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Short note

Probability of ectomycorrhizal infection in a declining stand of common oak

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Summary — The study was conducted on 50–55-year-old common oak (*Quercus robur* L) trees growing in a *Ornithogalo pyrenaici-Carpinetum betuli.* Four different classes of decline were determined. In each class, in a root sample belonging to ten common oaks, the number of tips with and without mycorrhizal mantle were counted and the probability of finding vital mycorrhizae was determined. The mycorrhizal morphotypes were described and their frequency calculated. The study demonstrated a significant decrease in the proportion of mycorrhizae between healthy and declining plants. Among damaged trees, the increase in decline did not correspond to a decrease in the probability of mycorrhizal infection. There was no correspondance between the probability of finding vital mycorrhizae and decline intensity. Most mycorrhizal morphotypes were found to be distributed homogeneously in the different classes of decline. Some of them, however, can be associated with the degree of decline in a variable manner. Relationships between growth anomalies and mycorrhizal infection are also discussed.

decline / probability of mycorrhizal infection / common oak

Résumé — Probabilité d'ectomycorhization du chêne pédonculé dans une forêt dépérissante. *L'étude a été menée sur des chênes âgés de 50–55 ans croissant dans un* Ornithogalo pyrenaici-Carpinetum betuli. *Quatre classes de dépérissement des arbres ont été déterminées dont la première comportait uniquement des arbres sains. Pour chaque classe, les apex racinaires mycorhizés et non mycorhizés ont été dénombrés dans des échantillons de racines prélevées à partir de dix arbres et la probabilité de trouver des mycorhizes vivantes a été déterminée. Les morphotypes de mycorhizes ont été séparés et décrits et leur fréquence a été calculée pour chaque classe de dépérissement. L'étude a démontré que la probabilité qu'un apex racinaire soit mycorhizé est significativement plus élevée pour les arbres sains que pour les arbres dépérissants. En revanche, parmi ces derniers, on n'a pas relevé de correspondance entre la probabilité de trouver des mycorhizes viables et la sévérité du dommage. La plupart des morphotypes sont distribués de façon homogène parmi les différentes classes de dépérissement. Toutefois, quelques morphotypes semblent être associés de façon variable avec la sévérité du dépérissement. Les relations entre les anomalies de croissance et la mycorhization sont discutées.*

dépérissement / probabilité de mycorhization / chêne pédonculé

INTRODUCTION

Common oak *(Quercus robur* L) was previously the dominant tree of hills and plains in northeast Italy; however, it was progressively rooted out to make room for agricultural crops. Today, only a few common oak stands are still found in this region.

The decline of common oak has been detected for many years in stands far apart from one another (Becker and Lévy, 1982; Ragazzi et al, 1986; Oosterban et al, 1990). Up to now it has not been possible to demonstrate a precise relationship of cause and effect between biotic or abiotic factors which might give rise to decline. Research on other species has evidenced a root problem characterized by a decrease in the biomass and vitality of fine roots, a reduction in the number of mycorrhizal apexes and reduced nutrient transport (Zak, 1964; Schütt et al, 1985; Wargo et al, 1988; Perrin and Estivalet, 1989). No studies have as yet been conducted in Italy on common oak decline in relation to mycorrhizal status. Thus, a preliminary study on the probability of mycorrhizal infection in an Italian area affected by decline was carried out.

MATERIALS AND METHODS

Site description

The study was carried out in a forest in the Veneto Region (Fagarè woods, northeast Italy), where a large number of common oaks were affected by varying degrees of decline. The most important site parameters are listed in table I.

Investigation of decline

The survey was performed on 50–55-year-old common oaks in which decline could not be imputed to parasites, silvicultural damage or climatic events. The age of the plants was determined by means of a core sample taken with a Pressler auger.

Four classes of defoliation were identified following the usual methods (Müeller and Bosshard, 1986; Keizer, 1993): class 0: < 20% defoliation; class 1: 20–45% defoliation; class 2: 45–70% defoliation; class 3: > 70% defoliation. Decline classes were subsequently obtained, adding one class to the first three classes of defoliation if additional symptoms of decline (yellow leaves, dead twigs and branches, etc) were detected (Keizer, 1993). Trees belonging to class 0 were considered to be nondeclining controls.

Probability of mycorrhizal infection of root tips

Collection of samples

Ten trees were randomly selected for each of the four decline classes and three equidistant major roots were chosen on each of these plants. A 15 cm soil cube beneath the litter layer was sampled at a distance of approximately 1.5 m from the collar close to a lateral root of 8–12 mm in diameter. Samples were collected during the last week of May. Within 24 h from sampling, roots were thoroughly and carefully washed in distilled water to remove soil particles. Roots belonging to each decline class were then pooled in 8% glutaraldehyde in 0.2 M phosphate buffer at pH 6.8 (Miller et al, 1991) and stored in a hermetically sealed glass box at 4 °C in the dark.

Quantitative analysis

Five hundred roots were randomly taken from the samples of each class of decline. On each root, ten tips were counted in succession, considering an uninterrupted mycorrhizal system (clustered mycorrhizae; Agerer, 1987) as one tip, starting from a random intermediate point and directing towards the distal extremity. In this way, 5 000 apexes *(TT)* were counted and distinguished between mycorrhizal *(M)* and not mycorrhizal *(NM)* tips. The number of uninterrupted mycorrhizal systems *(S)* and, for each of these, the number of tips *(ST)*, were also counted.

The number of vital mycorrhizae was determined both considering an uninterrupted mycor-

Table I. Site and stand characteristics of the declining *Quercus robur* trees surveyed for mycorrhizal infection.

Elevation mean (m)	157
Exposure	East–west
Precipitation (mm; average of last 10 years)	
Winter	218
Spring	375
Summer	332
Autumn	304
Temperature (°C; average of last 10 years)	
Annual mean	17.5
Hottest month mean	22.35
Coldest month mean	1.73
Bedrock	Arenaceous aggregate with clay and limestone
Soil (kind; pH)	Brown earth; 6–7
Texture	Clay
Humus	Mull
Forest stand	
Type	*Ornithogalo pyrenaici-Carpinetum betuli*
Structure	Even-aged high forest
Density	Normal
Regeneration	Low
Phytosanitary conditions	*Armillaria* sp; *Agrilus bilineatus*; *Fusarium eumartii*

Battisti and Covassi, 1991; Del Favero and Lasen, 1993; Martello, 1993; Ragazzi et al, 1993.

rhizal system as one tip (V_1) and counting all mycorrhizal tips, irrespective of their belonging to an uninterrupted mycorrhizal system (V_2). Dead mycorrhizae showed a dark brown colour, a scurfy surface and an easily detachable mantle. The vital ones were characterized by vivid colour, a firm and unscurfy surface (Keizer, 1993). Damaged and not fully developed apexes were excluded.

The differences between mycorrhizal infection of declining and nondeclining plants were pointed out by studying the variations of probability to find mycorrhizal tips and vital mycorrhizae. These probabilities were determined considering a single tip, or a single uninterrupted mycorrhizal system, as a replicate. The sampling technique provides data with a binomial distribution, so the significance of the comparisons between the different parameters (M, NM, $M - S + ST$, S, V_1, V_2) was tested using the χ^2 test (Camussi et al, 1986).

The following ratios were calculated:

$P_1 = M/TT$: probability of mycorrhizal infection of a tip, counting an uninterrupted mycorrhizal system as one mycorrhizal tip;

$P_2 = M - S + ST / TT - S + ST$: probability of mycorrhizal infection of a tip counting all mycorrhizal tips, irrespective of their belonging to an uninterrupted mycorrhizal system;

$L_1 = V_1/M$: probability of finding a vital micorrhizal tip, counting an uninterrupted mycorrhizal system as one mycorrhizal tip;

$L_2 = V_2/M - S + ST$: probability of finding a vital micorrhizal tip, counting all mycorrhizal tips irrespective of their belonging to an uninterrupted mycorrhizal system.

Frequency of mycorrhizal morphotypes

The living mycorrhizal tips counted in the quantitative analysis were characterized morphologically according to Agerer (1987). The frequency of recovery of each morphotype in the four decline classes was determined. Dissimilarity among ectomycorrhizal morphotypes with regard to their frequency of recovery was analysed and clustered using the Minkowsky's multiple distance algorithm (Gower, 1971).

RESULTS

Survey of decline

At Fagarè woods, 10% of the common oaks belonged to class 0, 20% to class 1, 35% to class 2 and 35% to class 3.

Probability of mycorrhizal infection in root tips

Table II shows the probability of mycorrhizal infection in root tips counting both an uninterrupted mycorrhizal system as one tip (P_1), and all mycorrhizal tips (P_2). It also reports the probability of finding a vital mycorrhizal tip counting both an uninterrupted mycorrhizal system as one tip (L_1), and all mycorrhizal tips (L_2). The proportion of mycorrhizal apexes in the roots collected from healthy plants was significantly larger than that collected from declining trees. Neither the proportions of mycorrhizal tips collected from plants belonging to classes 1, 2 and 3 were statistically different. The probabilities of finding vital mycorrhizal tips in the roots sampled from healthy and declining plants were significantly different.

Frequency of mycorrhizal morphotypes

The frequency of different mycorrhizal morphotypes and the distribution in the four decline classes are shown in table III. Forty-three mycorrhizal morphotypes were found in the samples collected. Morphotype 2 was present in all decline classes, but overall in the healthy one. Morphotypes 5, 11 and 22

Table II. Probability of mycorrhizal infection of a tip and probability of finding a vital mycorrhizal tip in the four decline classes.

Probability	*Percentages*			
	Class 0	*Class 1*	*Class 2*	*Class 3*
P_1	75.80^a	65.46^b	67.66^b	67.88^b
P_2	82.21^a	72.90^b	74.02^b	73.64^b
L_1	81.58^b	80.79^b	79.94^b	79.73^b
L_2	85.53^b	85.27^b	83.58^b	82.68^b

Probabilities refer to ratios described in *Materials and Methods*. P_1: probability of mycorrhizal infection of a tip counting an uninterrupted mycorrhizal system as one mycorrhizal tip; P_2: probability of mycorrhizal infection of a tip counting all mycorrhizal tips irrespective of their belonging to an uninterrupted mycorrhizal system; L_1: probability of finding a vital micorrhizal tip counting an uninterrupted mycorrhizal system as one mycorrhizal tip; L_2: probability of finding a vital micorrhizal tip counting all mycorrhizal tips irrespective of their belonging to an uninterrupted mycorrhizal system. ^{ab} Values in the same row with the same letter can be considered belonging to the same population (null hypothesis accepted according to χ^2 test; $P = 0.01$).

Table III. Frequency of recovering of mycorrhizal morphotypes in the four decline classes (% of total mycorrhized living tips).

Morphotypes	*Class of decline*			
	0	*1*	*2*	*3*
1	9.12	9.54	11.81	5.42
2	14.01	6.64	5.90	7.24
3	1.00	0.28	0.37	0.37
4	1.52	1.66	2.46	1.10
5	5.22	0.69	0.74	1.46
6	1.52	0.41	0.00	1.09
7	0.13	0.28	0.00	0.00
8	0.46	0.28	0.74	0.49
9	0.00	1.11	0.00	0.00
10	6.61	8.57	6.27	11.81
11	5.42	0.14	0.00	0.36
12	3.90	1.66	0.98	6.33
13	1.45	1.11	1.11	2.62
14	1.39	0.00	0.37	2.01
15	2.38	1.38	2.58	2.31
16	1.12	2.90	1.11	2.01
17	0.13	0.14	0.00	0.30
18	0.26	0.00	0.00	0.00
19	5.75	10.79	6.27	10.47
20	1.00	2.49	1.11	1.70
21	3.30	3.73	2.09	2.80
22	5.35	1.11	0.61	1.22
23	2.77	1.80	0.61	2.44
24	1.52	1.94	0.98	3.77
25	0.73	0.14	5.41	0.43
26	0.92	0.14	0.00	1.40
27	0.40	0.28	0.49	0.97
28	0.40	0.00	0.00	0.18
29	2.91	1.94	2.83	0.55
30	4.09	1.11	2.58	2.80
31	0.86	0.14	0.25	0.43
32	1.45	1.80	0.98	0.12
33	0.53	0.55	0.25	0.36
34	10.57	12.45	15.37	1.28
35	0.07	1.38	0.37	1.46
36	1.32	3.32	14.02	4.94
37	0.13	0.14	0.61	0.43
38	0.00	0.00	0.12	0.00
39	0.00	1.94	0.00	0.00
40	0.00	0.00	1.23	0.36
41	0.33	16.18	7.87	16.29
42	0.00	0.00	1.23	0.43
43	0.00	0.00	0.25	0.00

were mainly present in nondeclining trees, whereas morphotype 41 was especially collected from the declining plants. The morphological characteristics of the latter types are described in table IV and figure 1.

Minkowsky's algorithm applied to the frequencies of recovering (fig 2) indicated that distributions of morphotypes 5, 11 and 22 were comparable for a dissimilarity of 5%, but they became similar to the distributions of other 33 types when the dissimilarity level increased to 16%. The distribution of morphotype 2 was quite different from the others as the first cluster was found at a dissimilarity level of 34%. Morphotype 41 appeared distributed in a different way then all the other morphotypes.

DISCUSSION

In Fagarè wood, common oaks display serious and widespread symptoms of decline. In the root apexes examined, a large number of mycorrhizal morphotypes was detected in every class of decline, which suggests that this site is well provided with mycorrhizal fungi. Most were distributed individually with similar frequencies in the trees belonging to the different classes of decline. In most cases, it seems that the distribution of morphotypes cannot be associated with the visual criteria used in defining the different classes of decline. Nevertheless, some morphotypes show main frequency either in the nondeclining class or in the declining ones. This indicates that some of them can be associable with decline features. The results are referred to roots collected from trees of the same age in the same environmental conditions, therefore they must be confirmed on trees of a different age and living under different environmental conditions.

The quantitative analysis of root samples shows a significant decrease in mycorrhizae proportion between nondeclining and declin-

Table IV. Morphological characteristics (Agerer, 1987) of mycorrhizal morphotypes 2, 5, 11, 22 and 41.

Mycorrhizal morphotype	*2*	*5*	*11*	*22*	*41*
Shape and dimension					
Type of ramification	Unramified	Monopodial-pyramidal	Monopodial-pinnate	Irreg monopodial-pyramidal	Monopodial-pinnate
Length of ramified system (mm)	–	3.11	2.12;1.02	1.89	0.73;1.07
Length of unramified ends (mm)	1.15;0.64;0.81	0.47;0.20;0.30;0.24	0.29;0.10	0.62;0.24;0.22	0.28;0.25;0.16
Diam of unramified ends (mm)	0.22;0.17;0.18	0.34;0.25;0.25;0.20	0.26;0.15	0.24;0.19;0.19	0.23;0.19;0.16
Diam of axes (mm)	–	0.36	0.28;0.30	0.28	0.24;0.26
Shape of unramified ends	Bent	Large, short and straight	Straight or slightly bent	Bent	Straight or loosely bent
Features of surface					
Distinct mantle-surface visible	Yes	Yes	Yes	Yes	Yes
Cortical cells visible	No	No	No	No	No
Distinct features	Smooth	Smooth and cottony on axes, thick and cottony on ends	Densely fibrous	Smooth, loosely shiny	Densely grainy long-spiny
Colour					
Unramified	Yellow	White-yellow	Ochre-yellow with large white area	Brown-ochre	Black-brown
Very tips	Yellow	White-yellow	Ochre-yellow with large white areas	Brown-ochre	Brown
Older myccorhizae or older parts	Yellow	White-yellow	Ochre-yellow with large white areas	Brown-ochre	Black-brown
Rhizomorphs	No	Very rare	No	No	No
Diameter (mm)		0.036			
Occurrence		Basal			
Connections with mantle		Simple			
Colour		White			
Mode of ramification		Unramified			
Margin		Smooth			
Emanating hyphae					
Density; occurrence	Rare; not apical	Very frequent; overall on apexes	Frequent; ubiquist	Rare; never apical	Very frequent and widespread
Length; colour	Very short and thin; white	Long, very thin, cottony, white	Almost all close to mantle, the aerial ones rare; very thick and white	Thick; white	long, thin; white

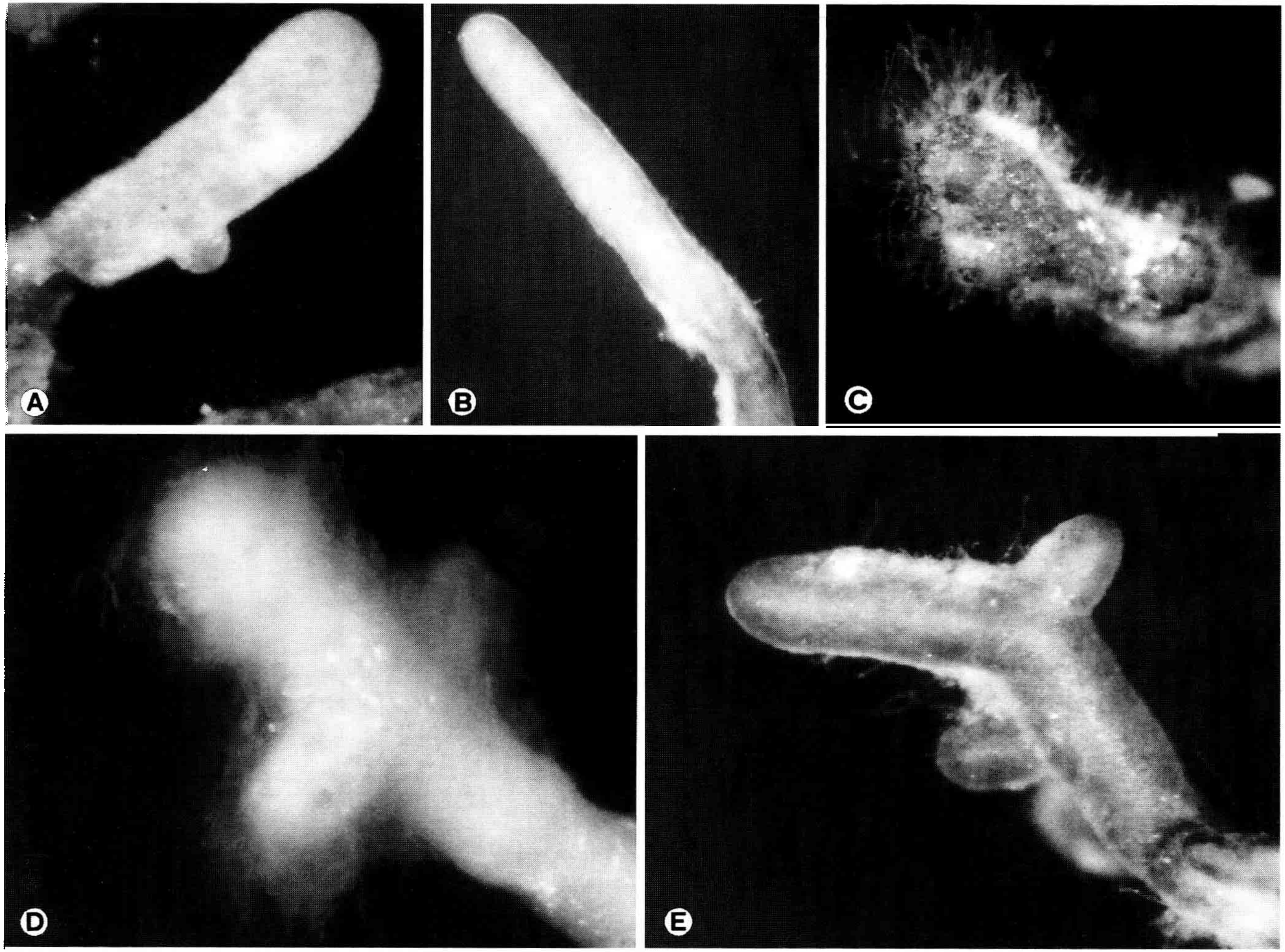

Fig 1. Photographs of mycorrhizal morphotypes described in table IV. (**A**) Morphotype 11 (80 x); (**B**) morphotype 2 (80 x); (**C**) morphotype 41 (80 x); (**D**) morphotype 5 (80 x); (**E**) morphotype 22 (80 x).

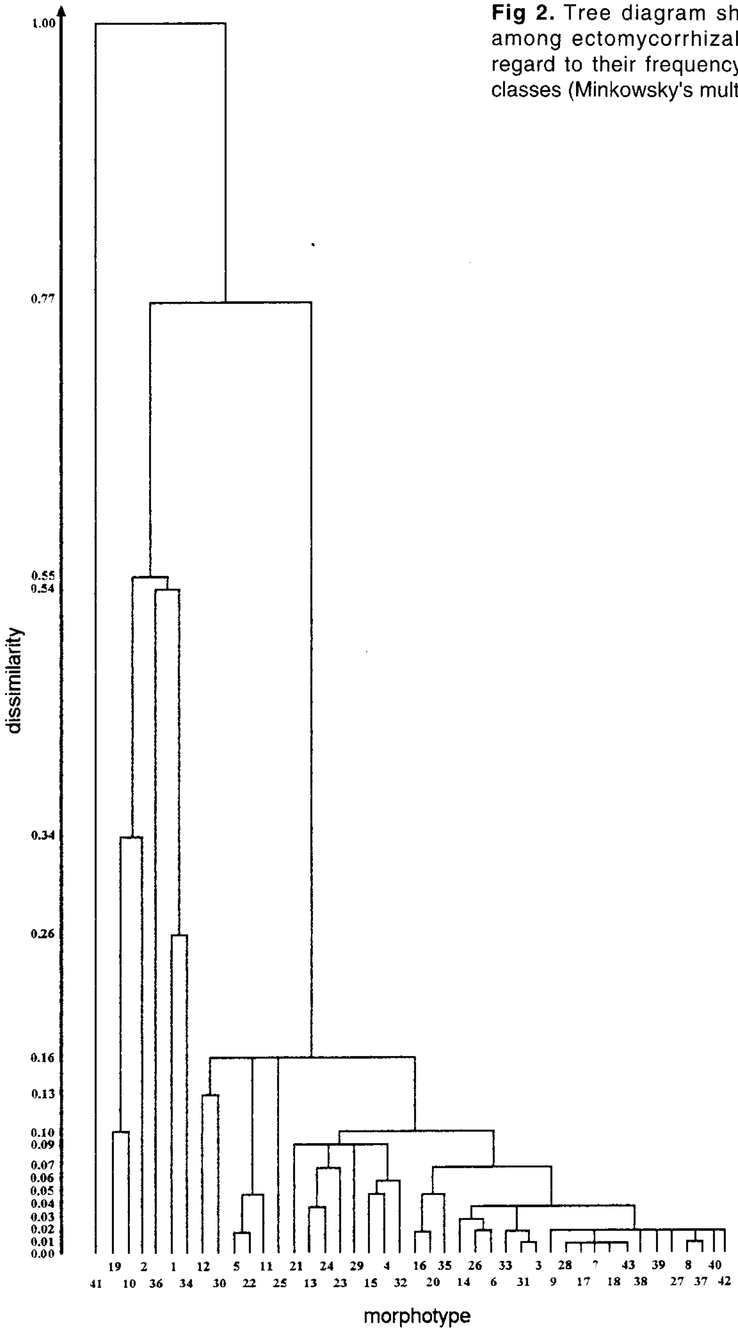

Fig 2. Tree diagram showing dissimilarity among ectomycorrhizal morphotypes with regard to their frequency in the four decline classes (Minkowsky's multiple distance).

ing plants. Although our research requires further investigation, both in the conditions described and during the different seasons, and on plants of different age, it is in agreement with the findings of other authors, in particular with the hypothesis that decline is associated with a variation in the quantity of mycorrhizae (Liss et al, 1984; Meyer, 1984; Fellner, 1988; Wargo et al, 1988; Termorshuizen, 1990; Jansen, 1991; Keizer, 1993).

However, in the samples examined, the increase of decline does not correspond to a decrease in the probability of mycorrhizal infection. Recent findings suggested that trees with a prevailing defoliation degree may recover and overcome decline (Kandler, 1992). Some of the selected trees belonging to classes 1, 2 and 3 produced new leaves at the onset of the vegetative period in 1994. Consequently, these trees were included in different decline classes as the original ones. These variations may be correlated with the probability to find vital mycorrhizae, which is not statistically different between declining and nondeclining trees. The occurrence of a high proportion of vital mycorrhizae could have allowed even the most damaged trees to produce a denser crown than in the previous year.

The decline classes were determined by evaluating the outward appearance of the trees. The visual parameters used varied from year to year, so they are not associable to the probability of mycorrhizal infection in the condition studied. More reliable parameters could be feeder root biomass and annual ring width, because in some cases annual ring growth starts earlier than changes in crown conditions (Kandler, 1992). Thus, these parameters will need to be examined further during the ongoing research.

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