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## Interaction between root rot basidiomycetes and *Phytophthora* species on pedunculate oak

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### Abstract

Forest declines are usually complex multifactorial phenomena that involve interactions between different factors. While interactions between insects and pathogens have been shown to often be of primary importance, the possible interaction between different types of pathogens has not yet been investigated. Through artificial inoculation on oak seedlings, we studied the interaction between two root rot basidiomycetes, *Collybia fusipes* and *Armillaria mellea* and two *Phytophthora* species, *P. cinnamomi* and *P. cambivora*. These pathogens were inoculated onto young *Quercus robur* saplings in greenhouse conditions, either alone or combining a root rot basidiomycete with a *Phytophthora* species. Three out of the four *Phytophthora* \* root rot basidiomycete combinations tested resulted in damages to oak host significantly larger than the sum of the damages induced by the pathogens on their own. This positive interaction could be significant in the oak decline syndrome.

**Key word:** root rot basidiomycete, *Phytophthora*, oak, forest tree, decline

### Introduction

Forest declines are usually described as complex multifactorial phenomena that require the combination of several factors to occur. Manion (1991) developed a conceptual model of forest decline that postulates a conjunction of 3 different types of factors that must occur for the onset of a decline: predisposing factors act over the long term to weaken the trees, while inciting factors are short term stresses that trigger the decline, and contributing factors, mostly opportunistic organisms, act on the weakened trees to increase or to speed up the level of decline and mortality. The importance of interactions between fungal pathogens and insects is often of prominent importance. Indeed, the interaction between bark beetles and their associated ascomycetous fungi have been well documented in pine declines (Aukema et al, 2010) while interactions between Lepidoptera defoliators and either root rot basidiomycetes or leaf pathogen is important in oak decline (Wargo and Harrington, 1991; Thomas et al, 2002; Marçais and Breda, 2006). By contrast, although declining trees are often faced with several pathogenic fungi (Guillaumin *et al*, 1985; Camy et al, 2003b), the potential interaction between those natural enemies has not been investigated.

Oak decline has been a chronic problem in Europe in the last 30 years and typically involves the interaction between several factors, biotic and abiotic (Landmann et al, 1993, Thomas *et al*, 2002). Such declines have been a growing concern, in particular because of the

possible relation with climatic change. In oak decline, fungal pathogens colonizing either the root system (*Armillaria* sp.) or the bole bark (*Biscogniauxia mediterranea*) and bark insects such as *Agrilus* species have been reported as important opportunistic parasites, contributing to tree mortality. As in most cases of forest decline, the importance of those opportunistic pathogens is controversial (Thomas *et al*, 2002). Other root pathogens such as *Collybia fusipes* have been shown to be important in some oak declines and show significant pathogenicity in inoculation trials (Marçais and Delatour, 1996). However, this pathogen destroys very slowly the root system of affected oak tree and its role in the decline is difficult to assess (Marçais and Caël, 2001, Camy *et al*, 2003c). In the last 10 years, the frequent presence of several *Phytophthora* species on declining oaks has been reported. In particular, *P. cinnamomi* and *P. quercina* were mentioned to be significantly associated with oak decline (Robin *et al*, 1998, Jung *et al*, 2000, Jönsson *et al*, 2003, Balci *et al*, 2008). However, although those *Phytophthora* species shows pathogenicity toward oak, inducing significant fine root mortality in inoculation trial on greenhouse grown oaks, they usually do not induce any seedling mortality or significant growth decrease (Marçais *et al*, 1996, Jung *et al* 1999, Jung *et al*, 2000, Jönsson *et al*, 2003). *Phytophthora* spp. and root rot basidiomycetes often occur together on the same trees (Camy *et al*, 2003b). They both are likely to be significantly influenced by host weakening by other natural enemies. Indeed, pathogens such as *Armillaria* sp or *P. cinnamomi* have been shown to be strongly influenced by host physiological condition (Blacker and Mc Donald, 1981, Wargo and Harrington, 1991, Marçais *et al*, 1993, Maurel *et al*, 2001, Marçais and Bréda, 2006).

The aim of this work was thus to determine through inoculation trials on young saplings whether significant interactions between *Phytophthora* spp. and root rot basidiomycetes might have some importance in oak decline.

## Material and Methods

### Plant material, fungal isolates and inoculum production

Studied *Q. robur* were 2-years-old seedlings grown from seed originating from the forest of Amance, nearby Nancy (NE France, Meurthe-et-Moselle). They were potted in 9 l pots filled with substrate composed of a 50/50 peat-sand mixture. In the 2 experiments, the seedlings were grown in glasshouses.

Two *Phytophthora* isolates were used. The first one, a *P. cinnamomi* isolate (P382) was an aggressive strain isolated by G. Strouts in 1980 from soil under *Nothofagus procera* in Surrey, UK, and was used in the two experiments. The second *Phytophthora* isolate used was P463 a *P. cambivora* isolated by C. Robin from *Castanea sativa* (Lot et Garonne, France). C41, the *Collybia fusipes* isolate used in both experiments was isolated by B. Marçais in 1994 from a root infection on *Q. robur* at Siarrouy (Hautes-Pyrénées, France). A69, the *Armillaria mellea* used in the second experiment was isolated by J.J. Guillaumin in 1969 from a root infection on a *Prunus* species (Pont-St-Esprit, France). The two root rot basidiomycete isolates have been inoculated and reisolated from their host several times since the first isolation.

Pieces of wood were colonised by *C. fusipes* or *A. mellea* (Marçais and Delatour, 1996). Briefly, stems of hazel, *Corylus avellana*, 1.5-2.5 cm in diameter, were collected and cut into segments 3 cm long. They were placed in glass jars filled with tap water and sterilised twice at 120°C for 30 min, 24 h apart. The water was drained at the end of each sterilisation. A liquid malt medium was added to cover half the height of the wood segments and a third sterilisation was done for 20 min at 120°C. To improve aeration, a hole was drilled in the jar

top and plugged with cotton wool. Each of the jars was aseptically seeded with blocks of inoculum (0.5 x 0.5 cm) from a *C. fusipes* or an *A. mellea* culture on malt agar (20 g l<sup>-1</sup> malt Difco, 15 g l<sup>-1</sup> agar). For *C. fusipes*, the jars were incubated for 30-45 days at 23°C, then all the liquid was drained from the jars with a syringe and the jars were further incubated at 23°C for 8-9 months. For *A. mellea*, jars were incubated at 23°C for 4 months.

*Phytophthora* inoculum for soil infestation was produced on a mixture containing 700 ml of whole millet grains, 50 ml of clarified multivitamin juice broth (14.1 g de CaCO<sub>3</sub> and 1000 ml vegetable Joker juice) and 200 ml of water which was autoclaved twice for 20 min at 120°C. The inoculum was incubated at 20°C in the dark for 3 weeks before use.

### Inoculation by the pathogens

Two inoculation experiments were conducted. In the first one, only 2 pathogens, *C. fusipes* and *P. cinnamomi* were used. Two inoculation times were included for *C. fusipes*, one before the *P. cinnamomi* inoculation and one after. *C. fusipes* was inoculated by placing on the collar of the 2-years-old seedlings a wood segment colonised by the pathogen. The collar of the seedling was carefully exposed to avoid wounding, a colonised hazelnut stem segment was tied to taproot with an elastic band at about 4-8 cm below the soil line and the soil was replaced on the inoculum. *P. cinnamomi* was inoculated in the summer by adding to the potting medium colonised millet seeds. The inoculum (40 ml per seedling) was mixed with the superficial substrate at 4 locations in the pots. Seedlings were then flooded just after the *Phytophthora* inoculation. Pots were placed in plastic bags and water was added until the level reached the soil surface. The water was allowed to drain after a period of 48h. The flooding was repeated 2 months later. Pots were separated by treatment during the flooding to avoid cross contamination by *Phytophthora*. Altogether, 6 treatments were compared: 3 *C. fusipes* treatments (no inoculation, inoculation in December 1998, inoculation in October 1999) x 2 *P. cinnamomi* treatments (no inoculation, inoculation in August 1999). Each treatment contained 20 seedlings (altogether 120 seedlings). The experiment was organised in a completely randomised design. The status of the root system was assessed in December 2000, i.e. 2 years after the first *C. fusipes* inoculation and 1.5 years after the *Phytophthora* inoculation. Seedling were 4-years-old at the end of the experiment.

In the second experiment, two *Phytophthora* spp., *P. cinnamomi* and *P. cambivora* and two root rot basidiomycetes, *C. fusipes* and *A. mellea* were included. The 2 root rot basidiomycetes were inoculated in November 2002 and the two *Phytophthora* spp. in May 2003 as previously explained. In particular, two periods of flooding 15 days apart were applied after the *Phytophthora* inoculation. Altogether, 9 treatments were compared: three root rot basidiomycete treatments (no inoculation, inoculation by *C. fusipes* and inoculation by *A. mellea*) x three *Phytophthora* treatments (no inoculation, inoculation by *P. cinnamomi* and inoculation by *P. cambivora*). Each treatment contained 25 seedlings (altogether 225 seedlings). The experiment was organised in a split plot design, with the root rot basidiomycete treatments randomised within the *Phytophthora* treatments, to avoid cross contamination between the different *Phytophthora* treatments. The status of the root system was assessed in March 2004, i.e. 17 months after the root rot basidiomycete inoculation and 5 months after the *Phytophthora* inoculation. Seedling were 3.5-years-old at the end of the experiment.

In both experiments, seedling diameter at 10 cm above substrate was measured in winter each year, starting at the time of the root rot basidiomycetes inoculation. In experiment 2, the height of seedlings was recorded each year as well.

## Assessment of root damage

Seedlings were monitored for above ground symptom development during the incubation time. For assessment of root status, seedlings were removed from the pots and all fine roots of less than 1mm diameter were cut. Fine roots were gently washed to remove all traces of substrate and oven dried at 65°C for 48 h. The dry weight of fine roots was recorded for each seedling. The collar area and inoculum were then examined. Width and height of dead areas of bark on the taproot and main lateral roots were recorded and the surface of the lesions was estimated as the geometric mean of those 2 diameters ( $\pi \times (\text{height} \times \text{width}) / 4$ ). Isolations of the parasites were attempted both from the inoculum and the host tissues. They were washed under water, immersed for 1 min in sodium hypochlorite at 3.75% active chlorine and rinsed 3 times in sterile water. The outer bark was removed and chips of dead bark or decayed wood were plated on either MAT medium (10 g.l<sup>-1</sup> of malt Difco, 100 mg.l<sup>-1</sup> penicillin, 100 mg.l<sup>-1</sup> streptomycin, 250 mg.l<sup>-1</sup> thiabendazole and 15 g.l<sup>-1</sup> agar) or BARPBHy medium (corn meal agar Difco with 200 mg ampicillin, 10 mg rifampicin, 10 mg pimaricin, 15 mg benomyl, and 50 mg hymexasol per liter). Inoculations by *C. fusipes* or *A. mellea* were recorded as successful when cambial death had occurred.

When no *Phytophthora* had been isolated from bark lesions on a seedling, a baiting was attempted from the substrate to determine whether the pathogen had established on the seedling root system. Baiting was performed under standard laboratory conditions (about 20°C, diffuse light). About 200 mL of substrate was flooded with 500 mL deionized water and baited by floating 2-3 rhododendron leaves on the surface. Baits were removed after 3 days and blotted dry; necrotic parts of the leaves were transferred to BARPBHy selective agar medium. Plates were examined daily and possible *Phytophthora* colonies were transferred to three different media for identification: corn meal agar amended with  $\beta$ -sitosterol, potato dextrose agar, and 200 mL Joker, (V-8 like clarified multivitamin juice) amended with 7 g per 500 mL CaCO<sub>3</sub>, 20 g agar and 800 mL deionized water.

## Data analysis

The effect of the inoculation treatments on seedling stem diameter or height growth was analysed by variance analysis (GLM, SAS/STAT 8.1).

Only data from inoculated seedlings where the root rot basidiomycetes or/and the *Phytophthora* had successfully established were used for the analysis (isolation of the pathogen from bark lesions, woody inoculum or successful baiting from the substrate). Frequency of successful establishment on the seedlings was analysed by logistic regression, assuming a binomial distribution and a logit link function (Genmod, SAS/STAT 8.1, SAS Institute Inc., Cary, NC).

The effect of the inoculation treatments on seedling fine root biomass was analysed by variance analysis. We tested the impact of the *Phytophthora* treatments, the root rot basidiomycete treatments and their interaction. When the root rot basidiomycete \* *Phytophthora* treatments interaction was significant, we tested whether the combined impact of each individual root rot basidiomycete \* *Phytophthora* association was only additive using linear functions of the fitted parameters. More specifically, for experiment 1 for example, the fitted model was:

$$DW_n = \mu + \beta_0 \times C_0P_0 + \beta_1 \times C_0P_1 + \beta_2 \times C_1P_0 + \beta_3 \times C_1P_1 + \beta_4 \times C_2P_0 + \beta_5 \times C_2P_1 + \varepsilon_n$$

with  $DW_n$ , the seedling fine root dry weight,  $C_iP_j$ , variables taking the value 1 for seedling not inoculated with *P. cinnamomi* or *C. fusipes* ( $i = 0, j = 0$ ), seedlings inoculated

with *P. cinnamomi* ( $j = 1$ ) or seedlings inoculated with *C. fusipes* in December 1998 ( $i = 1$ ) or in October 1999 ( $i = 2$ ). To test for an additive effect of the October 1999 *C. fusipes* inoculation and the *P. cinnamomi* inoculation, we tested whether  $(\beta_0 - \beta_1) - (\beta_2 - \beta_3)$  was equal to 0.

Lesion sizes on the seedling taproot were analysed using generalised linear model assuming a gamma distribution of the lesion areas (Genmod, SAS/STAT 8.1). The gamma distribution is a function taking only positive values and a retrospective analysis of many inoculation experiments with *C. fusipes* (Marçais and Delatour, 1996, Camy *et al.* 2003a) shows that it very well suits this type of lesion size data. We used the identity function as link function. The model validity was checked using the deviance/degree of freedom ratio, by plotting deviance residuals against the linear predictor and with a half-normal plot (Collett, 1991). We tested the impact of the *Phytophthora* treatments, the root rot basidiomycete treatments and their interaction. Whether the combined impact of each individual root rot basidiomycete \* *Phytophthora* association was only additive was tested as explained previously using linear functions of the fitted parameters.

**Table 1.** Impact of inoculation treatments on seedling fine root biomass (variance analysis).

	Source	Df	F value	Pvalue
Experiment 1	<i>C. fusipes</i> treatment (control / inoculation Dec. 98 / inoculation Oc. 99)	2	4.79	<0.011
	<i>P. cinnamomi</i> treatment (control / inoculated)	1	25.40	<0.001
	<i>C. fusipes</i> * <i>P. cinnamomi</i>	2	2.99	0.056
Experiment 2	Root rot basidiomycetes treatment (control / inoculation <i>C. fusipes</i> / inoculation <i>A. mellea</i> )	2	2.80	0.066
	<i>Phytophthora</i> treatment (control / inoculation <i>P. cinnamomi</i> / inoculation <i>P. cambivora</i> )	2	58.31	<0.001
	Root rot basidiomycetes * <i>Phytophthora</i>	4	2.39	0.055

## Results

In the first experiment, with inoculation only with *P. cinnamomi* and *C. fusipes*, no foliar symptoms or visible lesions at the stem base developed during the experiment, and the seedlings grew vigorously with no significant difference in stem diameter increment between treatments ( $F = 1.49$ ,  $pvalue = 0.2034$ ).

Survival of *C. fusipes* in woody inocula was 88 %, as evidenced by successful isolation. Survival was similar for the 2 inoculation dates and whether the seedlings were inoculated by *P. cinnamomi* or not (likelihood  $ch2 = 1.74$ ,  $pvalue = 0.6282$ ). Bark lesions were observed on all seedlings for which *C. fusipes* had survived on the inoculum. *P. cinnamomi* was recovered from either bark lesions or from soil by baiting for all the *Phytophthora* inoculated seedlings.

There was a very significant effect of the *C. fusipes* inoculation treatments on both fine root biomass and lesion area on the taproot (Table 1 and 2, Fig. 1a and 1b). Seedlings inoculated by *C. fusipes* alone had either similar or higher fine root biomass compared to controls (Table 1, Fig. 1a). Lesions on the taproot were significantly larger on seedlings inoculated with either *P. cinnamomi* or *C. fusipes* than on non-inoculated control seedlings. *C. fusipes* induced significantly larger lesions on seedling taproots than *P. cinnamomi* while the area of the *C. fusipes* induced lesions was not significantly different for the two inoculation dates (Table 2, Fig. 1b). The interaction between the impacts of *C. fusipes* and



*P. cinnamomi* was very significant for taproot lesion area and was close to significance for fine root biomass (Table 1 and 2). The two pathogens induced more damage in combination than what would have been expected from the addition of their individual impacts when *C. fusipes* was inoculated in December 1998 for both reduction in fine root biomass ( $t = -9.71$ ,  $pvalue = 0.017$ , Fig. 1a) and lesion area on the taproot (likelihood  $ch2 = 6.3$ ,  $pvalue = 0.012$ , Fig. 1b). However, for fine root biomass the significant *C. fusipes* x *Phytophthora* interaction is linked to an increase of the fine root biomass for the seedlings inoculated only with *C. fusipes* compared to control. The average size of the taproot lesion induced in the combined inoculation treatment was 2.6 fold higher than what would have been expected from the addition of the two pathogens individual impacts. By contrast, when *C. fusipes* was inoculated in October 1999, after the *P. cinnamomi* inoculation, their combined impact was not significantly different from what would have been expected from the addition of their individual impact whether for the fine root biomass ( $t = -5.39$ ,  $pvalue = 0.189$ ) or the taproot lesion area (likelihood  $ch2 = -0.90$ ,  $pvalue = 0.083$ ).

**Table 2.** Impact of inoculation treatments on lesion area on the taproot (generalised linear model assuming a gamma distribution of lesion areas).

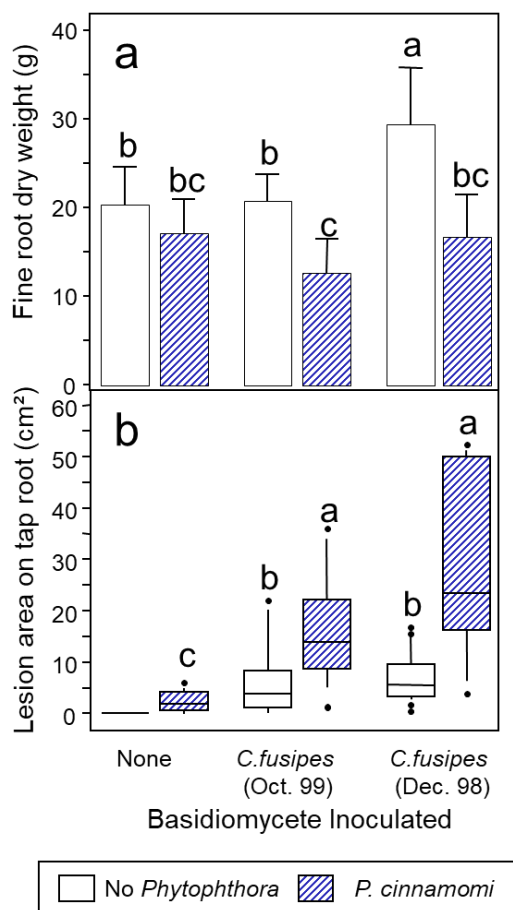
	Source	Df	Chi-square	Pvalue
Experiment 1	<i>C. fusipes</i> treatment (control / inoculation Dec. 98 / inoculation Oc. 99)	2	48.52	<0.001
	<i>P. cinnamomi</i> treatment (control / inoculated)	1	50.89	<0.001
	<i>C. fusipes</i> * <i>P. cinnamomi</i>	2	12.19	0.002
Experiment 2	Root rot basidiomycetes treatment (control / inoculation <i>C. fusipes</i> / inoculation <i>A. mellea</i> )	2	76.19	<0.001
	<i>Phytophthora</i> treatment (control / inoculation <i>P. cinnamomi</i> / inoculation <i>P. cambivora</i> )	2	72.15	<0.001
	Root rot basidiomycetes * <i>Phytophthora</i>	4	42.84	<0.001

In the second experiment, no foliar symptoms or visible lesions on seedling stems were observed. Again, no differences in diameter growth between the different inoculation treatments were significant ( $F = 1.75$ ,  $pvalue = 0.160$ ). However a significant impact of inoculation by *Phytophthora* was observed on height growth ( $F = 6.62$ ,  $pvalue = 0.002$ ), while *C. fusipes* and *A. mellea* had no impact ( $F = 0.20$ ,  $pvalue = 0.821$ ). *P. cinnamomi* and *P. cambivora* had a similar impact on height growth (height increment in 2 years of 150 cm, 145 cm and 188 cm respectively for seedlings inoculated with *P. cinnamomi*, *P. cambivora* or not inoculated with a *Phytophthora*).

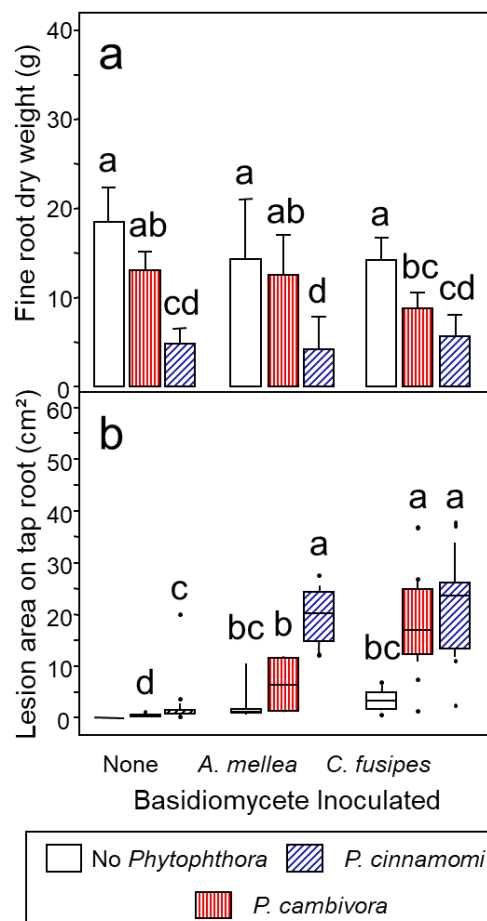
A significant difference in survival in the woody inocula was observed between *A. mellea* and *C. fusipes*, with survival of respectively 43 % and 96 % (likelihood  $chi2 = 1.74$ ,  $pvalue < 0.0001$ ). The survival of the root rot basidiomycetes was similar whether the seedlings were inoculated by *P. cambivora*, *P. cinnamomi* or not inoculated by a *Phytophthora* (likelihood  $ch2 = 3.57$ ,  $pvalue = 0.168$ ). Bark lesions were observed on all seedlings for which *C. fusipes* or *A. mellea* had survived on the inoculum. *P. cambivora* and *P. cinnamomi* were recovered from either bark lesion or from the soil by baiting for 90 % of the *Phytophthora* inoculated seedlings. The recovery was the same for the two *Phytophthora* species (likelihood  $chi2 = 1.08$ ,  $pvalue = 0.298$ ) and whether the seedling had been inoculated with a root rot basidiomycete or not (likelihood  $chi2 = 0.71$ ,  $pvalue = 0.700$ ).

There was a very significant effect of *Phytophthora* treatment on fine root biomass and of all inoculation treatments on lesion area on the taproot (Table 1 and 2, Fig. 2a and 2b).

Only *P. cinnamomi* significantly reduced fine root biomass compared to the control (Fig. 2a, reduction of the biomass of 60 %). Inoculation by *P. cinnamomi*, *C. fusipes* and *A. mellea* induced significantly larger lesions on the taproot than did *P. cambivora* (Fig. 2b). The interaction between the impact of root rot basidiomycetes, *A. mellea* and *C. fusipes*, and the *Phytophthora*, *P. cinnamomi* and *P. cambivora* was very significant for the lesion area on the taproot, but far less for the reduction in fine root biomass (Table 1 and 2).



**Fig. 1.** Impact of treatments on the root system of inoculated oak seedlings (experiment 1). a. fine root biomass (bars indicate the confidence interval on the mean). b. Lesion area on the taproot (the box indicates the 1<sup>st</sup> and 3<sup>rd</sup> quartile while the whiskers indicates the 1<sup>st</sup> and 3<sup>rd</sup> decile, the line in the box is the median).

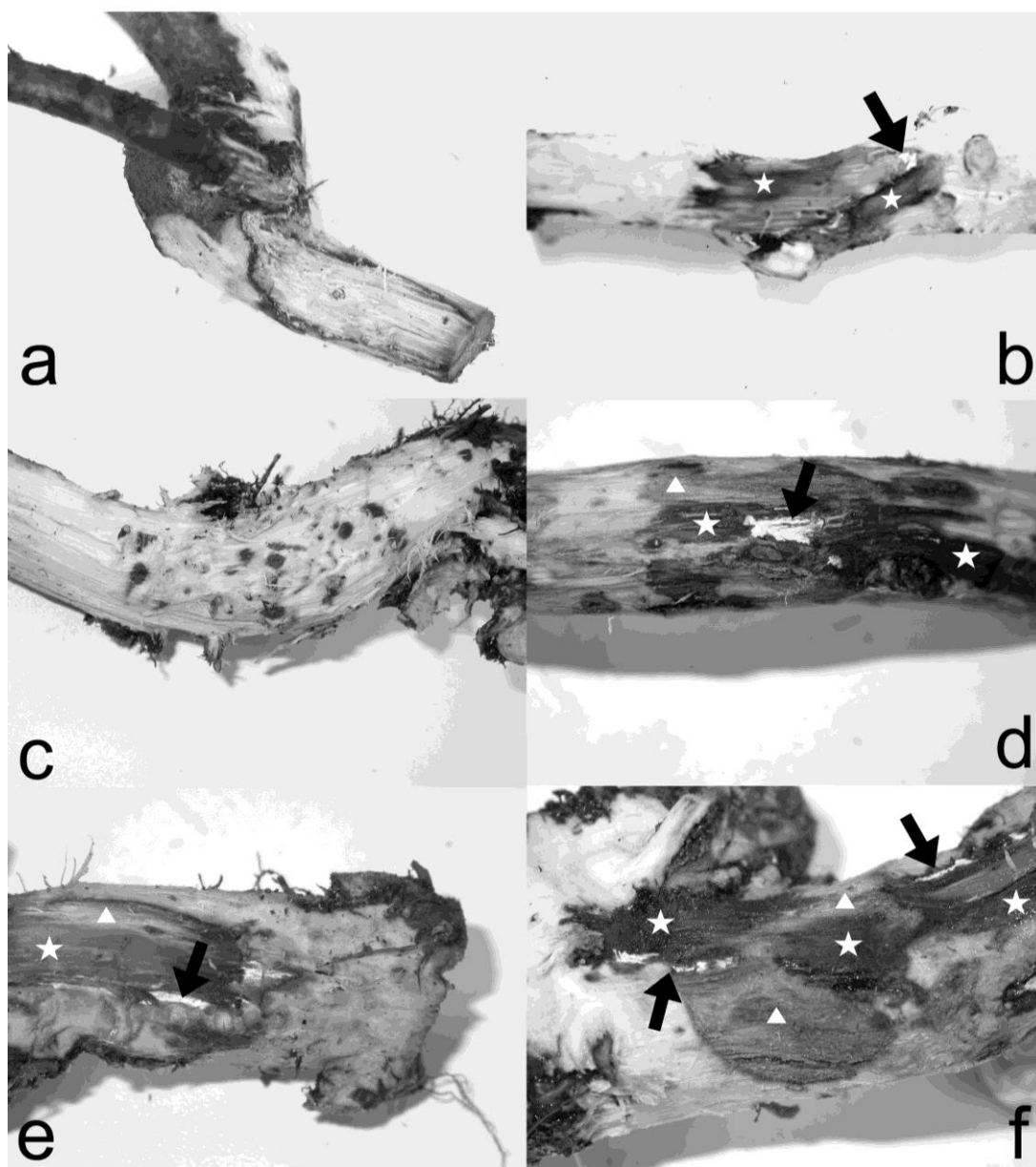


**Fig. 2.** Impact of treatments on the root system of inoculated oak seedlings (experiment 2). a. Fine root biomass (bars indicate the confidence interval on the mean); b. Lesion area on the taproot (the box indicates the 1<sup>st</sup> and 3<sup>rd</sup> quartile while whiskers indicates the 1<sup>st</sup> and 3<sup>rd</sup> decile, the line in the box is the median).

The reduction in fine root biomass induced in combination by the root rot basidiomycetes, *A. mellea* and *C. fusipes*, and by the *Phytophthora* spp., *P. cinnamomi* and *P. cambivora* was not significantly different from what would have been expected from the addition of their impact separately (respectively  $t = 0.34$ ,  $p\text{value} = 0.734$ ;  $t = 0.77$ ,  $p\text{value} = 0.441$ ;  $t = 1.83$ ,  $p\text{value} = 0.071$  and  $t = -0.75$ ,  $p\text{value} = 0.457$ ). The only combination close to significant was the *C. fusipes* x *P. cinnamomi* combination.



The impact of *C. fusipes* and *Phytophthora* spp. and of *A. mellea* and *P. cinnamomi* in combination on the taproot lesion area was much more than the addition of their impact separately (respectively likelihood  $\chi^2 = 13.35$ ,  $p$ -value  $< 0.001$ ; likelihood  $\chi^2 = 14.86$ ,  $p$ -value  $< 0.001$  and likelihood  $\chi^2 = 6.79$ ,  $p$ -value  $= 0.001$ , Fig. 2b). Depending on the root rot basidiomycete \* *Phytophthora* interaction, the average lesion size induced in combination was 3.6 to 4.9 fold higher than the addition of the average lesion size induced alone. By contrast the area of taproot lesion induced by *A. mellea* and *P. cambivora* together was not significantly different from what would have been expected from the addition of their impact separately (likelihood  $\chi^2 = 1.90$ ,  $p$ -value  $= 0.168$ ).



**Fig. 3.** Lesion on taproot induced by root rot basidiomycetes or *Phytophthora* spp. alone or in combination. A and B, *C. fusipes*; C, *P. cambivora*, note the numerous lesions remaining small in size; D, *A. mellea* + *P. cinnamomi*; E, *C. fusipes* + *P. cambivora*; F, *C. fusipes* + *P. cinnamomi*. arrow, *C. fusipes* or *A. mellea* mycelial fans; star, dark dry lesion associated with root rot mycelial fans; triangle, water-soaked olive brown lesion associated with *Phytophthora*.

When inoculated alone on the oak seedlings, the root rot basidiomycetes produced typical lesions with a central area with mycelial fans and outer margins with dry dark brown necrotic areas from which the pathogen could not be isolated (Fig. 3a and 3b). *Phytophthora* spp. produced numerous lesions that remained of a limited area located at the base of necrotic fine roots (Fig. 3c). When root rot basidiomycetes and *Phytophthora* were inoculated in combination, large areas of water soaked olive-brown dead bark tissues typical of *Phytophthora* induced lesions were present around necrotic tissues containing basidiomycete mycelial fans (Fig. 3d, 3e and 3f). *P. cinnamomi* was successfully isolated from water soaked olive-brown dead bark tissues in 85 % of cases (n = 54) while *P. cambivora* was isolated in only 13 % of the cases (n = 15). Almost all attempted isolation of *C. fusipes* and *A. mellea* from central necrotic area with mycelial fans were successful (65 out of 66).

## Discussion

We showed that, when inoculated on oak seedlings, root rot basidiomycetes such as *A. mellea* or *C. fusipes*, and *Phytophthora* are able to interact, inducing together much more damage than what would be expected from the addition of their impacts when inoculated separately. This was in particular the case for their ability to induce lesions on the taproots.

Indeed, the interaction between the studied basidiomycetes root rot and *Phytophthora* appeared to be most significant at the coarse root level. By contrast, their impact on fine root biomass was marginally different from what would have been expected from the addition of their separate impacts. The occurrence of the two types of pathogens associated within the same lesions suggests that they directly interact at the infection court to induce larger lesions. During the first experiment, this happened only when *P. cinnamomi* was inoculated after *C. fusipes*, which might suggest that the *Phytophthora* lesions are not acting by providing to the root rot basidiomycetes entry points into host tissues. This is in accordance with the very high inoculation efficiency, close to 100 %, observed when *C. fusipes* is inoculated alone in this work and in previous ones (Marçais and Delatour, 1996, Camy *et al.* 2003a). It also does not appear likely that the root rot basidiomycetes significantly provided entry points into host tissues to *Phytophthora* spp. Indeed, *Phytophthora* infected mainly the fine roots apices, and most lesions on the oaks taproot were induced at the basis of infected lateral rootlets when *Phytophthora* were inoculated alone. *Phytophthora* and in particular *P. cinnamomi* and *P. cambivora* are moderately aggressive on European oaks such *Q. robur* and *Q. petraea*. Indeed, although they very significantly decreased the fine root biomass of those oak species as was reported in the literature (Jung *et al.* 1999, Maurel *et al.* 2001, Jönsson, 2004), they do not have a strong impact on the seedling growth or survival. No impact on seedling growth of *Phytophthora* species has yet been observed on *Q. robur* (Maurel *et al.* 2001, Jönsson *et al.* 2003, Jönsson, 2004). We here showed an impact on height growth but not on stem diameter growth. During this work, *Phytophthora* spp. alone were unable to induce extensive lesions on the seedling coarse roots but when in the presence of *A. mellea* or *C. fusipes*, they produced large lesions on the taproot.

A more likely explanation of the interaction between the two types of pathogens would be that bark stressed at the periphery of root rot basidiomycete lesions was especially suitable for colonisation by *Phytophthora* spp., enabling *P. cinnamomi* and *P. cambivora* to develop large lesions. On the other hand, root rot basidiomycetes are able to colonise efficiently unstressed root tissues. In particular, *C. fusipes* was shown to be little influenced by host stress or vigour (Marçais and Delatour, 1996, Camy *et al.* 2003a). However, root rot basidiomycetes are slow growing and induced limited lesions in a 2-year period. It was shown that enlargement of *C. fusipes* induced root lesions on mature oaks was very slow in a six-year

period (Camy and Marçais, 2003c). By contrast, *Phytophthora* spp. are able to quickly induce large lesions provided that suitable conditions are met (Robin *et al*, 2001, Desprez-Loustau *et al*, 2006).

Whatever the mechanism of the interaction between the two types of pathogen was, the phenomenon was generic. Indeed, the interaction was significant in three of the four root rot basidiomycetes / *Phytophthora* combinations that we tested on oak seedlings. This interaction might prove to be very significant under natural forest conditions. Indeed, the impact of pathogens in tree declines and, in particular in oak decline have been underestimated (Thomas *et al*, 2002), in part because of the low impact of tested pathogens in inoculation trials. However, in natural situations, trees are never faced with one pathogen alone, but more typically to an array of several pathogens (specific to the host or generalist). Root rot caused by *Armillaria* and *Phytophthora* species are widespread in the oak forest (Balci *et al*, 2008, Jönsson, 2004, Tweery *et al*, 1990) and often occurs on the same trees (Camy *et al* 2003b). Thus, inoculation trials testing the impact of a pathogen alone on a tree species are probably inadequate to assess the impact of the pathogen when interactions with other frequently occurring pathogenic species are as important as shown during this work. This might explain why some *Phytophthora* species such as *P. quercina* have been shown to be significantly associated with oak decline in natural conditions although they show only moderate pathogenicity in inoculation trials (Jung *et al* 1999, Jung *et al*, 2000, Jönsson *et al*, 2003).

More generally, although microbial interactions in the rhizosphere have been extensively studied (Raaijmakers *et al*, 2009), few examples of interaction between pathogens on host plants have been documented in plant pathology (but see Nerey *et al*, 2010; Pieczarka *et al*, 1978). In particular, few positive interactions between pathogens have been documented although some cases have been reported in the ecology literature (Van der Putten *et al*, 1993, De Rooij-Van Der Goes, 1995; Holah and Alexander, 1999). However, positive interactions between pathogens could be important in explaining significant disease induction by pathogens showing low pathogenicity in inoculation trials or spatial variability in the impact of soil pathogens (Nerey *et al*, 2010).

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