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Mycorrhizal associations in Hong Kong Fagaceae

VI. Growth and nutrient uptake by *Castanopsis fissa* seedlings inoculated with ectomycorrhizal fungi

Paul C. F. Tam, D. A. Griffiths

Department of Botany, University of Hong Kong, Pokfulam Road, Hong Kong

Abstract. *Castanopsis fissa* Rehd. & Wils. is widely distributed from the tropics to the temperate regions of China and Japan and is an important forest component in Hong Kong. Pot-grown *C. fissa* seedlings inoculated with vegetative mycelial inocula of seven ectomycorrhizal fungi for 20 weeks were analysed for growth performance and mineral nutrient uptake of N, P, K and Ca. Shoot growth stimulation in all fungal treatments generally occurred in the first 4–8 weeks of seedling development. Uptake of P was generally enhanced by all fungi inoculated. Seedlings inoculated with *Pisolithus tinctorius* (Pers.) Coker and Couch and *Cenococcum geophilm* (Sow.) Fredinard et Winge, which colonized 22% and 33% of roots respectively, exhibited growth stimulation. The results indicate that *P. tinctorius* and *C. geophilum* are suitable for use in large-scale nursey inoculation.

Key words: *Castanopsis fissa* – *Pisolithus tinctorius* – *Cenococcum geophilum* – Nutrient uptake – Nursery inoculation

Introduction

The territory of Hong Kong, situated near the northern limit of the distribution of the tropical Southeast Asia flora, is characteristically diverse and rich in species. The total area of 1052 km^2 includes of only 13% woodland but surprisingly contains over 300 species (Thrower 1988). Most of the woodland was established on hill slopes by the direct sowing of tree seeds or by planting tree seedlings raised in nurseries. These forestry plantations, which occur within catchement areas of freshwater reservoirs, make up the principal areas of the country parks, protected and managed by the Agriculture and Fisheries Department of Hong Kong.

Correspondence to: P. C. F. Tam

Castanopsis fissa (chestnut oak) is a fast-growing, evergreen tree used in Hong Kong for reforestation programmes, and has become an important component of the mixed climax forest in most woodland areas. In a recent revision of the genus *Castanopsis,* this species was described by Griffiths (1991) as follows:

Castanopsis fissa Rehder et Wilson [Syn: *Quercus fissa* Champ & Benth; *Pasania fissa* Oersted; *Synaedrys fissa* Koidzumi].

Recent investigations at Yunnan Academy of Forest Sciences have shown that this species is a particularly fast-growing tree with many useful attributes.

It grows in natural stands in the S. E. of Yunnan on slopes between 700 m and 1700 m. It also occurs intermittently in the provinces of S. Kiangi, S. Hunnan, Guandong, Fujian and S. Kwaichow.

In Yunnan the tree grows in the wet monsoon evergreen forest and has the unique ability to withstand forest clearance, after which it becomes a pioneer species of the secondary forest and forms monocultural stands. It grows in deep, heavy, sticky soil at pH 4.5–6.5, and the trees can withstand temperatures as low as −5° C. It grows best in areas with average annual rainfall of 1000 mm and can thrive where this level reaches 2000 mm.

The species grows best on slopes bearing deep soil and can tolerate a certain amount of shade when young; it requires full illumination at maturity. It has strong regenerative powers by means of its secondary buds, and cut stumps yield thickets of new growth within months of forest clearing.

The growth rate of the tree is very fast and, for example, a 23-year-old specimen in secondary forest at 1470 m altitude was 22 m high with a girth of 300 cm. The peak rate of growth is around the tenth year and the trees normally reach 16 m after 15 years. At this stage it is ready for timber production. Trunks are relatively straight making milling easy. Growth slows after 30 years.

Seeds are first produced at years 7–8 and at peak production each tree yields 50 kg of seed per year.

The tree produces a heavy leaf fall which converts, in turn, to a substantial litter layer and this fact, coupled with its rapid growth rate, makes it an ideal tree for afforestation programmes on barren mountain slopes.

Seeds contain a high level of starch and the bark yields tannin.

During a recent survey of tree roots, it was observed that the roots of *C. fissa* bore a variety of ectomycorrhizas, though the fungal symbionts were often difficult to identify. Furthermore, our previous studies had shown that *Castanopsis*, together with two other genera of Hong Kong Fagaceae, were capable of forming ectomycorrhizas with some known fungal isolates (Tam and Griffiths 1993a). With these facts in mind, we attempted to investigate the effects a variety of ectomycorrhizal associations on growth parameters of *C. fissa* seedlings and to determine whether or not artificial inoculation by known symbionts would enhance seedling growth and mineral nutrient uptake, and thus be of some benefit in forest nursey practice.

Materials and methods

Fungal cultures

The fungi used were as described in Tam and Griffiths (1993a).

Inocula

Each fungal isolate was grown in a mixture of vermiculite and peat moss moistened with MMN nutrient solution (Marx and Bryan 1975). One-litre Erlenmeyer flasks containing 480 ml vermiculite, 20 ml peat moss and 250 ml MMN solution were autoclaved at 121° C for 40 min. Ten plugs (8 mm) of mycelial agar cut from the periphery of the 8-week-old fungal colony were aseptically introduced into each flask. The inoculated flasks were incubated at 20° C in the dark for 2 months. After incubation, the inoculum consisting of fungal mycelium, vermiculite and peat moss was leached in cool running tap water for 2–3 min in a double-layered cheesecloth bag to remove the remaining nutrient medium, and excess water was squeezed out by hand (Marx 1980).

Seedlings

Ripe acorns of *C. fissa* were collected in October 1990 from a single tree in the woodland area of Tai Po Kau Nature Reserve. After removal of the testa, the seeds were placed in a shallow tray and allowed to germinate in running tap water. Selected germinants bearing healthy cotyledons were planted in a deep plastic container containing a 9:1 mixture of steam-sterilized vermiculite and peat moss. Two weeks later, the seedlings were transferred to pots (three seedlings per pot) containing a 1:2 mixture of leached inoculum (see above): vermiculite/peat moss. Control seedlings were transferred to pots containing uninoculated vermiculite/peat moss. The treatments (10 pots per fungal isolate) were randomized on the shelves of a temperature-controlled propagation unit at 26–30° C with a photoperiod of 18 h under fluorescent lights. Seedlings were watered as needed. Four weeks after germination, each pot received 50 ml half-strength Hoagland's solution at weekly intervals throughout the study.

Growth parameters and chemical analysis of seedlings

The heights of all seedling shoots were measured at 4-week intervals after germination.

After 20 weeks, 10 seedlings from each of the inoculated and noninoculated treatments were randomly chosen for growth and nutrient analysis. Seedling measurements were made on shoot height, root collar diameter, leaf area, dry weight of roots and shoots (oven-dried at 80° C for 24 h).

The root systems of the seedlings were carefully washed free of particles and were visually examined for mycorrhizal colonization by each test fungus using the method described by Mitchell et al. (1984). Ectomycorrhiza formation was verified by removing the suspected mycorrhizal short roots, bleaching them in 1% sodium hypochlorite solution (to remove any polyphenol present in the roots), and mounting them in dilute glycerol for examination under a microscope.

Oven-dried seedling material was analyzed for mineral nutrients following digestion in sulphuric acid/hydrogen peroxide. Total nitrogen was determined by the indophenol-blue method and phosphorus determined colorimetrically by the molybdenum-blue method (Allen 1989). Potassium and calcium levels were determined with a flame photometer.

Data were subjected to an analysis of variance, and differences among means were evaluated using Duncan's multiple-range test ($P<0.05$).

Results

The growth parameters of *C. fissa* seedlings inoculated with seven ectomycorrhizal fungi and the noninoculated seedlings are summarized in Table 1. Four weeks after germination, all seedlings inoculated with the fungi generally exhibited growth stimulation; significantly greater shoot heights were shown in the treatments with *Pisolithus tinctorius, Cenococcum geophilum, Rhizopogon roseolus* and *Pisolithus* sp. Twelve weeks later, seedlings from these treatments still had shoot heights significantly greater than the controls. However, at the end of 20 weeks, only the seedlings inoculated with *P. tinctorius* and *C. geophilum* showed significant increases in shoot height (18%) over the controls.

Ectomycorrhizas were successfully formed in seedlings inoculated with *P. tinctorius, C. geophilum, Thelephora terrestris, Hymenogaster* sp and *Scleroderma* sp., though the percentage of mycorrhizal colonization varied significantly among these treatments. Seedlings inoculated with *R. roseolus* or with *Pisolithus* sp. and the noninoculated control seedlings were free of mycorrhizal colonization.

The growth performances of the seedlings after 20 weeks also differed greatly among the various treatments. However, seedling growth was generally positively correlated with the percentage mycorrhizal colonization. Seedlings inoculated with *C. geophilum* exhibited the highest percentage infection (33%) and had significantly higher root collar diameters, leaf areas and shoot dry weights compared to the controls. Seedlings with a lower percentage infection (22%) also had significant increases in root collar diameter, but no significant differences were observed in leaf area and shoot dry weight compared with the controls. Seedlings inoculated with the remaining five isolates (with per-

Table 1. Growth parameters of pot-grown *Castanopsis fissa* seedlings with and without inoculated ectomycorrhizal fungi. Pt, *Pisolithus tinctorius*; Cg, *Cenococcum geophilum*; Tt, *Thelephora terrestris*; Rr, *Rhizopogon roseolus*; H, *Hymenogaster* sp.; S, *Scleroderma* sp.; P, *Pisolithus* sp. Values within a column bearing a common letter are not significantly different ($P = 0.05$) in Duncan's multiple-range test

Fungal treatment	Shoot height (cm)			Growth after 20 weeks[b]						
	4[a] weeks	12[a] weeks	20[b] weeks	Root collar diameter (mm)	Leaf area (cm^2)	Shoot dry wt. (g)	Root dry wt. (g)	Total dry wt. (g)	Shoot to root ratio	Percentage mycorrhizal colonization
Control	5.3 e	6.4 b	6.7 c	2.1 c	122 b	0.63 b	0.26 c	0.89 bcd	2.5 a	0 d
Pt	6.5 a	7.3 a	8.0 a	2.4 b	114 bc	0.60 bc	0.41 a	1.00 b	1.5 c	22 b
Cg	6.2 ab	7.5 a	7.9 a	2.6 a	140 a	0.75 a	0.40 a	1.15 a	1.9 b	33 a
Tt	5.5 de	6.5 b	7.0 c	2.2 bc	86 de	0.47 de	0.34 ab	0.81 cd	1.4 c	10 bc
Rr	6.0 abcd	7.0 a	7.2 bc	2.1 c	103 cd	0.51 de	0.38 a	0.89 bcd	1.4 c	0 d
H	5.6 cde	6.4 b	7.1 bc	2.2 bc	97 cd	0.52 cd	0.40 a	0.92 bc	1.3 c	16 b
S	5.7 bcde	6.5 b	7.2 bc	2.1 c	77 e	0.42 e	0.34 ab	0.76 d	1.3 c	5 c
P	6.2 abc	7.2 a	7.2 bc	2.1 de	88 de	0.46 de	0.31 bc	0.75 d	1.5 c	0 d

[a] Means of 30 seedlings per treatment
[b] Means of 10 seedlings per treatment

centage infections lower than 20% or zero) were not significant different in root collar diameter and even showed decreases in leaf area and shoot dry weight compared with the controls. It is interesting to note that seedlings from all fungal treatments generally had significantly higher root dry weights than the control and may, therefore, have had lower shoot to root dry weight ratios than the controls. In general, there was no significant difference between the total dry weights of seedlings in any of the fungal treatments and those of the controls; however, seedlings inoculated with *C. geophilum* had total dry weights significantly higher (29%) than those of the controls.

The nutrient uptake in the various treatments after 20 weeks of growth is summarized in Table 2. There was a wide variation among the treatments; however, in general inoculated seedlings had significantly lower nitrogen contents than the controls but the higher P contents. There was no significant difference between the K contents of the inoculated seedlings and the controls, but the Ca contents of seedlings from all fungal treatments were generally lower than those of the controls.

Table 2. Analysis of nutrient uptake of pot-grown *Castanopsis fissa* seedlings with and without inoculated ectomycorrhizal fungi. Values within a column having a common letter are not significantly different ($P = 0.05$) in Duncan's multiple-range test. Means of 3 seedlings per treatment

Fungal treatment	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Calcium (%)
Control	1.21 a	0.36 c	0.96 a	0.64 a
Pt	1.01 bc	0.53 a	1.01 a	0.52 d
Cg	1.09 ab	0.51 ab	0.94 ab	0.58 bc
Tt	1.01 bc	0.48 ab	0.94 ab	0.53 cd
Rr	0.99 bc	0.42 bc	0.84 bc	0.50 d
H	0.93 c	0.45 bc	0.91 abc	0.53 cd
S	1.01 bc	0.47 ab	0.97 a	0.50 d
P	1.04 bc	0.50 ab	0.83 c	0.63 ab

Discussion

The genus *Castanopsis* is an important forest component of zones extending from the tropics to the cooler regions of China and Japan. The trees are fast-growing and can tolerate a wide variety of soil types. They are ideal for reforestation schemes where their rapid root growth stabilizes soil and the trees themselves yield a wide variety of products such as timber, edible nuts and tannins (Griffiths 1991). Any possible measures that enhance seedling growth could play an important role in nursery practice and provide fast-growing trees for remedial situations.

In this study, we found that 20-week-old seedlings readily formed mycorrhizal associations with five test fungi, viz *P. tinctorius, C. geophilum, T. terrestris, Hymenogaster* sp. and *Scleroderma* sp. *Rhizopogon* failed to form mycorrhiza, probably due to its conifer-specific nature (Trappe 1962; Harley and Smith 1983), as did the slow-growing local isolate of *Pisolithus* sp.

The overall mycorrhizal colonization of the seedlings by the compatible ectomycorrhizal fungi was relatively low in this study, possibly because most of the rapidly growing lateral roots escaped infection by the fungal inocula. *C. geophilum* has been reported to be slow- growing (Mitchell et al. 1984), but was previously demonstrated to be very efficient in mobilizing polyphenol compounds in vitro (Tam and Griffiths 1993b). Since a high level of polyphenols occurs in the root exudation of *C. fissa* seedlings, this ability of the fungus may enhance its compatibility with the host root and thus increase mycorrhizal colonization.

It has been suggested that ectomycorrhizal infection of 30–50% of the root system is required to obtain root and shoot growth responses. Our results show that 20-week-old seedlings inoculated with *C. geophilum* had over 30% mycorrhizal infection and were stimulated in both shoot height and total dry weight. Seedlings inoculated with *P. tinctorius* had about 20% infection and only slight enhancement of growth, and seedlings with infection rates below 20% showed growth responses lower than those of the controls. However, during the early stages of seedling development, i.e. the first 4–12 weeks, seedlings inoculated with all fungal species tested showed significant increaes in shoot height, possibly because most of the fungal inocula was still viable during this period.

Of the four mineral nutrients (N, P, K and Ca) analysed in this study, only P uptake by the seedlings was enhanced. This can be explained by the active surface phosphatases of the ectomycorrhizal fungi (Theodoru 1968, 1971; Ho and Zak 1979), which may facilitate P uptake even though they only form 'superficial' associations with host roots. Enhancement of P uptake by ectomycorrhizas has been reported by Harley and Smith (1983).

This study showed that inoculation of 20-week-old *C. fissa* seedlings with species of *P. tinctorius* and *C. geophilum* benefits seedling growth and enhances nutrient uptake of P. Therefore, both of these fungal species may be suitable for use in large-scale nursery inoculation. However, the ultimate effectiveness of any mycorrhizal inoculum lies in the growth responses following outplanting (Dixon et al. 1981).

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