

PRESENCE OF VESICULAR-ARBUSCULAR  
MYCORRHIZAE IN *EUCALYPTUS* SPP. AND  
*ACACIA* SP., AND THEIR ABSENCE IN *BANKSIA* SP.  
AFTER INOCULATION WITH *GLOMUS*  
*FASCICULATUS*

BY NICHOLAS MALAJCZUK

*Division of Land Resources Management, C.S.I.R.O., Private Bag, P.O.  
Wembley, Western Australia 6014*

ROBERT G. LINDERMAN

*U.S. Dept. of Agriculture, SEA-AR, Ornamental Plants Research Laboratory,  
Corvallis, Oregon 97330, U.S.A.*

JOHN KOUGH

*Oregon State University, Corvallis, Oregon, 97331, U.S.A.*

AND JAMES M. TRAPPE

*U.S. Department of Agriculture, F.S., Pacific Northwest Forest and Range  
Experiment Station, Forestry Sciences Laboratory, Corvallis,  
Oregon, 97331, U.S.A.*

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SUMMARY

Vesicular-arbuscular (VA) mycorrhizae occurred in seedlings of *Eucalyptus marginata*, *E. diversicolor* and *Acacia pulchella* grown in soil inoculated with *Glomus fasciculatus*. Infection levels were 30 to 35% of the root length of the eucalypts and 50 to 70% of the *Acacia*. When *A. pulchella* was grown in the same pots with the two eucalypts, its infection was not increased. *Banksia grandis* did not form VA mycorrhizae when grown alone or in association with *A. pulchella*. These results are discussed in relation to the evolution of plants in Australia and the ecological significance of VA mycorrhizae in the eucalypt forest ecosystem.

INTRODUCTION

Eucalypts, the major forest species in Australia, typically have an understory composed predominantly of legumes that develops as an aftermath of high-intensity fires (Christenson and Kimber, 1975; Floyd, 1976). However, recent low-intensity controlled fires have led to an increase in proteaceous species as components of the understory (Peet, 1971). Chilvers and Pryor (1965) reported that eucalypts typically form ectomycorrhizae. However, Asai (1934) and Maeda (1954) observed 'endomycorrhizae' on eucalypts, and recently Khan (1978) reported vesicular-arbuscular (VA) mycorrhizae of *Eucalyptus pilularis* Sm. and *E. paniculata* Sm. growing on mine spoils. Legumes, including *Acacia* spp., are generally reported

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to have VA mycorrhizae (Khan, 1978). On the other hand, proteaceous species, including *Banksia grandis* Willd., are reportedly non-mycorrhizal (Lamont, 1972; Khan, 1978).

The sharing of a common VA fungal symbiont by eucalypts and leguminous understory species could promote ecosystem stability. An increase in non-mycorrhizal proteaceous species in the understory, in contrast, might seriously affect the maintenance of VA fungal populations in eucalypt forest soil, with potential implications for nutrient cycling and regeneration. We therefore designed experiments to determine (1) whether the Western Australian forest eucalypts *E. marginata* Donn. ex Sm. and *E. diversicolor* F. Muell. form VA mycorrhizae, although they are typically ectomycorrhizal; and (2) whether the reportedly non-mycorrhizal *Banksia grandis* can form VA mycorrhizae. Hirrel, Mehraveran and Gerdemann (1978) reported mycorrhizal fungus hyphae and vesicles in the roots of usually non-mycorrhizal species grown with VA mycorrhizal 'companion plants'. In addition to growing the aforementioned species alone in VA fungus-inoculated soil, therefore, we included the additional treatment of growing each with *Acacia pulchella* R. Br. plus VA fungus inoculum.

#### MATERIALS AND METHODS

Pregerminated seedlings of *E. marginata*, *E. diversicolor*, *A. pulchella* and *B. grandis* were planted in 250 cm<sup>3</sup> plastic tubes (Leach Cone-Tainers, Inc., Aurora, Oregon, USA) filled with inoculum from asparagus *Glomus fasciculatus* (Thaxter *sensu* Gerd.) Gerd. and Trappe sand-pot cultures which included roots, spores, and hyphal fragments. The seedlings were planted alone or with *A. pulchella* as a companion plant. In all treatments with *A. pulchella* we added *Rhizobium* strains isolated from *Acacia* root nodules (WA 336 and WA 400, University of Western Australia Soil Science Department). All treatments were replicated three times.

Inoculated plants were harvested after 4 months of growth in a temperature-controlled glasshouse (25/15 °C day/night temperatures). Roots were washed and separated according to species; only intact root systems were kept for examination. Roots were cleared and stained in lactophenol trypan blue (Phillips and Hayman, 1970), and mycorrhizal fungus infection was quantitatively assessed by determining the percentage of the root length with mycorrhizal fungus structures in 25 randomly selected 1 cm root segments per plant or from the entire root system if it was less than 25 cm in total length.

#### RESULTS

Typical vesicles, arbuscles and internal hyphae were formed in *A. pulchella* inoculated with *G. fasciculatus*; arbuscles filled most cortical cells. About 60% of the *A. pulchella* roots were occupied by VA fungal structures [Table 1; Fig. 1(a), (b)].

Eucalypts also formed typical VA mycorrhizae (about 30 to 35% of the root length), although with less intensity than *A. pulchella*. Colonization levels in the eucalypts were not increased when they were grown with *A. pulchella* as a companion plant. Arbuscles in the eucalypts were morphologically distinct from those in *A. pulchella*; they were more compact, occupying only a portion of the cortical cells [Fig. 1(d) to (f).] Most arbuscles were in the eucalypt short roots, and they were abundant close to the root tips [Fig. 1(e)]. In addition, more hyphal coils were present in the eucalypts than in *A. pulchella*.

Table 1. *Extent of vesicular-arbuscular (VA) mycorrhiza formation by Glomus fasciculatus on Eucalyptus spp. and Banksia grandis grown singly or with Acacia pulchella as a companion plant*

Host plant examined	Percentage of root length with VA mycorrhizae*		
	Grown alone	Grown with <i>Acacia</i>	<i>Acacia</i> companion plant
<i>Eucalyptus diversicolor</i>	31 ± 2.5	30 ± 2.5	52 ± 4.4
<i>E. marginata</i>	30 ± 2.1	34 ± 5.3	55 ± 3.0
<i>Banksia grandis</i>	0.0	0.0	66 ± 7.0
<i>Acacia pulchella</i>	61 ± 3.0	—	—

\* Data are mean percentages ± standard deviations of three replications, each based on observations of 25 root pieces approximately 1 cm long.

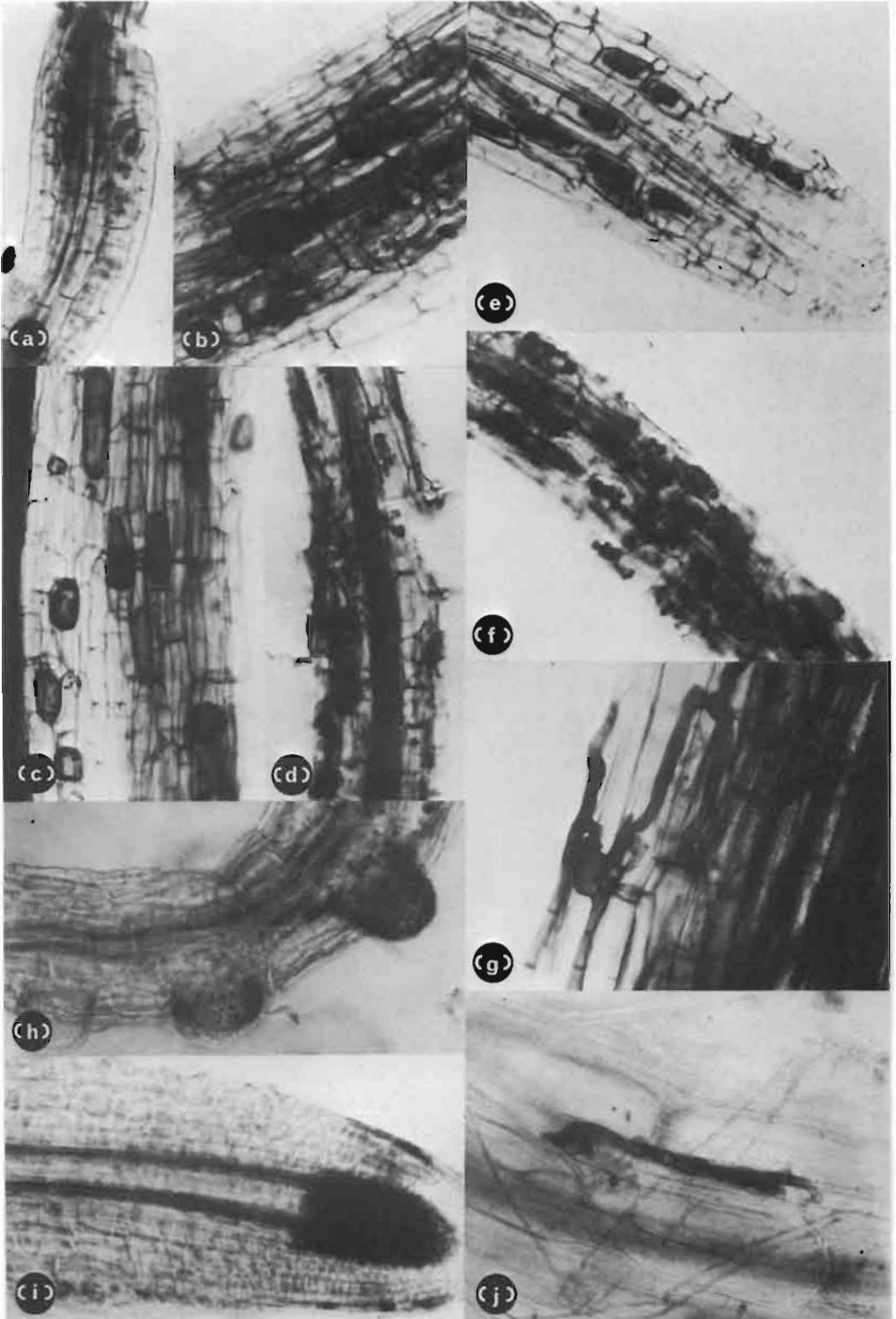
*Banksia grandis* did not form VA mycorrhizae, even when grown with *A. pulchella* as a companion plant. Neither proteoid nor non-proteoid roots contained fungal tissue [Fig. 1(h) and (i)]. Although occasional spores derived from the inoculum and surface hyphae of *G. fasciculatus* occurred on the rhizoplane [Fig. 1(j)], no intercellular or intracellular fungal structures were observed.

#### DISCUSSION

This first experimental evidence that eucalypts form VA mycorrhizae confirms the earlier observations from field collections (Asai, 1934; Khan, 1978; Maeda, 1954). The VA mycorrhizal structures in eucalypt roots are typical of those in other VA hosts, so it can be inferred that eucalypts utilize dual mycorrhizal associations in nature. The Salicaceae have been known to form both VA endomycorrhizae and ectomycorrhizae for some time (e.g. Dominik, 1956), but the phenomenon is emerging as more common than was previously recognized. Baylis (1971) and Sward (1978) found that *Leptospermum* spp., members of the Myrtaceae along with *Eucalyptus* and also commonly ectomycorrhizal, could form VA mycorrhizae. Other plants with this dual capability include species of *Quercus* (Grand, 1969), actinorrhizal plants in seven genera of five families (Trappe, 1979), and several ferns (Cooper, 1976).

The prerequisite conditions for formation of ectomycorrhizae *v.* VA mycorrhizae are yet to be determined. Fire in the forest environment may influence the type formed in that it removes accumulated litter that serves as a nutrient base favouring formation of ectomycorrhizae (Meyer, 1973; Harvey, Larsen and Jurgensen, 1976). More specifically, fire is a natural part of the Western Australian forest ecosystem (Churchill, 1968). Malajczuk and Hingston (manuscript in preparation) observed fewer ectomycorrhizae and more unsubserved, non-mycorrhizal roots on eucalypts in burned-over areas than in unburned areas. Possibly a colonization of the eucalypt roots by VA mycorrhizal fungi could occur after fire, because the VA mycorrhizal legume understory also develops then. As the litter again accumulates and the legumes die out, conditions become increasingly conducive to activity of ectomycorrhizal fungi.

The sharing of common VA fungal symbionts by eucalypts and understory legumes might have profound ecological implications. For example, the possibility



exists that the VA fungal symbiont might be involved in transfer of nitrogen and other materials between legumes and eucalypts.

Several non-mycorrhizal plant families such as Chenopodiaceae, Cruciferae, Cyperaceae, and Juncaceae (Hirrel, Mehraveran and Gerdemann, 1978; Read, Koucheki and Hodgson, 1976) can be colonized by VA mycorrhizal fungi when they are grown in the presence of VA mycorrhizal companion plants. In our study, *B. grandis* did not form VA mycorrhizae, even when grown in the same container with heavily mycorrhizal *A. pulchella* plants. It is known that proteaceous plants have evolved the proteoid root system as a means of enhancing uptake of phosphorus (Malajczuk and Bowen, 1974) and possibly other nutrients. In addition, proteoid roots are sites of greatly enhanced metabolic activity, as demonstrated by  $^{14}\text{CO}_2$  autoradiography (Malajczuk and Bowen, unpublished data). Thus the proteoid root system appears to function in members of the Proteaceae as mycorrhizae function symbiotically in other plants.

An important feature of the mycorrhizal symbiosis is the symbiont's ability to acquire and store phosphorus as polyphosphate granules in the roots of plants, an adaptation to long-term survival in phosphate-deficient soil (Ashford, Ling-Lee and Chilvers, 1975; Ling-Lee, Chilvers and Ashford, 1975; Callow *et al.*, 1978). Jeffrey (1964, 1968) reported that *Banksia* spp. have developed a means of synthesizing polyphosphates in the absence of a fungal symbiont, a feature previously thought to be unique to microbial metabolism (Jeffrey, 1964; Harold, 1966). Perhaps the high internal phosphate levels in roots of proteaceous plants prevent colonization by VA fungi (Sanders, 1975; Menge *et al.*, 1978).

From an evolutionary viewpoint, plant species that can form either VA endomycorrhizae or ectomycorrhizae may be connecting links between the more primitive VA mycorrhizal habit (Pirozynski and Malloch, 1975) and the more advanced ectomycorrhizal habit. Proteaceous plants, found almost exclusively in Australia, are apparently a case of divergent evolution from primitive Angiosperms (Johnson and Briggs, 1963). They have evolved a nutrient support system alternative to the mycorrhizal system of other plants.

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Fig. 1. All photomicrographs are of cleared and stained root tissue. (a) Arbuscules of *Glomus fasciculatus* in root of *Acacia pulchella*. (75 ×). (b) Vesicles of *Glomus fasciculatus* in root of *Acacia pulchella* (75 ×). (c) Vesicles of *Glomus fasciculatus* in long root of *Eucalyptus marginata* (75 ×). (d) Arbuscules of *Glomus fasciculatus* in short root of *Eucalyptus marginata* (75 ×). (e) Arbuscules of *Glomus fasciculatus* in root tip of *Eucalyptus diversicolor* (75 ×). (f) Arbuscules and vesicles of *Glomus fasciculatus* in root of *Eucalyptus diversicolor* (75 ×). (g) Penetration site of *Glomus fasciculatus* on root of *Eucalyptus diversicolor* (187.5 ×). (h) Proteoid root initials on root of *Banksia grandis* (75 ×). (i) Nonproteoid root tip of *Banksia grandis* (75 ×). (j) External hypha of *Glomus fasciculatus* on root of *Banksia grandis* (187.5 ×).

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