

Original article

## Patterns of arbuscular- and ecto- mycorrhizal colonization of *Eucalyptus dunnii* in southern Brazil

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**Summary** – After planting *Eucalyptus dunnii*, virtually free of mycorrhizal colonization, at six sites in southern Brazil, three distinctive patterns of root colonization by arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) fungi were discerned during the ensuing 13 months which seemed to be very strongly related to previous cropping: 1) pattern A followed the AM-forming soya bean: the relatively large incidence of AM 5 months after planting progressively decreased while that of ECM increased; 2) pattern B followed the AM/ECM-forming *Eucalyptus viminalis*: the incidence of AM remained minimal while that of ECM relatively rapidly reached a high plateau; and 3) pattern C followed the ECM-forming *Pinus taeda*: both AM and ECM progressively increased but were never abundant. Although the results do not fully explain the three patterns of colonization, it is suggested that the inoculum potential and the specificity fungi–host are implicated.

**Eucalyptus / ectomycorrhizas / arbuscular mycorrhizas / Preceding crop / inoculum potential**

**Résumé** – Séquences de colonisation endo- et ectomycorhizienne chez *Eucalyptus dunnii* au sud du Brésil. La colonisation d'*Eucalyptus dunnii* par des champignons endomycorhiziens à arbuscules (MA) et ectomycorhiziens (ECM) a été suivie pendant 13 mois après transplantation dans six plantations à Santa Catarina, au sud du Brésil. Les résultats indiquent que la colonisation MA et ECM est influencée par la plante précédemment cultivée dans le site et a été représentée par trois séquences différentes. Dans un site à soja, un hôte endomycorhizien, les MA ont été plus importantes au 5<sup>e</sup> mois mais elles ont pro-

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gressivement diminué tandis que les ECM ont augmenté rapidement jusqu'à la fin des observations. Dans quatre sites précédemment cultivés avec *E viminalis*, un hôte endomycorhizien, les ECM ont augmenté rapidement avec le temps et ont atteint un plateau tandis que la colonisation MA est restée très faible avec des fluctuations. Dans un site auparavant cultivé avec *Pinus taeda*, un hôte ectomycorhizien, les deux types ont augmenté pendant les observations mais les taux de colonisation sont restées plus faibles que dans les autres sites. Bien que les résultats ne permettent pas d'expliquer l'occurrence de ces trois séquences de colonisation, il est suggéré que le potentiel d'inoculum et la spécificité champignon-plante hôte y sont impliqués.

### ***Eucalyptus* / endomycorhizes / ectomycorhizes / plantation précédente / potentiel d'inoculum**

## **INTRODUCTION**

Roots of *Eucalyptus* spp can be colonized by two types of mycorrhizal fungi, namely those forming i) arbuscular mycorrhizas (AM) and ii) those forming ectomycorrhizas (ECM) (Asai, 1934). As a result of studies made in controlled conditions it was suggested that the occurrence of AMs followed by ECMs is determined by the age of these plants (Lapeyrie and Chilvers, 1985; Chilvers et al, 1987). More recently, observations made on plantations of *Eucalyptus viminalis* in Brazil seemed to confirm this suggestion. The occurrence of vesicles attributable to AM fungi was more intense in roots of young plants, up to 7 to 8 months after planting, than the occurrence of ectomycorrhizas, which gained in frequency thereafter (Bellei et al, 1992).

Thus, the observations made by Bellei et al (1992) confirm the suggestion made by Chilvers and co-workers (Lapeyrie and Chilvers, 1985; Chilvers et al, 1987). However, these observations were made simultaneously at several stands of *E viminalis* that differed in age and possibly in management regime. To eliminate this possible problem and in an attempt to confirm the patterns and age events recorded by Bellei et al (1992), this paper describes successive observations, made over 13 months following transplanting, in six plantations of *Euca-*

*lyptus dunnii* Maiden, in the states of Santa Catarina and Paraná in southern Brazil.

## **MATERIAL AND METHODS**

### **Site description**

The study was carried out between November 1990 and January 1992 on six industrial plantations of *E dunnii* located in the states of Santa Catarina and Paraná in southern Brazil (table I). The soils of five sites, which had previously carried stands of *E viminalis*, namely Formiga, Bugre, Paredão and Experimental, or of *P taeda*, namely Mafra, were similar (Oxisols) with organic matter varying between 4.2 and 6.5%, pH 3.8–4.0 and extractable P 2.6–3.8 ppm. At the sixth site, Laginski (an agricultural site), the soil was markedly different (Inceptisol) with organic matter at 2.7%; pH 5.4 and extractable P 11.0 ppm.

At all of the forest sites, the adjacent vegetation was composed of *Eucalyptus* and *Pinus* plantations and native forest presenting *Araucaria angustifolia* and *Ilex paraguayensis* as the dominant species. At Laginski, however, the adjacent vegetation was dominated by *Brachiaria plantaginea*, *Bidens pilosa*, *Amaranthus hybridus* and *Euphorbia heterophylla* but plantations of *P taeda* and *E viminalis* could be found at 3–4 km from this site.

Weather records were maintained at one location within 1–20 km of the five forest sites. It was 60 km from the agricultural site. During 1991 there was a total of 1 183 mm precipitation, mean daily temperatures exceeded 25 °C for 5 months of the year (January to March and

**Table I.** Locations of preceding crop and mycorrhizal type at six sites planted with *Eucalyptus dunnii* in southern Brazil.

| Sites        | Preceding crop              | Mycorrhizal type | Location               |
|--------------|-----------------------------|------------------|------------------------|
| Laginski     | Soya bean                   | AM               | São João do Triunfo-PR |
| Bugre        | <i>Eucalyptus viminalis</i> | AM-ECM           | Três Barras-SC         |
| Formiga      | <i>Eucalyptus viminalis</i> | AM-ECM           | Mafra-SC               |
| Paredão      | <i>Eucalyptus viminalis</i> | AM-ECM           | Três Barras-SC         |
| Experimental | <i>Eucalyptus viminalis</i> | AM-ECM           | Três Barras-SC         |
| Mafra        | <i>Pinus taeda</i>          | ECM              | Mafra-SC               |

AM: arbuscular mycorrhizal; ECM: ectomycorrhizal; SC: state of Santa Catarina; PR: state of Paraná.

November to December) with the minimum air temperature falling one evening in August to  $-8^{\circ}\text{C}$ . In general the months from November to February, when seedlings of *E. dunnii* were transplanted, were the warmest and also consistently had more rain than at other times of the year.

### Planting procedures

At the forest sites (Formiga, Bugre, Paredão, Experimental and Mafra) the seedlings were transplanted to the field following clear-cutting of the previous plantations. The vegetation growing at all sites between clearcutting of previous forest plantations – or harvesting of soya bean – and planting of *E. dunnii* seedlings was composed mainly of *Sida* sp, *Baccharis dracunculifolia* and *Panicum maximum*. These plants were also the main invading species (about 90%) in the sites during the first months of *E. dunnii* seedlings growth. They were eliminated by several applications of the herbicides: glyphosate isopropylammonium, oxyfluorfen and haloxyfop-methyl.

Seeds were sown in mid-August 1990 at one forest nursery using a fumigated (methyl bromide) mixture of peat/ash/vermiculite (1:1:1, v/v/v) into plastic conical containers (60 mL). In December 1990, when they were 30–35 cm tall, the seedlings, together with the substrate, were transplanted to the field sites at a spacing of  $2 \times 2$  m apart both within and between rows.

### Sampling

To assess the activity of mycorrhizal fungi during the nursery phase ten seedlings were taken

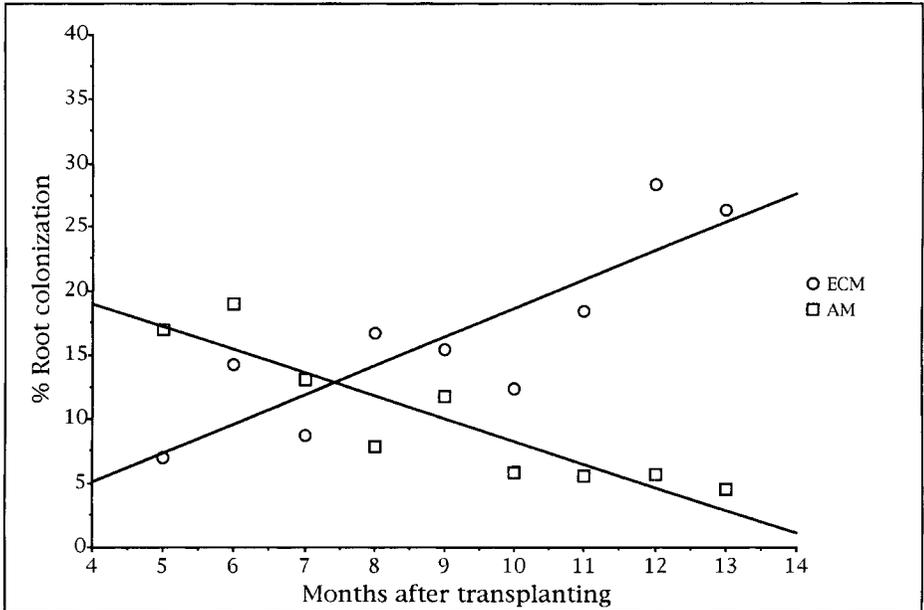
from each of ten batches of seedlings prior to being transplanted; the seedlings were lifted very carefully so as to minimize damage to roots. At each field site, where areas in excess of 10 ha were planted, one plot of 1 ha was identified on the basis of visual uniformity, avoidance of edge effects and convenient access. Thereafter, seedlings roots were sampled from the field at intervals of 1 month usually starting within 2 months of transplanting; the last samples were taken 13 months after transplanting. At each site and on each occasion ten seedlings were sampled at random.

### Root examination

Roots of the randomly selected seedlings were lifted carefully (with a trowel) and put into bags for transport from the field to the laboratory where they were stored at  $4 \pm 1^{\circ}\text{C}$ . When being processed, the roots were washed in tap water and stained using the technique of Philips and Hayman (1970) modified by Koske and Gemma (1989). Colonization – the occurrence of intracellular structures (AM) or ectomycorrhizal root tips (ECM) – was estimated microscopically ( $\times 30$ ) using the intersection method of Giovanetti and Mosse (1980): estimates of percentage root colonization were calculated from observations of 400 intersections per root sample.

## RESULTS

Immediately before transplanting from the nursery to the field, root colonization by the different mycorrhizal fungi was negligible



**Fig 1.** Successive changes in the incidence of ecto- (ECM) and arbuscular (AM) mycorrhizas on roots of *Eucalyptus dumii* planted at Laginski after AM-forming soya bean (pattern A).

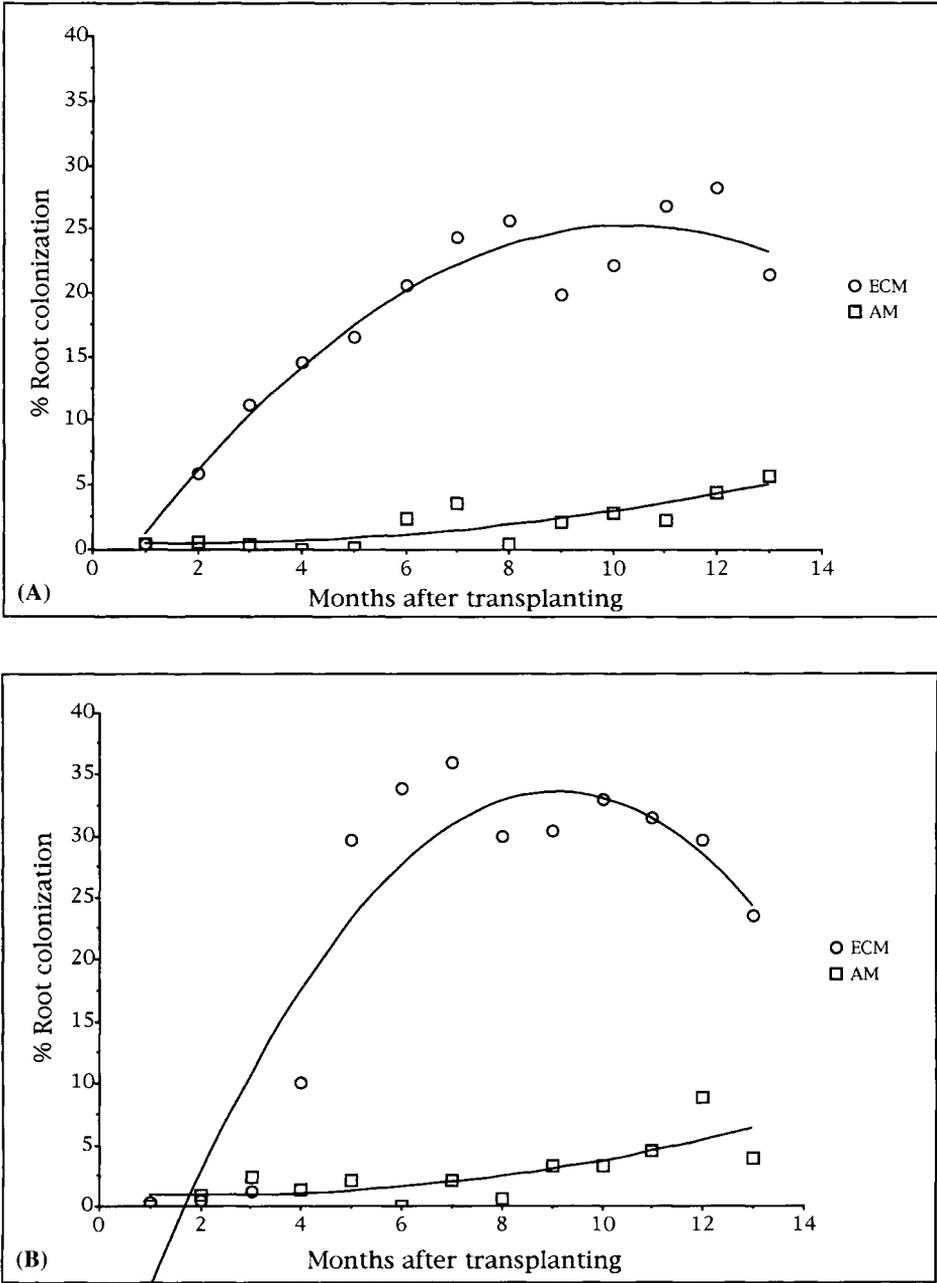
(0.001%). Thereafter, three different patterns of mycorrhizal colonization were observed, which are described here.

**Pattern A, following AM-forming soya bean (location: Laginski) (fig 1)**

At Laginski the percentage AM root colonization was 17% in the fifth month after transplanting. Thereafter, and despite fluctuations, colonization by AM fungi decreased to 4.5% by month 13. In contrast, the abundance of ECM progressively increased reaching 26.4% after 13 months. These changes in the abundance of the two types of fungi are effectively described by linear regressions (% AM =  $25.78 - 1.70x$ ,  $r^2 = 0.82^{**}$ ; % ECM =  $-3.51 + 2.23x$ ,  $r^2 = 0.73^{**}$ ).

**Pattern B, following AM/ECM-forming *Eucalyptus viminalis* (locations: Bugre, Formiga, Paredão and Experimental) (fig 2)**

Unlike events at Laginski, colonization by AM fungi at these four sites rarely exceeded 5%. In the months after transplanting the percentage of endomycorrhizas fluctuated, possibly reflecting seasonal changes. In contrast, the build-up of ECM was rapid and exceeded the rate at Laginski, with 25% of colonization after 7 instead of 13 months. There was a further distinction: whereas the percentage of ectomycorrhizas continued to increase throughout the period of observation at Laginski, the more rapid development at Bugre, Formiga, Paredão and Experimental was not sustained: a plateau was reached. The non-linear ECM curves are of



**Fig 2.** Successive changes in the incidence of ecto- (ECM) and arbuscular (AM) mycorrhizas on roots of *Eucalyptus dunnii* planted at Bugre (A), Formiga (B), Paredão (C) and Experimental (D) after AM/ECM-forming *E. viminalis* sites (pattern B).

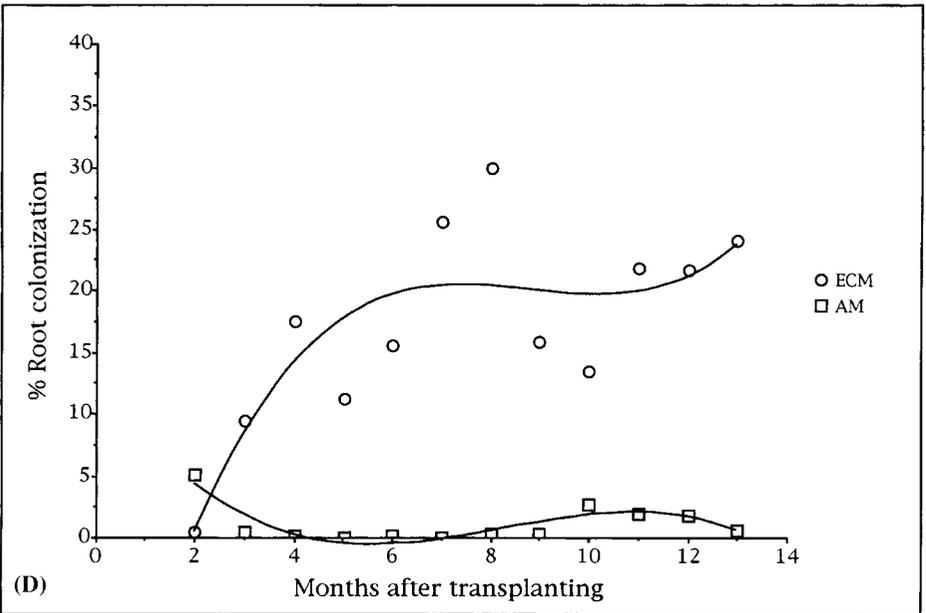
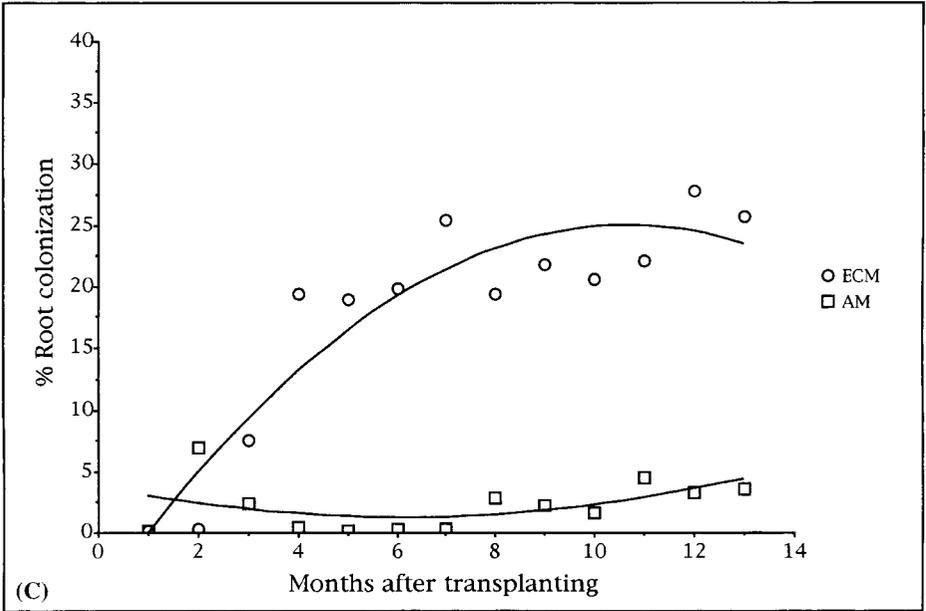


Fig 2. (continued)

the following forms: Bugre: % ECM =  $-4.32 + 5.731x - 0.278x^2$ ,  $r^2 = 0.92^{**}$  (fig 2a); Formiga: % ECM =  $-17.366 + 11.185x - 0.614x^2$ ,  $r^2 = 0.86^{**}$  (Fig 2b); Paredão: % ECM =  $-5.564 + 5.73x - 0.269x^2$ ,  $r^2 = 0.85^{**}$  (fig 2c); Experimental:  $-24.999 + 16.262x - 1.899x^2 + 0.072x^3$ ,  $r^2 = 0.65^*$  (Fig 2d).

**Pattern C, following ECM-forming *Pinus taeda* (location: Mafra) (fig 3)**

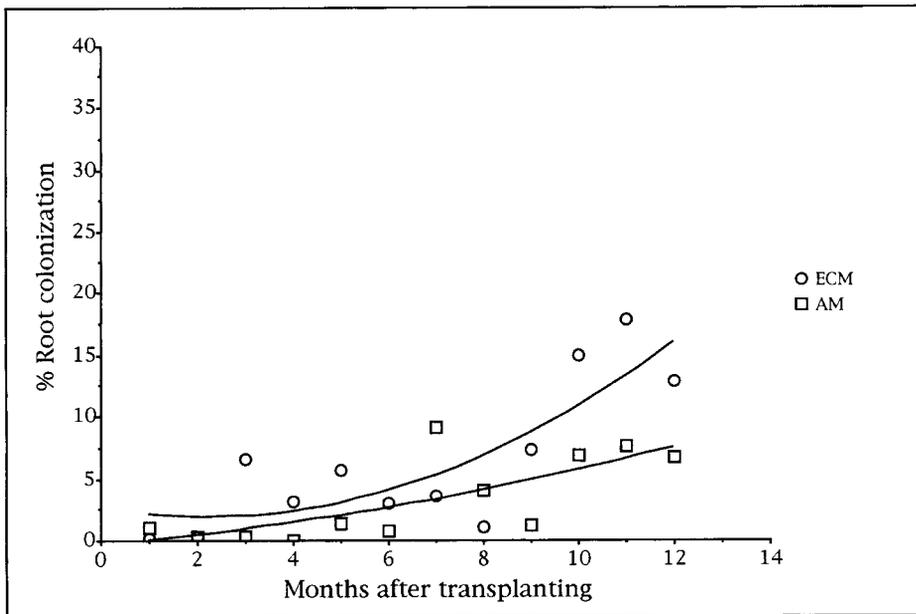
Unlike the sequence of events in patterns A and B, the recorded incidences of AM and ECM colonization at Mafra both progressively increased, non-linearly, after transplanting reaching maximal values at the end of the period of observation (13 months after transplanting). However, the largest value of AM colonization was less than that at Laginski (pattern A following soya bean):

the maximal incidence of ectomycorrhizas was less than elsewhere (% ECM =  $2.59 - 0.65x + 0.15x^2$ ,  $r^2 = 0.68^{**}$ ; % AM =  $-0.33 + 0.33x + 0.03x^2$ ,  $r^2 = 0.54^*$ ).

**DISCUSSION**

This examination of events at six sites has produced evidence of three distinctive patterns of ECM and AM colonization on roots of *E dunnii*, with pattern A at Laginski following the AM-forming soya bean (*Glycine max*), pattern B at Bugre, Formiga, Paredão and Experimental following the AM/ECM-forming *E viminalis* and pattern C at Mafra where the ECM-forming *P taeda* had been grown.

In pattern A, the initially high frequency of AM decreased while that of ECM progressively increased. In pattern B the inci-



**Fig 3.** Successive changes in the incidence of ecto- (ECM) and arbuscular (AM) mycorrhizas on roots of *Eucalyptus dunnii* planted at Mafra after ECM-forming *Pinus taeda* (pattern C).

dence of AM remained minimal while that of ECM increased more rapidly than in pattern A; it reached a plateau. In pattern C both AM and ECM increased progressively but amounts of AM and ECM never attained the levels recorded in pattern A.

According to these results, it seems that these different patterns directly reflect the pre-planting history of earlier vegetation. But, caution is essential: while pattern B was found at four sites where *E dunnii* immediately followed *E viminalis*, patterns A and C were each exhibited at only one site, A being associated with previous soya bean and C with *P taeda*.

Interestingly, pattern B was not identified by Bellei et al (1992), who investigated sites that carried either *P elliottii* (ECM) or semi-natural (AM) vegetation. In the event the only pattern recorded by Bellei et al (1992) was reminiscent of pattern A. Thus, like us, Bellei et al (1992) reported pattern A where *E viminalis* was planted on AM sites but unlike us they also recorded pattern A on ECM sites.

There are therefore similarities and differences between the present study and that reported by Bellei et al (1992). At present there is not a convincing explanation of the occurrence of pattern A on sites that previously carried vegetation with either AM or ECM. More replicate sites must be investigated before predictions can be made legitimately: the increased number of sites should include the range of soil types subject to plantation forestry in the region.

Nevertheless, we hypothesize that the existence of high levels of AM propagules associated with a lower level of ECM inoculum in soil was probably responsible for pattern A, observed in the agricultural site, Laginski. It is known that high levels of AM propagules in the soil are quite important in the initial colonization by these fungi because they are efficient in the primary colonization. On the other hand, the increase of ectomycorrhizal colonization with age

observed in the six plantations, is the result of secondary colonization of the ECM fungi. As these fungi establish their colonization most points of entry may be blocked, preventing subsequent AM colonization (Chilvers et al, 1987).

Futhermore, the stronger ECM colonization of *E dunnii* on land that had previously carried *E viminalis*, occurred presumably because old stands of eucalypts are strong inocula sources of ectomycorrhizas as observed by Estrada et al (1993) in the state of Minas Gerais, Brazil. But why should ECM formation following ECM-forming *P taeda* be less vigorous? It may be possible that each host species has a preferred strain, if not species, of ectomycorrhizal symbionts as pointed out by Lei et al (1990), Malajczuk et al (1990) and Molina et al (1992). The concurrent increases in both types of mycorrhizas may have resulted from the ability of the endomycorrhizal fungi to colonize the sites that remained available owing to the low ectomycorrhizal colonization.

The data suggest that the occurrence of arbuscular- and ecto- mycorrhizas on the roots of eucalypts is responsive to local conditions. Having established the range of responses in the field of *E dunnii* to natural inocula it would probably be more 'cost-effective' to simulate them in glasshouse conditions where soil moisture and air temperatures can be controlled.

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