Measuring the impact of Collybia fusipes on the root system of oak trees

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Measuring the impact of *Collybia fusipes* on the root system of oak trees

Benoît Marçais*, Olivier Caël, Claude Delatour

Unité des ecosystèmes forestiers, laboratoire de pathologie forestière, Inra, 54280 Champenoux, France

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Abstract – This work describes the aetiology of *Collybia fusipes* root rot and the impact of the parasite on the structure of mature oak root systems. The collar roots were examined and rated for *C. fusipes* infection at the base of 26 *Quercus robur* and 20 *Q. rubra* trees. Trees were then felled and their root systems were up-rooted with a mechanical shovel. Number and infection status of the roots present were recorded at 40, 60 and 80 cm from the trunk base. *C. fusipes* drastically reduced the number of living roots. At 80 cm from the trunk base, on cylinder 3, *Q. robur* rated as lightly and heavily damaged had only 52 and 25 %, respectively, the frequency of living roots of undamaged trees; the values were 72 and 25 %, respectively, for lightly and heavily damaged *Q. rubra* trees. *C. fusipes* impacted especially the vertical roots just under the collar. (© Inra/Elsevier, Paris.)

***Quercus* / *Collybia fusipes* / root rot / incidence**

Résumé – Mesure de l'impact de *Collybia fusipes* sur le système racinaire des chênes. Ce travail décrit l'étiologie du pourridié à *Collybia fusipes* et l'impact du parasite sur le système racinaire des chênes. Le départ des racines maîtresses a été examiné et noté pour l'infection par la collybie chez 26 *Quercus robur* et 20 *Q. rubra*. Les arbres ont ensuite été abattus et leur système racinaire extrait avec une pelle mécanique. Le nombre de racines présentes et leur état sanitaire ont été déterminé à 40, 60 et 80 cm du collet. La collybie diminuait fortement le nombre de racines vivantes présentes. Les arbres gravement attaqués à l'examen précédant l'arrachage n'avaient plus, à 80 cm de la base du tronc, que 25 % du nombre de racines vivantes des arbres non attaqués. Ceux jugés faiblement attaqués n'en avaient plus que 52 à 72 % selon l'espèce. La destruction par le parasite touchait plus particulièrement les racines verticales situées sous le tronc. (© Inra/Elsevier, Paris.)

***Quercus* / *Collybia fusipes* / pourridié / impact**

1. INTRODUCTION

Oak decline has been a chronic problem in Europe in the past decades. The causes of this decline are not completely clear. Climatic stress, in particular droughts, are widely accepted to be important factors as well as defoliation by insects [4, 5]. Fungal parasites have also been shown to be involved. One of them, *Collybia fusipes* (Bull. ex Fr.) Quel. is a basidiomycete that has been known by European mycologists for a long time, but has only recently been reported to be a pathogen of mature oak roots [1, 3]. It was often found associated with declining oaks in France [2]. Moreover, it was shown to behave as a primary pathogen on *Quercus robur* L. (pedunculate oak) and *Q. rubra* L. (red oak) seedlings [8]. *C. fusipes* can also be found on *Castanea sativa* Miller, *Carpinus betulus* L., *Corylus avellana* L. and *Fagus sylvatica* L.

* Correspondence and reprints
marcais@nancy.inra.fr

As very little was known about this apparently common root rot fungus, research was started to determine the impact of *C. fusipes* in oak forests in France. Preliminary results showed that the fungus is frequently present, most of the time not in connection with decline [6]. In two of the three surveyed forests, 20–30 % of the trees with *C. fusipes* fruit bodies had poor crown conditions, while in the third, only 1 % of the trees with fruit bodies had poor crowns. Other observations suggest that the relationship between crown condition and root infection in *C. fusipes* infected trees is poor. In some examined red oaks where most of the main lateral roots were dead, the crown did not show any pronounced decline in the following 7 years (Delatour, unpublished results). Also, the collar roots are apparently not often killed in pedunculate oak and *Q. petræa* (Matt.) Liebl. (sessile oak). They can have bark heavily infected by *C. fusipes*, but still exhibit little evidence of cambial death. Therefore, it is not very clear whether the parasite is having a significant impact on the tree (e.g. radial growth, decline status). To clarify this question, it is necessary to quantify the disease in the roots. Therefore, we wanted to know if we could predict the infection status of the entire root system using a quick rating of the collar roots. For that, we examined the main collar roots of a sample of pedunculate and red oak trees and rated them for infections, then up-rooted them and studied the entire root system in more detail.

2. MATERIALS AND METHODS

2.1. Study plots

Trees were sampled in two stands from central and north-eastern France. *Quercus rubra* trees were located at Les Barres (Loiret). The soil consisted of a 60–90 cm layer of podzolic sand, over a layer of soft red clay in which a fairly large number of roots was present. In winter the water table is close to the surface. There was no major physical limit to vertical root growth in this soil. Tree age ranged between 40 and 70 years. The pedunculate oaks were located at Les Aynans (Haute-Saône), in a pure *Q. robur* stand. The soil consisted of a 0.5–1 m layer of sandy loam over a deep layer of gravel. Most roots over 1 cm in diameter did not extend into the gravel. Tree age ranged from 80 to 100 years. Incidence of *C. fusipes* in both stands was known to be high, with 43 % of the trees with fruit-bodies at the trunk base at Les Barres and 25 % in Les Aynans [6, 7].

2.2. Sampling of the trees

About 35 trees with diameter of 20–33 cm at breast height were chosen in each stand. On most trees, *C. fusipes* infection could be detected quickly by scraping the collar roots with a knife to reveal bark necrosis. Root systems were studied for *C. fusipes* infection in the following way: the root collar was partially excavated to a depth of 20–30 cm and a distance of 80–100 cm from the trunk base. The infection status of each major root was assessed as: 0) no necrosis detected; 1) necrosis present, but covering less than half of the root circumference (usually superficial for *Q. robur*, with penetration of *C. fusipes* in the bark of about 1–2 mm); 2) necrosis covering one side of the root entirely (usually 2–5 mm thick for *Q. robur*); 3) *C. fusipes* infection over the entire root circumference but root still alive (usually more than 4–5 mm thick for Q. *robur)*; 4) root dead with decayed wood. Diameter of the root was measured at about 10 cm from the trunk base. The root infection index of a tree was computed as: Σ(root diameter $\times$ root rating)/Σ(root diameter). This index therefore takes values from 0 to 4. Trees with a rating of 0–0.5 will be referred to as 'not damaged', having no or very limited infection by *C. fusipes*. Those with a rating of 0.5–2 and 2–4 will be referred to as lightly and heavily damaged trees, respectively.

A sub-sample of 20 red oaks and 26 pedunculate oaks was selected for further study. It consisted of nine trees undamaged (five *Q. robur* + four *Q. rubra*), 21 lightly damaged (12 *Q. robur* + nine *Q. rubra*) and 16 heavily damaged (9 *Q. robur* + 7 *Q. rubra*). Trunk diameter at breast height was recorded. Tree crowns were rated as damaged if large dead branches were present in the upper part of the crown, undamaged otherwise. This rating was performed in March, when trees had no leaves.

2.3. Study of root system structure and of infection status

Trees were felled to leave a stump 40 cm tall. A trench 1 m deep and about 2 m radius was dug around each stump. The root system was then extracted by pulling up on the stump with a mechanical shovel and vigorously shaking it to remove most of the soil (*figure 1a*). The root systems were washed with water at low pressure and all small roots (< 1 cm in diameter) were cut and discarded.

Root system structure was studied using a method adapted from Nielsen [10]. Briefly, root systems were placed upside down on a board and characterised at the level of three imaginary surfaces located at increasing

Figure 1. Up-rooted *Q. rubra* root systems. a) Mechanical shovel up-rooting the tree. b–d) Root systems at different stages of infection by *C. fusipes*. All roots under 1 cm in diameter have been removed. B) Root system lightly damaged by *C. fusipes*; lesions are present only on the left side of the picture where broken and rotten roots can be observed. c) Intermediate stage of infection by *C. fusipes*. Most of the vertical roots are killed and rotten. d) Root system heavily damaged with all main roots dead. The poor development of the right part of the root system indicates that the attack by *C. fusipes* of that part might be very old.

distance from the trunk base, cylinders 1, 2 and 3 (*figure 2*). Cylinders were 80, 120 and 160 cm in diameter and extended 40, 60 and 80 cm below ground, respectively. The vertical part of the cylinder was referred to as the wall and the horizontal part as the floor. Cylinders were outlined by sticks marked at the level of the floor and placed at the level of the wall (*figure 1b*, *c*). All the roots passing through cylinder 3 were cut at the level of cylinder 3 floor or wall, and the position, diameter and infection status of each root cross-section were recorded. Position of the root sections was recorded as: i) floor or wall of the cylinder; and ii) azimuth (position within eight compass sectors). The largest and smallest diameters of each root section were measured and the root cross-section was estimated as the geometric mean of those two diameters. Finally, the infection status of the section was recorded as healthy, infected or dead. This procedure was repeated for cylinder 2 and then for cylinder 1 (*figure 2*). At cylinder 1, the roots were cut at about 10–20 cm from the place where they join the stump.

Thirty-seven root pieces with lesion margins were sampled from six different root systems (five *Q. rubra* and one Q. *robur*). They were taken to the laboratory, washed under water; surface sterilised for 1–2 min in sodium hypochlorite at 3.75 % active chlorine and rinsed twice in sterile water. Chips of dead bark and pieces of the black cord-like fungal structures found on the root surface were placed 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, 15 $g.L^{-1}$ agar).

2.4. Data analysis

The frequency (no. per m^2) and total cross-section area of living roots was computed for each of the three cylinders and for wall and floor of the cylinder. The root

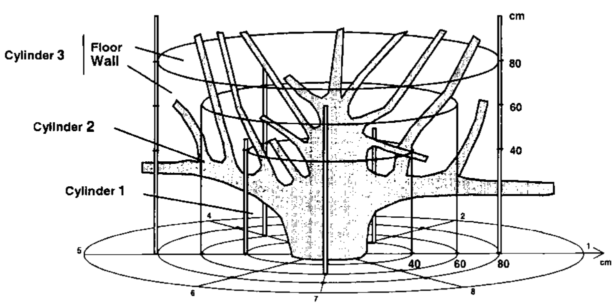

Figure 2. Illustration of the system used to describe and measure the structure, root abundance and infection status of uprooted trees.

frequency for a cylinder floor was considered to be zero if the absence of roots could not be explained by an obvious local limit to root extension. When it could be explained by a clear local limit to root extension, i.e. all roots suddenly changing direction or branching to small diameter roots at a lower depth, then the data were considered missing. This occurred only for trees from Les Aynans. Root frequencies and proportion of root dead were log transformed and analysed by linear regression analysis using SAS Inc. software [11]. Differences in root frequencies between trees with crown damaged or undamaged were analysed by Student's *t*-test.

3. RESULTS

On standing trees, lesions of *C. fusipes* could be easily detected on the major roots as patches of dead bark that were orange in colour with small white fans of mycelium scattered within the necrotic inner bark, as was previously mentioned by Guillaumin et al. [3]. The development and appearance of lesions on pedunculate oaks were very different from lesions on red oaks. Lesions could be very extensive on pedunculate oak roots before the cambium was attacked (*figure 3a*). Severely attacked large roots had their entire surface covered with thick bark lesions, while most of the cambium appeared to be still alive. A hypertrophy response of the bark to infection could be observed as the infected bark was usually thickened up to 3–4 cm, most of it being necrotic. The cambium was first reached and killed at several scattered locations, then areas of dead cambium enlarged and coalesced, and the root was ultimately killed. By contrast, on red oak *C. fusipes* induced lesions in the bark were always associated with a similar amount of cambial death. Also, no thickening of attacked bark tissues was observed (see *figure 3b*).

C. fusipes was isolated from 68 % of the sampled symptomatic root pieces. *Armillaria* was isolated from two root pieces of one of the *Q. rubra* trees from which *C. fusipes* was also recovered. It was determined as *A. mellea* (Vahl: Fr.) by pairing with testor monokaryons of known *Armillaria* species. The extension of *A. mellea* in the root system was far less than that of *C. fusipes,* and it was a located on small root at the periphery of the root system. No other pathogenic basidiomycete was isolated. At the lesion margin, an area of brown necrotic bark 1–10 cm wide was usually present between the typical orange coloured infected bark and the healthy bark tissues. Isolation success of *C. fusipes* from the brown necrotic tissue was poor (six successful isolations out of 30 attempts). Black appressed cord-like structures (about 0.5 mm in diameter) with globular thickenings (about 2–3 mm) were observed on the surface of attacked roots

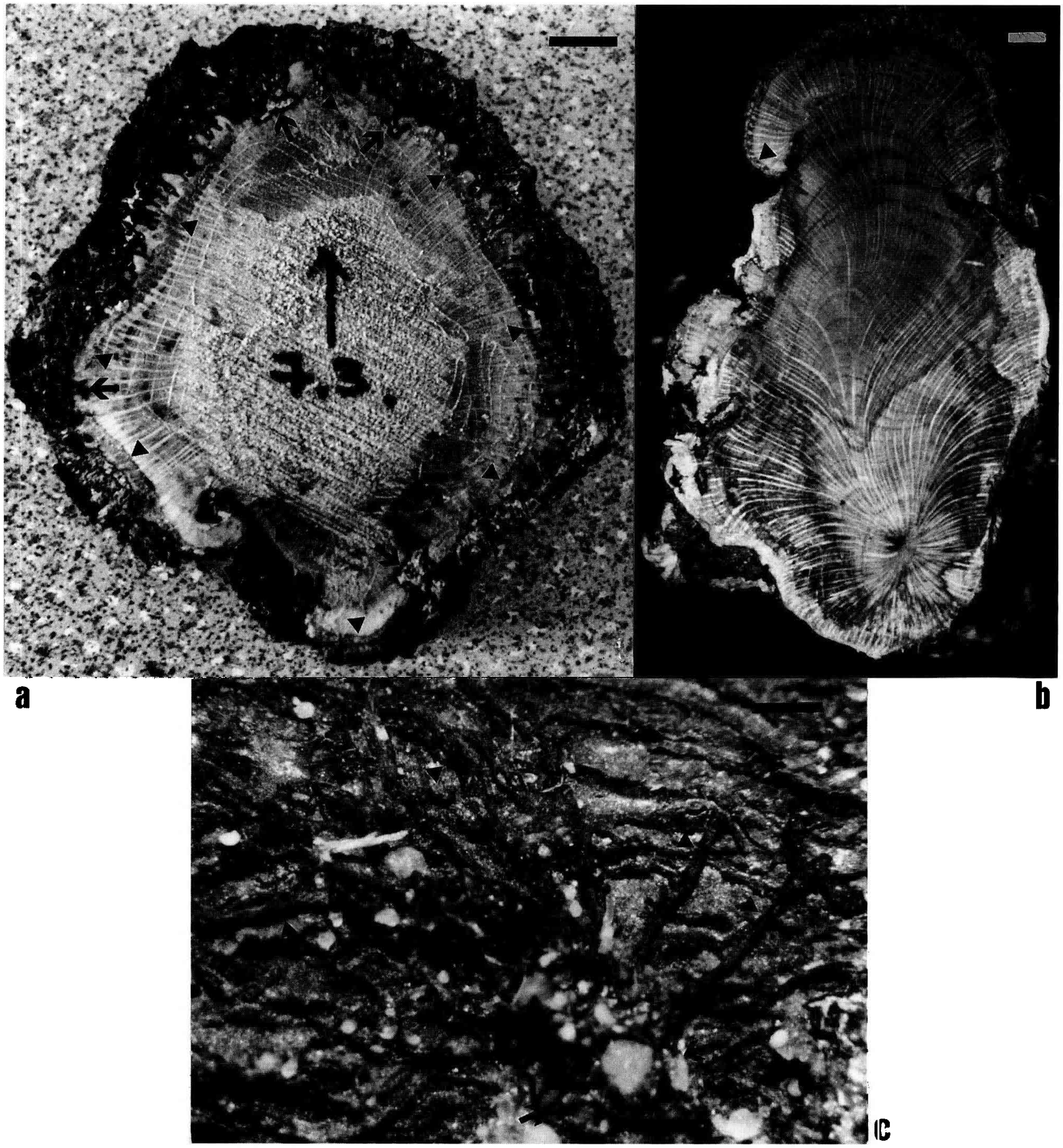

Figure 3. *C. fusipes* lesions on oak roots. a) Transverse section of a *C. fusipes* infected *Q. robur* root. Despite presence of bark lesions all around the root, cambium (▼) is killed by *C. fusipes* only at a few places (➔); note the abnormally thick inner bark (bar = 1 cm). b) Transverse section of a *C. fusipes* infected *Q. rubra* root. Little of the cambium is alive (▼) (bar = 1 cm). c) *C. fusipes* cords on the surface of the root (▼). Some parts of the cords are thickened (➔) (bar = 2 mm).

both of pedunculate oak and red oak (*figure 3c*). This ectotrophic mycelium was present over all the necrotic bark. In particular, it was present over the brown necrotic tissues, closer to the lesion margin than the orange coloured infected bark. *C. fusipes* was difficult to isolate from the very thin cords (four of 96 attempts). However, it was isolated more frequently from the thickened part of the cord structures (14 of 34 attempts).

On lightly damaged trees of both species, all lesions were found in the root collar area, either on the collar itself, or on a large horizontal root near the trunk base. No *C. fusipes* lesions were on peripheral roots in the absence of root collar infection. As the infection increased, lesions quickly reached the part of the root system just beneath the trunk and apparently spread from there to the entire root system. On seven out of the nine lightly damaged red oaks investigated, lesions were clustered on one part of the root system (*figure 1b*). Two red oak trees had infections located in two distinct parts of the root system that were not connected. In contrast, no unique point where the infection might have started could be distinguished on the lightly damaged pedunculate oaks and small infections were usually present on several scattered large collar roots.

Despite a similar level of bark infection in the two oak species, only 5 % of the collar roots more than 10 cm in diameter were killed on the damaged pedunculate oaks, while 32 % were killed on the damaged red oaks. In contrast, the proportions of small roots (diameter < 10 cm) found dead and colonised by *C. fusipes* on the damaged pedunculate and red oaks were similar (28 and 29 %, respectively). The total proportion of dead roots was much higher for trees of both species with high root infection index (*figure 4*), whereas the frequency of living roots decreased (*figures 1b, c* and *5*, *table I*). At 80 cm from the trunk base, on cylinder 3, *Q. robur* rated as lightly and heavily damaged had only 52 and 25 %, respectively, the frequency of living roots of undamaged trees; the values were 72 and 25 % for lightly and heavily damaged *Q. rubra* trees, respectively. In the most heavily damaged trees, the only remaining living roots were recently formed adventitious roots while all the original root system was killed (*figure 1d*). For the wall of cylinders 1–3 and for the floor of cylinder 1, there were no significant differences between the two oak species in the relationship between frequency of living roots and infection index, and the data were pooled for the regression analysis. The decrease in living root frequency was of a similar order of magnitude in wall of

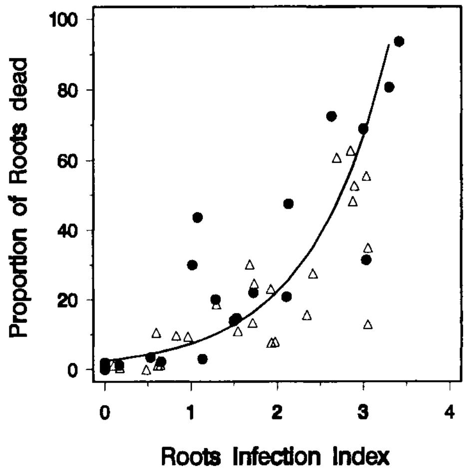

Figure 4. Proportion of dead roots on the up-rooted trees. △ = *Q. robur*; ● = *Q. rubra*; —— = regression line for *Q. robur* and *Q. rubra*. The data were pooled because the differences between the two oak species were not significant. Log (% dead root) = 0.9 + 1.1 × root infection index.

Table I. Relationship between root frequency at walls and floors of the three cylinders and root infection index.

		Oak species	Relationship*	R^2
Cylinder 1	wall	*Q. robur* + *Q. rubra***	$RF = 37.3 \times e^{-(0.44 \times RII)}$	0.50
	floor	*Q. robur* + *Q. rubra*	$RF = 47.9 \times e^{-(0.86 \times RII)}$	0.49
Cylinder 2	wall	*Q. robur* + *Q. rubra*	$RF = 32.1 \times e^{-(0.58 \times RII)}$	0.61
	floor	*Q. robur*	$RF = 13.9 \times e^{-(0.40 \times RII)}$	0.20
	floor	*Q. rubra*	$RF = 34.5 \times e^{-(0.78 \times RII)}$	0.47
Cylinder 3	wall	*Q. robur* + *Q. rubra*	$RF = 16.6 \times e^{-(0.47 \times RII)}$	0.55
	floor	*Q. robur* and *Q. rubra*	no relationship	0.09 and 0.12

* RF = root frequency in no.m^{-2}; RII = root infection index derived from observation of the major horizontal collar roots of the tree before up-rooting.
** When differences between the two oak species were not significant, data were pooled.

cylinders 1, 2 and 3 (*table I*). Frequency of living roots decreased very quickly for low root infection index (*figure 5 a–c*). Just beneath the trunk, on the floor of cylinder 1, the decrease was more drastic (*figure 5d*). At greater depth, on the floor of cylinder 2, the undamaged red oaks had a higher frequency of roots, compared to undamaged pedunculate oaks. Decrease in root frequency at that level was greater for *Q. rubra* attacked by *C. fusipes* than for damaged *Q. robur* (*figure 5e*). On the floor of cylinder 3, root frequency was low for all trees and even some undamaged trees had no roots larger than 1 cm in diameter at that level. No relationship between infection index and root frequency was evident at that depth (*figure 5f*).

Trees with major dead branches in the crown had much fewer living roots compared to trees with undamaged crowns (*table II*). However, the relationship between root infection and crown damage was not very strong because some trees heavily damaged by *C. fusipes* and with few living roots had crowns with no major damage, i.e. no dead branches (*table II*).

4. DISCUSSION

For both oak species, the root infection index was well correlated with the frequency of living roots left on the tree, and thus adequately represented the state of the entire root system. The main reason for this was that the part of the root system just beneath the trunk is colonised by *C. fusipes* early in the infection process and so the root infection index, measured close to the trunk, reflects well what occurs deeper in the soil. Indeed, if *C. fusipes* causes major damages in all the root system, its maximum impact occurred on the floor of the first cylinder, 40 cm below soil level (*figure 5d*).

On lightly damaged trees, the infection was always limited to the central part of the root system, and thus appeared to start from the collar area. This is in agreement with previous work showing that in infected stands each tree was attacked by a different genet of *C. fusipes* and thus the fungus does not spread from tree to tree by root contacts [7].

C. fusipes lesions, as described in this work, correspond well to what was observed on inoculated young and mature oaks ([8]; Marçais, unpublished results). In particular, both the ectotrophic mycelium (cord-like structure) and the brown necrotic area at the lesion margin were present in artificially induced infections. *C. fusipes* spreads at the bark surface, and secondarily toward the cambium. Perhaps the ectotrophic mycelium is involved in the spread of the fungus at the bark surface, as for *Phellinus noxius* G.H. Cunn., *P. weirii* (Murr.) Gilberson and *Rigidoporus lignosus* (Kl.) Imaz [9, 12]. However, the ectotrophic mycelium is always a few centimetres back from the lesion margin.

Root destruction by *C. fusipes* is obvious in both *Q. robur* and *Q. rubra*. The proportion of roots dead was sometimes very high in the heavily damaged trees investigated, and the total living root biomass was drastically reduced, which is in good agreement with the results of Guillaumin et al. [3]. Although pedunculate oaks showed greater capacity than the red oaks to keep the cambial area of the large horizontal collar roots alive, their smaller roots were killed by *C. fusipes* as readily as those of *Q. rubra*. As a result, the root system of heavily damaged pedunculate oaks was reduced to a skeleton of large, infected, but living and undecayed large roots. This might explain why, despite widespread occurrence of *C. fusipes* in oak forests in France [6], problems of wind thrown infected trees have never been reported for pedunculate oaks. In contrast, the main problem induced by *C. fusipes* in red oak stands is wind thrown trees [2].

Despite differences in disease development between the two species, the relationship between the root infection index and the frequency of living roots was the same

Table II. Number of living roots more than 1 cm in diameter on oaks with damaged or undamaged crowns.

	Crown	No.	Mean no. of living roots (range)		
			Cylinder 1	Cylinder 2	Cylinder 3
Q. robur	undamaged	15	41 (11–79)	55 (15–77)	43 (14–112)
	damaged	11	21 (6–69) ND*	28 (8–43) SD	25 (8–70) SD
Q. rubra	undamaged	15	36 (10–79)	62 (16–103)	55 (6–97)
	damaged	5	14 (7–41) SD	22 (0–42) SD	23 (1–53) SD

* ND, no significant difference between trees with crown damaged and trees with crown undamaged, based on a Student's *t*-test; SD, difference significant at the 0.05 level.

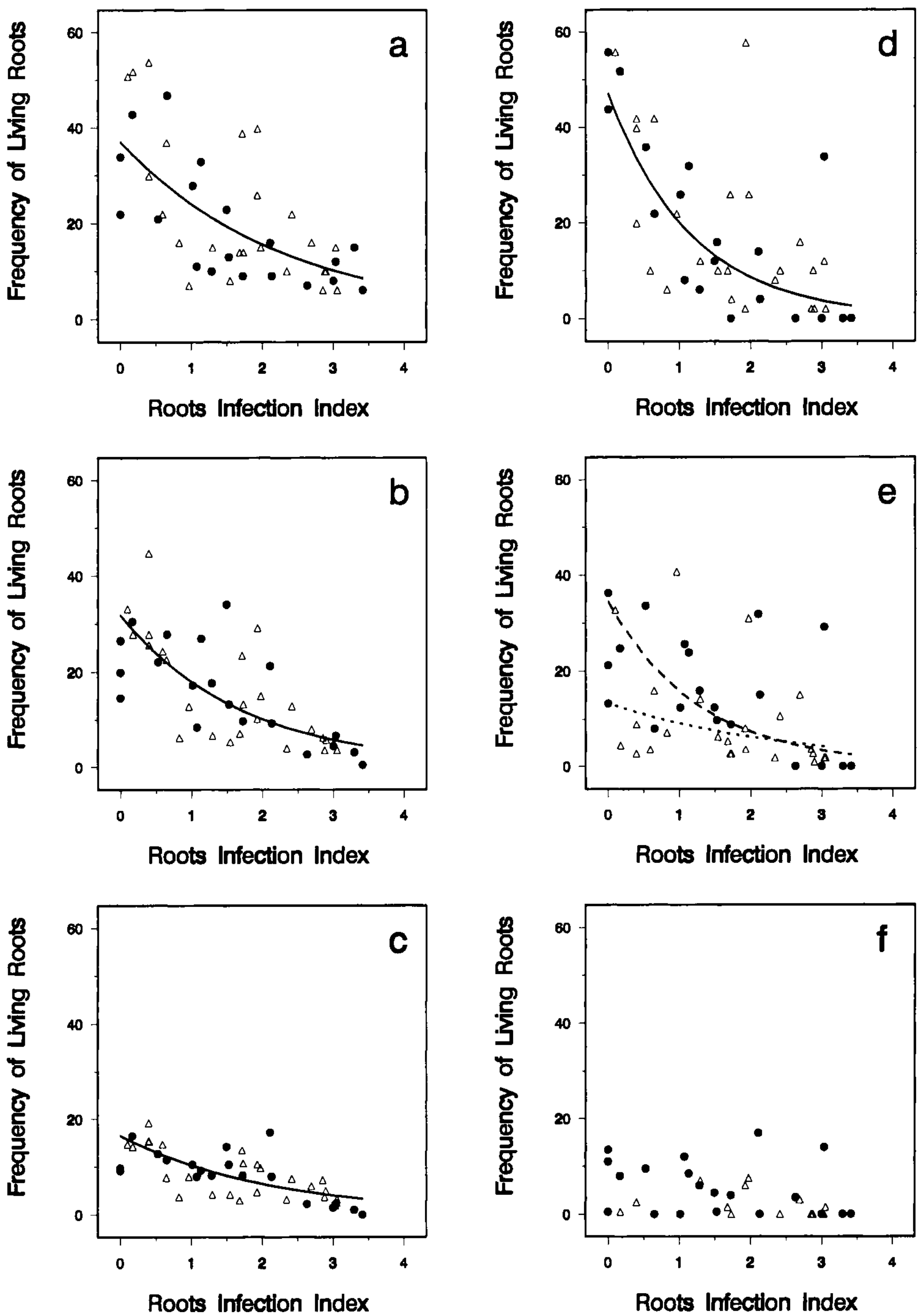

Figure 5. Number of living roots more than 1 cm in diameter present on up-rooted *Q. robur* and *Q. rubra* trees with different levels of *C. fusipes* infection. a) Cylinder 1 wall; b) cylinder 2 wall; c) cylinder 3 wall; d) cylinder 1 floor; e) cylinder 2 floor; f) cylinder 3 floor. △ = *Q. robur*; ● = *Q. rubra*; —— = regression line for *Q. robur* and *Q. rubra*; ------ = regression line for *Q. rubra* only; ······ = regression line for *Q. robur* only.

for pedunculate and red oak in almost all parts of the root system. The only exception to this was on the floor of cylinder 2 (the horizontal surface 60 cm below the soil surface), where the impact of *C. fusipes* was higher for red oaks than for pedunculate oaks (*figure 5e*). This can probably be explained by the presence in Les Aynans stand of a gravel layer at 50–100 cm beneath the soil surface that constituted a strong physical limit to rooting for the pedunculate oaks. Since even the undamaged pedunculate oaks have a rather low root frequency 60 cm below soil level, the impact of the infection there is not so high.

There was a relationship between crown status and root infection. However, there were a number of exceptions, i.e. trees with very few living roots and no marked symptoms at the crown level (*table II*). Although the total reduction in root amount is important, type and distribution in the soil of the remaining roots could be decisive for the future of the infected tree. Our results demonstrate that the pathogen destroys the central part of the root system, which is mainly composed of roots penetrating deep into the soil. However, other roots survive, developed from the large lateral roots, which are able to pump deep soil water. The weak connection between decline symptoms and root reduction suggests that the remaining roots can be sufficient for heavily infected trees to live for a long time in the absence of stressful conditions without obvious decline symptoms. Also, adventitious roots often develop after large collar roots are killed and could mitigate the effect of root loss. However, such trees are probably unable to overcome abnormal situations such as water shortage.

During this study, we rated the crown status in winter and thus, we might have not adequately described crown decline. Therefore, one cannot make definitive conclusions from our study on this point. As the infection index we tested appears to measure well the destruction of the entire tree root system by *C. fusipes*, we now have a tool to investigate the relationship between root infection and crown decline in infected oaks for a large number of trees.

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