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Effectiveness of mycorrhizal inoculation in the nursery on root colonization, growth, and nutrient uptake of aspen and balsam poplar

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ABSTRACT

Aspen and balsam poplar seedlings were inoculated with six species of ectomycorrhizal fungi (*Hebeloma longicaudum*, *Laccaria bicolor*, *Paxillus involutus*, *Pisolithus tinctorius*, *Rhizopogon vinicolor*, and *Suillus tomentosus*), one species of endomycorrhizal fungus (*Glomus intraradices*), two species of bacteria (*Agrobacterium* sp. and *Burkholderia cepacia*), treated with a growth hormone (SR3), and co-inoculated with a combination of *Paxillus* and *Burkholderia*. The seedlings were grown in a greenhouse under three different fertility regimes. Bacterial inoculation alone did not affect seedling growth and nutrition as observed when co-inoculated with ectomycorrhizal fungus. The biomass and root collar diameter of aspen and balsam poplar were significantly increased when adequate mycorrhizas are formed and more prominent when co-inoculated with *P. involutus* and *B. cepacia* and grown at the 67% fertilizer level. Except for *R. vinicolor* and *S. tomentosus*, the other four species of ectomycorrhizal fungi and *G. intraradices* formed symbiotic associations with both plant species. Both ectomycorrhizal and endomycorrhizal colonization were observed at all fertilizer levels and fertilizer applications did not affect the colonization rates. Nitrogen and phosphorus concentrations were significantly improved in both aspen and balsam poplar compared with control only when co-inoculated with *P. involutus* and *B. cepacia*. However, plant net nitrogen uptake (content) increased significantly in all successful inoculation treatments and co-inoculated treatment when compared with control. These results hold promise for incorporation of inoculation of *Populus* sp. with appropriate mycorrhizal fungi and selected bacteria into commercial nursery system to improve the establishment of *Populus* in various sites.

1. Introduction

The genus *Populus* has a widespread geographic distribution and is most abundant in temperate regions of northern hemisphere with about 120 species in North America. *Populus* are recognized as fast-growing tree species and therefore possess substantial commercial importance [1]. It is one of the most economically important hardwood genera, and is an important source of raw material for both the pulp and oriented strand board industries in Canada and expected to play a crucial role in forestry practices in prairie province [2]. Because of its rapid growth, plantation of fast-growing trees

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species can provide opportunities for quick biomass production in marginal agricultural lands or abandoned farmlands for different purposes [3,4] and can contribute to positive carbon balance [5,6]. It is also used in reforestation programs for biomass production and for reclamation of spoil banks [2,7–9]. Recently, the oil sand industries have shown interest in growing fast-growing *Populus* for reclaiming more difficult tailing sands produced after mining activities [10].

Aspen (*Populus tremuloides* Michx.) and balsam poplar (*Populus balsamifera* L.) produced in the nursery for reforestation purposes reach plantable size within a few months. Because of the generous fertilizer and pesticide used in nurseries to promote rapid initial growth, seedlings root system can be devoid of beneficial symbiotic mycorrhizal fungi. It has been shown in several studies that the appropriate mycorrhizal fungi can improve the tree growth and establishment in forest ecosystem, particularly on adverse site by facilitating nutrient and water availability [11–17]. Mycorrhizas can also play an important role in the protection of plants against root pathogens [18]. Much of the information on nursery inoculation mainly comes from studies on pines, spruces, and oaks or other hardwoods. However, compared with conifers, not much information is available about mycorrhizae in *Populus* [2]. Although ectomycorrhizal (ECM) fungi are already present in most natural forests, seedlings planted on routine reforestation sites and disturbed land may benefit from pre-inoculation [19,20]. Therefore, controlled inoculations of nursery seedlings are useful as a nursery culture method to increase field performance of outplanted seedlings.

Populus is one of the few genera known to form both ECM and endomycorrhizal associations [21], and this flexibility in colonization capacity may contribute to its widespread distribution [10]. However, mycorrhizal dependency of poplar species, the degree to which they are dependent on mycorrhizas to produce maximum growth, and effectiveness of nursery inoculation of *Populus* with mycorrhizal fungi has not been fully assessed. Available reports demonstrate that the host range of fungal associates with *Populus* species is very broad [21–23]. Godbout and Fortin [22] reported that 29 species of ECM fungi form mycorrhizae with *P. tremuloides*; 43 ECM species associated with aspen [21], and 23 different morphotypes associated with aspen clones [23]. Another area of research on mycorrhizal symbiosis showed recently that many rhizosphere bacterial strains stimulate mycorrhiza formation, plant growth, and seedling emergence [24–30]. Arbuscular mycorrhizal (AM) fungi and bacteria can interact synergistically and benefit plant growth and nutrient acquisition [30]. However, the effect of bacterial inoculation alone or co-inoculation with mycorrhizal fungi on *Populus* growth and mycorrhiza formation has not been investigated. Therefore, an attempt was also made to explore if there exists any relationship between poplars and rhizosphere bacteria and growth hormone.

In this study, we tested the effectiveness of six species of ECM fungi, one species of AM fungus, two species of bacteria, treated with a growth hormone to produce mycorrhizal aspen and balsam poplar seedlings. We present the effectiveness of artificial inoculation of seedlings grown in a commercial greenhouse under three levels of fertilizer to determine the effect of these isolates upon seedling growth response, mycorrhiza formation, and nutrient uptake.

2. Materials and methods

2.1. Organisms

Six species of ECM fungal isolates were used: *Hebeloma longicaudum* Pers.: Fr. (UAMH 9317), *Laccaria bicolor* R. Mre. (NOF 2290), *Paxillus involutus* Batsch: Fr. (NOF 2340), *Pisolithus tinctorius* (Mich.: Pers.) Coker and Couch (commercial vegetative inoculum grown in vermiculite was obtained from Plant Health Care, Inc. PA, USA), *Rhizopogon vinicolor* A.H. Smith (UAMH 6200), and *Suillus tomentosus* Kauffman (UAMH 6252). An endomycorrhizal fungus *Glomus intraradices* Schenck and Smith (commercial inoculum mixed in vermiculite was obtained from Premier Peat Moss, Quebec, Canada), two species of bacteria, *Agrobacterium* sp. and *Burkholderia cepacia* Burkholder (liquid inoculum of *B. cepacia* was obtained from Agrium Biologicals, Saskatoon, Saskatchewan, Canada), and a growth hormone SR3 (Stim Root No. 3, which contains 0.8% IBA rooting powder, manufactured by Plant Products Co. Ltd., Brampton, Ontario, Canada) were also used in this study. The SR3 growth hormone often used to promote rooting of plant cuttings in nurseries to grow seedlings with better root system. We used this growth hormone to examine if there is any advantage to use in inoculation practice.

2.2. Inoculation and seedling production

The experiment was conducted at Woodmere commercial Nursery, Fairview, Alberta under a standard nursery environment. A single seedlot was used and each of aspen and balsam poplar seeds were sown in styrofoam blocks (77 cavities/block) containing 170 ml peat:vermiculite nursery mixture (3:1, vol/vol). All inoculation was performed just before sowing seeds. The liquid inoculum of *H. longicaudum*, *L. bicolor*, *P. involutus*, *R. vinicolor*, and *S. tomentosus* was prepared by growing these fungi in liquid modified Melin Norkrans medium [31] for 8 weeks and mycelial slurry was harvested. In total, 600 ml of blended mycelial slurry was resuspended in 8 l of water to a final density of ca. 5×10^5 colony forming units (cfu)/ml (viable propagules/ml). The inoculation was performed by injecting 5 ml of each ECM fungal liquid inoculum into each cavity containing seeds of either aspen or balsam poplar. The commercial vegetative inoculum of *P. tinctorius* grown on vermiculite: peat and the commercial inoculum of *G. intraradices* were applied at a rate of 10 ml per cavity at about 2–3 cm below the seeds. For *Agrobacterium* sp. inoculum, a 5 ml of the bacterial cell suspension (5×10^6 cfu/ml) was injected into each cavity. For *B. cepacia* inoculum, the proprietary liquid formulation was applied at a rate of 0.3 ml (1×10^8 cfu/ml) per cavity. For SR3 growth hormone, about 500 mg of powder was applied to each cavity just below the seeds. Control treatment received no inoculum. Styroblocks were placed on the greenhouse bench in a split-plot randomized factorial design with fungal treatment and fertilizer levels in three blocks (replicates). The following 12 treatments were used: control, *H. longicaudum*,