BIOTROPIA No. 8, 1995: 45-52

EFFECTS OF *PISOLITHUS TINCTORIUS* AND *LACCARIA FRATERNA* ON THE GROWTH AND MYCORRfflZAL DEVELOPMENT OF *PINUSPATULA* SEEDLINGS*)

M. SUDHAKARA REDDY and K. NATARAJAN
Centre for advanced Studies in Botany, University of Madras,
Guindy Campus, Madras 600 025, India

ABSTRACT

Vegetative inoculum of *Pisolithus tinctorius* and *Laccariafraterna* were inoculated to *Pinuspatula* seedlings grown in both steam sterilized and unsterilized shola soil. After 4 months of seedling growth, 10 seedlings from each treatment were harvested and various growth parameters were studied. Inoculation of these two fungi resulted in the production of ectomycorrhizas and increase in growth of *P. patula* seedlings when compared to uninoculated seedlings. *Laccariafraterna* inoculated seedlings showed more number of mycorrhizas than *P. tinctorius* inoculated seedlings at the end of one year. Both these fungi poorly colonized the root system in both soil treatments. There was no significant difference between these two fungi in improving the seedling growth in the nursery.

Key words: *Pisolithus tinctorius/Laccaria fraterna/Pinus patula*/Inoculum/Seedlings/Growth.

INTRODUCTION

Extensive work on nursery inoculation has been done with *Pisolithus tinctorius* because of its ecological adaptation to adverse soil conditions, wide geographic distribution and broad host range, tolerance to a variety of environmental conditions and its easy propagation and manipulation in pure culture (Marx *et al.* 1984). Eventhough *P. tinctorius* is worldwide in distribution and has a broad tree host range it has not been found in the Nilgiri hills either in pine or eucalypt plantations. The other potential ectomycorrhizal fungus *Laccariafraterna* is wide spread throughout the world where *Eucalyptus* and other ectomycorrhizal hosts have been introduced (Tommerup *et al.* 1991). At certain places in Nilgiri hills where eucalypt and pine plantations occur side by side, *L. fraterna* is mostly found to be associated with the eucalypts but rarely with pines (Natarajan 1977). But under *in vitro* condition these two fungi were able to form mycorrhizas with *P. patula* seedlings. The present research study was undertaken to study the effects of these two fungi on the growth and mycorrhizal development of *P. patula* seedlings in the nursery.

*)Paper presented at the Second Symposium on Biology and Biotechnology of Mycorrhizae and Third Asian Conference on Mycorrhizae (ACOM III), 19-21 April 1994, Yogyakarta, Indonesia.

MATERIALS AND METHODS

Pisolithus tlnctorius culture was isolated from basidiomata collected from *Eucalyptus tereticornis* plantations, near Madras coast, Tamilnadu and the culture of *L. fraterna* was isolated from the basidiomata collected from *E. globulus* plantations in Nilgiri hills, Tamilnadu, South India. The cultures were maintained at 25°C on potato dextrose agar medium.

The mycelial inoculum of *P. tinctorius* and *L. fraterna* were prepared according to the method of Marx and Bryan (1975). Inoculum of each fungus was grown aseptically in one litre Erlenmeyer flasks containing 750 ml of vermiculite moistened with 375 ml of MMN liquid medium. The flasks were incubated at 25°C in dark. After 12 weeks of incubation, inoculum was removed from the flask and leached with cool running tap water to remove the unused nutrients. Excess free water was removed by gently squeezing the inoculum wrapped in cheese cloth. Fungus free vermiculite wih MMN served as control.

The shola soil collected under natural vegetation of the upper region of Nilgiri hills was used in the present study. The chemical constituents of the soil are as follows: pH - 5.3; Organic matter content -4.79%; NPK levels - 245, 5.2 and 35 kg/acre, respectively. The nursery experiment was conducted at the Forest Department Nursery, Ootacamund, Nilgiri hills during July 1992 to June 1993. Both steam sterilized and unsterilized soils were filled in polybags (about 2 kg/bag) and 10% inoculum by volume was added to the polybags separately and mixed into the upper 8-10 cm of the soil. Fungus free vermiculite added to the polybags served as controls. The surface sterilized seeds of *P. patula* (30% H_2O_2 for 30 minutes) were sown in all the bags. Five (5) seeds were sown in each bag. After germination, seedlings were thinned to one per bag. Ten (10) replicates were kept for each treatment. After 12 months of seedling growth, 10 seedlings from each sample were harvested and the growth and mycorrhizal development were studied. The shoot height, root length, and root collar diameter were measured. After counting the mycorrhizal and non-mycorrhizal tips, the shoots and roots were dried in a hot air oven at 85°C for 48 hours. Dry weights of shoot and root were determined by obtaining constant weights. The method used by Zak (1973) and Agerer (1986) were followed for studying the macroscopic and microscopic features of ectomycorrhizas. The colour terminology used is that of Kornerup and Wanscher (1978). All the data were analysed by analysis of variance and the means were compared by least significant difference (Snedecor and Cochran 1967) at P = 0.05 level.

RESULTS

The root examinations revealed that both the ectomycorrhizal fungi viz., *P. tinctorius* and *L. fraterna* were able to form mycorrhizas with *P. patula* seedlings in the nursery.

The *P. tinctorius* type of mycorrhizas are mostly dichotomous, tetrapodials and coralloid forms, 3-6 mm long and 0.4-0.5 mm in diameter. Colour of mycorrhizas are brownish yellow (5C8) to light brown (6D6). Loose hyaline hyphae are associated with the surface of the mycorrhizal system. The rhizomorphs are light brown (6D6) in colour and 150-550 µm wide, composed of closely packed parallel hyphae and covered with radiating hyphae (Fig. la). The transverse section showed that the mantle is 15 - 20µrn thick. It consists of a simple prosenchymatous tissue. Cystidia, setae or sclerotia are not observed. The Hartig net is composed of one or two rows of oval to globose hyphal cells which measure 5 — l0 µm in thickness and penetrate up to 4 cortical cell layers deep (Fig. Ib).

Laccaria fraterna type of mycorrhizas are mostly bipodial, rarely monopodial, 3-5 mm long and 0.3 - 0.4 mm in diameter. Colour of the mycorrhizas are orange white (6A2) when young and brown (6D7) when old. The surface is smooth. Rhizomorphs are absent (Fig. Ic). The transverse section showed a mantle of 10- 15µm thick and consists of a simple prosenchymatous tissue. The Hartig net is composed of a single row of oval to globose hyphal cells which measure 3 - 6 µm in thickness and penetrate up to 3 cortical cell layers deep (Fig. Id).

Inoculation of *P. tinctorius* and *L. fraterna* increased the number of mycorrhizas both in sterilized and unsterilized soil. The number of mycorrhizas were more in sterilized inoculated soil than in unsterilized soil. *Laccaria fraterna produced* more number of mycorrhizas than *P. tinctorius*. But there was no significant difference between these two fungi. The percentage of mycorrhizas did not differ significantly between *P. tinctorius* and *L. fraterna* inoculated seedlings in both soil treatments. The percent colonization of both inoculated fungi were lower in both soil treatments. When compared to *P. tinctorius, L. fraterna* inoculated seedlings showed higher percent colonization at the end of one year. No basidiomata production was seen in the case of *P. tinctorius* whereas *L. fraterna* inoculated seedlings produced basidiomata in the bags at the end of one year.

The growth of *P. patula* seedlings was improved in both sterilized and unsterilized soil by the inoculation of the fungi. The shoot height was more in sterilized inoculated soil than in unsterilized inoculated soil. But there was no significant difference between the two fungi in improving the shoot height. The root length has increased in both soils by the inoculation of these two fungi. *Laccaria fraterna* inoculated seedlings showed a higher root collar diameter when compared to *P. tinc-*

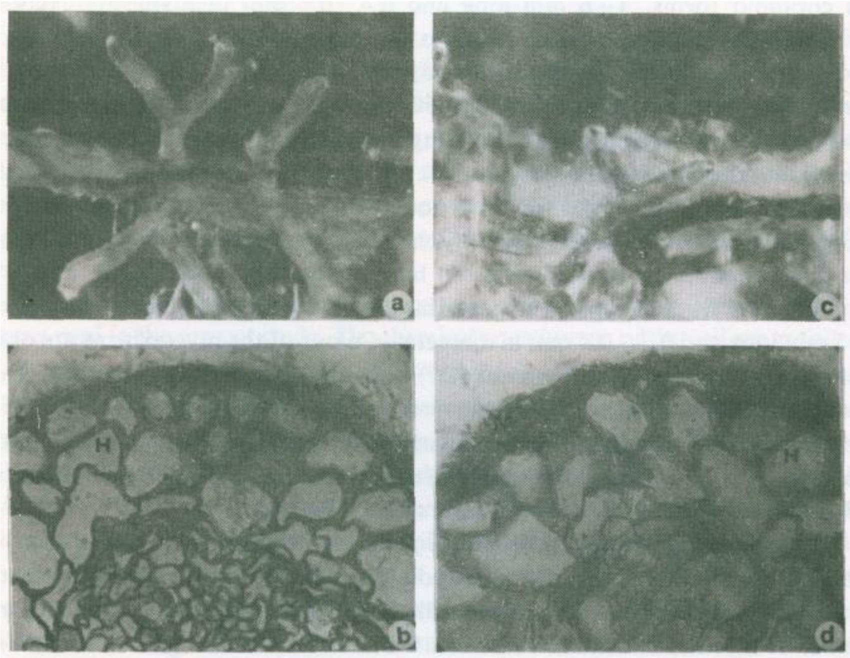

Figure 1. Ectomycorrhizas of *Pinus patula* produced by *Pisolithus tinctorius* and *Laccaria fraterna.*
a. Morphology of *P. tinctorius* ectomycorrhizas x 240.
b. Transverse section of *P. tinctorius* ectomycorrhizas x 320.
c. Morphology of *L. fraterna* ectomycorrhizas x 200.
d. Transverse section of *L. fraterna* ectomycorrhizas x 325.
M - Mantle; H - Hartig net.

torius inoculated seedlings. The shoot dry weight was more in sterilized soil than in unsterilized soil in inoculated seedlings. The seedlings inoculated with *L. fraterna* showed maximum shoot dry weight in both soils. But there was no significant difference between the two fungi in improving the shoot dry weight. The root dry weight also did not differ significantly between the two fungi. The shoot/root ratio differed significantly in control seedlings than in inoculated seedlings in sterilized soil. In unsterilized soil there was no significant difference between the two fungi and control seedlings with respect to the shoot/root ratio (Table 1).

Seedlings grown in sterilized soil inoculated with the two fungi showed more growth and mycorrhizal development than the seedlings grown in unsterilized soil inoculated with these fungi. *Laccaria fraterna* and *P. tinctorius* did not differ significantly in improving the growth and mycorrhizal development of *P. patula* seedlings in the nursery.

DISCUSSION

Since *P. tinctorius* and *L. fraterna* are known to be early stage ectomycorrhizal fungi (Marx 1991; Tommerup *et al.* 1991), these two species have been selected to evaluate their effects on the growth and mycorrhizal development of *P. patula* in the present study. Vegetative inoculum of these two fungi improved the growth and mycorrhizal development of *P. patula* seedlings when compared to uninoculated seedlings in the nursery in both steam sterilized and unsterilized soil. But the difference in various growth parameters is not significant in the inoculated seedlings. The seedlings inoculated with *L. fraterna* seem to be marginally better than the seedlings inoculated with *P. tinctorius.* Tommerup *et al.* (1991) reported that *L. fraterna,* an early colonizing mycorrhizal fungus of *Eucalyptus* is a potential competitor when the seedlings were inoculated with other selected ectomycorrhizal fungi. It is interesting to note that basidiomata of *L. fraterna* very rarely occur in *P. patula* plantations in Nilgiri hills and found mostly associated with *Eucalyptus* plantations which are adjacent to the *P. patula* plantations. It has also been noticed that basidiomata of *L. laccata,* the most predominant fungus in *P. patula* plantations, were seldom found in the *Eucalyptus* plantations. The results of the present experiment suggest that *L. fraterna* and *P. tinctorius* are capable of improving the growth of nursery seedlings in Nilgiri conditions. The colonization of these two fungi was poor in both treatments.

Marx and Cordell (1987) found that pH between 4.5 and 5.5 is adequate for *P. tinctorius,* but that a pH above 6.0 inhibits ectomycorrhizal formation. The pH of the soil used in the present study is 5.3 and hence it may not be the factor for

Table 1. Effect of vegetative inoculum of *P. tinctorius* and *L. fraterna* on the growth and mycorrhizal development of *P. patula* (one year old) seedlings in the nursery

	Shoot height (cm)	Root length (cm)	Root collar (mm)	Shoot dry wt. (gm)	Root dry wt. (gm)	S/R ratio	Total myco-tips	% of mycor-rhizas	% of inoculated fungi
				STERILIZED SOIL					
P. tinctorius	17.2a	35.6a	3.2b	1.16a	0.65a	1.8b	1211a	86.5a	44.3b
L. fraterna	17.6a	29.9a	3.9a	1.37a	0.70a	1.9ab	1420a	85.5a	50.7a
Control	9.7b	21.9b	1.3c	0.25b	0.08b	3.6a	103b	57.8b	0.0c
				UNSTERILIZED SOIL					
P. tinctorius	11.7a	28.1b	1.7b	0.37a	0.15a	2.7a	433a	85.6a	30.9a
L. fraterna	13.0a	27.5a	2.3a	0.42a	0.17a	3.0a	496a	86.0a	36.8a
Control	11.6a	26.7a	2.0ab	0.32a	0.14a	2.2a	461a	83.2a	0.0b

Means sharing a common letter in the same column within the soil treatment are not significantly different at P = 0.05.

poor colonization of *P. tinctorius* in the nursery. Marx *et al.* (1970) reported that *P. tinctorius* grew rapidly at 28 - 30°C and capable of growing at 40 - 42°C in pure culture and formed more mycorrhizas with *P. taede* at 34° C than at lower temperatures. Marx and Bryan (1971) reported that aseptically grown *P. taede* seedlings with *P. tinctorius* ectomycorrhizae had better survival and growth at 40°C in laboratory tests than non-mycorrhizal seedlings or those mycorrhizal seedlings with *T. terrestris.* Temperature may be the main reason why *P. tinctorius* is not occurring in Nilgiri hills where the temperature ranges between 5 and 20° C during different parts of the year. The isolate of *P. tinctorius* used in the present study was obtained from the basidiomata associated with *E. tereticornis* in coastal Madras where the temperature in summer will raise up to 40°C. Further research is needed to evaluate different environmental conditions on the growth and mycorrhizal development of *P. patula* by *P. tinctorius.* In spite of the positive growth response shown by the seedlings in the present study only further research will reveal whether these two fungi will survive when seedlings are outplanted since they are conspicuous by their absence in *P. patula* plantations in the Nilgiri hills.

REFERENCES

AOERER, R. 1986. Studies on ectomycorrhizae. II. Introducing remarks on characterization and identification. Mycotaxon, 26: 473-492.

KORNERUP, A. and J.H. WANSCHER. 1978. Methuen Handbook of Colour 3 rd ed. Methuen and Co. Ltd. London, p. 243.

MARX, D.H. 1991. The practical significance of ectomycorrhizae in forest establisment. *In:* Ecophysiology of ectomycorrhizae of forest trees. The Marcuswallenberg Foundation, Symposia proceedings, 7: 54-90.

MARX, D.H. and W.C. BRYAN. 1971. Influence of ectomycorrhizae on survival and growth of aseptic seedlings of loblolly pine at high temperature. For.Sci. 17: 37-41.

MARX, D.H. and W.C. BRYAN. 1975. Growth and ectomycorrhizal development of loblolly pine seedlings in fumigated soil infested with the fungal symbiont *Pisolithus tinctorius.* For. Sci. 21: 245- 254.

MARX, D.H. and C.E. CORDELL. 1987. Ecology and management of ectomycorrhizal fungi in regenerating forests in the eastern United States. *In:* Mycorrhizae in the Next Decade: Practical applications and research priorities. Seventh NACOM, May, 3-8, 1987. Gainesville, FL. Ed. D.M. Sylvia, L.L. hung and J.H. Graham. Int. of food and agric. Sciences, Univ. of Florida, Gainesville.

MARX, D.H., W.C. BRYAN and C.B. DAVEY. 1970. Influence of temperature on aseptic synthesis of ectomycorrhizae by *Thelephora terrestris* and *Pisolithus tinctorius* on loblolly pine. For. Sci. 16: 424-431.

MARX, D.H., C.E. CORDELL, D.S. KENNEY, J.G. MEXAL, J.D. ARTMAN, J.W. RIFFLE and R.J. MOLINA. 1984. Commercial vegetative inoculum of *Pisolithus tinctorius* and inoculation techniques for development of ectomycorrhizae on bare-root tree seedlings. For. Sci. Monogr. 25: 1-101.

NATARAJAN, K. 1977. South Indian Agaricales III. Kavaka. *5:* 35-39.

SNEDECOR, G.W. and W.G. COCHRAN. 1967. Statistical Methods. The Iowa State University Press. Iowa USA p. 593.

TOMMERUP, I.e., N.L. BOUGHER and N. MALAJCZUK. 1991. *Laccaria fraterna* a common ectomycorr- hizal fungus with mono-and bisporic basidia and multinucleate spores: Comparison with quadri- sterigmate, binucleate spored *L. laccata* and the hypogeous relative *Hydnangium cameum.* Mycol. Res. 95: 689-698.

ZAK, B. 1973. Classification of ectomycorrhizae: *In:* Ectomycorrhizae: ecology and physiology. G.C. Marks and T.T. Kozlowski eds. Academic Press, New York and London, p. 43-78.