraino et al., 2005). Among the possible control measures, selection and breeding for resistance are primary goals to reduce the impact of the disease in new plantations and nursery stock. Interspecific hybrids (*C. sativa x C. crenata* or *C. mollissima* or the reciprocals) are known to be tolerant and are used as rootstock in orchards (Vietez, 1960; Salesses et al., 1993; Fernandez-Lopez et al., 2001). However, breeding for resistance in *C. sativa* and conservation of genetic resources require ascertaining the genetic variation in susceptibility of wild and cultivated populations.

The objective of this study was to study the genetic variation in susceptibility to Ink Disease in chestnut populations and possible relationships to geographic origin and level of domestication. Shoot inoculation assays were designed to study among-population variation, whereas root inoculations were used to analyse the susceptibility of open-pollinated families from these same populations.

2. Material and methods

2.1. Chestnut populations

Twenty-three chestnut populations representing different domestication levels (natural populations, coppice forests and orchards) were studied. They were distributed in five countries (England, France, Spain, Italy and Greece) and 10 natural regions (Table 1). When the three domestication levels were present within a region, one site from each level was sampled.

Table 1
Castanea sativa populations studied in population and progeny tests for the variability of chestnut susceptibility to Ink Disease

Country	Natural region	Sites	DL[a]	Longitude	Latitude	Annual rainfall (mm)	T_{mean} (°C)	Report of Ink Disease[b]	Population test trial number	Number of trees	Progeny test trial number	Number of families
Spain	Sierra de Ronda	EU7	NP	5°18′W	36°32′N	1214	13.7	No	NI[c]	0	5	8
		EU8	O						NI	0	5	4
	Galicia	EU11	NP	8°22′W	43°17′N	1148	13.2	Yes	2	23	NI	8
		EU12	O						2	25	NI	8
France	Cévennes	EU14	NP						1	25	4	7
		EU15	C	3°49′E	44°01′N	1479	12.2	Yes	1	26	4	7
		EU16	O						1	25	4	8
	Maures	EU17	NP						1	23	4	8
		EU18	O	6°20′E	43°18′N	1231	13.4	Yes	1	26	4	8
		EU19	C						1	25	4	8
Italy	Sicily	EU35	C						2	24	5	8
		EU36	O	14°05′E	37°54′N	867	13.6	No	2	22	5	7
		EU37	NP						2	26	5	9
	Valle Pellice	EU56	NP						2	17	5	9
		EU57	C	7°15′E	44°81′N	1215	10.7	Yes	2	22	5	7
		EU58	O						2	20	5	8
UK	Gloucestershire	EU63	C	2°30′W	51°46′N	795	10.3	No	1	26	4	7
	Suffolk	EU64	C	1°03′E	51°59′N	584	9.7	No	1	26	4	8
Greece	N. Macedonia	EU65	NP						3	21	6	8
		EU66	C	23°09′E	40°22′N	417	14.7	Yes	3	24	6	1
		EU67	O						3	18	6	8
	Central Macedonia	EU68	NP	22°22′E	40°57′N	558	12	No	3	24	6	6
		EU69	C						3	23	6	8

[a] LD, domestication level; C, coppice; NP, naturalized population; O, orchard.
[b] From Vettraino et al., 2005.
[c] NI, not included.

In all the studied sites, symptoms of Ink Disease were assessed and *Phytophthora* spp. detection was attempted in soil and tissue (Vettraino et al., 2005). *P. cinnamomi* was detected in the plots EU11, EU12 (in Galicia, Spain) and EU17 (in the Maures, France) and *P. cambivora* in site EU66 (Greece).

Chestnut populations were represented by a maximum of 26 trees and by open-pollinated families harvested under mother trees (most often eight families per population, Table 1). Within a site, studied trees were selected along transects crossing the site and according to the availability of easily harvested shoots and nuts. To avoid the possibility of relationships between families, seeds (an average of 15 per tree) were harvested under distanced mother trees and we assumed that these families were true half-sib families.

2.2. Excised shoot test

The tolerance of parents to Ink Disease was assessed by inoculating excised shoots. Branches (at least 1 cm in diameter at the base and up to 100 cm long) were harvested at the end of winter while they were still dormant, about 2 weeks before flushing. In orchards, care was taken to cut branches from the canopy to be sure to sample shoots from the scion variety rather than the root stock. In all sites, the samples were wrapped in plastic bags to limit their desiccation as much as possible during transport to the laboratory. There, all branches were cut into 40 cm segments, their bases put into jars filled with water, and placed randomly in an environmentally controlled chamber (20 °C, 12 h photoperiod). When all buds were opened, shoots were inoculated with a *P. cambivora* isolate using the method described by Vettraino et al. (2000). This isolate (P15FC2), stored in the culture collection of the Department of Plant Protection, University of Tuscia, Italy, was recovered from the rooting zone of a diseased chestnut tree in Italy. The inoculum disc consisted of a plug of *P. cambivora* culture (5 mm in diameter). It was placed onto a horizontal incision made with a sterile razor blade in the cortical tissue of the stem and covered with a plastic film. The water level was kept constant in the jars throughout the experiment. In France, England and Greece, three shoots per tree were inoculated. Two to four inoculations and one control were performed on each shoot. In Spain and Italy, up to eleven shoots were inoculated per tree and two inoculations per shoot were performed. In all cases, the average lesion length was calculated per shoot. Shoots from two clonal hybrid varieties, CA15 (Marigoule) and CA125 (Bouche de Bétizac), grown at the INRA Center of Bordeaux, France, were also inoculated.

Seven days after inoculation, shoots were debarked in order to assess visually the margins of lesions which were measured and lesion length (LL) was recorded. Isolation of *P. cambivora* in inoculated stems was attempted in symptomless tissue above and below. Stem sections were cut at 0–5 cm from both ends of the lesions and plated on PARBHy selective medium (Robin et al., 1998). After 7 days of incubation in the dark at 25 °C, sections were examined under the microscope to look for *P. cambivora* mycelium and maximal distance between successful isolations was recorded as the colonization length.

2.3. Root inoculation

The tolerance of chestnut seedlings to Ink Disease was estimated by root inoculation. After harvest, seeds were surface sterilized and sown in pot trays with 20 individual cells (of 800 cm^3 volume) in a mixture of sphagnum peat (75%) with perlite (15%) and clay granules (10%). Seedlings were grown in glasshouses where pot trays were randomised. When plants reached a height of 20 cm and their lower stems were lignified, 10 ml of millet seeds contaminated with *P. cambivora* isolate P15FC2 were placed in each pot (at 10 cm depth). Infected millet seeds were obtained following the method described by Maurel et al. (2001). Nothing was added to control plants (about five plants per family). Each pot was flooded to water capacity just after the inoculation, and then regularly flooded depending upon the water consumption of each plant. Seeds from a CA15 (Marigoule) and of a *Castanea crenata* trees were harvested at the INRA center (Bordeaux, France) and used as tolerant controls.

At the end of the experiments, when more than 30% of seedlings were wilted, invasion by the pathogen was assessed as the percentage of infected taproot (PIT, obtained by dividing the length of the taproot lesion by the total root length) and stem lesion length (SLL). Four parameters related to seedling growth such as shoot diameter increment at the collar SDI (mm), shoot height increment SHI (cm), leaf and stem dry weight (LDW) and root dry weight (RDW) were also measured at the end of the experiments. To obtain the dry weight, leaves and roots were cut at the end of the experiment and incubated at 60 °C for 7 days.

2.4. Data analysis

All statistical analyses were performed with SAS software (SAS Institute Inc., Cary, NC, USA). Analyses of variance were carried out on data from each trial separately using the GLM procedure. For the shoot inoculation trials, the following model was used:

$$LL_{ijk} = \mu + P_i + I(P)_{ij} + e_{ijk}$$

with LL_{ijk} the mean lesion length measured on the shoot k of the tree j from the population i, μ the overall mean, P_i the effect of population i, $I(P)_{ij}$ the random effect of tree j nested in population i and e_{ijk} the error term. This model was also tested on restricted data sets, keeping populations coming from the same geographical region or with the same level of domestication. For the root tests, the family effect F was nested within population i. The linear model tested was written as: $PIT_{ijk} = \mu + P_i + F(P)_{ij} + e_{ijk}$. Variance components were derived for PIT using restricted maximum likelihood analyses (method REML in the VARCOMP procedure of SAS).

3. Results

3.1. Development of lesions in shoots excised from parents

At 7 days after the inoculation, *P. cambivora* induced visible necrotic lesions of varying length, limited by a black line and

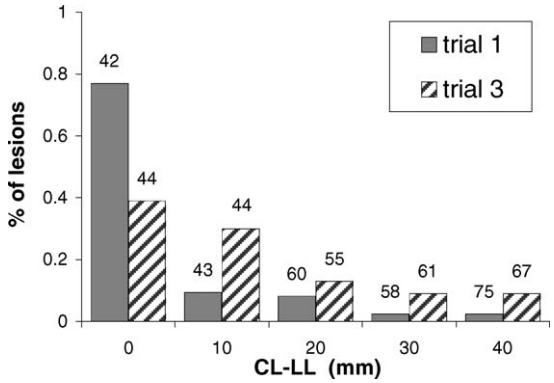

Fig. 1. Frequency of lesions in relation with the difference between lesion length (LL) and colonization length (CL, maximal distance between successful isolations of *Phytophthora cambivora*). The average lesion length (mm) of each lesion class is indicated on the top of the bars.

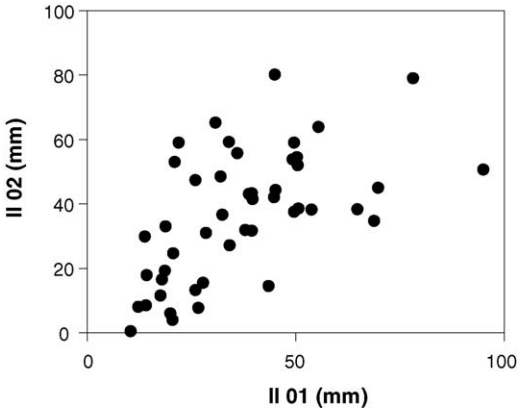

Fig. 2. Relationship between stem lesion lengths measured after inoculation with *P. cambivora* of shoots excised in 2001 and in 2002 in six French chestnut populations ($n = 48$).

developing in the cortical tissues, the phloem and the xylem, where a slight discoloration was visible. In the control inoculations, the wounding only caused a slight reaction. In 4.4% of the inoculated shoots, lesion length was as short as the damage caused by the wounding in the control inoculated shoots. However, in these shoots, the inoculum disc was not in contact with the incision or was dry. Isolations attempted at the inoculation point confirmed that *P. cambivora* had not penetrated the cortical tissues, except in only two of these stems. When the other inoculations performed on the same branch resulted in distinct lesions, these failed inoculations were attributed to the death of the pathogen in the inoculum disc and were removed from the data set. This allowed us to cull several outliers.

Isolation of *P. cambivora* in inoculated stems was attempted in 132 shoots. Frequency of recovery of *P. cambivora* decreased with the distance from the front of the lesion (Fig. 1). Colonization length was significantly correlated with the measured lesion length (Pearson coefficient of correlation $R = 0.96$ for French and English shoots, $R = 0.78$ in Greek shoots, $P < 0.01\%$ in both trials).

Forty-eight trees from the French populations were inoculated in 2001 and in 2002 (Fig. 2). The Pearson correlation coefficient between the two measurements was highly significant ($R = 0.58$, 46 d.f., $P = 0.00002$) suggesting a satisfactory repeatability of the screening method.

3.2. Variation in shoot susceptibility

It was not possible to include all the chestnut populations in a single trial. Hence, English and French populations were tested in France (Trial 1), Spanish and Italian ones in Italy (Trial 2) and Greek ones in Greece (Trial 3). In each trial, the average lesion length was calculated for each shoot. The overall means of lesion lengths were 48, 35 and 47 mm in Trials 1, 2 and 3, respectively. Population average lesion length varied from 82.5 (English coppice, EU63) to 20 mm (Sicilian orchard, EU36, Fig. 3).

In the three trials there was a significant effect of population on LL (Table 2). The strongest population effect was observed in Trial 1, in which trees from the two English coppice populations (EU63 and EU64) produced significantly longer lesions than those of trees from the six French populations. In Trial 2, two populations from Sicily (EU35 and EU36) and one from Galicia (EU12) were significantly more resistant

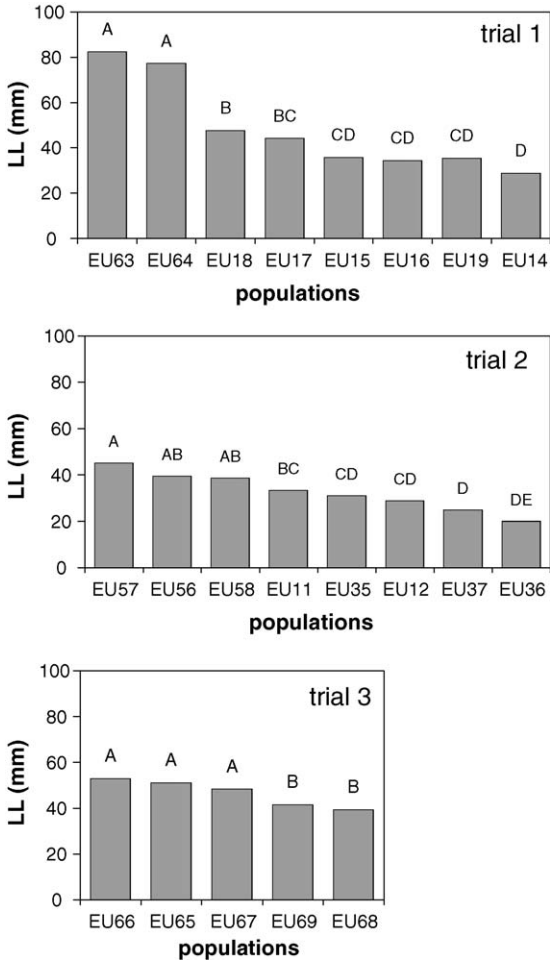

Fig. 3. Average population stem lesion length (LL in mm) measured after excised-shoot inoculation with *P. cambivora* in the three trials. Histogram bars having different letters are significantly different according to a Scheffe mean comparison test ($P = 0.05$).

Table 2
Analyses of variance of lesion length within and among populations of chestnut trees after shoot inoculation with *Phytophthora cambivora*

Populations	Source	d.f.	Mean square	*F*	*P*
English and French	Population	7	29110.55	98.11	<0.0001
	Tree (population)	193	911.64	3.07	<0.0001
Italian and Spanish	Population	7	7158.78	31.31	<0.0001
	Tree (population)	171	1140.30	4.99	<0.0001
Greek	Population	4	2287.14	29.94	<0.0001
	Tree (population)	104	274.84	3.60	<0.0001

than the other populations. In Trial 3, the three populations from Northern Macedonia were more susceptible than the two populations from Central Macedonia. The population effect was examined further to see if susceptibility was related to domestication or geographic origin. The origin was always significant (see Table 3 which shows, as examples, the results of analyses of variance for the four coppice populations in Trial 1, the three naturalized populations and three orchards in Trial 2). Alternatively, within a geographical region, the domestication level effect was always significant except in Valle Pellice (Table 4). However, the ranking of the domestication levels was not consistent in the different regions. Orchard populations from Galicia, Sicily and Northern Macedonia were more tolerant than coppice populations. In the Maures, we observed the opposite result whereas in the Cévennes the most tolerant population was the naturalized one.

Individual tree mean lesion length varied from 4 to 90 mm in all populations, except in the English ones (the range of LL was 40–130 mm). The mean length of lesions produced by the two hybrid varieties used as reference was 1 and 6 mm for Bouche de Bétizac and Marigoule, respectively. *P. cambivora* was isolated from these branches at the inoculation point. The variation within populations was significant in the three trials (Table 2). Moreover, within a geographic origin, variance among trees was always higher than among populations. One or more trees with LL < 20 mm were identified in 15 of the 21 populations. The percentage of tolerant trees (LL < 10 mm) per population varied from 0 (populations from England, Valle Pellice and Greece) to 20 (coppice population in the Maures, Fig. 4).

3.3. Root inoculation test

In Trial 4, populations and families from France and England were tested, in Trial 5, populations from Italy and Spain and in Trial 3, those from Greece (Table 1). In the three trials, data were recorded only from plants higher than 7 cm at the time of inoculation. At the end of the experiments (15, 21 and 28 days after inoculation in Trials 4, 5 and 6, respectively) no evidence of contamination was observed in non-inoculated plants, showing that cross contaminations did not occur. The average shoot height increment (SHI) of the inoculated plants was 4, 0.3 and 0.2 cm, and the average shoot diameter increment (SDI) was 0.5, 0.2 and 0.35 mm, in Trials 4, 5 and 6, respectively. In comparison, the average SHI of the non-inoculated plants was 10, 3 and 2 cm and the average SDI was 1.3, 0.9 and 0.5 mm in Trials 4, 5 and 6, respectively. Most of the inoculated plants exhibited a lesion in the taproot (76%,

Table 3
Analysis of variance of stem lesion length within and among chestnut populations with the same domestication level, after shoot inoculation with *Phytophthora cambivora*

Populations	Source	d.f.	Mean Square	*F*	*P*
English and French coppices	Population	3	48747.99	144.75	<0.0001
	Tree (population)	99	958.99	2.85	<0.0001
Spanish and Italian natural populations	Population	2	6111.82	27.70	0.0018
	Tree (population)	63	1135.61	5.15	<0.0001
Orchards	Population	2	105767.68	52.24	<0.0001
	Tree (population)	64	959.96	4.74	<0.0001

Table 4
Analysis of variance of lesion length (LL) among chestnut populations from the same geographic origin, after shoot inoculation with *Phytophthora cambivora*

Geographical origin	F_{pop}	*P*	Mean comparison		
			Coppice	Natural population	Orchard
Cévennes	3.54	0.0072	34.6 AB	28.9 B	35.9 A
Maures	8.93	0.0002	35.6 B	44.2 A	47.8 A
Sicily	23.05	<0.0001	31.2 A	25.1 B	20.1 C
Valle Pellice	1.66	0.1923	45.2 A	39.6 A	38.7 A
Galicia	6.96	0.0088	Not available	33.5 A	28.9 B
Northern Macedonia	4.12	0.0187	53.1 A	51.2 AB	48.5 B

Means (in mm) within rows having different letters are significantly different according to a Scheffe mean comparison test ($P = 0.05$). Means are presented by geographical origin and domestication level.

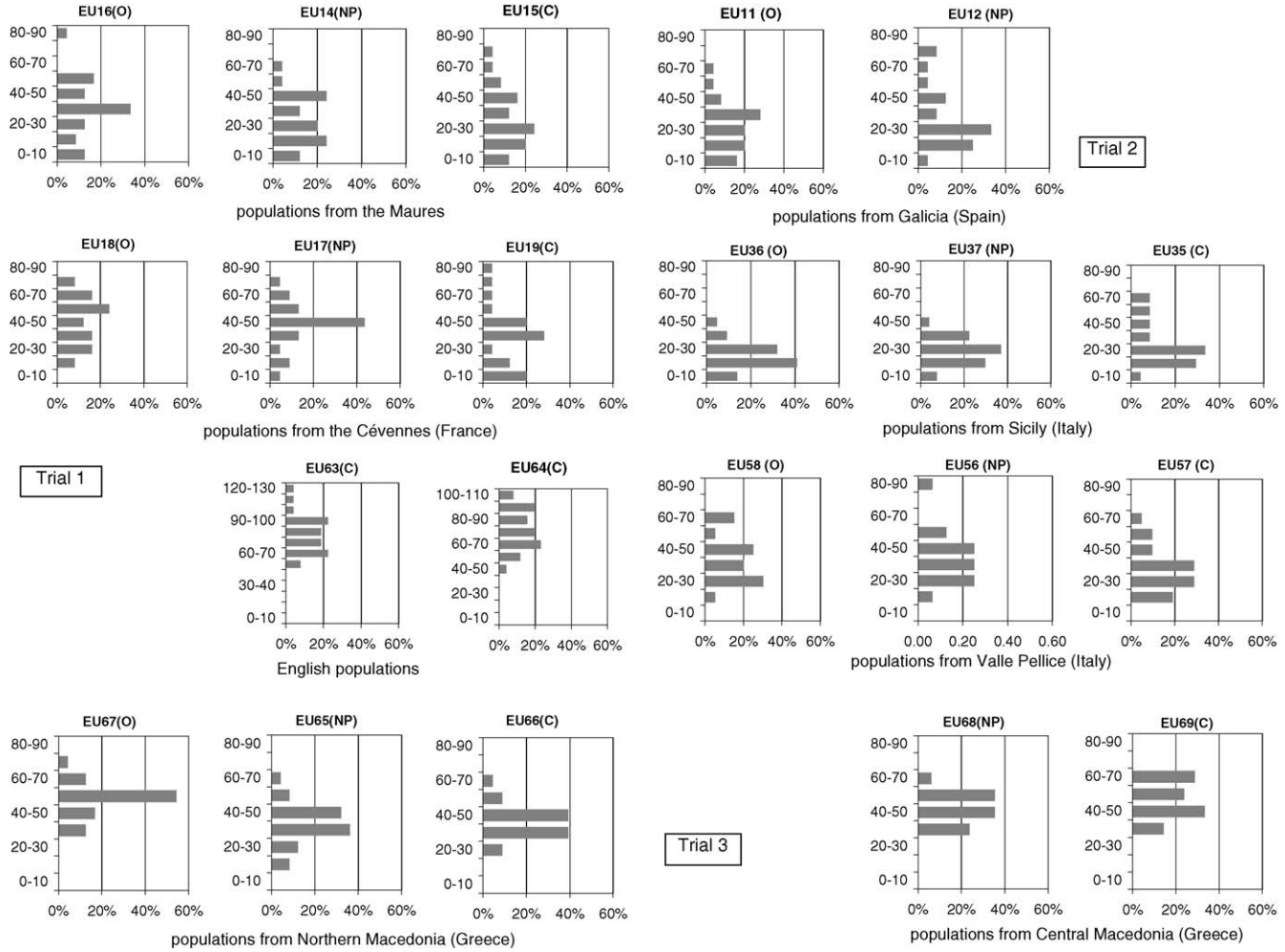

Fig. 4. Distribution of chestnut trees of each population in percentage classes according to the stem lesion length LL (in mm) measured after excised-shoot inoculation with *Phytophthora cambivora*. Note the different scale used for English populations.

84% and 84% of the plants in Trials 4, 5 and 6, respectively). The lesions were either limited (and then located at the secondary root insertions) or invasive in the entire taproot, causing wilting of the seedling. A stem lesion developed in 54, 68 and 48% of inoculated seedlings in Trials 4, 5 and 6, respectively. Most of these plants (83, 60 and 70% in the three respective trials) had their taproot completely colonized by *P. cambivora*.

Pearson correlation between the family mean values of PIT and of the other traits measured at the end of the experiment was calculated (Table 5). PIT was significantly correlated with SDI and SLL in the three trials and with SHI or RDW in only one of the three trials. Only PIT was chosen for further statistical analyses.

3.4. Variation of root susceptibility

The average PIT of the *C. crenata* family was 0, 0.03 and 0.08 in Trials 4, 5 and 6, respectively. The family of Marigoule was slightly more susceptible with average PIT values of 0.10, 0.05 and 0.15 in Trials 4, 5 and 6, respectively. Only three families (from populations EU16, 58 and 69) had a mean value of PIT lower than or equal to 0.15. For Trials 5 and 6, mean susceptibility of the families increased with the susceptibility of their mother trees assessed with LL (Fig. 5B and C). For the Trial 4, all the families, except one which was obtained from a tree assessed as tolerant, had a mean PIT superior to 0.4 (Fig. 5A). The relationship between susceptibility of mother trees and stem lesion lengths measured on seedlings was

Table 5
Pearson correlation coefficients between percent of infected taproot (PIT) and parameters measured for the progeny tests after root inoculation with *Phytophthora cambivora*

Families	Number	SLL	SDI	SHI	RDW	LDW
French and English	41	0.61**	−0.61**	−0.37*	−0.09	−0.21
Italian and Spanish	75	0.62**	−0.32*	−0.07	−0.25*	−0.04
Greek	30	0.61**	−0.39*	−0.28	0.00	0.33

SLL, stem lesion length, SHI, stem height increment, SDI, stem diameter increment, RDW, root dry weight, LDW, leaf dry weight.
* Significant at the 5% level.
** Significant at the 1% level.

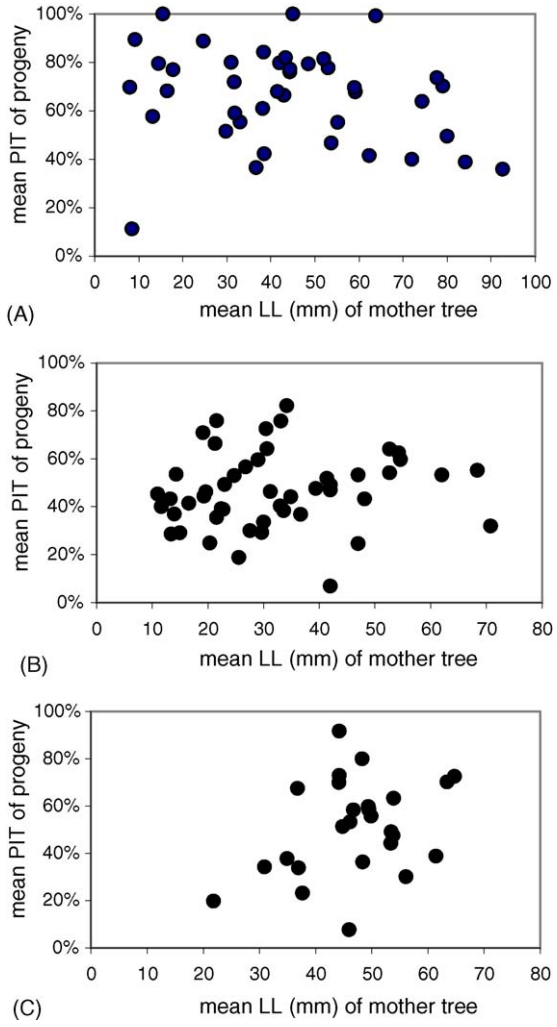

Fig. 5. Relationship between the mean percentage of infected taproot (PIT) of each family and the mean stem lesion length (LL) of their mother trees, after root inoculation and excised shoot inoculation with *Phytophthora cambivora*, respectively. (A) English and French families, (B) Spanish and Italian families, C: Greek families.

Table 6
Analysis of variance of the percentage of infected taproot for different chestnut families, after root inoculation with *Phytophthora*, and estimates of variance components for this parameter

Families	d.f.	MS	*F*	*P*	Variance components
French and English					
Population	7	0.4053	2.22	0.0322	0.0003
Family	39	0.2956	1.662	0.0132	0.0287
Greek					
Population	4	0.4242	2.89	0.0222	0.0020
Family	26	0.5381	3.67	<0.0001	0.0131
Italian and Spanish					
Population	9	0.6890	4.22	<0.0001	0.0028
Family	71	0.3351	2.05	<0.0001	0.0105

significant for Greek chestnut plants (Trial 6, Pearson coefficient of correlation $r = 0.38$, $P = 0.05$).

In the three trials there was a significant effect of population and of family on PIT (Table 6). The family component of variance was at least four-fold greater than the population component (Table 6).

4. Discussion

4.1. Assessment of susceptibility to Ink Disease in chestnut

Two methods, differing in age of the plant and the inoculum, were used to investigate intraspecific variation in susceptibility to *P. cambivora*. In the absence of chestnut provenance tests to study the among-population variation in this adaptive trait we inoculated shoots excised from adult trees sampled in different sites. With such a method we were able to estimate the component of Ink Disease resistance which controlled the invasion of shoots by the pathogen. We showed that this excised shoot test was reliable as a screening method and that lesion length was a good estimator of shoot susceptibility. Although *P. cambivora* was re-isolated in tissue at the front of the necrotic lesion, this underestimation of the invasion did not bias our screening for resistance to Ink Disease since the length of colonization was positively correlated with the length of the visible lesion. This ability to invade plant tissue without causing any lesion was also observed in *P. cinnamomi* infecting pine and eucalyptus. In those wound-inoculated trees, *P. cinnamomi* was recovered in symptomless phloem and wood at least 1 cm away from the ends of the lesion (Davison et al., 1994; Huberli et al., 2000). Moreover, the shoot tests that we carried out in two consecutive years were correlated ($r = 0.48$) and showed that trees with significantly lower susceptibility can be detected by this screening method, as has been observed with a similar test on *Q. agrifolia* and susceptibility to *P. ramorum* (Dodd et al., 2005).

To study the within-population variation, open-pollinated families were harvested and root-inoculated. With this method, *P. cambivora* present in the potting mix had to penetrate the fine roots before invading the taproot and eventually the collar and the stem. We used PIT to assess susceptibility to *P. cambivora* as it is more discriminating than the percentage of mortality assessed in previous studies (Schad et al., 1952) since taproot infection had a significant impact on seedling growth (Maurel et al., 2001). This parameter enables the genetic analysis of a wide component of resistance since the family mean values were negatively correlated with mean values of growth parameters (stem diameter in the three trials and stem height in one trial). The family mean values of PIT were also positively correlated with the mean values of SLL in all trials, suggesting that taproot and stem resistance were well correlated in chestnut, as has been shown for *E. marginata* (Hüberli et al., 2002a).

4.2. Among-population differentiation

Our results suggest a large amount of genetic variation of resistance to Ink Disease among European chestnut populations

originated from different gene pools and falling into different domestication levels. A significant among-population variation of susceptibility to *P. cambivora* was detected in all trials. It is in agreement with the among-population variation reported in chestnut for other adaptive traits, like growth, biomass traits and resistance to drought (Pliura and Eriksson, 2002; Lauteri et al., 2004) and in other species for susceptibility to a similar Phytophthora induced disease (Stukeley and Crane, 1994).

In novel epidemics, it is expected that resistance of native hosts infected by an invasive pathogen evolve rapidly depending on the effect of the pathogen on the host (Parker and Gilbert, 2004). Thus, resistance to Ink Disease in chestnut populations may have evolved after the introduction of *P. cambivora* or *P. cinnamomi* in Europe. In agreement with this selective pressure hypothesis, in regions where Ink Disease is thought to be present since at least the 1900's (i.e. the Cevennes and the Maures), chestnut populations exhibit higher resistance, in average, than populations from England where Ink Disease is not described or much more recent (Vettraino et al., 2005). Moreover, the rate of evolution of resistance is expected to be dependent on the strength of selection (Parker and Gilbert, 2004). Thus, evolution is more likely to occur in coppice and naturalized populations than in the populations of grafted varieties since in infested forest plots, only chestnut trees tolerant to *Phytophthora* could survive Ink Disease epidemic waves and regenerate. On the contrary, fruit varieties are not exposed to the pathogen. This hypothesis is supported in the Cévennes and in the Maures but not in Sicily, where Ink Disease has not been detected so far and the orchard population exhibited the highest level of resistance. However, this population appeared genetically different from the coppice and naturalized Sicilian populations (Mattioni, personnal communication) and could be the result of a private collection of chestnut varieties from different geographic origins.

4.3. Within-population variation

Evolution for resistance can occur when there is enough genetic variation for the trait to respond to selection (Parker and Gilbert, 2004). We report a large amount of within-population variation of susceptibility to Ink Disease in chestnut. Tolerant trees and families were detected in several populations suggesting that resistance genes could exist in *C. sativa* as already reported by Schad et al. (1952). We found up to 20% of tolerant *C. sativa* trees (with LL < 10 mm) in which the invasion of stem cortical tissue by the pathogen was restricted to a limited area, as is the case with chestnut hybrid clones (Salesses et al., 1993; Robin et al., 1994; Pereira et al., 1995; Fernandez-Lopez et al., 2001). With the progeny tests, we detected only one tolerant family (mean PIT < 0.15) which is less than the percentage of resistant plants reported by Schad et al. (1952) in different *C. sativa* families. However, these authors considered as resistant those plants which survived after inoculation and they did not look at the percentage of infected roots. Only one of the three mother trees which were assessed as tolerant provided a tolerant family. On the contrary, families exhibiting a good tolerance were obtained from two "susceptible" mother trees. This difficulty in predicting progeny performance with the lesion length of the mother tree may be explained by the complexity of the mechanisms involved in susceptibility or resistance and by the fact that different components were assessed in the mother tree and in its progeny. The same difficulty was reported in *Eucalyptus marginata*, although high values of heritability (0.74–0.85) have been estimated for susceptibility to *P. cinnamomi* (Stukeley and Crane, 1994). In P*inus radiata* heritability values for susceptibility to *P. cinnamomi* varied from 0.86 to 0.90 (Butcher et al., 1984). Heritability estimates could not be assessed with our experimental design. However, the family component of variance being, at least, four-fold greater than the population component and chestnut families being well discriminated for susceptibility to Ink Disease, it is highly likely that a strong differentiation of susceptibility within chestnut populations and a high heritability for this adaptive trait occur in *C. sativa*. Future studies focusing on heritability of susceptibility to Ink Disease and performed with a sufficient number of seedlings and different environmental conditions (resistance to *Phytophthora* could be temperature dependant as shown by Hüberli et al., 2002b) are needed to confirm this. Although, Fernandez-Lopez et al. (2001) did not find any significant difference between chestnut clonal susceptibility to *P. cambivora* and to *P. cinnamomi*, resistance to Ink Disease should also be tested with several isolates from both species.

The large amount of variation detected for resistance to Ink Disease suggest that breeding for this trait might be successful in *C. sativa*. Developing a genetic control against this disease might be an important topic in the context of climate change. Indeed, incidence of Ink Disease in chestnut is increasing (Saintonge, 2004). Moreover functional disease models and climatic change scenarios have suggested that *P. cinnamomi* might spread in disease-free areas like Brittany in France or England in the UK (Brasier, 1996; Bergot et al., 2004). Our evaluation of intraspecific variability of chestnut susceptibility showed that shoot excised inoculations can be used as a routine test to screen adult chestnut trees for susceptibility to Ink Disease. In spite of the satisfactory repeatability from year to year of this method, it could be necessary to confirm resistance suspected in trees exhibiting smaller lesions by a second year test. To screen families, a routine test easier than root inoculation can be performed by stem inoculation, since susceptibility of the stem appeared related to the susceptibility of the root system. Such a test would allow a quantitative assessment of susceptibility integrating both defence mechanisms and passive invasion by the pathogen and the estimation of heritability.

Acknowledgements

The authors are grateful to Xavier Capdevielle, Karen Russel, David Moodley, Josefa Fernandez-Lopez, Raphael Zas-Arregui for their helpful contribution for collecting stems and seeds, to Claudia Mattioni for sharing her results with us and to Antoine Kremer for reviewing an early draft of this paper. This study was carried out with financial support from the European project EVK2-CT1999-00006 "Securing gene

conservation, adaptive, breeding potential and utilization of a model multipurpose tree species (*Castanea sativa* Mill.) in a dynamic environment''.

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