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SHORT NOTE

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Occurrence of arbuscular mycorrhiza on aged *Eucalyptus*

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Abstract Arbuscular mycorrhiza have never been described within roots of aged *Eucalyptus* despite the fact that *Eucalyptus* seedlings do form both endomycorrhiza and ectomycorrhiza early during their developement. In the present study, all the structures of arbuscular mycorrhiza were observed within roots of four *Eucalyptus* species of 15, 17 and more than 50 years old at three different sites in northern Algeria. Arbuscular mycorrhiza frequency was assessed in roots of 15-years old *Eucalyptus camaldulensis* species, during two periods in 2 consecutive years (July and November of 1996 and 1997). Intensity of root colonization was dependent on the time of sampling and attained 42% in July 1997.

Key words Arbuscular mycorrhiza · Aged *Eucalyptus* · Natural conditions

Introduction

The genus *Eucalyptus* encompasses some of the few woody plants known to form both arbuscular mycorrhiza (AM) and ectomycorrhiza (Lapeyrie and Chilvers 1985; Chilvers et al. 1987; Boudarga et al. 1990). Numerous studies have dealt with ectomycorrhiza on samples collected from old trees in the field as well as with ectomycorrhiza obtained after in vitro synthesis or after inoculation of seedlings grown in controlled conditions (Chilvers and Pryor 1965; Chilvers 1968, 1973, 1974; Ashford et al. 1975; Malajczuk et al. 1982; Grenville et al. 1986; Bougher and Malajczuk 1990). Conversely, papers on *Eucalyptus* AM are relatively scarce, despite early reports on *E. globulus* by Asai (1934) and Maeda (1954). Up to now, there has been no report of AM on aged trees. The few existing studies on *Eucalyptus* AM have been carried out on young seedlings grown in experimental conditions (Malajczuk et al. 1981; Lapeyrie and Chilvers 1985; Boudarga and Dexheimer 1988; Adjoud et al. 1996), or on *Eucalyptus* from young plantations (Gardner and Malajczuk 1988; De Mendonça Bellei et al. 1992). Almost all studies dealing with the dual symbiosis of *Eucalyptus* report a temporal replacement of AM by ectomycorrhiza with host ageing, and AM are generally considered as the predominant mycorrhizal form of the early growth stages of *Eucalyptus* (Boudarga and Dexheimer 1988). In this paper, we report the occurrence of typical AM on aged eucalypts as part of a field survey of the mycorrhizal status of *Eucalyptus* planted in northern Algeria.

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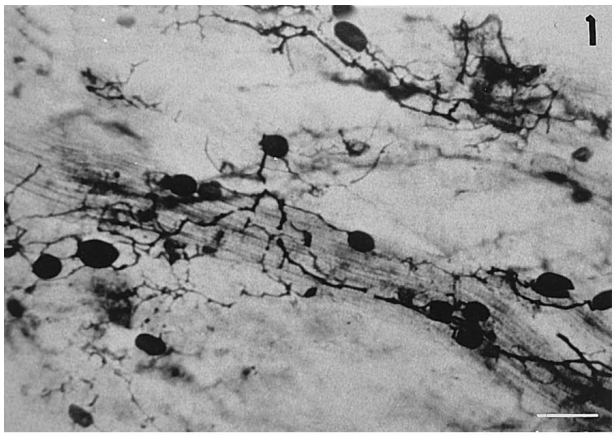
Materials and methods

The three *Eucalyptus* plantations surveyed are located in Kabylia, a region of northern Algeria with a subhumid climate type. The first stand was located at the seaside near Azzefoun at an altitude of 400–1200 m with a slope of 15–20%. Average annual rainfall was 800–1200 mm and the average temperature varied from 4 °C in the coldest month to 35° in the hottest. The soil had a clayey-silt texture. *Eucalyptus globulus*, *E. maideni*, and *E. sideroxylon* 17-year-old trees were sampled here. The understorey consisted of herbaceous plants and shrubs typical of mediterranean climates. Stands 2 and 3 were on flat lands (about 80 m in altitude) located in the south periphery of Tizi-ouzou about 10 km apart. These two stands are separated from the sea by a mountain range which results in locally continental microclimates with large daily temperature variation. The average annual rainfall was about 700 mm and an average annual temperature of 18 °C with January as the coldest month and August as the hottest. The soils were of a silty sand texture in the second stand and sandy silt in the third stand. The eucalypts in these stands were *E. camaldulensis.* The trees sampled were more than 50 years old in the second stand and about 15 and 40 years old in the third stand. The understorey at both sites was made up of herbaceous plants.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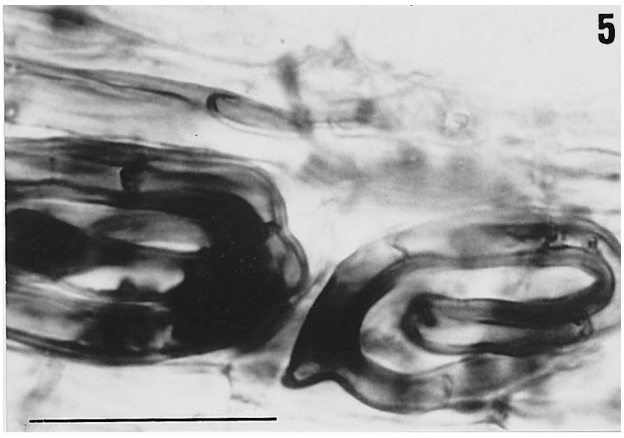
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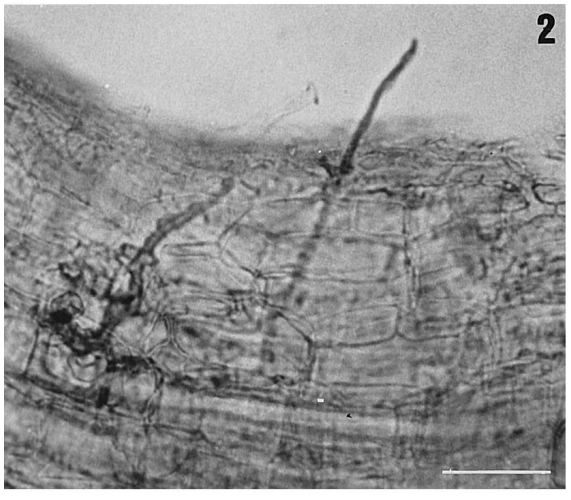
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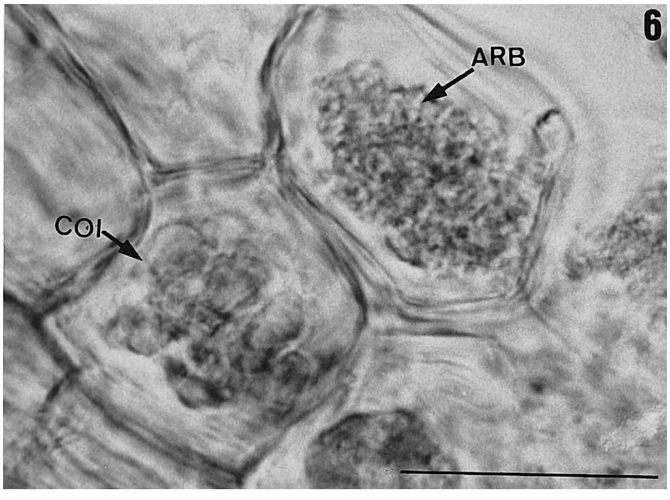
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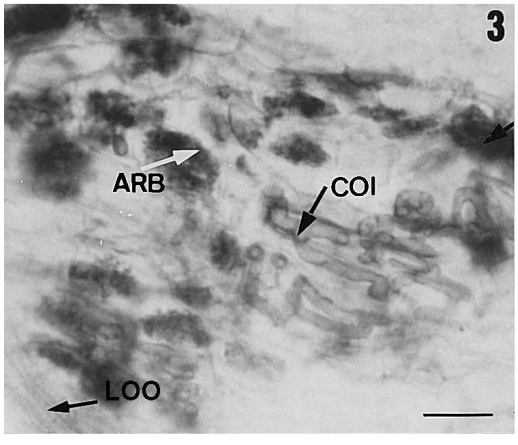
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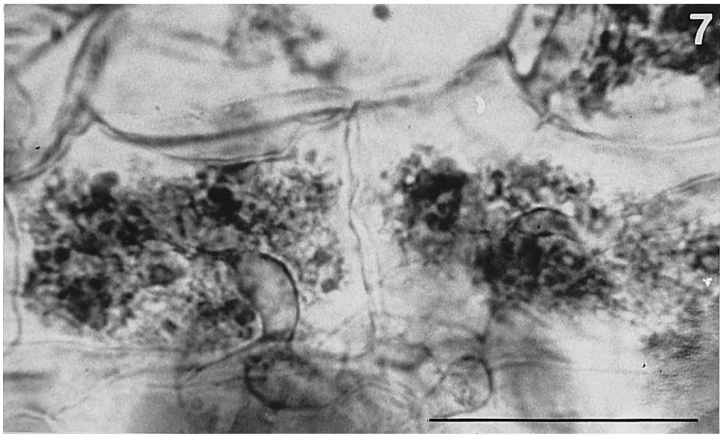
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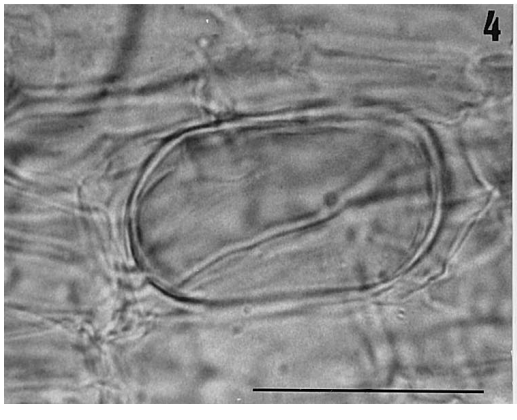
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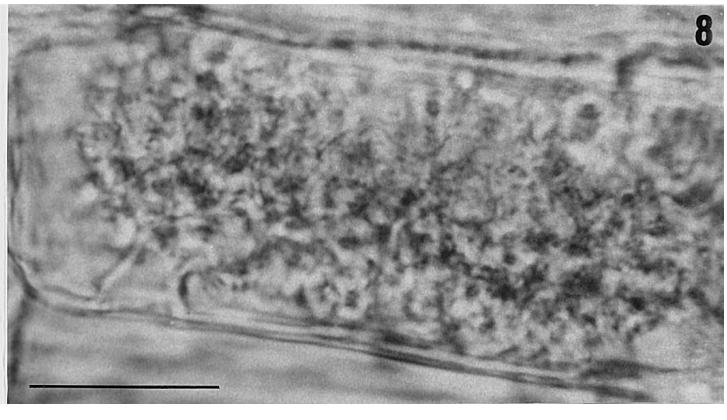
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Roots of the four *Eucalyptus* species were examined for occurrence of AM. Ten trees were randomly selected in each stand. Samples were collected over 2 consecutive years at about 2-month intervals. These were taken at about 1.5 m from the base of the trunk, in 5–6 radii around the trees and to a depth of 25 cm. To make sure that only E*ucalyptus* roots were taken, thick roots were chosen and only rootlets which could be traced from them were collected. Rootlets were washed in running water, cleared in 10% KOH and stained with trypan blue (Phillips and Hayman 1970) or acid fushin (Berch 1979). Random subsamples of stained roots were observed under a dissecting microscope before light microscope study.

AM assessments were performed on roots of 15-year-old *E. camaldulensis* sampled at the third site. Five trees were randomly selected in the stand. To ensure minimal heterogeneity, small soil volumes ($15 \times 15 \times 20$ cm deep) containing the roots were taken. Around each tree, 15 samples were collected at approximatively equidistant points. Great care was taken to collect only *Eucalyptus* roots, as described above. Samples were collected twice a year for 2 consecutive years (1996 and 1997), after the highest rainfall (November) and at the dryest period (July). After clearing and staining, root lengths colonized by AM fungi were estimated using the grid line intersect method (Giovannetti and Mosse 1980). Data from AM colonization were subjected to Anova analyses and Newman Keuls test ($P < 0.05$).

Results and discussion

All the *Eucalyptus* trees sampled from the different sites had AM structures within their roots and all the root systems sampled had both AM and ectomycorrhiza. All the typical AM features, such as arbuscules, vesicles, intracellular hyphal coils, extra and intraradical hyphae, were observed in the samples (Figs. 1–8). Arbuscules were present in all the samples observed, providing unequivocal evidence of an AM association within the *Eucalyptus* roots. The presence of arbuscules is a s*ine qua none* for identification of an AM infection in roots (Bonfante-Fasolo 1984), since these structures are formed by all AM fungi whilst vesicles are not (Gerdemann and Trappe 1974). Vesicles were observed within roots where they were intra- (Fig. 4) or extracellular and on extramatrical hyphae. Intracellular hyphae, which varied in diameter, also formed coils or loops inside the cortical cells (Figs. 3, 5).

Arbuscules showed either fine or coarse branching (Figs. 6–8). The morphological diversity of the different fungal structures observed within the same roots could indicate that the *Eucalyptus* roots were colonized by several different fungal species. Such typical AM fungal structures have not been described within roots of aged *Eucalyptus* before, despite the fact that *Eucalyptus* forms AM on seedlings in controlled conditions or on roots from young plantations (Malajczuk et al. 1981; Lapeyrie and Chilvers 1985; Boudarga and Dexheimer 1988). Ashton (1976), studying mycorrhiza of *Eucalyptus regnans* roots from the field, reported only hyphae within epidermal and cortical cells. Khan (1978), surveying a recently revegetated coal spoil area, found AM within roots of *E. pilularis* and *E. paniculata*.

Assessments of percentage AM root length are shown in Fig. 9. The extent of root colonization varied from about 19% to 42% depending on the time of sampling. The highest values were registered in July, which is in the dryest period of the 2 sample years. These results are in accordance with those obtained for other AM plants. Several authors reported that

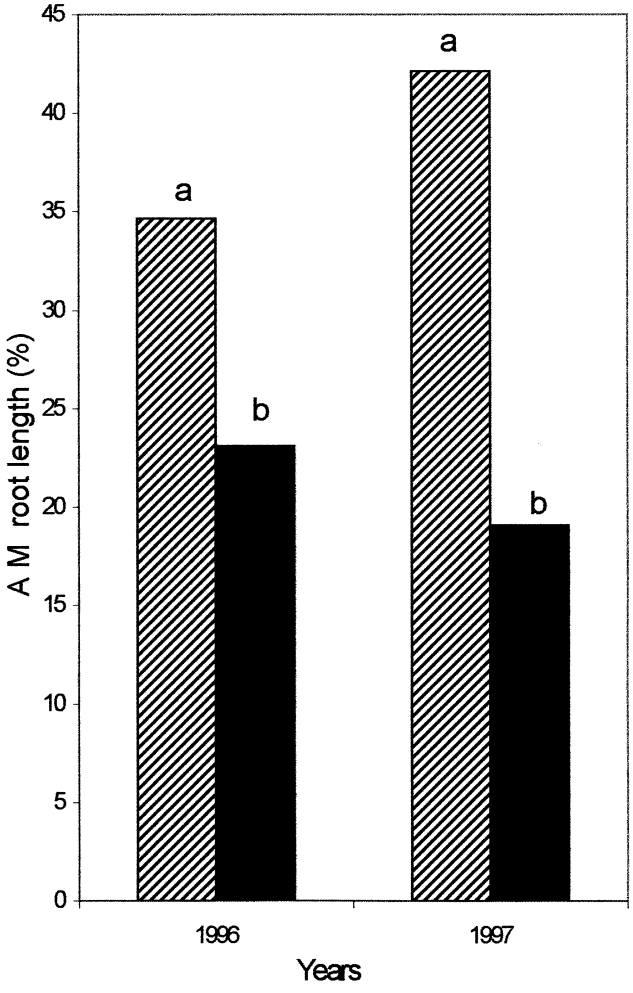

Fig. 9 Percentage mycorrhizal root length of 15-year-old *Eucalyptus camaldulensis* trees at site 3 (▨ July sampling, ■ November sampling). For each year, columns labelled with different letters differ significantly (Newman Keuls test, $P < 0.05$)

Figs. 1–8 AM structures observed within *Eucalyptus* roots collected from different sites; bars for all figures 5 μ
Fig. 1 Extra and intra radical hyphae bearing vesicles
Fig. 2 Entry points with different hyphal sizes
Fig. 3 Numerous arbuscules (*arb*), hyphal coils (*coi*) and loops (*loo*) in the inner root layers near the vascular stele (arrow)
Fig. 4 Intracellular vesicle
Fig. 5 Hyphae forming loops inside the cortical cells
Fig. 6 Hyphal coil (*coi*) and arbuscule (*arb*) inside two adjacent cortical cells
Fig. 7 Large hyphae penetrate the cortical cells where they branch repeatedly to fill the lumen of the cell
Fig. 8 An arbuscule extends the length of the cell. Hyphal clumps appear compact

soil moisture levels influence AM colonization and that most intense development of AM is registered under dry conditions (Rabatin 1979; Liberta et al. 1983). Likewise, endomycorrhiza have been reported to form in arid soils (Khan 1974).

Studies dealing with the dual symbiosis (AM and ectomycorrhiza) of *Eucalyptus* seedlings in pots or from young plantations have reported a successional pattern of colonization, with ectomycorrhiza taking over in older plants (Lapeyrie and Chilvers 1985; Chilvers et al. 1987; De Mendonça Bellei et al. 1992; Gardner and Malajczuk 1992). In contrast, our study revealed the existence of AM fungi associated with fine roots of different *Eucalyptus* species of different ages, up to more than 50 years. This study also provides evidence that replacement of AM by ectomycorrhiza during plant growth is not a general phenomenon in the genus *Eucalyptus,* under natural conditions where eucalypts are exotic.

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