Mycorrhizal status of indigenous tree species in a forest biome of the Eastern Cape, South Africa

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Mycorrhizal fungi are intimately associated with plant roots, affecting plant growth, health and increasing the plants' tolerance to environmental stress. Several mycorrhizal types are recognized based primarily on morphological characteristics within plant roots. When considering propagation and management of an indigenous plant species, it is essential to know its mycorrhizal status. Root samples from 17 tree species common to the pockets of forest in the Eastern Cape province, and representing the families Rubiaceae, Scrophulariaceae, Oleaceae, Podocarpaceae, Myrsinaceae, Anacardiaceae, Caesalpinoideae, Papilionoideae, Rutaceae, Meliaceae, Celastraceae, Flacouticeae and Ebenaceae, were sampled and examined for mycorrhizal colonization. Microscopic examination of all the species produced evidence of morphological structures indicative of endomycorrhizal associations as indicated by the presence of intercellular hyphae combined with vesicles, arbuscules or hyphal coils. Hyphal coils (also known as *Paris*-type associations) appeared to be abundant, especially within the *Cassine* genus. Arbuscules (also known as *Arum*-type associations) were scarce but sometimes present, and vesicles were prolific in *Olea capensis.* Most of the tree species examined have been assigned arbuscular mycorrhizal status. No ectomycorrhizal associations were recorded.

Introduction

The management and conservation of forest biomes is a recognized priority on a global scale. Integral to the understanding of forest ecology is the rhizosphere and in particular the mycorrhizal symbiotic associations between plant hosts and fungi. Mycorrhizal relationships are an example of mutual symbioses, involving plants and fungi, whereby both organisms benefit through an exchange of nutrients at the root–soil interface.[1] A number of different types of mycorrhiza can be identified by the hyphal structures they form, but only two types that are relevant to this study are discussed here.

Arbuscular mycorrhizas (AM), sometimes referred to as endomycorrhizas, are formed predominantly by the fungal group Glomeromycota.[2] The association is identified by intracellular dichotomously branching haustorial structures called arbuscules, found in the cortical plant root cells and hyphal coils, as well as intercellular hyphal networks and external hyphae that extend into the soil. AM are found on a wide range of host species, predominantly colonizing herbaceous shrubs and tree species. AM colonization has no visible effect on root morphology.[3] Ectomycorrhizas (ECM), on the other hand, form an outer sheath (mantle), an internal, intercellular network of hyphae (Hartig net) and extra-radial hyphal networks and rhizomorphs. ECM fungi have a visible effect on root morphology. Root tip branching often becomes dichotomous or irregular. The root tip also tends to swell and the mantle may colour the area of

colonization. ECM are formed predominantly by the group Basidiomycotina, to a lesser extent by Ascomycotina and only rarely by Zygomycota,[1] and are largely associated with tree species. ECM fungi are more host specific, with associations being more dominant in temperate and tropical forest regions. AM fungi, on the other hand, are generalists, occurring in most vegetation types.[1] There are other mycorrhizal types but these are generally more specific in their host range and fungal partners and are not mentioned in this paper.

Mycorrhizal associations play important roles in the ecological functioning of ecosystems, forming an integral part of the nutrient cycle. Mycorrhizal fungi break down organic nutrients, assimilate inorganic nutrients and transport these to the plant. This is necessary for the survival of over 90% of all flowering plants.[1] Mycorrhizal associations are essential to forest biome ecology,[1] and have been shown to support the diversity of higher plants in experimental models.[4] Recent research in the rainforests and Miombo forests of central, east and southern Africa reveal a large diversity of ECM fungi.[5–9] Hogberg[5] detailed the mycorrhizal status of species representative of a number of plant families, namely, Caesalpiniaceae, Dipterocarpaceae, Euphorbiaceae, Papilionaceae, Proteaceae (*Faurea saligna*), Mimosaceae and Stilaginaceae. Such studies have been concentrated in Tanzania, Zambia and Zimbabwe, with little research carried out in South Africa. Allsopp and Stock[10] and Skinner[11] have outlined the mycorrhizal status of some Western Cape fynbos species and Eastern Cape shrubs, respectively. Most of these plant species were recorded as having AM associations. Although both studies included some tree species, most of the accounts related to herbaceous shrubs. Fungal records[12] indicate that ECM fungi, such as *Amanita, Cantherellus* and *Boletus,* have been collected in Eastern Cape forests, in particular Knysna and Tsitsikamma.

The objective of this study was to determine the mycorrhizal status of selected indigenous tree species in the Eastern Cape. Tree species that predominantly make up the Eastern Cape forest biome were chosen and identified in the field. Root samples were taken for macroscopic and microscopic examination and the assessment of mycorrhizal status.

Materials and methods

Sampling site and tree selection

Grahamstown (26°31′E, 33°18′S) is situated on the eastern border of the Cape Fynbos region, in the Eastern Cape.[13] The areas sampled around the city lie within the 'Albany Hotspot',[14] where five major African phytochoria meet. Having formed part of a conservancy, this area has retained a high plant species biodiversity.[14] The natural pockets of forest surrounding Grahamstown, and particularly in the Featherstone Kloof, are relatively young. The Grahamstown area is approximately 700 m above sea level and receives an annual rainfall of 400–600 mm. The mean summer temperature (November–May) is 22–24°C, with a mild mean winter temperature (June–October) of 12–14°C.[15] Samples were taken during the summer (December 2003 – January 2004), the growing season for the vegetation, but

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this was also a relatively dry period, in which only 38 mm fell.[13]

Seventeen species representative of 16 genera from 13 families were examined in this study, each selected on the likelihood of finding ECM within certain tree families (Table 1).[16] The tree species were identified taxonomically in the field, and root samples were collected. The soil at the base of the tree was loosened using a hand fork and roots were followed from the base of the tree to the feeder roots to ensure accuracy of identification. Approximately 15–20 g of root material was collected in each case.

Processing and examination of root samples

Freshly collected roots were examined under a dissecting microscope (Leica S4E) to identify any external mycorrhizal features, such as mantles, rhizomorphs and hyphal networks. The presence of these features provides preliminary indication of ECM associations.[17] The roots were washed gently with water to remove excess soil particles. A sub-sample of the roots was then cleared and stained using the procedure described in Brundrett *et al.*[18] Ten grams of root material was heated to 90°C in 5% KOH for 30 min to soften the root, then bleached in alkaline H_2O_2 for 30–40 min. Some of the root samples had high phenolic concentrations and required longer bleaching times. The roots were then acidified with 0.1 M HCl overnight and heated to 90°C with lactoglycerol trypan blue stain for 30 min; this was followed by destaining in lactoglycerol for 24 h. The remaining roots were stored in 50% ethanol for preservation, should additional analyses become necessary.

Stained roots were examined for both external and internal hyphal structures under a compound microscope (Leica CME). Each slide examined contained 4–5 root pieces 2 cm long. For each species, five slides were prepared, such that between 20 and 25 root pieces and 40–50 cm of root were examined per tree species. All the fungal structures were recorded and tabulated, and the mycorrhizal status determined. The presence of a mantle and Hartig net would be evidence of ECM status. Roots were regarded as AM only if either vesicles and/or arbuscules were found. The presence of hyphae alone was not regarded as evidence of AM status, although hyphal networks were noted. Photographs were taken on black and white film, as well as digitally, under the DIC microscope (Olympus BX50).

The most defining morphological characteristic of AM associations is the presence of arbuscules within cortical root cells. These are regarded as the interface of nutrient exchange between plant and fungus.[1,25] Two types of arbuscules are recognized and are termed *Arum* type and *Paris* type. *Arum*-forming mycorrhizas are typical of fast-growing plants. The fungus colonizes the plant rapidly through intercellular hyphae and the intracellular arbuscules are short-lived. Coils may form, but are not common.[1] *Paris*-forming mycorrhizas have a slower infection rate, passing from cell to cell within the plant cortex, and can be characterized by extensive intracellular coiled hyphae with arbuscules forming from the coils. The coil structures are suspected of functioning as active nutrient exchange interfaces, as the surface area the coil creates is comparable to that of *Arum*-type arbuscules in a cortical cell.[1,26]

Results

Examination of roots using a dissecting microscope indicated no external structures, and therefore no obvious ECM associations. Observing the roots under the compound microscope revealed several mycorrhizal fungal structures in the root as presented in Table 2; examples of these are shown in Fig. 1a–h. The presence of nodules was recorded on *Podocarpus latifolius* (Fig. 2). Table 2 indicates that *Psoralea pinnata* roots also bore nodules that are associated with nitrogen-fixing bacteria.[19] Examples of coils representing *Paris*-type associations (Fig. 1g,h) were found in roots of *Cassine aethiopica* and *Cassine papillosa*. This AM type was

Table 1. List of families, genera and species sampled and examined for mycorrhizal status.

Family	Genus and species
Rubiaceae	*Burchellia bubaline* (Linn. F.) Sims
Scrophulariaceae	*Halleria lucida* L.
Oleaceae	*Olea capensis* L.
Podocarpaceae	*Podocarpus latifolius* (Thunb.) R. Br ex Mirb.
Myrsinaceae	*Rapanea melanophloeos* (Mez)
Anacardiaceae	*Harpephyllum caffrum* Bernh. ex Krauss; *Rhus chirindensis* Baker f.
Caesalpinoideae (Fabaceae)	*Schotia afra* (L.) Thunb.
Papilionoideae (Fabaceae)	*Psoralea pinnata* L.
Rutaceae	*Vepris lanceolata* (Lam.) G. Don
Meliaceae	*Ekebergia capensis* Sparrm.
Celastraceae	*Cassine aethiopica* Thunb.; *C. papillosa* (Hochst.) Kuntze
Flacourtiaceae	*Kiggelaria africana* L.; *Scolopia mundi* (Eckl. & Zeyh.) Warb.
Ebenaceae	*Diospyros dichrophylla* (Gand.) De Winter; *D. scabrida* (Harv. ex Hiern) De Winter

Table 2. Fungal features found in the roots of each species examined, in terms of mycorrhizal characteristics.

Species	Hyphae	Vesicles	*Arum* arbuscules	*Paris* coils	Intraradical spores	Other structures	Type of association
Burchellia bubalina	+	+	–	+	–		AM
Halleria lucida	+	+	–	+	–		AM
Olea capensis	+	++	+	+	–		AM
Podocarpus latifolius	+	+	–	+	–	Nodules	AM
Rapanea melanophloeos	+	+	+	+	+		AM
Harpephyllum caffrum	+	–	+	+	–		
Rhus chirindensis	+	+	+	+	–		AM
Schotia afra	+	–	–	+	–		
Psoralea pinnata	+	+	+	+	+	Nodules	AM
Vepris lanceolata	+	–	–	+	–		AM
Ekebergia capensis	+	–	+	+	–		AM
Cassine aethiopica	+	–	–	++	–		AM
Cassine papillosa	+	+	–	++	–		AM
Kiggelaria africana	+	+	+	+	–		AM
Scolopia mundii	+	+	+	+	–		AM
Diospyros dichrophylla	+	+	–	–	–		Endo
Diospyros scabrida	+	–	+	+	–		AM

Results are presented as features absent (–) or present (+). Species displaying large frequency of certain features are designated by (++).
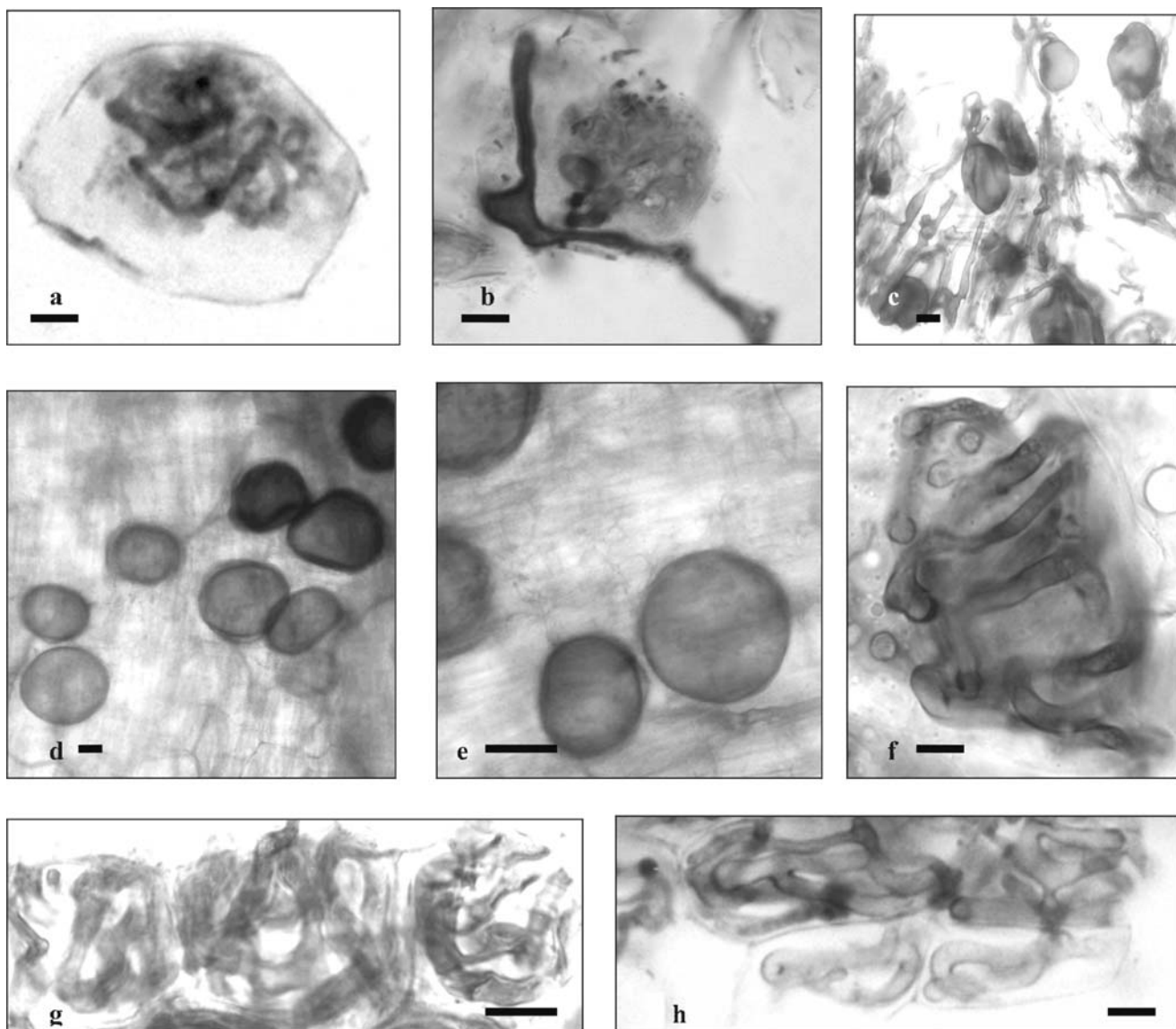
Fig. 1. **a**, **b**, *Arum*-type arbuscules found in *Psoralea pinnata*; **c**, vesicles of *P. psoralea*; **d**, **e**, vesicles of *Kiggelaria africana*; **f**, *Paris*-type coils found in *Burchellia bubaline*; **g**, **h**, *Paris*-type coils in *Cassine papillosa*. Scale bars = 1 μm.

prevalent in most species examined with the exception of *Diospyros dichrophylla* (Table 2). *Arum*-type associations (Fig. 1a,b) were less common but found together with coils in the same root. Vesicles were found within most of the species with the exception of *Vepris, Ekebergia, Cassine aethiopica* and *Diospyros scabrida* (Table 2).

Discussion

This study found that the majority of tree species examined were associated with AM fungal structures and were therefore given AM status (Table 2). Similar results have been found in other investigations, a summary of which is provided in Table 3. Within the family Rubiaceae, Skinner,[11] Allsopp and Stock,[10] Harley and Harley,[20] and Högberg[5] found that the species examined were AM, or endomycorrhizal. The exception, *Rubia peregrina,*[20] was the only ECM species out of 11 species examined.

Skinner[11] reported the family Scrophulariaceae, in the Eastern Cape, to be AM. Our study and Allsopp and Stock[10] confirm the AM status given to *Halleria lucida* (Tables 2 and 3). Harley and Harley[20] suggest that the many species they listed may either have AM fungi or be non-mycorrhizal, having no fungal association at all (Table 3). *Olea capensis* (Oleaceae), reported by Allsopp

Fig. 2. Nodules on *Podocarpus latifolius*. Scale bar = 10 μm.

Table 3. Comparative results from previous studies within the same plant families.

Family	Skinner[11]	Allsopp and Stock[10]	Harley and Harley[20]	Wubet *et al.*[21]	Högberg[5]	This study
Rubiaceae	AM	AM	1 of 11 spp. is ECM (*Ruba peregrina*); the rest are AM	–	Endo	AM
Scrophulariaceae	AM	AM	AM or absent (many spp.)	–	–	AM
Oleaceae	–	Endo	*Fraxinus excelsor* AM and ECM	AM	–	AM
Podocarpaceae	–	AM	–	AM	–	AM
Anacardiaceae	AM	AM	–	–	AM	AM
Caesalpinoideae	–	–	–	–	*Bautinia* spp. AM *Brachystegia* spp. ECM *Julbernardia globiflora* ECM	AM
Papilionoideae	–	–	–	–	AM	AM
Rutaceae	AM	–	–	–	–	AM
Meliaceae	–	–	–	AM	–	AM
Celastraceae	–	AM	–	–	AM	AM
Flacourtiaceae	–	–	–	–	AM	AM
Ebenaceae	–	AM	–	–	AM	AM

–, No data; AM, arbuscular mycorrhizal; ECM, ectomycorrhizal; Endo, endomycorrhizal.

and Stock[10] as AM, is confirmed by our study. Other species of Oleaceae examined by Wubet *et al.*[21] indicated AM colonization. Harley and Harley,[21] however, report *Fraxinus excelsor* to be either AM or ECM.

Wubet *et al.*[22] and Allsopp and Stock[10] recorded that *Podocarpus falcatus* (Podocarpaceae) associates with AM fungi (Table 3); this was confirmed for *P. latifolius* (Table 2). Nodule-like structures (Fig. 2) were observed in the roots of *Podocarpus* species. *Podocarpus henkelii* (Stapf ex Dallim & Jacks) as well as other species sampled from different regions in the country (results not presented) also displayed root nodules. These nodules were not of ECM origin, although ECM fungi such as *Scleroderma* sp. and *Amanita* sp. have been observed growing below or near *Podocarpus* species in forestry areas in Sabie, Mpumalanga, and Hogsback, Eastern Cape. These nodules have been referred to as mycorrhizal when mycorrhizal fungi are present and/or actinorrhizal when they contain N-fixing bacteria such as *Frankia*.[22] They have been recorded for a number of gymnosperm families such as Podocarpaceae[23] and angiosperm families such as Casuarinaceae.[22]

The family Myrsinaceae was not included in any of the comparative studies, but in our study the species *Rapanea melanophloeos* was found to be AM (Table 2). The *Rhus* species (Anacardiaceae) examined in this study as well as species examined by Skinner[11] and Allsopp and Stock[10] all record AM colonization (Tables 2 and 3). AM status was also assigned to *Lannea schimperii*.[5]

Several species within the family Caesalpinoideae were examined by Högberg[5] and recorded as being either AM or ECM (Table 3). He also noted that none of these species bore nitrogen-fixing nodules (although they are members of the Leguminaceae), but that all Papilionoideae species examined were nodulated. *Schotia afra* (Caesalpinoideae) did not bear any nodules, which concurs with Högberg's[5] findings, and was found to be AM. Members of genera within this family deserve more thorough examination, as the presence of ECM seems to be established.[5]

Psoralea pinnata (Papilionoideae) was found to be AM, in agreement with Högberg,[5] who recorded this status for all five species examined (Table 3). Nodules were also observed on roots (Table 2) and this was confirmed to be an association with nitrogen-fixing rhizobia.[19] Tripartite associations between the N-fixing bacteria, AM and legumes are well known[24] and indeed evidence of this was found on the roots of *P. pinnata*, where

hyphae and vesicles were present inside root nodules (Table 2).

Skinner[11] examined species from the family Rutaceae and recorded that this family was AM (Table 3); this was confirmed in *Vepris lanceolata* (Table 2). This study also confirms the status of *Ekebergia capensis* (Meliaceae) as AM[21] (Tables 2 and 3). Celastraceae species were documented by Allsopp and Stock[10] and Högberg[5] as being AM (Table 3) and this was confirmed by the presence of AM structures observed in *Cassine aethiopica* and *C. papillosa* (Table 2).

Högberg[5] found *Oncoba spinosa* (Flacourtiaceae) to be AM; two other species within the same family, *Kiggelaria africana* and *Scolopia mundi,* confirmed this status. All the *Diospyros* species (Ebenaceae) examined by Allsopp and Stock[10] and Högberg[5] reveal AM colonization. *D. dichrophylla* roots that were investigated showed no evidence of arbuscule structures, but the presence of vesicles indicate that the plant is more than likely AM. More intense sampling is needed to confirm AM status convincingly.

Although a defining characteristic, arbuscules may be difficult to find under natural conditions. *Arum*-type associations were observed in low frequency and this is generally attributed to their short life span.[1] The results from this investigation show that *Paris*-type associations were more frequently encountered overall and were particularly prolific in the genus *Cassine*. Table 2 provides an indication of their presence or absence and some idea of their prolific nature. *Paris*-type coils were found in the majority of species and were generally more common (Table 2). The dominance of the *Paris* type may imply that coiled hyphae live longer and are more tolerant to environmental stress conditions such as drought, suggesting that external factors create responses that allow for the dominance of one type of mycorrhiza (*Paris* vs *Arum* type) over another. However, the arbuscular types may also be fungal species specific, with environmental conditions affecting the fungal-species composition. If arbuscular types are not fungal species specific as suggested by Kubota *et al.*,[27] then host plants may determine dominant arbuscular type, indicating that the different structures are adaptations for fungal survival. Both *Arum*- and *Paris*-type associations were observed in several plant species examined (Table 2) and this indicates that the arbuscular types are fungal-species specific, as more than one AM fungal species would be expected to colonize the host plant; this is difficult to detect when examining colonized roots. The presence of either or both types is more likely to be dictated by a combination of fungal and

host species[27] with possibly environmental influences; the relative dominance of arbuscular type is poorly understood and requires further investigation.

The family of AM fungus that has colonized the root will determine the presence (Glomineae) or absence (Gigasporineae) of vesicles and their presence is affected by the soil environment.[1] Vesicles vary from species to species in morphology and position of occurrence in the root. They contain lipids and nuclei and are generally considered to be storage organs.[1] In our study, the absence of vesicles could be attributed to either the species of fungus present, environmental factors, or simply under-sampling. *Olea capensis* roots had large numbers of vesicles (Table 2), possibly ruling out the environmental factors and suggesting either an effect of host hospitality or of under-sampling.

Conclusion

Allsopp and Stock[10] and Högberg[5] use the term endomy-corrhiza, the former adopting it with caution. Högberg's use of the term satisfies the general definition of AM, requiring the presence of vesicle and arbuscules.

Our study provides the first account of mycorrhizal status for the following species: *Burchellia bubalina, Podocarpus latifolius, Rhus chirindensis* and *Harpephyllum caffrum, Schotia afra,* and *Vepris lanceolata.* The prevalence of AM associations reported here reflects a similar pattern to that found by Allsopp and Stock[10] and Skinner.[11] Even though these studies have not encompassed even a small percentage of the tree species of South Africa, the results confer an initial status on those investigated. No ECM associations were found in this study, although there are other species that could be considered for this possibility, for example, other genera within the Caesalpinoideae and Papilionoideae. *Afzelia quanzensis* is another promising species whose taxonomic relatives have ECM associations.[16] Special attention should be directed at the Knysna/Tsitsikamma forests and Miombo woodlands, where the presence of ECM fungi such as the species mentioned above and, in addition, species of *Clavulina* (14 species), *Lactarius* (3 species), *Cantherellus* (4 species), *Russula* (4 species), *Cortinarius* (5 species), amongst others, have been recorded.[12]

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