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Susceptibility of common alder (*Alnus glutinosa*) seeds and seedlings to *Phytophthora alni* and other *Phytophthora* species

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Abstract

Phytophthora alni is a highly destructive host specific pathogen to alders (*Alnus* spp.) spreading all over Europe. Recently this pathogen has been reported to cause diseases in common alder (*Alnus glutinosa*) in Spain. Seeds and seedlings of *A. glutinosa* were tested *in vitro* for their susceptibility to alder *Phytophthora* and other *Phytophthora* species. Isolates of *P. alni* ssp. *alni*, *P. cinnamomi*, *P. citrophthora*, *P. nicotianae* and *P. palmivora* were used in the experiments. Seeds and seedlings were inoculated with a zoospore suspension and uniform mycelial blocks of agar of the *Phytophthora* species. Susceptibility was calculated in terms of pathogen virulence on seed germination and seedling mortality 42 and 67 days after inoculation respectively. Seed germination and seedling mortality rates varied differently among the isolates used. Results implied that common alder and its seeds and seedlings are at risk to be infected by *P. alni*. In addition, other *Phytophthora* species are able to infect this kind of material showing their relative host non-specificity. This is one important finding concerning alder regeneration in infected areas, and the possibility of disease spread on this plant material.

Key words: Alder diseases; virulence; zoospore suspension; V8 agar; forest pathology.

Resumen

Susceptibilidad de las semillas y brotes de aliso común (*Alnus glutinosa*) a *Phytophthora alni* y otras especies del género *Phytophthora*

Phytophthora alni es un patógeno muy destructivo de los alisos (*Alnus* spp.) que se está expandiendo por toda Europa. Recientemente este patógeno ha sido citado como causante de enfermedades del aliso común (*Alnus glutinosa*) en España. Semillas y plántulas de *A. glutinosa* fueron analizadas *in vitro* para ver su susceptibilidad a *Phytophthora alni* y otras especies del género *Phytophthora*. En el experimento se usaron aislamientos de *P. alni* ssp. *alni*, *P. cinnamomi*, *P. citrophthora*, *P. nicotianae* y *P. palmivora*. Las semillas fueron inoculadas con una suspension de zoosporas mientras que las plántulas fueron inoculadas con bloques uniformes de micelio de agar de las especies de *Phytophthora* utilizadas. La susceptibilidad fue calculada evaluando la germinación de las semillas y la mortalidad de las plántulas después de 42 y 67 días tras la inoculación respectivamente. Los ratios de germinación de las semillas y de mortalidad de las plántulas variaron significativamente entre los aislamientos utilizados. Los resultados demostraron que el aliso común y sus semillas y plántulas tienen riesgo de ser infectados por *P. alni.* Además, otras especies de *Phytophthora* fueron capaces de infectar, lo que evidenció una relativa falta de especificidad por el hospedante. Estos datos son importantes por su transcendencia para la regenaración del aliso en las áreas infectadas, y la dispersión de la enfermedad en este material vegetal.

Palabras clave: Enfermedades del aliso; virulencia; suspensión de esporas; Agar V8; patología forestal.

Introduction

The genus '*Alnus*' is characterized by their capability to colonize on bare land and tolerate high groundwater table and flooding (Gibbs *et al.*, 2003). Common alder (*Alnus glutinosa* (L.) Gaertn) is the most widespread species among alders occurring in Europe which has been used for reforestation and stabilizing river banks. This species is occurring extensively in Spain and is distributed along streams and rivers.

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An extensive and rapid mortality of common alder was observed along many rivers of northern Spain (Tuset *et al*., 2006). Some years later of that observation, *Phytophthora alni* (Brasier and S.A. Kirk) was associated to cause that mortality in *A. glutinosa* (Solla *et al*., 2010; Varela *et al*., 2010). *P. alni* is an interspecific hybrid between *Phytophthora cambivora* (Petri) and an unknown *Phytophthora* similar to *P. fragariae* Hickman (Brasier *et al*., 1995, 2004), and it has been described as a host specific pathogen to alders spreading all over Europe. Three different types have been recognized within *P. alni* based on morphological characteristics and aggressiveness (Brasier and Kirk, 2001; Brasier *et al*., 2004): the standard type of the pathogen, which is the most aggressive one, has recently been named as *P. alni* ssp. *alni*, the hybrid types collectively known as *P. alni* ssp. *uniformis* and *P. alni* ssp. *multiformis*, which are locally very damaging and could represent a serious threat to alder population and stability of riparian ecosystems (Brasier *et al*., 2004).

The association of *P. alni* in the decline and mortality process of alders has been the center point of several studies in different parts of the world (Gibbs, 1995; Gibbs *et al*., 1999; Santini *et al*., 2001; Brasier and Kirk, 2001; Brasier, 2003; Gibbs *et al*., 2003; Santini *et al*., 2003; Jung and Blaschke, 2004; Ioos *et al*., 2005; Cerný and Strnadová, 2010; Solla *et al*., 2010; Varela *et al*., 2010). *Phytophthora* decline of the riparian alder population has recently become an important problem in Spain because of the rapid spreading of the causal pathogen. According to Santini *et al*. (2003), the way of spreading of alder *Phytophthora* may be related to its introduction with the planting materials, which may become infected in nurseries where the cross infection between different hosts is frequent due to asymptomatic infections. According to this, alder population may be in danger as they co-exist with *Phytophthora* contaminated hardwood and ornamental woody species in the same nurseries. In addition to that, container grown seedlings in nurseries may act as a prime carrier facilitating further dispersion of *Phytophthora* to the natural ecosystems which may hamper alder regeneration. Zoospores of the alder *Phytophthora* swim freely in water and therefore most likely spread far distance using river system. In young alders, infection and bark killing often start at the collar region where zoospores are attracted. However, the infections in the middle of the trunk in the inner bark might indicate that environmental factors may also play a prominent role in the occurrence of the disease which may be associated with flooding events (Brasier, 2003).

Another *Phytophthora* species have been found causing damage in a wide tree host range. Among those, *Phytophthora nicotianae* Breda de Haan (= *Phytophthora parasitica* Dastur) which is a soilborne pathogen, has been recorded causing dieback in *Eucalyptus* species in South Africa where *Eucalyptus* species are planted commercially (Maseko *et al*., 2001); and damaging several woody ornamental plants in nurseries (Schwingle *et al*., 2007; Donahoo and Lamour, 2008); but it has been also detected in another 298 plant species (Erwin and Ribeiro, 1996). *Phytophthora cinnamomi* Rands, which is an invasive soil-borne roots pathogen, has been stated as one of the causal agents of the decline of holm oak (*Quercus ilex* L.) and cork oak (*Q. suber* L.) in Southwestern Iberian Peninsula (Brasier *et al*., 1993; Sanchez *et al*., 2002). And *P. citrophthora* (R.E. Sm. and E.H. Sm.) Leonian and *P. palmivora* (E.J. Butl.) have been recorded in nurseries causing diseases on several woody ornamental plants (Schwingle *et al*., 2007; Donahoo and Lamour, 2008).

Although few studies have been conducted to examine the susceptibility of alders to *P. alni* and other *Phytophthora* species (Brasier and Kirk, 2001; Santini *et al*., 2003; Santini *et al*., 2006), the Spanish isolates *P. alni* have not been tested yet in terms of aggressiveness on common alder. In addition to that, due to the frequent occurrence of other *Phytophthora* species in the forest ecosystems of Spain (including nurseries), the main objective of the present study was to evaluate the susceptibility of common alder seeds and seedlings to Spanish isolates of *P. alni* and the other most common *Phytophthora* species present in nurseries which could prompt the failure of the common alder regeneration.

Materials and Methods

Phytophthora isolates and inoculum production

Two isolates of *P. alni* ssp. *alni* recovered in 2009 from diseased *A. glutinosa* trees growing along river Miño (Spain), which were isolated as described by Solla *et al*. (2010), were used to inoculate seeds and seedlings. Besides, one isolate of each *P. cinnamomi*, *P. citrophthora*, *P. nicotianae* and *P. palmivora* (Supplied by the Instituto Agroforestal Mediterráneo [Mediterranean Institute of Agroforestry], Universidad Politécnica de Valencia, Valencia, Spain) were used in the experiments. Both, mycelia and zoospores were used in the experiments. For

mycelial agar plugs, colonies of the *Phytophthora* species were sub-cultured for 1 week at 20 °C in the dark onto 90-mm Petri dishes containing sterilized V8 agar (V8 agar: 100 ml/L V8 Campbell Grocery Products, 3 g/L CaC03, 20 g/L Agar Technical DIFCO, Detroit, MI, USA). For zoospores production, colonies of the *Phytophthora* species were sub-cultured for 14 days at 20 °C in the dark onto 90-mm Petri dishes containing sterilized V8 agar exposed to 16 h light per day. Sporangia were obtained by flooding the colonies with sterile distilled water, and then transferred into sterile water by rubbing surface of the culture with a sterile bent glass rod. The liquid was poured off the plates and again collected in a sterile beaker which was placed in a refrigerator at 7 °C for 1 h, then returned to room temperature (20 °C) during another 75 min to promote zoospore releasing. Zoospore concentration was determined by using a haemocytometer, and suspension was adjusted to 3×10^5 zoospores per ml (Denman *et al*., 2005).

Plant material

Disease free seeds of common alder were supplied by the Centro Nacional de Mejora Forestal [National Centre for Forest Breeding] El Serranillo (Guadalajara, Spain) in order to be used in the experiments. Before use, seeds were sterilized in the following way: they were firstly washed several times with sterilized distilled water, then dipped into hydrogen peroxide (3%) for 20 minutes. Finally seeds were washed twice with sterilized distilled water to remove excess hydrogen peroxide and dried aseptically. Those sterilized seeds were ready to be used in the seed inoculation experiment. For seedling inoculation experiment, sterilized seed were plated onto water agar Petri dishes and sealed with Parafilm® (American National Can, Greenwich CT, USA) to avoid contamination. Plates were kept into growth chamber at 24 °C and photoperiod (16/8) to promote germination. After that, eight seedlings per plate were aseptically transferred to Petri dishes containing potato-dextrose-agar (PDA) to get the seedling hardening prior to inoculation.

Experimental design

Seed inoculation experiment

Eight sterilized seeds per plate were transferred onto the surface of water agar contained into 90-mm Petri dishes. Each one of the six *Phytophthora* isolates (two of *P. alni* ssp. a*lni*, and one of each *P. cinnamomi*, *P. citrophthora*, *P. nicotianae* and *P. palmivora*) were inoculated in the Petri dishes following two different methods: (1) Individual seed inoculation treatment (noted as IS) when a zoospore suspension of 0.1 ml (3×10^5 zoospores per ml) was sprayed at the base of each seed; and (2) central inoculation treatment (noted as CE) when a zoospore suspension of 1 ml was sprayed very precisely at the centre of Petri dishes keeping equal distances from each seed and later shaken gently for the uniform spreading of suspension over the surface to facilitate the zoospore suspension contact with the seeds. In the controls (CO) 0.1 ml of sterile distilled water was sprayed instead the zoospore suspension. Four replications for each *Phytophthora* species and for each inoculation method were used in the assay. After inoculation, Petri dishes were sealed with Parafilm and kept at 24 °C and photoperiod (16/8). Seeds were inoculated on June 2010. Data of seed germination percentage were taken at every 5 days interval (except the first record which was taken at 7 days) until 42 days.

Seedling inoculation experiment

Seedling were inoculated on April 2010 with the six *Phytophthora* isolates following two different methods: (1) individual seedling inoculation (noted as IT) when a 2-mm size mycelial agar plug was put at the base of each seedling; and (2) central inoculation method (noted as CE) when a 2-mm size mycelial agar plug was placed precisely at the centre of Petri dishes keeping equal distances from each seedling. In the controls, seedlings were inoculated with sterile V8 agar plugs. Four replications for each isolate and method were used in the experiment. After inoculation, plates were sealed with Parafilm and kept at 24 °C and photoperiod (16/8). Data of seedling mortality percentage were taken at every 5 days interval (except the first record which was taken at 7 days) until 67 days.

Statistical analysis

Seed germination and seedling mortality were analyzed by repeated measures ANOVAs ($p < 0.05$) to examine significant differences among the *Phytophthora* isolates and inoculation methods, as well as the time period when different measurement data were taken. The differences between means were considered significant ($p < 0.05$) according to Tukey multiple range

test. All statistical analyses have been performed with the software Statistica 6.0 for Windows (StatSoft Inc., Tulsa, Oklahoma, USA).

Results

Seed inoculation experiment

Repeated measures ANOVA applied to seed germination percentage (Table 1) showed that both the isolate of *Phytophthora* species and the treatments used in the experiment produced significant effect on seed germination. Interactions between the factors as source of variation were significant with the exception of time. Seeds started to germinate during the first week, regardless of the treatment, although in the controls a higher percentage was obtained after 7-12 days after the inoculation. In case of seeds inoculated, comparatively a low germination was achieved 22-27 days after the inoculation depending on the isolates of *Phytophthora* species and pursued almost a steady rate up to 37-42 days (Figure 1). The complete progression of seed germination after the inoculation with the isolates of *Phytophthora* species with respect to number of days has been indicated in Figure 1. Forty two days after inoculation, all the isolates of the *Phytophthora* species tested hampered significantly germination regardless of the method used. When zoospore suspension was applied at the centre of the plate (method CE), no differences were found among the isolates, but when suspension was applied in each seed (method IS), *P. cinnamomi* caused a significant lower reduction of the germination percentage than that caused by rest of the species (Figure 2). On average, seed germination percentage when inoculation was made for each seed, was

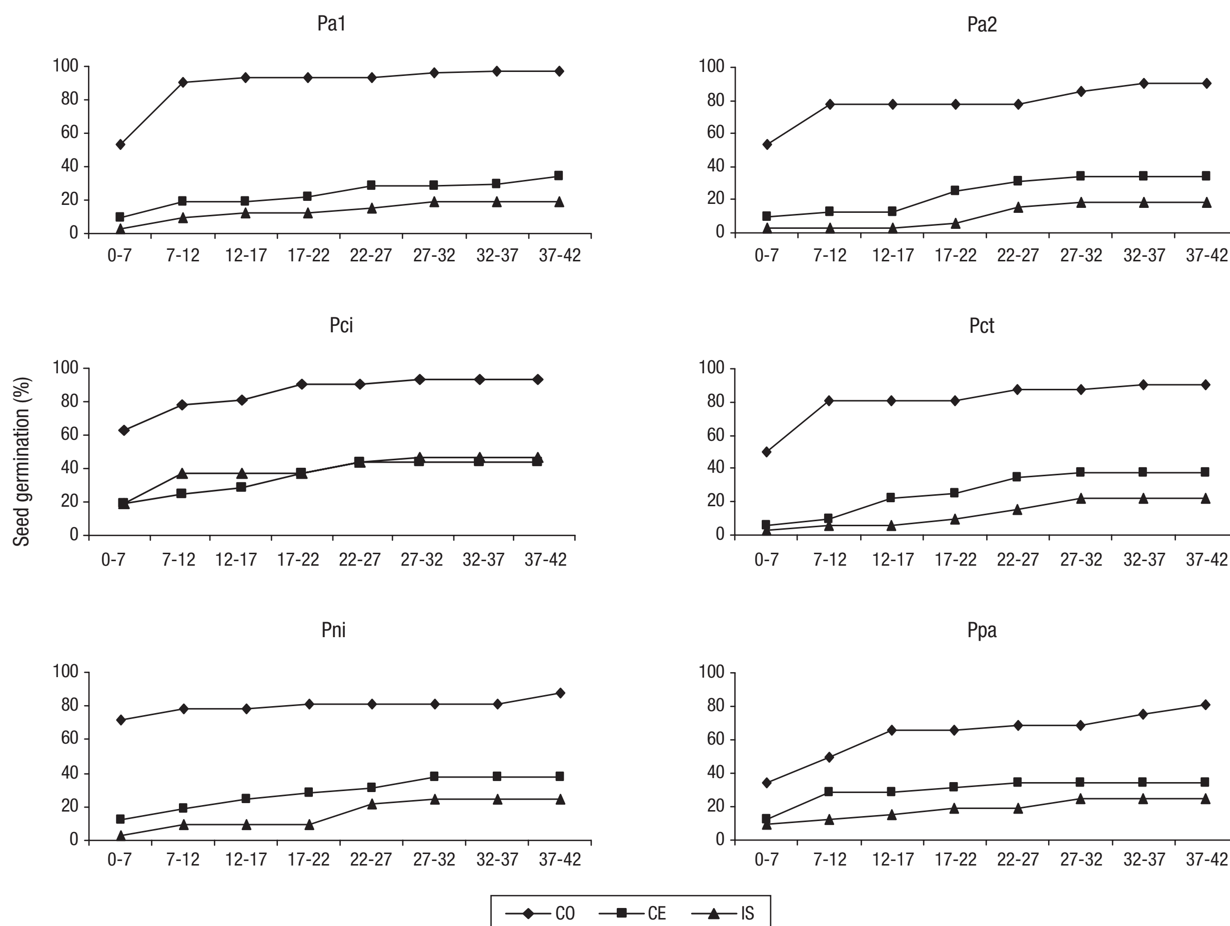

Figure 1. Mean seed germination percentage after inoculation with each one of the six *Phytophthora* isolates, Pa1=*P. alni* (isolate 1); Pa2 = *P. alni* (isolate 2); Pci = *P. cinnamomi*; Pct = *P. citrophthora*; Pni = *P. nicotianae*; Ppa = *P. palmivora*. CO = Control; CE = zoospore suspension sprayed at the centre of the Petri dishes; IS = zoospore suspension sprayed at the base of each seed.

Table 1. Repeated measures analysis of variance for seed germination (%) that considers *Phytophthora* species, treatment and time, and their interactions as source of variation

Source of Variation	SS	df	MS	F	p
Intercept	1009397	1	1009397	1483,077	0,000000
Species	17509	5	3502	5,145	0,000624
Treatment	402502	2	201251	295,692	0,000000
Species*$_{Treatment}$	16632	10	1663	2,444	0,017391
Error	36753	54	681		
TIME	40796	7	5828	104,029	0,000000
TIME*$_{Species}$	1452	35	41	0,740	0,860560
TIME*$_{Treatment}$	3926	14	280	5,006	0,000000
TIME*$_{Species}$*$_{Treatment}$	4387	70	63	1,119	0,254916
Error	21177	378	56		

SS = sum of square; df = degree of freedom; MS = Mean square.

26.04%; and when zoospore suspension was applied at the centre, the percentage was 36.97%. In controls, germination percentage was higher than 80% in all cases (Figure 2).

Seedling inoculation experiment

Repeated measures ANOVA (Table 2) revealed that the seedling mortality percentage was significantly influenced by the *Phytophthora* isolates, the inoculation methods, the time period, and by their interactions. Most of the seedlings inoculated with the different isolates of *Phytophthora* started to die 7 days after inoculation, with a progressive mortality increment, reaching the maximum after 15-20 days of the inoculation (Figure 3). However this maximum was achieved earlier by both isolates of *P. alni* and *P. citrophthora*. At the end of the experiment all the isolates, excepting that of *P. cin-*

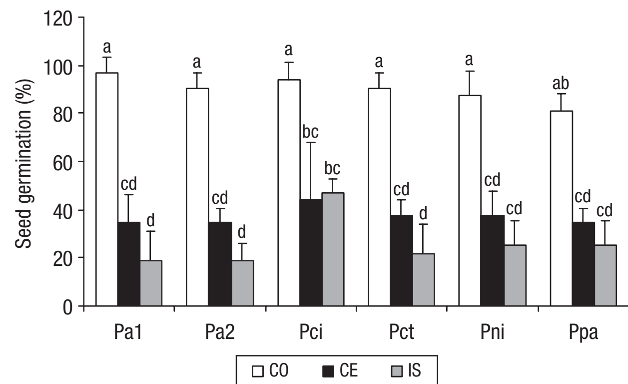

Figure 2. Mean (± SE) seed germination percentage after 42 days of inoculation with each one of the six *Phytophthora* isolates (Pa1 = *P. alni*, isolate 1; Pa2 = *P. alni*, isolate 2; Pci = *P. cinnamomi*; Pct = *P. citrophthora*; Pni = *P. nicotianae*; Ppa = *P. palmivora*). CO = Control; CE = zoospore suspension sprayed at the centre of the Petri dishes; IS = zoospore suspension sprayed at the base of each seed. Different letters indicate significant differences ($p < 0.05$) according to Tukey test.

Table 2. Repeated measures analysis of variance for seedling mortality (%) that considers *Phytophthora* species, treatment and time, and their interactions as source of variation

Source of Variation	SS	df	MS	F	p
Intercept	2674948	1	2674948	2927,116	0,000000
Species	105666	5	21133	23,125	0,000000
Treatment	1364249	2	682125	746,429	0,000000
Species*$_{Treatment}$	63397	10	6340	6,937	0,000001
Error	49348	54	914		
TIME	112953	12	9413	223,165	0,000000
TIME*$_{Species}$	9336	60	156	3,689	0,000000
TIME*$_{Treatment}$	81528	24	3397	80,538	0,000000
TIME*$_{Species}$*$_{Treatment}$	19211	120	160	3,796	0,000000
Error	27332	648	42		

SS = sum of square; df = degree of freedom; MS = Mean square.

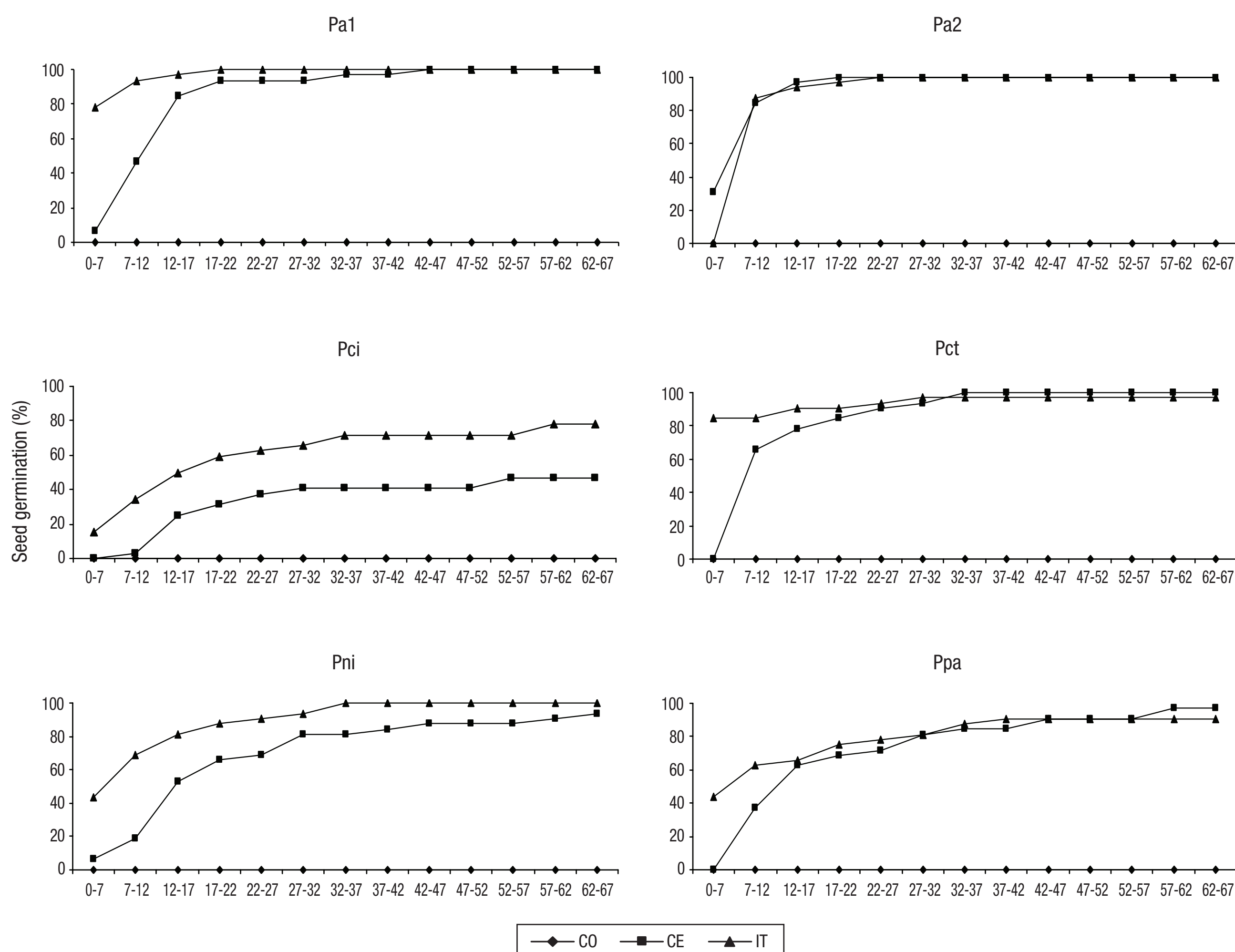

Figure 3. Mean seedling mortality percentage after inoculation with each one of the six *Phytophthora* isolates, Pa1=*P. alni* (isolate 1); Pa2 = *P. alni* (isolate 2); Pci = *P. cinnamomi*; Pct = *P. citrophthora*; Pni = *P. nicotianae*; Ppa = *P. palmivora*. CO = Control; CE = mycelial blocks of agar placed precisely at the centre of the Petri dishes; IT = mycelial blocks of agar put at the base of each seedling.

namomi, produced a seedling mortality rate higher than 90%, regardless of the inoculation method (Figure 4). *P. cinnamomi* isolate caused a seedling mortality percentage of 46.9% when it was inoculated in the center of the plate (method CE), and 78.1% when it was inoculated in each seedling (method IT). In controls were not observed any seedling mortality (Figure 4).

Between inoculation methods, differences were found mainly at the beginning of the experiment (in the first 2-5 measurements depending of the isolate). In those first records, the inoculation of the isolate plugs in each seedling (method IT) caused a greater seedling mortality than the inoculation in the centre of the plate (method CE) (Figure 3). The exception was found for the *P. alni* isolate 2; in this case the method CE was more aggressive than the method IT in the first record interval. At the end of the experiment, no differences were found between inoculation methods, except in the *P. cinnamomi* isolate which caused a significant higher mortality when was inoculated separately in each seedling (Figure 4).

Discussion

Pathogenicity of *Phytophthora alni* ssp. *alni*, *P. cinnamomi*, *P. citrophthora*, *P. nicotianae* and *P. palmivora* on common alder (*Alnus glutinosa*) seeds and seedling was examined *in vitro*. This is the first study of this type performed so far in Spain with the aim to examine

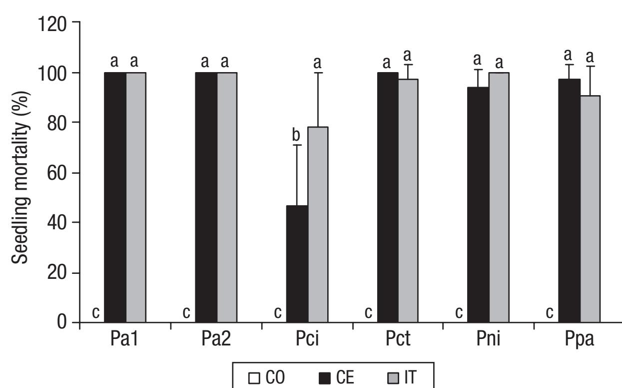

Figure 4. Mean (± SE) seedling mortality percentage after 67 days of inoculation with each one of the six *Phytophthora* isolates (Pa1 = *P. alni*, isolate 1; Pa2 = *P. alni*, isolate 2; Pci = *P. cinnamomi*; Pct = *P. citrophthora*; Pni = *P. nicotianae*; Ppa = *P. palmivora*). CO = Control; CE = mycelial blocks of agar placed precisely at the centre of the Petri dishes; IT = mycelial blocks of agar put at the base of each seedling. Different letters indicate significant differences ($p < 0.05$) according to Tukey test.

the interaction between *A. glutinosa* and host specific pathogen *P. alni* along with non-host specific pathogens *P. cinnamomi*, *P. citrophthora*, *P. nicotianae* and *P. palmivora* under laboratory conditions. Both inoculation methods applied have confirmed their ability to infect seeds and seedlings. Our findings have revealed that all the *Phytophthora* spp. tested in the present assay could represent a serious threat to *A. glutinosa* which may cause failure of the alder regeneration.

In our present inoculation tests, *P. alni* ssp. *alni* has appeared as a highly aggressive pathogen on seeds and seedlings of *A. glutinosa*. The high aggressiveness of *P. alni* has been already stated by other authors in several European countries (Brasier *et al*., 1995; Gibbs, 1995; Szabó *et al*., 2000; Santini *et al*., 2001; Streito *et al*., 2002; Nagy *et al*., 2003; Jung and Blaschke, 2004; Cerný and Strnadová, 2010; Solla *et al*., 2010; Varela *et al*., 2010). Pathogenic ability of *P. alni* on seeds of *A. glutinosa* has been revealed from a similar laboratory test made by Schumacher *et al*. (2006), where seeds were inoculated with a zoospore suspension. The finding has strengthened the speculation that *P. alni* could infect alder seeds under natural conditions. Results of seedling inoculation test are consistent with a laboratory test conducted by Santini *et al*. (2003) where seedlings of *A. glutinosa*, *A. cordata* and other hosts were inoculated with one isolate of *P. alni*. In that study, the maximum seedling mortality was observed in *Alnus* species. Infecting ability of *P. alni* has been revealed from baiting tests which confirmed the involvement of the pathogen on *A. glutinosa* seedlings at nurseries in Germany (Jung and Blaschke, 2004). In Italy, *P. alni* was found infecting *A. cordata* both in young plantations and nurseries (Santini *et al*., 2003). Several other authors have also reported the association of *P. alni* with alder seedlings in plantations and nurseries (Santini *et al*., 2001; Gibbs *et al*., 2003; Schumacher *et al*., 2006). So, we assume that *P. alni* ssp. *alni* is able to infect and contaminate seeds and seedlings of *A. glutinosa* both in artificial and natural conditions.

Another *Phytophthora* species tested is *P. cinnamomi*, which has not been detected naturally in alders yet, but in our study it has proved its ability to reduce seed germination and cause seedling mortality under *in vitro* conditions. Similar results were obtained from a previous laboratory test by Santini *et al*. (2003) where an isolate of *P. cinnamomi* caused mortality of *A. glutinosa* seedlings after its inoculation using two different methods. In general, *P. cinnamomi* has been described as a soil-borne pathogen and it has been reported causing root rot in *Quercus suber* and *Q. ilex* under Mediterranean conditions and stem canker of *Q. rubra* in France (Brasier *et al*., 1993; Marcais *et al*., 1993; Linde *et al*., 1999; Sanchez *et al*., 2002). Hardy and Sivasithamparam (1988) recorded the involvement of *P. cinnamomi* and several other *Phytophthora* species with root rot of container grown seedlings from 14 nurseries in Western Australia. In contrast to our study, Australian and South African isolates of *P. cinnamomi* showed a considerable variation in virulence when were inoculated into seedlings of *Eucalyptus* (Dudzinski *et al*., 1993; Linde *et al*., 1999). Robin and Desprez-Loustau (1998) observed a wide range in aggressiveness of *P. cinnamomi* isolates to *Q. rubra*. Host range of *P. cinnamomi* was examined by Tippet *et al*. (1985) who assessed differences in susceptibility of *P. cinnamomi* to the hosts examined in inoculation tests. On the basis of the findings, we draw conclusion that the alders are under risk to be infected as sharing same environmental conditions to grow with other plants hosting *P. cinnamom.*

Among the other pathogen species tested, *P. citrophthora* has shown its virulence on *A. glutinosa* seeds and seedlings. *P. citrophthora* has been described as a pathogen of *Citrus* and it has been reported causing crown rot of peach, plum and cherry rootstocks after artificial inoculations (Thomidis, 2001). Association of *P. citrophthora* in nurseries of woody ornamental plants has been surveyed (Donahoo and Lamour, 2008). Although *P. citrophthora* is non-host specific to alders, in

our present inoculation tests, the pathogen has showed a great aggressiveness on *A. glutinosa* seeds and seedlings. In support to our findings, a separate inoculation test conducted by Santini *et al*. (2006), showed a certain seedling mortality of *Alnus* spp. after inoculation with *P. citrophthora*, although a lower mortality was obtained. In contrast, *P. citrophthora* was the most aggressive pathogen out of eleven *Phytophthora* species tested in a pathogenicity experiment conducted *in vivo* on cherry (Thomidis and Sotiropoulos, 2003). Being an aggressive pathogen, we could assume that alders growing in nurseries and mixed plantations may be at risk to be infected by *P. citrophthora*.

P. nicotianae and *P. palmivora* are occurring frequently in nurseries with container grown seedlings. Donahoo and Lamour, (2008) reported the presence of *P. nicotianae and P. palmivora* in nurseries of woody ornamentals. In addition, Hardy and Sivasithamparam (1988) found the presence of *P. nicotianae* in 9 nurseries out of 14 surveyed in Western Australia. Besides, a baiting bioassay detected *P. nicotiana* and several other *Phytophthora* in naturally infested container mixes from South Carolina nurseries in United States (Ferguson and Jeffers, 1999). Up to date, no report was available on the pathogenic ability of *P. nicotianae and P. palmivora* on *Alnus* but, in our tests it has been revealed their capacity to show virulence on *A. glutinosa* seeds and seedlings. That's why it is assumed that container grown seedlings of *Alnus* spp. may be vulnerable to *P. nicotianae* and *P. palmivora* if there is enough inoculum as it occurs with other plants in nurseries. In support of the hypothesis, several non-host specific pathogens *P. citricola*, *P. cactorum* and *P. gonapodyides* were reported to be present in a nursery soil in the vicinity of alders (Oszako *et al*., 2007). Several other *Phytophthora* species such as *P. cambivora*, *P. cactorum*, *P. citricola*, *P. megasperma* and *P. quercina* were baited from flooding alder plants (Jung *et al*., 1999, 2003). All these observations may suggest that under suitable conditions, *P. nicotianae* and *P. palmivora* could be potentially harmful to *A. glutinosa*. The phenomenon gives a serious implication for future establishment of forest stands with infested nursery stock.

Production of zoospores in the presence of water significantly contributes to the dispersal of *Phytophthora* via irrigation (Yamak *et al*., 2002; Hong *et al*., 2006). Transportation of nursery stock and use of natural rivers and other water courses to irrigate nurseries give ideal opportunities to *Phytophthora* to infect and spread in those nurseries. Out-planting with *Phytophthora* infected nursery stock have added further more to the dissemination process of *Phytophthora* to natural ecosystems. Falling of seeds, young shoot or leaves onto the contaminated water or soil and later disseminating far distances through water ways. For the alder seeds and seedlings, which are vulnerable to *P. alni* and other *Phytophthora* species, is certainly a new threat. In addition to that, the emergence of a new *Phytophthora* species provides additional threat, as it has been the case of *Alnus* by a hybrid pathogen *P. alni* ssp. *alni* (Brasier *et al*., 2004). For instance, a new *Phytophthora* species, *P. polonica*, was isolated from rhizosphere soil samples when surveys conducted in declining alder stands grown naturally by the riversides in Poland (Belbahri *et al*., 2006). New pathogens can be brought into nurseries and distributed with seeds and seedlings to plantations or riparian ecosystems. In Great Britain, spreading of alder *Phytophthora* has occurred through watercourses (Gibbs *et al*., 1999) whereas in Bavaria, Germany *P. alni* has been introduced into many places either by planting infected nursery stock or by irrigation water (Jung and Blaschke, 2004).

Artificial inoculation methods applied in an artificial environment may not provide precise information on the real interactions between hosts and pathogens. Besides, it may not possible to get exact results on the virulence of the *Phytophthora* species on hosts. Nevertheless, experiments done in an artificial environment may avoid interactions with other organism or minimize host natural defense mechanism. In this sense, the present study provides consistent clue of how seed germination and seedling mortality are affected by different *Phytophthora* isolates. Extrapolation of laboratory results to natural environment may not always be valid. However, results obtained from those *in vitro* inoculation methods can be useful for the identification of potential hosts and contribute to pathogens risk assessment. The results of the present study provided us a rough estimation on the susceptibility of an important tree species towards *Phytophthora*. As other *Phytophthora* spp. are able to infect alder seeds and seedlings, it is important to apply further controls on nursery management and irrigation system to avoid dissemination of *Phytophthora*. As a whole, additional dissemination of inoculum with diseased nursery stock will hamper control measures of the diseases on alders. There is an immediate need of a molecular-based detection protocol and safer conditions for *A. glutinosa* to grow in nurseries and plantations. It is important to apply effective and immediate actions to prevent spread and transfer of *Phytophthora* spp.

In order to reduce the spread of *Phytophthora* diseases in plantations and nurseries the following few measures could be adapted: (a) careful selection of plant material (seeds and seedlings) free of diseases, (b) avoidance of frequent seedling transportation with soils from nurseries to planting sites, (c) monitoring of plant health growing in nurseries at a regular interval, (d) careful inspection and testing of nursery soil and water reservoir used to irrigate nurseries, (e) sterilization of nursery tools before performing silvicultural treatments, (f) planting of *Alnus* in the stands where nursery plants have not been planted for a long period of time, and (g) development of molecular based detection protocol for a rapid and effective identification of *Phytophthora* species.

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