Mycorrhiza (1995) 5:283–287

ORIGINAL PAPER

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Ectomycorrhizal synthesis on seedlings of *Afzelia quanzensis* Welw. using various types of inoculum

Abstract Ectomycorrhizal synthesis on seedlings of *Afzelia quanzensis* was initiated in the greenhouse and in the field using basidiospores or soil inoculum originating from fungi associated with adult trees of *A. quanzensis*, *Brachystegia microphylla*, *B. spiciformis*, and *Julbernardia globiflora*. Of the spore inocula used, only a *Pisolithus* sp. associated with adult *A. quanzensis* formed mycorrhizae. Seedlings raised in contact with all soil inocula formed mycorrhizae; however, the mycorrhizal types formed differed between soil inoculum used in the greenhouse and soil inoculum directly used in the field. *A. quanzensis* has a low specificity for mycorrhizal association. The concepts of ectomycorrhizal succession are also applicable to African savanna ecosystems.

Key words Miombo · Ectomycorrhizal legumes
Afzelia quanzensis · Specificity
Mycorrhizal succession

Introduction

Afzelia quanzensis Welw. (Leguminosae – Caesalpinioideae) occurs naturally in dry African woodlands (miombo). Together with *Pterocarpus angolensis* D.C. (Leguminosae – Papilionoideae), *A. quanzensis* is a tree producing timber of high commercial value (Bryce 1967; Mgeni and Malimbwi 1990) and for that reason has been overcut. Today, it enjoys the special protection of the Tanzanian government, and efforts to artificially regenerate it are being deployed through the DANIDA/Tanzania National Tree Seed Programme.

Högberg (1989) suggested that African savanna ecosystems are acidic and phosphorus limited at the moist end of the spectrum, and near neutral and nitrogen limited at the dry end. Due to recurrent fires (Trapnell et al. 1976) nitrogen present in the vegetation volatilizes, and this exacerbates nitrogen deficiency. Trees growing on poor sites possess several survival strategies, amongst which is mycorrhizal formation (Oldeman 1989; Högberg 1989, 1992).

Whereas most tree species form only one type of mycorrhiza, species of *Afzelia* have been found to possess both arbuscular mycorrhiza and ectomycorrhiza (*A. africana* Sm., Alexander 1985; *A. pachyloba* Harms., Newberry et al. 1988). However, *A. quanzensis* is only known to be ectomycorrhizal (Alexander and Högberg 1986).

Recent studies in Tanzania (Härkönen et al. 1993; Munyanziza and Kuyper, in preparation) have shown that trees of the genera *Afzelia*, *Brachystegia*, and *Julbernardia* (Leguminosae – Caesalpinioideae) are associated with a large number of ectomycorrhizal fungi occurring both i n the short rains of November to January and in the long rains from March to the end of May. Repeated occurrence of mushrooms around a particular tree in the field is an indication of a possible mycorrhizal association between the tree and the fungus concerned. However, not all ectomycorrhizal fungi found associated with adult trees in the field form mycorrhizae with young seedlings, especially in the nursery (Last et al. 1992). Only when young seedlings are grown together with adult trees can the ectomycorrhizal fungi associated with adult trees easily colonize seedlings (Fleming 1983). For large-scale afforestation with indigenous species on marginal land, it is important to gather information on mycorrhizal associations of seedlings in artificial environments and in the field.

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The aim of the present study was to examine the ability of various fungi associated with adult, introduced and miombo ectomycorrhizal trees to form mycorrhizae with *A. quanzensis* seedlings.

Materials and methods

Three different trials were set up: (1) inoculation of *A. quanzensis* seedlings with spores in the greenhouse; (2) inoculation of seedlings with soil inocula in the greenhouse; and (3) raising seedlings in the root zone of adult ectomycorrhizal trees in the field. All trials took place in Morogoro, Tanzania.

Inoculation of seedlings with spores in the greenhouse

Sporophores frequently seen in the field in association with species of *Afzelia*, *Brachystegia*, and *Julbernardia* (indigenous), *Pinus* or *Eucalyptus* (both introduced) were collected, air dried and stored for 3 weeks before use. They were then mixed with sterile soil at the rate of 10 mg l^{-1}. This mixture was used as a substrate for raising *A. quanzensis* seedlings in the greenhouse. The fungi used were *Pisolithus* sp. (collected under *A. quanzensis*), *Lactarius chromospermus* Pegler & Piearce (under *B. microphylla* Harms.), *Russula* sp., [under *J. globiflora* (Benth.) Troupin], Pisolithus *tinctorius* (Pers.: Pers.) Coker & Couch (under *Eucalyptus* spp.), and *Suillus granulatus* (Fr.: Fr.) O. Kuntze (under *Pinus caribaea* Morr.). Seedlings (25 per fungal species) were watered as needed, depending upon weather conditions. After 5 months, the infection rate was determined and mycorrhizal types present were sorted.

Inoculation of seedlings with mycorrhizal soil in the greenhouse

Soil inocula from various sources were used in the greenhouse to raise *A. quanzensis* seedlings. The soil came from the rhizosphere of the following indigenous tree species growing in various localities in Morogoro: *J. globiflora* (miombo woodlands in Uluguru), *B. spiciformis* Benth. (isolated trees in a yearly cropped farm), and *A. quanzensis* (two isolated trees at the edge of miombo woodlands, Uluguru and Mikese).

Each soil type was packed fresh into plastic pots and used unamended to raise seedlings (25 per soil type) of *A. quanzensis*. Watering was done as needed. At the end of the experiment, the seedling root system was examined for mycorrhizal formation.

Raising seedlings directly under adult trees in the field

Germination plots were established within a radius of 5 m around the stem base of adult trees of *A. quanzensis*, *J. globiflora*, and *B. microphylla*, all three in the miombo woodlands, Uluguru. Seedlings of *A. quanzensis* were also directly raised within a radius of 5 m around adult trees of *Pinus caribaea* growing at the edge of a nursery in Morogoro. These pines were planted to provide mycorrhizal inoculum for pine nursery seedlings. The field trials comprised 18–120 seedlings of *A. quanzensis* per tree species. Watering was continually adjusted to the prevailing weather conditions. After 8 months, seedlings were carefully uprooted and their mycorrhizal status was assessed.

Results

Inoculation of seedlings with spores in the greenhouse

Only *Pisolithus* sp. associated with adult *A. quanzensis* formed mycorrhizae with *A. quanzensis* seedlings. All seedlings were colonized. The mean infection percentage of the fine roots was 64± 13%. The mycorrhiza of this fungus is represented in Fig. 1a (Type 1). Mycorrhizae were ochre, the colour of the spore mass of the fungus involved. They were completely sunk in ochre, long hyphae through which ran ochre brown large rhizomorphs connected mainly to mycorrhizae. Long roots were also covered by hyphae and bore rhizomorphs. Hyphae appeared easily removable and were often stripped off or bent backwards when the roots were dipped in running water. In still water mycorrhizae floated. When undisturbed, hyphae pointed forwards or outwards. Mycorrhizae had a characteristic smell. Clamp connections were present.

Mycorrhizal formation on seedlings raised in mycorrhizal soil in the greenhouse or directly raised in the field

In the greenhouse, seedlings in contact with an inoculum from any source formed mycorrhizae regardless of the associated tree species. Several mycorrhizal types were formed; some types were common to several sources, but others were exclusively found in association with a given soil. The infection rate in the greenhouse was 92±7% (n=43) and did not differ signifi cantly among the different types of soil inoculum. In the field, as in the greenhouse, several ectomycorrhizal types formed; four types were formed in the field but not in the nursery. The infection rate in the field was 100%. In the field, several mushrooms formed during the short rain and the long rain periods. In the nursery, in the plot around *Pinus caribaea* trees, sporophores of *S. granulatus* developed near *A. quanzensis* seedlings. However, *A. quanzensis* did not form *S. granulatus* mycorrhizae. In the greenhouse, only one fungus, presumably an *Inocybe* sp. (Fig. 2), produced sporophores varying in number in several pots; this mushroom was not seen in the field.

The common feature of all mycorrhizal types observed is the high level of hairiness of the mycorrhizae. This is illustrated by three types (Fig. 1a–c). Type 1 (Fig. 1a) formed in the nursery. Type 2 (Fig. 1b) formed in the nursery in soil associated with *J. globiflora* and *A. quanzensis* and in the field around *A. quanzensis*. Mycorrhizae were dark brown, the mantle was covered by thick, long hyphae and extensive rhizomorphs. The surface of rhizomorphs was very hairy, and hyphae bore many clamp connections.

Type 3 (Fig. 1c) formed only in the field and only on seedlings raised in the rhizosphere of *A. quanzensis*. Mycorrhizae were visible to the naked eye as white balls, giving the impression of underground puffballs. In the infection process, the mycelium of the fungus grew and hardened around a given lateral root; lateral elongation was then hindered. The lateral root then produced more branches which grew in a spiral inside the mycelium. The lateral root in the centre of the spi-

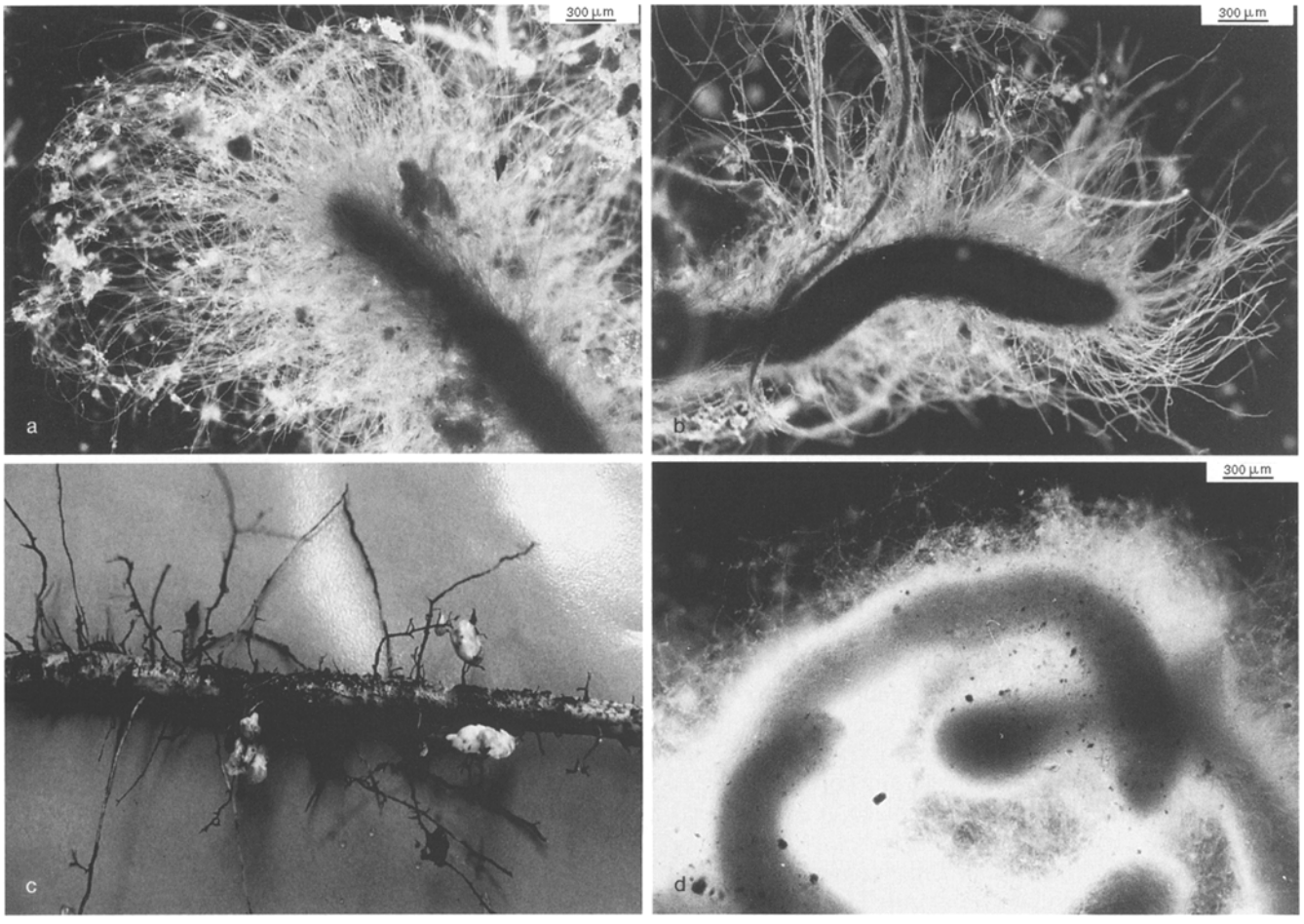

Fig. 1a–d Some ectomycorrhizal types that formed on *Afzelia quanzensis* in the greenhouse and in the field. **a** Type 1, developed in the nursery. Basidiospores of a *Pisolithus* sp. were used as inoculum. **b** Type 2, developed in the nursery and in the field. Soil/vegetative inoculum associated with *Julbernardia globiflora* and *A. quanzensis* was used. **c** Type 3, developed in the field on seedlings raised in the root zone of *A. quanzensis*. The mycorrhizal system is shown before dissection. **d** Detailed view of **c**

Fig. 2 Ectomycorrhizal mushrooms produced in the nursery around *A. quanzensis* seedlings raised in *A. quanzensis* soil

ral was thicker than those of the periphery. It was mainly the inner lateral roots that were mycorrhizal. The mycelium of the fungus involved was white and had very thin hyphae. Large, white rhizomorphs ran through the mycorrhizal system. This mycorrhizal type was suspected to be connected to mushrooms of *Leccinum foetidum*.

Discussion

Spore inoculum has been used to produce specific ectomycorrhizae on tree seedlings (Trappe 1977). This technique has also been successfully applied to pines in Kenya (Ivory and Munga 1983). Mycorrhizal formation by *A. quanzensis* seedlings inoculated with spores of *Pisolithus* sp. confirmed that this fungus was symbiotically associated with *A. quanzensis* trees in the field. The fruit bodies of this fungus, which were produced in both the short and the long rains, were seen at five different locations around isolated trees of *A. quanzensis*, but not with other miombo tree species.

The spore inoculum of *Pisolithus tinctorius*, associated with eucalypts, failed to develop mycorrhizas. Examination of *Pisolithus* collections from southern Africa has shown three distinct types differing in spore

ornamentation (Van der Westhuizen and Eicker 1989). Even though *Pisolithus tinctorius* has been reported in association with a high diversity of host genera (Marx 1977), strains from exotic provenances and trees might well be incompatible with indigenous trees (Bâ et al. 1994; Dell et al. 1994). Incompatibility is also likely between A. quanzensis and *S. granulatus*, which is restricted to pines.

Failure to produce mycorrhizae may also have been caused by the unsuitability of the substrate for spore germination or insufficient time for the symbiosis to develop. Bâ et al. (1994) found that seedlings of *A. africana* inoculated with basidiospores of *Pisolithus* sp. required at least 6 months for mycorrhizae to form in an artificial environment.

Soil inoculum is the commonest method of inoculation (Harley and Smith 1983). Its main advantage is that it ensures the formation of mycorrhizae and thus meets the first premise that any mycorrhiza on tree roots is far better than none at all (Marx 1980). It has, however, disadvantages, one of which is that the fungi dealt with are unknown.

While *A. quanzensis* seedlings were mycorrhizal irrespective of the type of soil inoculum, mycorrhizal types differed depending whether the trial was in the field or in the greenhouse. These observations are consistent with the hypothesis of ectomycorrhizal succession (Last et al. 1992) also taking place in savanna ecosys tems.

Brachystegia, *Julbernardia*, and *Afzelia* species occur naturally in the miombo woodlands (Lind and Morrison 1974). The likelihood that they share some fungal symbionts is therefore high. In Senegal, Thoen and Bâ (1989) found that A. a*fricana* and *Uapaca kirkiana* Müll. Arg. (Euphorbiaceae) had a number of mycorrhizal types in common. Surveys conducted in the miombo woodlands on mushroom occurrence (Munyanziza and Kuyper, in preparation) indicated that many fungi fruited around *A. quanzensis*, *B. spiciformis*, *B. microphylla*, and *J. globiflora*.

Examination of the root systems showed that some mycorrhizal types earlier seen on seedlings of other tree species, namely *J. globiflora* and *B. spiciformis* seedlings, were present on *A. quanzensis* seedlings. An example is type 2, which developed on seedlings of *J. globiflora* inoculated with *J. globiflora* soil. Seedlings of *A. quanzensis* raised in the rhizosphere of pines also formed well-developed ectomycorrhizae of type 2. This type was not seen on pine roots in this study and may have originated from contamination due to routine soil mixing in the nursery.

The mean mycorrhizal root diameter was 282 ± 38 μm ($n = 19$), and the mean sheath thickness was 40 ± 6 μm ($n = 19$). The sheath represented on average almost 50% of the cross-sectional area, which is higher than the cross-sectional area indicated by Hall and Swaine (1976) for *A. bella* Harms. (22%) and by Högberg and Nylund (1981) for *A. quanzensis* (33%). Alexander and Högberg (1986) suggested that the fungal contribution to ectomycorrhiza in the tropics is distinctly larger than commonly found in temperate ecosystems (about 20–30%), which is confirmed by our observations. The chief feature of these mycorrhizae is t he presence of extensive hyphae and thick rhizomorphs, which can be as thick as 150 μm. Such types have repeatedly been noticed on mycorrhizae of tropical dry areas (Redhead 1980; Thoen and Bâ 1989; Bâ and Thoen 1990). The effectiveness of such types for water transport has been demonstrated by Duddridge et al. (1980).

Acknowledgements The present work was sponsored by the International Foundation for Science. Preparation of the research proposal and data processing were funded by Wageningen Agricultural University, and Professor R.A.A. Oldeman gave suggestions during field work and writing up. We thank the Faculty of Forestry of the Sokoine University of Agriculture and scientists there for cooperation during the field work. This is communication 537 of the Biological Station Wijster.

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