Front. For. China 2008, 3(4): 475–479
DOI 10.1007/s11461-008-0077-9

RESEARCH ARTICLE

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Mycorrhizal formation of nine ectomycorrhizal fungi on poplar cuttings

Abstract In order to discover which ectomycorrhizal-(ECM) fungi have better growth-promoting effects on poplars, cuttings from four poplar species were inoculated with nine species of ECM fungi by three methods. We investigated the status of mycorrhizal formation and the effects of these fungi on the growth of the poplars. The results show that *Xrocomus chrysentero* (Xc), *Boletus edulis* (Be), *Pisolithus tinctorius* (Pt) and *Laccaria amethystea* (La) formed clear ectomycorrhizal symbiosis with the poplar seedlings. Among these four ECM fungi, Xc had the greatest ability to develop mycorrhizae with all four poplar species. Be shows a greater ability to form mycorrhizae with *Populus deltoides* Bartr cv. 'Lux' (Poplar I-69). Pt and La had relatively weaker abilities of colonization. The other five ECM fungal species, i.e., *Scleroderma luteus* (Sl), *Leccinum scabrum* (Ls), *Boletus speciosus* (Bs), *Calvatia craniiformis* (Cc) and *Rhizopogen luteous* (Rl) could not easily form mycorrhizae with poplar seedlings grown in sterilized substrates, but could do so in non-sterilized soil. With the method of drilling and injecting liquid inoculum, a simple operation, the mycorrhizal infection rates were higher than with the other two methods, applying solid inoculum as fertilizer at the bottom of the pots and dipping roots in the inoculum slurry. *P. simonii* Carr. formed mycorrhizae with most of the nine ECM fungi. *P.* × *euramericana* (Dode) Guinier cv. 'San Martino' (Poplar I-72) and *P. deltoids* Harvard × *P. deltoids* Lux (Poplar NL-351) had the highest compatibility with Pt. Poplar I-69 shows the highest compatibility with Xc. The study indicates that the optimal ECM fungi for poplars I-69, I-72 and NL-351 were Be, Xc and Pt, respectively. The optimal fungi for *P. simonii* Carr. were Xc and Be. These ECM fungi promoted the growth of the poplar seedlings significantly.

Translated from *Journal of Nanjing Forestry University* (*Natural Sciences Edition*), 2007, 31(6): 29–33 [译自: 南京林业大学学报(自然科学版)]

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Keywords poplar, ectomycorrhizal fungi, mycorrhizal formation, mycorrhizal morphology

1 Introduction

Populus is an important, fast-growing tree genus for afforestation and is widely cultivated in China. The species of this genus occur mostly in pure plantations for their commercial importance and, besides the large demand for water and fertilizer, the trees are vulnerable to attacks by pests and diseases and are easily affected by changes in environmental conditions, such as soil and climate, resulting in a decline of growth and vigor. The ectomycorrhizal (ECM) fungi of poplar absorb required nutrients from host trees and in return, the trees obtain more water, phosphorus and other needed substances through these fungi (Lee and Koo, 1985; Zhou and Qi, 1993; Luo, 1996; Gong, 1997). Liang et al. (2003) inoculated potted cuttings of *Populus 'beijingensis'* and the field cuttings of *P. euramericana* with *Boletus edulis* Bull. ex Fr. and *Leccinum scabrum* Bull. Gray and studied the effect of mycorrhizal formation on poplar growth and resistance to adverse conditions. The results showed that mycorrhiza effectively increased the survival rates of the cuttings and promoted height growth, root collar diameter and the formation of a suitable root-shoot ratio. However, different fungi had various effects on the growth of poplars (Kikuchi et al., 1991; Zhao and Liu, 1994). For our study, we inoculated four species of poplars, widely grown in eastern and northern China, with nine species of ECM fungi. The mycorrhizal formation and its growth-promoting effects were examined in order to select the optimal ECM fungi for poplars and to provide some references for sustained management of poplar plantations.

2 Materials and methods

2.1 Poplar and ectomycorrhizal fungi

The four poplar species, widely planted in Jiangsu Province, were *P. deltoides* Harvard × *P. deltoides* Lux

(Poplar NL-351), *P. deltoides* Bartr cv. 'Lux' (Poplar I-69), *P.* × *euramericana* (Dode) Guinier cv. 'San Martino' (Poplar I-72), and *P. simonii* Carr. Cuttings were used as seedlings.

The nine ECM fungi were *Pisolithus tinctorius* (Pt), *Boletus speciosus* (Bs), *Boletus edulis* (Be), *Laccaria amethystea* (La), *Rhizopogen luteous* (Rl), *Scleroderma luteus* (Sl), *Leccinum scabrum* (Ls), *Xrocomus chrysentero* (Xc) and *Calvatia craniiformis* (Cc). The first eight fungi originated from southwest and northeast China and are preserved in the pathology laboratory of the Nanjing Forestry University. The last fungus was collected from the Nanjing Purple Mountain. These fungi were cultivated in petri dishes for about one week at 25°C.

2.2 Inoculum preparation

Solid inoculum: The solid culture substrate was a mixture of vermiculite, cotton-shell and peat (8:1:1; v/v/v) and was sterilized for 1 h under standard conditions of 121°C and 1.01×10^6 Pa, repeated three times. The substrate was moistened with a modified Melin Norkrans (MMN) liquid medium and its water content determined by the following method: by clenching the mixture, water exudation could be seen between fingers, but no obvious water drops were formed. The substrate was placed in polypropylene bags and sterilized for 1 h at 121°C. The nine ECM fungi from the petri dishes were inoculated in the bags and incubated for 4–6 weeks in the dark at 25°C.

Liquid inoculum was prepared by inoculating these fungi from petri dishes into culture flasks with a MMN liquid medium and then incubating on vibrating beds and shaken at a speed of 120 r/min at 25°C. After 4–6 weeks, the mycelial balls or slurry were harvested.

2.3 Inoculation of poplar cuttings

The two growth media for nurturing poplar cuttings were a non-sterilized soil and a sterilized substrate, a mixture of sand, peat and vermiculite (1:1:1; v/v/v), sterilized for 2 h. The poplar cuttings were grown in pots (30 cm in height, with a 28 cm diameter) in either the soil or substrate. There were 30 seedlings for each treatment. After growing for 40 d, the seedlings were inoculated separately with the nine species of ECM fungi by three methods.

Method I—after taking the seedling from the pot, we applied solid inoculum (10 g/seedling) as base fertilizer at the bottom of each pot and replanted the seedling. Method II—we drilled and injected a liquid inoculum (10 mL/seedling) into the holes around the root base. Method III—we took the seedling out, dipped its roots in the inoculum slurry, a blend of liquid inoculum (10 mL/seedling) and sterilized sand, then planted it again. The control seedlings were injected with MMN medium (without any fungi, 10 mL/seedling). There were five replications for each treatment.

2.4 Seedling growth indices and ectomycorrhizal formation assessment

At harvest, six months after inoculation, the lengths and basal diameters of all coppice shoots from the cuttings were measured and their ectomycorrhizal status examined. For mycorrhizal formation assessment, the poplar seedlings were randomly harvested, with roots carefully washed. Thirty root tips, about 5 cm long, of each seedling were randomly chosen for observing mycorrhizal morphologies and colors. Ectomycorrhizal formation was quantified by counting the colonized root tips under a stereomicroscope. The mycorrhizal infection rates were calculated as follows: mycorrhizal infection rate = (number of infected root tips/total number of root tips) × 100%. The data were analyzed by the SPSS statistical software.

3 Results

3.1 Ectomycorrhizal morphology

After a six-month growth, the colonized short roots of inoculated poplar seedlings were thicker than those of the control. These mycorrhizal seedlings formed developed lateral roots with more bifurcations. The tender mycorrhizae were mostly white or yellow. Mature mycorrhizae were deeper colored, mostly yellow brown and dark brown. The dominant form of ECM was club-shaped, dichotomous branching and monoaxial branching mycorrhizas were fewer. Root hairs were rarely seen on the mycorrhizal short roots, but were developed in the control seedlings. The hyphae wrapped root tips to form a fungal mantle, which was sparse in the ectomycorrhizae developed by Be, but was thick in the Pt group. A large amount of extra-radical hyphae were seen extending from the mantle, especially at the bifurcations of the mycorrhizae. The hyphae, rather than some root hairs, played an important role in taking up nutrients.

3.2 Abilities of ectomycorrhizal formation

Among the tested ECM fungi, four species (Xc, Be, Pt, and La) formed clear mycorrhizal associations with poplar seedlings, but their abilities to develop mycorrhizae varied. In method I, the application of solid inoculum, the mycorrhizal infection rates with the four fungi of poplar seedlings were all over 25% in both the sterilized substrate and non-sterilized soil. The highest rate was 65% (Fig. 1). This indicated that the four fungal species had good symbiotic relationships with the tested poplars. Among the fungi, Xc and Be had greater abilities than Pt and La. The other five fungal species, Sl, Ls, Rl, Bs and Cc also formed a few mycorrhizae with poplar seedlings in non-sterilized soil, but did not do so in the sterilized substrate. It might be that

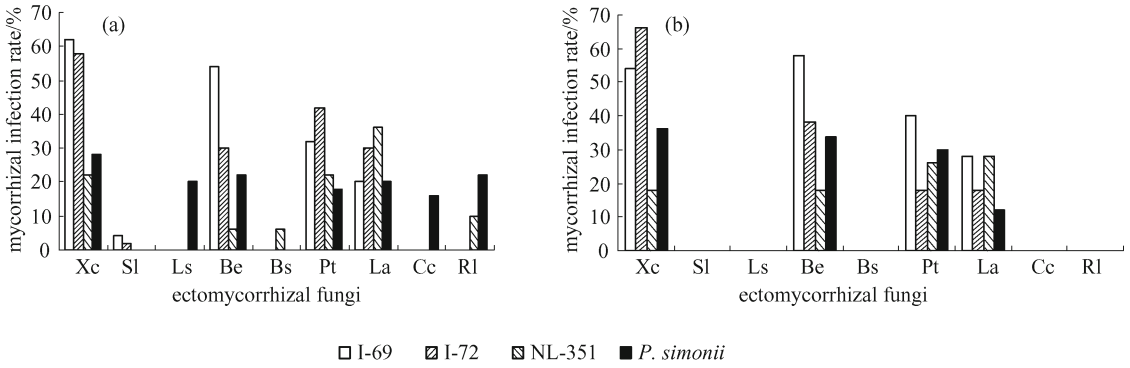

Fig. 1 Status of mycorrhiazal formation of different ECM fungi and poplar seedlings
Note: (a) non-sterilized soil, (b) sterilized substrate.

some rhizosphere microorganism in the non-sterilized soil interacted with these ECM fungi and promoted mycorrhizal formations.

The status of the mycorrhizae varied among the four poplar species. Overall, the abilities of these poplars in developing mycorrhizae strengthened in non-sterilized soil in the following order: poplar I-69 < I-72 < NL-351 < *P. simonii*. As the dominant species cultivated in Jiangsu Province, Poplar I-69 (America black poplar strain) and I-72 (European and American poplar strains) formed clear associations with five kinds of ECM fungi, especially with Xc, Be, Pt and La treatments, with higher infection rates. Poplar NL-351 formed combinations with six kinds of ECM fungi. Its infection rate was the highest in the La group. *P. simonii*, a species of the Cathay Poplar Group, could be colonized by seven kinds of ECM fungi in spite of their generally lower infection rates (Fig. 1a). In the sterilized substrate, all the poplars formed mycorrhizae with the same four ECM fungi, i.e., Xc, Be, Pt and La. In general, poplar I-72 had the best mycorrhizal status; Poplar I-69 showed the highest infection rate with Be at nearly 60%. Poplar NL-351 had higher infection rates with Pt and La and the infection rates of *P. simonii* were higher with Xc and Be (Fig. 1b).

3.3 Effects of different inoculating methods on mycorrhizal formation

The methods of inoculating had some effects on the formation of mycorrhizae. The mycorrhizal infection rates, by applying solid inoculum (method I) and drilling and injecting liquid inoculum (method II), were higher than that by dipping roots in the inoculum slurry (method III) (Table 1). For poplar I-69, the mean infection rate by method I was 35%, higher than by method II an III. For Poplars I-72, NL-351 and *P. simonii*, the average infection rates were 39%, 33% and 32% by method II, 30%, 30% and 25% by method I and 24%, 25% and 22% by method III, respectively.

Among the four ECM fungi Xc, Be, Pt and La, which formed distinct mycorrhizae with poplar seedlings, the mean infection rate for Xc was 50% by method I, 31% by method III and the rate reached 55% by method II. For Be, the rate was 51% by method II, rather higher than the 37% by method I and 33% by method III. For Pt, the rate was 48% by method II and about 34% by methods I and III. In the La treatment, the rate was 32% by method II and both 23% by methods I and III. Thus, drilling and injecting liquid inoculum (method II) increased the colonization rates.

Table 1 Mycorrhizal infection rates of poplars by different inoculating methods (unit: %)

mycorrhizal fungi	soil treatment	poplar I-69			poplar I-72			poplar NL-351			*Populus simonii*		
		method I	method II	method III	method I	method II	method III	method I	method II	method III	method I	method II	method III
Xc	A	62	48	20	58	72	30	22	40	36	28	60	30
	B	54	30	14	66	70	32	18	30	20	36	42	22
Be	A	54	46	34	30	50	31	6	22	12	22	40	18
	B	58	70	66	38	44	40	18	50	33	34	50	26
Pt	A	32	16	20	42	38	20	22	42	32	18	24	22
	B	40	40	16	18	44	40	26	20	44	30	30	24
La	A	20	32	34	30	26	30	36	50	18	20	32	24
	B	28	10	10	18	50	20	28	44	32	12	8	10
CK	A	0	0	2	0	0	0	0	0	0	4	0	0
	B	0	0	0	0	0	0	0	0	0	0	0	0

Note: A was non-sterilized soil; B was sterilized substrate.

3.4 Effect of different ECM fungi on poplar seedling growth

After six months of growth, the poplar seedlings which developed clear mycorrhizae showed a definite increase in length and basal diameter of the coppice shoots, compared with the control seedlings. The maximum increase in the seedlings inoculated with Xc and Pt was nearly 20%, these seedlings had developed root systems, dark green leaves and grew better. Poplar NL-351 and *P. simonii* showed higher growth rates up to 30.3%. The growth-promoting effects were more distinct in sterilized substrates (Fig. 2). The fungi Sl, Ls and Bs were not found to form obvious mycorrhizae with poplars, but improved growth in length and basal diameter of shoots in some inoculated seedlings.

We found that most poplar seedlings grew better in the sterilized substrate than in the non-sterilized soil. One possible reason was that the substrates were loose and the soil used was rather thick and had poor air permeability. Another reason might be that there were large numbers of microbes in the non-sterilized soil and a certain antagonistic competition between some microbes and ECM fungi affected the functioning of these fungi.

Analyses of variance indicated that in sterilized substrates, the lengths of the shoots showed statistically significant differences both among the ECM fungi and among the poplar species, and so did the basal diameters of the shoots. In non-sterilized soils, the lengths showed significant differences both among the ECM fungi and among the poplar species, but there were no distinct differences in basal diameters (Table 2).

4 Conclusions and discussion

Four poplar species were inoculated with nine species of ECM fungi in this study. As a result, four species of the tested fungi formed good symbiotic relationships with poplar seedlings. Xc had the greatest ability to develop mycorrhizae, followed by Be, which shows greater ability to form mycorrhizae with Poplar I-69. Pt and La had relatively weaker abilities to form combinations. Poplars in sterilized substrates were difficult to colonize for the other five fungal species Sl, Ls, Bs, Cc and Rl, but formed associations in the non-sterilized soil. Considering the compatibility of poplar species with ECM fungi, *P. simonii* formed mycorrhizae with most of the nine ECM fungi. Poplar I-72 and NL-351 had the highest compatibility with Pt and Poplar I-69 had the highest compatibility with Xc. All nine ECM fungi promoted length and basal diameter growth of the coppice shoots to different degrees. Growth-promoting effects were the best in the Pt and Xc groups. The optimal ECM fungi for poplars I-69, I-72, and NL-351 were Be, Xc, and Pt, respectively and that for *P. simonii* were Xc and Be. These ECM fungi promoted the growth of the poplar seedlings significantly.

Different inoculating methods had some impacts on mycorrhizal formation. Drilling and injecting liquid inoculum resulted in higher mycorrhizal infection rates than

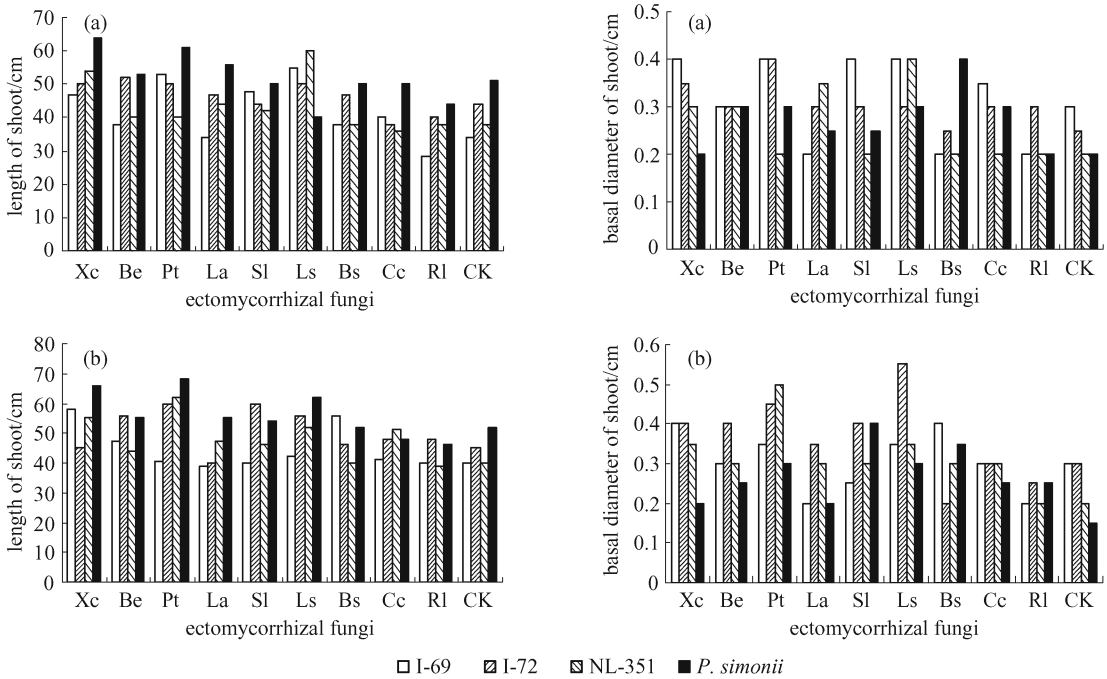

Fig. 2 Effect of different ECM fungi on the growth of poplar cuttings
Note: (a) non-sterilized substrate, (b) sterilized substrate.

Table 2 Analyses of variance of lengths and basal diameters of poplar shoots under different growth conditions

soil treatment	growth index	variation sources	*df*	*SS*	*MS*	*F*	*p*
sterilized substrate	length	between poplars	3	694.025	231.342	6.150	0.003**
		between fungi	9	855.025	95.003	2.525	0.030*
	basal diameter	between poplars	3	0.045	0.015	3.102	0.043*
		between fungi	9	0.123	0.014	2.807	0.018*
non-sterilized soil	length	between poplars	3	631.969	210.656	5.838	0.003**
		between fungi	9	931.506	103.501	2.868	0.016*
	basal diameter	between poplars	3	0.024	0.008	1.774	0.176
		between fungi	9	0.053	0.006	1.298	0.284

Note: * means significant difference ($p < 0.05$); ** means extremely significant difference ($p < 0.01$).

the other two methods. This was because this method had little effect on the root zone which might be helpful for the colonization of ECM fungi. Furthermore, it was found in our study that although the five fungal species Sl, Ls, Rl, Bs and Cc could not form clear mycorrhizae with poplar seedlings, they did promote seedling growth to some extent, with the exception of Sl. This indicates that the growth-promoting effect was not only owed to mycorrhizal formation which changed root morphology and enlarged the absorption area, but also due to some extra-cellular substances secreted by the ECM fungi, which were supplied to the seedlings directly or improved the rhizosphere environment to increase absorption, thus promoting seedling growth. The mechanism of growth-promotion, induced by ECM fungi on poplars, needs further study in the future.

Acknowledgements This study was financially supported by the National Natural Science Foundation of China (Grant No. 30571471) and the Natural Science Foundation of Jiangsu Province, China (Grant No. BK2004217).

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