European Journal of Plant Pathology **106:** 227–232, 2000.

Comparison of the susceptibility of *Quercus petraea, Q. robur* and *Q. rubra* to *Collybia fusipes*

Benoit Marçais and Olivier Caël
Laboratoire de Pathologie Forestière, Institut National de la Recherche Agronomique, 54280 Champenoux, France (Fax: +03383 39 40 69; E-mail: marcais@moncy.inra.fr)

Accepted 6 December 1999

Key words: root rot, oak, *Quercus petraea*, *Quercus robur, Quercus rubra, Collybia fusipes*, artificial inoculation

Abstract

Collybia fusipes is the cause of a root rot of *Quercus petraea* (sessile oak), *Q. robur* (pedunculate oak) and *Q. rubra* (red oak). This parasite is often reported to cause problems in stands of red oaks and field evidence suggests that this North American oak species is more susceptible than the two European oak species. Young saplings of the three oak species and of chestnut, *Castanea sativa*, in the glasshouse, and also mature sessile and red oaks growing in the same stand were inoculated with *C. fusipes* to compare their susceptibility. Red oak, both as young seedlings and mature trees, was more susceptible to *C. fusipes* than sessile oak. Chestnut seedlings were as susceptible as sessile oak. Susceptibility of pedunculate oak seedlings was intermediate between red oak and sessile oak. In one experiment this species was significantly less susceptible than red oak, and in the other it was as susceptible.

Introduction

Collybia fusipes (Bull. Ex Fr.) Quél. has been reported to cause root rot in beech, *Fagus sylvatica* L., pedunculate oak *Quercus robur* L. and sessile oak *Q. petraea* L., Hornbeam, *Carpinus betulus* L. and hazelnut, *Corylus avellana* L. (Buller, 1958; Kreisel, 1961; Delatour and Guillaumin, 1984; Guillaumin et al., 1985). The fungus is widespread in oak forests of north east France where it develops most often at the base of pedunculate oaks (Marçais et al., 1998b). It is reported by the forest health service of France to occur mainly on oak trees and to a lesser extent on chestnut, *Castanea sativa* Miller. Artificial inoculations showed that *C. fusipes* is a primary pathogen capable of infecting vigorous young oak saplings (Marçais and Delatour, 1996).

Red oak, *Q. rubra* L., is an important species in plantations in France. *C. fusipes* root rot often occurs on this oak species, particularly in the Pyrénées piémont where red oak is widely planted (Département de la Santé des Forêts, 1994). More detailed studies show that the parasite is able to infect as many as 60% of the trees in some mature red oak stands (Marçais et al., in press). Several field studies suggest that red oak is more prone to the disease than are pedunculate or sessile oaks. *C. fusipes* readily colonises and kills large roots of red oak while, on large roots of affected pedunculate and sessile oaks, the parasite usually infects large portions of the bark, but less commonly penetrates the cambial zone (Marçais et al., 1999). *C. fusipes* also causes problems in young stands of red oak but not in young stands of pedunculate and sessile oaks. Severely affected stands of pedunculate and sessile oaks are usually over 110 years old while severely affected stands of red oak can be as young as 50 years old.

The aim of this work was to use artificial inoculation to compare the susceptibility of red oak, pedunculate and sessile oaks to *C. fusipes*. This was done both on young seedlings and mature trees. Because chestnut is

occasionally reported as a host of this parasite, it was included in one of the experiments.

Materials and methods

Plant material and fungal isolates

In experiment I, five-year-old oak seedlings were inoculated. Pedunculate oaks were from the Azeirex provenance (Hautes-Pyrénées, France) and red oaks were from the Saint Florentin provenance (Deux-Sevres, France). They were transplanted into 9-L pots containing a mix of an equal volume of sand and forest soil (from Amance forest, Meurthe-et-Moselle, France) when two years old and subsequently maintained in a glasshouse. In experiment II, two-year-old seedlings of pedunculate, sessile and red oak and chestnut (from NE of France) were inoculated; they were transplanted in November 1997 into 9-L pots containing a mix of one part peat and two parts sand. The seedlings were kept in a glasshouse and watered daily. No artificial light was used in the glasshouse, heating was used to prevent frost in winter and temperature was prevented from rising above 25 °C in summer by cooling. Seedlings in experiment II were fertilised with 45 g of Nutricot per pot (13N–13P–13K) each year. *C. fusipes* strain C49, isolated from an infected red oak in 1992 at les Barres (Loiret, France) was used for experiments I and II.

In experiment III, mature oaks of about 50 years old were inoculated in a mixed stand of sessile and red oaks at Les Barres, which had originated by natural regeneration. The soil was podzolic loamy sand 60–90 cm deep over a layer of soft red clay, and showed traces of waterlogging at a depth of 30–80 cm. Trees selected for inoculation were dominants and co-dominants. Each was inoculated with three isolates of *C. fusipes*, C49, C41, isolated from an infected pedunculate oak in 1994 at Siarrouy (Hautes-Pyrénées, France) and C62, isolated from an infected sessile oak in 1994 at Amance.

Production of inoculum

Pieces of wood were colonised by *C. fusipes* according to the method described by Marçais and Delatour (1996). Briefly, stems of hazel, *Corylus avellana* L., 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 then added to cover half the height of the 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. Ten blocks of inoculum (0.5 × 0.5 cm) from a *C. fusipes* culture on malt agar (20 g l^{-1} malt Difco, 15 g l^{-1} agar) were aseptically added to the glass jar and incubated for 30–45 days at 23 °C. Subsequently, all liquid was drained from the jars with a syringe and they were further incubated at 23 °C for 7–9 months.

Inoculation experiments

In experiment I, 23 seedlings of both pedunculate and red oak were inoculated in May 1995 with the colonised hazel stem segments. Soil from the base of the seedlings was removed and the collar area was brushed carefully and washed with water. The inoculum was attached tightly to the unwounded collar at 2–5 cm under the soil level by a rubber band and the soil replaced. Four additional seedlings of each oak species were inoculated with uncolonised wood segments. Two years after inoculation, in August 1997, the seedlings were removed from the pots and the collar area and inoculum were examined. The condition of the inoculum was recorded: survival of *C. fusipes* was assumed when black crusts covered the inoculum and white mycelium was present underneath and/or when the wood had a bright orange colour. Otherwise *C. fusipes* was assumed to have disappeared from the inoculum. Isolations of the parasite were attempted both from the inoculum and the host tissues; these were washed under water, surface sterilised for 1 min in sodium hypochlorite at 3.75% active chlorine and rinsed three times in sterile water. The outer bark was removed and chips of dead bark or decayed wood were plated on MAT medium (10 g l^{-1} of malt Difco, 100 mg l^{-1} penicillin, 100 mg l^{-1} streptomycin, 250 mg l^{-1} thiabendazole and 15 g l^{-1} agar). Inoculation was recorded as successful when cambial death had occurred. Width and height of dead areas of bark and cambium were recorded and the surface of the lesions was estimated as the geometric mean of those two diameters ($\pi \times$ (height $\times$ width)/4).

In experiment II, 20 seedlings of all the four species, *Q. petraea*, *Q. robur*, *Q. rubra* and *C. sativa*, were inoculated in May 1997 as described in experiment I. Ten additional seedlings per species were inoculated with uncolonised hazel segments. In January 1999,

20 months after inoculation, the seedlings were removed from the pots and the inoculum and the collar area were examined.

In experiment III, ten mature trees each of sessile oak and red oak were inoculated in May 1995. Mean diameter at breast height of the trees was 23 cm. The base of the tree collar was exposed and three roots, 3–15 cm in diameter were selected for inoculation. The surface of the bark was brushed and washed with water and a hazel tree stem segment colonised by one of the three isolates, C41, C49 and C62 was fastened firmly in contact with the unwounded bark on one of the three selected roots. Each tree was inoculated with the three isolates. Six additional inoculations had uncolonised hazel stem segments attached to the roots. Root diameter was recorded and the soil was replaced. In March 1999, 4 years after inoculation, inoculated roots were excavated and examined for infection. Only inoculations resulting in a lesion with a depth of penetration in the bark of at least 2–3 mm were considered successful. It was difficult to be sure that smaller lesions were caused by *C. fusipes* because of their non-specific appearance and because isolation was usually not successful. The maximum length and width of lesions in the bark and in the cambium was recorded as well as their depth of penetration within the bark. Pieces of necrotic root bark and wood putatively colonised by the fungus as well as the inoculum segment were brought back to the laboratory for reisolation of *C. fusipes*. Isolates that were successfully recovered were paired on malt agar medium with a control isolate of C41, C49 and C62 to check that the isolate recovered was indeed the one that had been inoculated.

Data analysis

Lesion sizes were log transformed and subjected to an analysis of variance using the SAS Inc. software (1989). The lesions in the bark and the cambium were analysed separately. The lesion areas in the different species were compared using Newman–Keuls test. Infection success on mature trees was analysed by generalised linear analysis, using the procedure Genmod of SAS. A binomial distribution of the data was assumed and the logistic link function was used. 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).

Results

Experiment I: seedlings of pedunculate and red oak

A few months after inoculation (autumn 1995), waterlogging developed in the pots of a few seedlings. Because *C. fusipes* seems to be intolerant of anoxia *in vitro*, it was suspected that waterlogging would strongly influence the development of the disease. Steps were therefore taken to avoid further development of waterlogging in the pots. Indeed, at the end of the experiment, *C. fusipes* was recorded as surviving in only one out of the 13 hazel stem segments used as inocula in pots suffering from waterlogging and the seedling with the viable inoculum was the only one infected. Only one of the seedlings that escaped waterlogging had none viable inoculum; it also remained uninfected. Wherever *C. fusipes* had survived on the hazel stem segment, lesions were present on the seedlings. Thus, seedlings that lacked viable inocula at the end of the experiment were not used in further analysis. No seedlings died during the experiment. None of the eight control seedlings were infected. Eighteen pedunculate oaks and 15 red oaks were infected. In both oak species, lesions were typical of those described in the literature, i.e. bark necrosis with a greater extension near the outer bark surface than at the cambium level. The margins of the lesions were brown while the central part was orange in colour. White mycelium fans were usually present where the lesion reached the cambium. Lesions were circular in shape and tended to girdle the tap root. Isolations were successful from eight hazel stem segments (24%), and from nine *Q. robur* and six *Q. rubra* (45% of the successful inoculations). Size of the lesions in both the bark surface and cambium level was not significantly different between the two oak species (Student's t-test $t = 1.00$, df $= 31$, P value $= 0.324$). The mean surface area of the lesions in the bark was $11.0 \pm 4.2\,cm^2$ for the pedunculate oaks and $8.5 \pm 3.2\,cm^2$ for the red oaks. At the cambium level, the mean lesion area was $3.4 \pm 1.2\,cm^2$ for the pedunculate oaks and $3.0 \pm 1.2\,cm^2$ for the red oaks.

Experiment II: seedlings of chestnut and pedunculate, red and sessile oak

No seedlings died during the course of the experiment. At the end of the experiment, *C. fusipes* was recorded as viable on 70 hazel stem segments (88%) and was isolated from 69 of them. Where the parasite was still

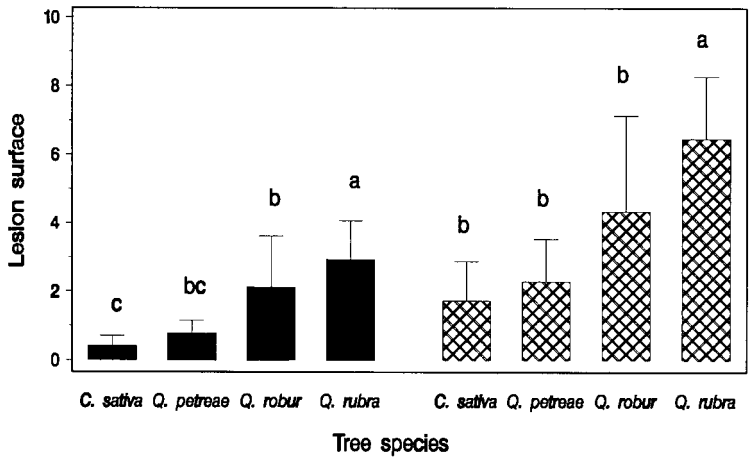

Figure 1. Susceptibility of three oak species and of chestnut seedlings to *C. fusipes* as indicated by the lesion size (cm^2) in the cambium (■) and outer bark (⊠). Species labelled by the same letter within each lesion category are not statistically different. The confidence interval of the mean is given.

present in the wood segment at the end of the experiment, the inoculation was successful, i.e. the lesion reached the cambium. This situation applied to 66 of the inoculated seedlings (94%): 15 chestnuts, 16 sessile oaks, 17 pedunculate oaks and 18 red oaks. Successful infection did not occur on seedlings where *C. fusipes* had not persisted in the inocula, and those seedlings were not included in further analysis. *C. fusipes* was reisolated from 56 of the infected seedlings (85%). The size of the lesions in the outer bark and in the cambium differed significantly between the different species (Figure 1). Lesions were significantly larger on red oak than on the three other species. On average, lesions on pedunculate oak were larger than on sessile oak and chestnut, particularly at the cambium level. However, the difference between sessile and pedunculate oaks was not significant. Sessile oaks and chestnuts had lesions of a very similar size.

Experiment III: mature red and sessile oak trees

After about four years in the soil on mature trees, *C. fusipes* was still recorded as viable on 46 hazel stem segments (77%) and was reisolated from 41 of them (68%). Lesions were not present on any of the 14 inoculation points where *C. fusipes* was not recorded as alive or reisolated from the hazel stem segment. Lesions were present on 27 of the inoculated roots and the parasite was isolated from all the lesions. The morphology of the lesions was typical of *C. fusipes* infection on the two oak species, i.e. bark necrosis of an orange colour with white mycelial fans scattered within the necrotic tissues. The lesions were active with little sign of healing or callusing. In three cases on red oaks, a small root of about 1 cm diameter in the vicinity of the inoculation point was found dead and colonised by the *C. fusipes* isolate used for the inoculation although it was not connected with the main lesion. Five lesions yielded *C. fusipes* isolates that were somatically incompatible with the one that had been used for inoculation so only 41 inoculations could be used for the analysis. Infection success was significantly different on the two oak species (Table 1). About 82% (14 out of 17) of the inoculations resulted in successful infections on the red oaks, but only 33% (8 out of 24) were successful on the sessile oaks. Root diameter and the *C. fusipes* isolate did not significantly affect the infection success. Neither oak species nor isolate significantly influenced the lesion size of the

Table 1. Factors affecting the infection success on mature sessile and red oaks (experiment III): analysis of deviance

Source	Deviance	Df	χ^2	*P* value
Intercept	56.22	0	—	—
Root diameter	54.25	1	1.974	0.160
Tree species	45.58	1	8.671	0.003
Isolate	44.18	2	1.401	0.496
Species × isolate	43.78	2	0.400	0.819

Note: Model deviance was 43.78, with 34 df and a probability of 0.122.

Table 2. Factors affecting the lesion size of successful infections on mature sessile and red oaks (experiment III): analysis of variance

Source	Df	Mean Square	*F* value	*P* value
Model	5	0.73	0.50	0.775
Error	18	1.48	—	—
Tree species	1	0.78	0.63	0.477
Isolate	2	1.63	1.11	0.352
Species × isolate	2	0.36	0.24	0.788

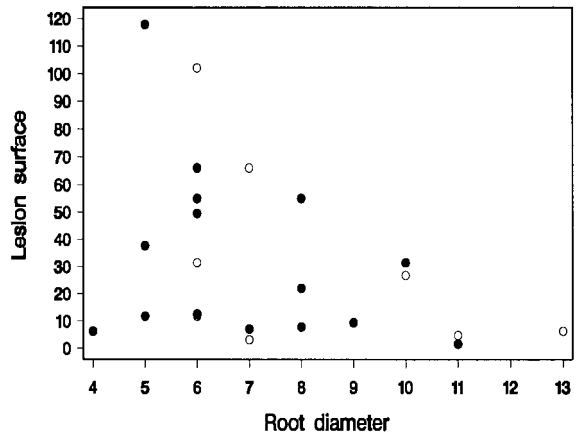

Figure 2. Lesion size (cm^2) caused by *C. fusipes* in the bark of roots of different diameter (cm) on mature sessile (○) and red (●) oak trees.

successful infections in the bark (Table 2). The surface area of the lesions at the bark level was $30.1 \pm 21\ cm^2$ for sessile oaks and $32.7 \pm 16.4\ cm^2$ for red oaks. However, lesion size was significantly and negatively correlated with root diameter; lesions were larger on small diameter roots (Spearman correlation coefficient 0.421, *P* value = 0.046, Figure 2). Despite a similar lesion size in the outer bark of the two oak species, penetration of the parasite to the cambium was greater in the red oaks than in the sessile oaks. Twelve of the 14 successful infections reached the cambium in the red oaks (lesion size in the cambium was $10.6 \pm 6.2\ cm^2$) while one out of eight did so in the sessile oaks.

Discussion

Results in the seedling experiments clearly show that red oaks are more susceptible to *C. fusipes* than sessile oaks, that pedunculate oaks are intermediate between red and sessile oaks, being in one experiment of a similar susceptibility as red oak and in the other of a lower susceptibility and that sweet chestnut appeared to have a susceptibility to *C. fusipes* similar to sessile oak.

The results of inoculations on mature trees confirmed the higher susceptibility of red oaks compared to sessile oaks. Infection success (% of root infected) as well as lesion size was higher for red oaks. The capacity of mature sessile oaks to prevent the establishment of the parasite at the cambium in this experiment is consistent with observations made on naturally infected trees (Marçais et al., 1999). These showed that, even if sessile and pedunculate oaks have extensive lesions at the root collar, cambial infection on the large roots is often limited. In contrast, *C. fusipes* readily infects the cambial tissues of mature red oaks and kills the larger roots. Small roots of all three oak species were readily killed by the parasite. Mature pedunculate oaks could not be compared with the other oaks because none were present in the stand that was used. However, in another experiment, mature dominant pedunculate oaks were inoculated and, after 2.5 years, approximately three-quarters of the inoculations had resulted in infection and half the successful infections had reached the cambium (Marçais et al., 1998b). So, mature pedunculate oaks can show quite a high susceptibility to *C. fusipes*. This agrees with the finding that most of the stands where *C. fusipes* was associated with poor health were pedunculate oak stands. Similarly, in a large-scale survey, the parasite was found to occur more frequently on *Q. robur* than on *Q. petraea* (Marçais et al., 1998b).

In experiment III on mature red oak trees, the infection success was high, but the lesion sizes remained limited after four years. The parasite did not kill any of the inoculated roots, although it did occasionally kill a small diameter root in the vicinity of the inoculation point. However, the lesions were still active and the trees were apparently not able to wall-off the parasite in the bark tissues. It is quite surprising that the fungus was not very aggressive on the red oaks of Les Barres because *Collybia* root rot is very active in this stand: it is present on 60% of the red oaks and 25% of them are severely infected, with over half of their root system destroyed (Marçais et al., 1999; in press). Moreover, each tree is usually infected by one large clone of *C. fusipes* (Marçais et al., 1998a), indicating that the parasite is able to spread readily through the root system of a tree. Inoculations of mature trees with other primary root rot pathogens such as *Armillaria mellea, A. ostoyae* or *Phellinus weirii* usually give similar

results, with lesions of the same order of magnitude in 2–3 years (Davidson and Rishbeth, 1988; Entry et al., 1991; Goheen and Hansen, 1994; Hansen, 1986; Wahlström and Barklund, 1994). Perhaps *C. fusipes* requires a very long time to infect and destroy the root system of mature trees. The infection process may start very slowly, but speeds up significantly when the parasite has killed and colonised large roots and can develop a large inoculum base. The infection process could be aided by a faster spread of the pathogen along small diameter roots, as they show a higher susceptibility.

The higher susceptibility of red oak to *C. fusipes*, compared to sessile oak and possibly to pedunculate oak confirms that this parasite represents a risk for red oak stands in Europe. Because of the slow rate of disease development, the impact in first generation stands, generally free of the disease at the outset, might not be high, with most of the trees only becoming diseased late in life. This could explain why, despite the abundance of the parasite in affected stands, decline of the trees has not yet been reported (Marçais et al., in press, Département de la Santé des forêts, 1994). Second generation stands of red oaks following stands that were heavily infected could be especially at risk because they would be exposed to a high inoculum potential from the outset. It seems that in such situations, as many as half of the trees can be infected after 20 years (Marçais, unpublished results).

Acknowledgements

We want to thank G. Maréchal for his technical assistance and P. Wargo and C. Delatour for reviewing the manuscript. Part of this work was done thanks to the support of the Office National des Forêts, France.

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