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Effect of ectomycorrhizal fungal species on the competitive outcome of two major forest species

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Mycorrhizae-mediated processes are known to influence the growth performances of host species in plant communities, but not much is known about their role in competitive outcome of host species. We show that the outcome of competition between the seedlings of two major Indian Himalayan tree species, viz. ban oak (*Quercus leucotrichophora*) and chir pine (*Pinus roxburghii*) is changed with the change in ectomycorrhizal fungal species. While oak does better than pine when grown in a mixed culture in the presence of *Russula vesca*, the outcome is reversed in the presence of *Amanita hemibapha*.

Keywords: Biodiversity, biofertilisers, ecosystem, ectomycorrhizae, Himalaya, productivity.

MYCORRHIZAE are known to influence plant performance through the benefits they confer on their hosts. Their benefits, for example, lead to improved growth of host plants and increased tolerance to drought and disease[1]. Mycorrhiza-mediated processes are likely to influence plant nutrition, plant competition and soil nutrient cycling[2]. More than 90% of plant species have association with mycorrhizal fungi[3], but not much is known about the effects of mycorrhizal symbiosis on plant species composition and competition[4]. The importance of mycorrhizal fungi in determining plant diversity relative to other mechanisms such as species competition and species coexistence has been little studied. The role of mycorrhizal fungi in nutrient uptake by host plant may vary from one group of fungi to another, and with changing environmental condition. Through a conceptual model, Aerts[5] schematically showed that the type of mycorrhizal association, such as ericoid mycorrhizal fungi and arbuscular mycorrhizal fungi, determines the plant species which dominate in heathland ecosystem. It is likely that the mycorrhizal effect between species, on the nutrient uptake of the host plant also varies from one species to another within the same group of mycorrhizal association. Different species of ectomycorrhizal fungi differ in their responses to host plants[6,7]. Colonization of mycorrhizal fungi is reported to reduce competitive dominance between host species and promote species diversity[8,9], so as to increase competition between them[10].

The main objective of the present study is to examine whether competitive outcome of the tree species occur-

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ring together varies with the change in associated ectomycorrhizal fungal species. We hypothesize that the ectomycorrhizal fungal species generally associated with a given host species in natural forest communities should enable it to out-compete the other host species with which it is relatively less associated. Here the assumption is that in the course of evolution, the most beneficial fungal associate of the host is selected.

The tree species studied were *Quercus leucotrichophora* (ban oak) and *Pinus roxburghii* (chir pine; hereafter referred to as oak and pine respectively). Having considerable overlaps in the altitudinal distribution (generally *P. roxburghii* between 500 to 1800 m and *Q. leucotrichophora* between 1200 to 2000 m), these tree species tend to dominate their respective forests. The ectomycorrhizal fungi selected for the study are *Amanita hemibapha* and *Russula vesca*. Globally, the genus *Amanita* is more commonly known to be associated with conifers and *Russula* with temperate broadleaved forest species. In the Himalaya, though both *Amanita* and *Russula* are found in pine as well as in oak forests, the latter is more abundant in oak forest than pine and the former in pine forest than in oak[11]. We further hypothesize that oak seedlings would have a competitive advantage over pine seedlings in the presence of *R. vesca* and pine over oak in the presence of *A. hemibapha*.

A glasshouse experiment was conducted to assess the in-growth and fitness of oak and pine seedlings with artificial inoculation of ectomycorrhizal fungi. For this, sporocarps of two species, *A. hemibapha* and *R. vesca*, were collected from various study sites of mixed forests in Nainital, the Central Himalayan region of Uttaranchal State. The sporocarps were brought to the laboratory and after surface-sterilization with 30% H_2O_2, the spores were transferred to modified Melin Norkran's (MNM) agar medium plates (previously autoclaved) under aseptic conditions[12]. The plates were incubated at 25°C. The mycelium was transferred to freshly sterilized MNM plates and pure cultures were obtained after repeating the process two to three times (i.e. within 10–12 days).

Bulk inocula were produced as mycelia on paddy husk substrate, supplemented with MNM broth medium. For this, 2 l conical flasks, each containing 200 g paddy husk mixed with 1 l MNM broth medium were sterilized. Pure cultures of the two species were prepared and inoculated separately in different conical flasks, under aseptic conditions. The cultures were allowed to grow for 60 days to get dense growth of the mycelia on the paddy husk.

Seeds of ban oak (*Q. leucotrichophora*) and chir pine (*P. roxburghii*) were germinated on moist, sterilized sand in a glasshouse after surface-sterilization. Potting mixture was prepared by mixing sand and soil in the ratio 1 : 2.

For each fungus, 10 g of paddy husk inoculum was thoroughly mixed with 3 kg of sterilized sand–soil mixture and was filled in nursery bags sterilized with alcohol. For control, no fungal inoculum was added in the sand–soil mixture. In each bag, two seedlings were transferred in the following combinations.

(A) Oak + oak – (I) Control
(II) Inoculated with *R. vesca* (III) Inoculated with *A. hemibapha*
(B) Oak + pine – (I) Control
(II) Inoculated with *R. vesca* (III) Inoculated with *A. hemibapha*
(C) Pine + pine – (I) Control
(II) Inoculated with *R. vesca* (III) Inoculated with *A. hemibapha*

All the treatments were replicated five times with irrigation applied at an interval of 72 h. After six months, the seedlings were carefully removed by brushing up the poly bags, and washed with gently flowing tap water. Different growth parameters, viz. root length, shoot length, collar diameter, dry mass of shoot, root and the number of mycorrhizal and non-mycorrhizal fine roots were recorded.

A study was also carried out to observe any change in the water potential and photosynthetic rate of the seedlings inoculated with mycorrhizal fungi. For this, seedlings grown in different sets were divided into four lots and subjected to different levels of water stress. For both oak and pine seedlings, predawn and midday water potential measurements were made on five seedlings each of every lot. A pressure chamber imported from Corvallis, USA (PMS Instrument Company, Corvallis, Orgegon) was used to determine the water potential. When a leaf or twig is severed, water columns in the xylem are pulled back into the xylem elements away from the cut surface. The twig or leaf is then sealed into the chamber, with only the cut xylem surface exposed to atmospheric pressure. The pressure in the chamber is increased until water columns in the xylem reach the cut surface. The amount of excess pressure applied to the leaves, just enough to cause water to refill the xylem elements, is called a balance pressure (BP). Photosynthetic rate was also recorded on five seedlings of each stress level of ban oak for each set during morning and afternoon using a portable photosynthesis system (LICOR 6200). The LICOR consists of a CO_2 analyser, a system consol and a sensor housing with interchamber leaf chamber. The LI 6200 CO_2 analyser is anon-dispersive, infrared-type (NDRI), calibrated for measurement of 0–1500 ppm.

The net change in CO_2 concentration between a leaf and the atmosphere is measured by enclosing the leaf in a chamber, and monitoring the rate at which CO_2 concentration changes over a short time interval (typically 10–20 s). The net photosynthesis rate is then calculated using this rate of change and other factors such as the amount of leaf area enclosed, volume of the enclosure, and temperature. The low noise level of the LI6250 (typically 0.2 ppm peak-to-peak) means that the CO_2 concentration need change only a few ppm during a measurement. Because

of instrumental limitation, it was not possible to record photosynthetic rate of pine. Hence we have not compared photosynthetic rate of pine in this study.

Both in oak and pine, the seedlings inoculated with ectomycorrhizal fungi showed significantly more growth in all parameters (shoot length, root length, collar diameter) than the un-inoculated ones. The ectomycorrhizal fungi, *Russula* and *Amanita*, significantly increased the number of fine roots (Figure 1). Oak seedlings inoculated with *Russula* showed more mycorrhizal roots (32.3%) as compared to those inoculated with *Amanita* (22.8%), whereas *Amanita* favoured the production of mycorrhizal roots in pine seedlings (18.1% with *Russula* and 39.61% with *Amanita*). In oak, the dry mass of seedlings inoculated with *Russula* and *Amanita* was seven- and fivefold greater than that of un-inoculated seedlings respectively. A sixfold and elevenfold increase in the total pine seedling mass was observed when inoculated with *Russula* and *Amanita* respectively.

In both species, seedling dry mass was greater when the seedlings were grown separately than when grown in a mixture (Figure 2). Evidently, competition reduced the growth of seedlings both with and without inoculation. However, the competitive outcome between oak and pine seedlings changed with the change in fungal associate, i.e. *R. vesca* favouring oak, and *A. hemibapha* favouring pine. Since our experiment dealt with a very early stage of the seedlings, it only hints at the competitive outcome.

Measurements of the photosynthetic rate and water potential of seedlings subject to various treatments indicated the favourable effect of mycorrhizal association on both oak and pine seedlings. In the presence of *Russula*, oak seedlings seemed to keep stomata open even after 15 days of watering interval when predawn water potential was –0.75 MPa. At that level the daily change in water potential was 2.05 MPa. On the other hand, pine seemed to close down its stomata relatively early when afternoon water potential approached close to –2.0 MPa (Table 1).

The photosynthetic rates in *Russula*-inoculated oak seedlings were significantly higher ($P < 0.001$) than the un-inoculated ones (Table 2). Even after 21 days of water stress, oak seedlings could maintain photosynthetic rate of 5.2 μmol CO_2 m^{-2} s^{-1} and 3.2 μmol CO_2 m^{-2} s^{-1} in the morning and afternoon respectively, compared to zero values in un-inoculated seedlings. In brief, *Russula* helps oaks in maintaining higher predawn water potential, a larger daily change in morning and afternoon photosynthetic rates.

Both predawn water potential and mean photosynthetic rates were higher in inoculated seedlings than in un-inoculated ones. These values in oak were higher in *Russula*-inoculated seedlings than in *Amanita*-inoculated seedlings. The pattern was reversed in pine seedlings when predawn water potential was measured.

Evidences suggest that mycorrhizal fungi may be involved in the regulation of competition between plant species, particularly when neighbouring individuals differ in responses to mycorrhizal association[13]. They may affect competition between plants in more than one way and often by triggering a chain of reactions. For example, mycorr-

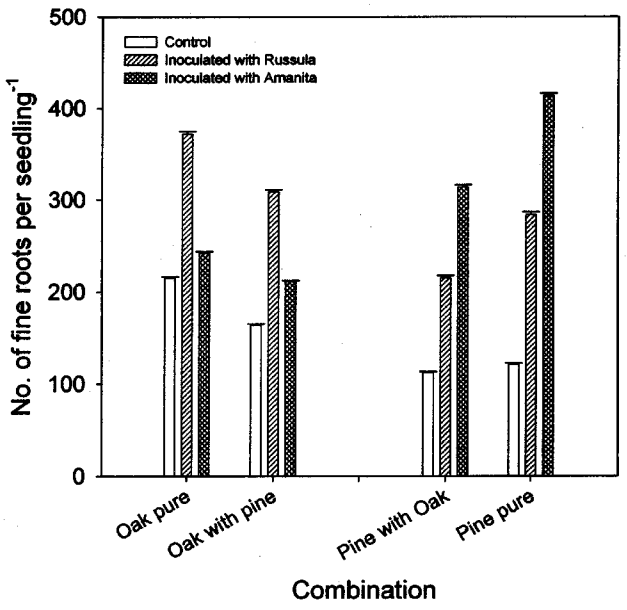

Figure 1. Effect of mycorrhizal fungi on competitive outcome in total fine roots production of six-month-old oak and pine seedlings. 'Oak with pine' indicates oak seedlings grown with pine and compared with pure oak seedlings, whereas 'pine with oak' indicates their comparison with pure pine.

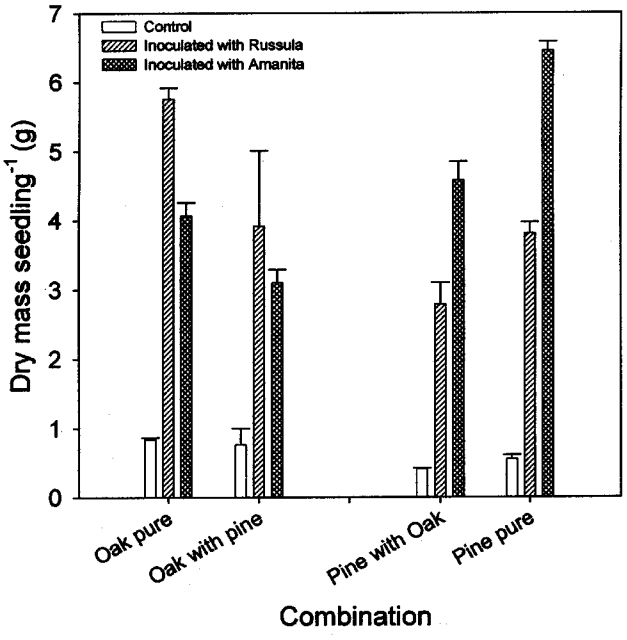

Figure 2. Effect of mycorrhizal fungi on competitive outcome in total dry mass of six-month-old oak and pine seedlings. 'Oak with pine' indicates oak seedlings grown with pine and compared with pure oak seedlings, whereas 'pine with oak' indicates their comparison with pure pine.

Table 1. Water potential (MPa) of oak (*Quercus leucotrichophora*) and pine (*Pinus roxburghii*) seedlings in relation to water stress, mycorrhizal association and competition

	Oak/Oak		Oak/Pine		Pine/Pine	
Stress level (watering interval)	Predawn	Afternoon	Predawn	Afternoon	Predawn	Afternoon
Un-inoculated seedlings						
Control	–0.40	–2.30	–0.65/–0.70	–2.50/–1.80	–0.60	–1.65
7 days	–0.86	–1.60	–0.87/–0.75	–2.40/–1.75	–0.65	–1.6
15 days	–1.00	–1.80	–1.10/–1.15	–2.80/–1.80	–1.10	–1.8
21 days	–1.50	–2.70	–1.65/–1.35	–2.85/–1.80	–1.25	–1.7
Russula vesca inoculated seedlings						
Control	–0.35	–2.30	–0.40/–0.58	–2.35/–1.85	–0.50	–1.60
7 days	–0.45	–2.10	–0.65/–0.70	–2.15/–1.55	–0.65	–1.70
15 days	–0.75	–2.80	–0.80/–1.10	–2.90/–1.85	–1.05	–1.70
21 days	–1.10	–3.15	–1.35/–1.30	–3.20/–2.00	–1.20	–1.90
Amanita hemibapha inoculated seedlings						
Control	–0.40	–1.80	–0.60/–0.70	–2.00/–1.5	–0.38	–1.40
7 days	–0.68	–1.90	–0.80/–0.75	–2.22/–1.5	–0.45	–1.30
15 days	–0.90	–2.10	–0.95/–1.10	–2.20/–1.75	–0.90	–1.80
21 days	–1.45	–3.00	–1.50/–1.35	–3.48/–2.00	–1.40	–1.95

Table 2. Photosynthetic rates (μmol CO_2 m^{-2} s^{-1}) of oak (*Q. leucotrichophora*) seedlings in relation to water stress, mycorrhizal association and competition

	Oak		Oak grown with pine	
Stress level (watering interval)	Predawn	Afternoon	Predawn	Afternoon
Un-inoculated seedlings				
Control	12.2	6.5	10.0	5.0
7 days	12.0	5.0	11.5	6.0
15 days	6.0	2.5	6.5	0.0
21 days	0.0	0.0	0.5	0.0
R. vesca inoculated seedlings				
Control	15.6	10.2	14.0	8.5
7 days	17.0	8.3	12.5	6.3
15 days	11.5	3.6	8.9	4.6
21 days	5.2	3.2	4.1	2.9
A. hemibapha inoculated seedlings				
Control	13.0	9.2	11.6	6.2
7 days	12.5	5.3	11.0	5.2
15 days	7.5	5.2	7.0	3.3
21 days	2.7	2.5	1.2	1.4

hizal fungi may increase soil nutrients and water availability, which in turn may cause greater leaf expansion and ability to compete for light[14]. They may also modify the outcome of competition through inter-plant mycelial connections and transfer of material[15,16] and changes in the rhizosphere chemistry[17]. A fungal species that modifies competitive outcome between neighbouring species must facilitate supply of nutrients and water at different relative rates[18,19]. In our experiment, the two ectomycorrhizal fungi differed in their effect on the hosts, which thereby brought about a change in their competitive outcome. The fungal species, the association of which favoured the host species in competition seemed to give a relative advantage to it through a better access of resources such as water, over other forest species. Such modifications can have implications for community dynamics.

Since the host species differ in their successional stage, the pine being early and the oak late successional[20,21], fungal association is likely to affect the rate of successional changes. *Russula* may hasten the growth of oak, while *Amanita* may enable pine to withhold the site against the growth of oak.

Streitwolf-Engel *et al.*[22] have shown strong differential effects of the fungal species on morphology and pattern of clonal growth of plants, thus affecting their spatial arrangement in communities. Observations such as these and of the present study suggest that mycorrhizae play a significant role in determining the organization of plant communities. Host plants also bring about difference in life-history traits, such as sporulation or infection of different mycorrhizal fungi[23–25]. Our experiment dealt with a very early phase of seedling growth, and the results of competitive outcome between the host species may change as time progresses. Nevertheless, it throws light on the importance of fungal flora involved in mycorrhizal association to plant community dynamics.

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ACTN3: Athlete gene prevalence in North India

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Alpha actinin is an actin-binding protein involved in anchoring thin filaments of actin to the Z-line of myofibrils. Two structural isoforms of alpha-actinin (ACTN2 and ACTN3) are present in fast twitch (type 2) fibres of the skeletal muscle. *ACTN3* gene has two alleles, R and X; the R allele is able to code for full length protein, while no functional protein results from the X allele due to a nonsense mutation (R577X) in exon 16 of *ACTN3*. The presence of X and R alleles of *ACTN3* has been reported to affect the sprinting and endur-

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