Mycorrhizal Fungal Inoculation of Woody Seed Propagation Substrate©

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Incorporation of mycorrhizal inoculum into a range of soilless propagation substrate did not result in colonization of either ectomycorrhizal or endomycorrhizal tree species grown from seed. Numerous factors may have accounted for or contributed to the lack of colonization, including bark substrate phenolic compounds, excessive substrate moisture and nonviable mycorrhizal inoculum.

INTRODUCTION

Mycorrhizal fungi are naturally occurring soil fungi that form symbiotic relationships with over 95% of the green plants of the world (Marx, 2000). These fungi are unique in that they colonize fine absorbing roots as ectomycorrhizas or endomycorrhizas. Once colonized mycorrhizal plants benefit via greater water and nutrient uptake, increased disease protection, and increased tolerance to soil temperature extremes.

In healthy forest soils, mycorrhizal associations are the norm because the soils generally have physical and chemical characteristics that are conducive to fungal growth. In soils used for nursery production and landscapes, however, mycorrhizal fungi may not be present due to soil compaction, topsoil removal or subsoil addition, unfavorable soil pH levels, soil saturation and low aeration, excessive soil temperatures, phytotoxic chemicals, and other conditions adverse to the fungi.

Benefits could potentially be derived from transplanting colonized plants into landscapes with less than ideal soil conditions. Interest in the possibility of initiating colonization during nursery production has therefore increased. Fungal inoculants could be introduced at one or more stages including propagation, initial container or field production, and even postharvest inoculation into containers or field root balls (Appleton et al., 1999). It is most cost-effective and efficient to apply mycorrhizal inoculum during propagation since relatively small amounts of inoculum are required (Brooke, 1998).

Brooke (1998) outlined several important considerations relative to mycorrhizal fungal inoculation of growing substrates, including inoculum formulation and its activity, application procedure and timing. Mycorrhizal associations are generally absent in growing substrates or substrate components such as perlite, vermiculite, composted bark, and other naturally or artificially sterile, soilless materials. In addition, the large quantities of water, fertilizer, and chemical pesticides used in the nursery industry can reduce survival of mycorrhizal fungi. More environmentally friendly practices need to be utilized.

Much of the propagation research involving mycorrhiza has been for forestry, not nursery or landscape applications. A good review of tree seedling colonization by

ectomycorrhizal fungi (beech, birch, hickory, pines and other conifers, oak, poplar, willow) in both field and container propagation is provided by Cordell and Marx (1994). They state that ectomycorrhizal fungi, like their tree seedling hosts, are favored by coarse-textured nursery soils and container substrate components (vermiculite, pine bark) that promote good internal water drainage and aeration. They further state that artificially introduced ectomycorrhizal fungi generally respond quite differently in "artificial container media" than in nursery soil. While the best natural ectomycorrhizal development has occurred in field nursery soil, to get consistent success with the introduction of ectomycorrhizal fungi into nursery soils requires soil pasteurization or fumigation (Marx et al., 1991). Cordell and Marx (1994) felt that inoculation of container substrates can be successful because natural-origin ectomycorrhizal fungi and other potentially competing soil microorganisms (bacteria, fungal parasites, etc.) are frequently absent.

The majority of the seedling inoculation research has been narrowly focused on tree species in the *Pinaceae* family as hosts, with the ectomycorrhizal *Pisolithus* species as the inoculum fungi (Castellano, 1994). Most of the seed-propagated trees and shrubs used for landscapes, however, are colonized by endomycorrhizal fungi, and more specifically, by AM fungi (arbuscular mycorrhizae). Much of the research dealing with AM fungal inoculation of horticultural crops has centered on production in field soil, not soilless container substrates (Smith and Read, 1997).

With regard to inoculation of tree seeds, the type of substrate components used often are not reported. Substrate components that have been reported include composts, composted coniferous and hardwood barks, expanded shale, peat, perlite, sand, sewage sludge, soil, turface, vermiculite, and specific commercial formulations (Brundrett et al., 1996). These components have been used for the seed propagation of a variety of ecto- and endomycorrhizal tree hosts, with mixed results often due to confounding factors such as fertility (Guttay, 1982; Johnson et al., 1980; Maronek et al., 1980; Maronek et al., 1982; Ruehle and Wells, 1984).

There are many unanswered questions relative to the biological, chemical, physical, and economical feasibility of inoculating and colonizing seed-propagated tree species during container propagation in soilless substrate. Therefore the purpose of this preliminary research was to begin to systematically assess substrate selection and inoculum application timing.

MATERIALS AND METHODS

Seeds were collected from trees at the Hampton Roads Agricultural Research and Extension Center, Virginia Beach, Virginia and the Norfolk Botanical Garden, Norfolk, Virginia, during September and October, 1999, and were subjected to cold stratification. Four ectomycorrhizal species – white oak (*Quercus alba*), swamp white oak (*Q. bicolor*), bur oak (*Q. macrocarpa*), and live oak (*Q. virginiana*) — and four endomycorrhizal species – flowering dogwood (*Cornus florida*), sweetgum (*Liquidambar styraciflua*), American sycamore (*Platanus occidentalis*), and lacebark elm (*Ulmus parvifolia*) — were selected.

On 13 March 2000, a propagation substrate of milled sphagnum moss, peat, and perlite (2 : 1 : 1, by volume) was prepared. No pine bark was used in the substrate because it has been hypothesized that phenolic compounds in the bark might be inhibitory to the endomycorrhizal fungi which in nature are more frequently found in lower organic matter, highly mineral soils.

The propagation substrate was subdivided and used either uninoculated or inoculated with the appropriate mycorrhizal fungi. Inoculum was provided by Becker Underwood (Ames, Iowa), but instead of using their commercial Rhizanova™ product containing a blend of ecto- and endomycorrhizal spores, we were provided with the spores as fungal-type-specific inoculum. The ectomycorrhizal fungal inoculant was *Pisolithus tinctorius* (cut with silica sand to aid in distribution), and the endomycorrhizal fungal inoculant was an equal mixture of *Glomus claroideum*, *G. diaphanum*, *G. etunicatum*, and *G. intraradices* (undiluted, a combination of spores and vegetative propagules). Inoculum was incorporated into the propagation substrate at the manufacturer recommended rate of 10 g liter^{-1} and 100 g liter^{-1}, respectively, a rate considered to be in excess of actually needed. Spore counts and infectivity were verified by Dr. Joe Morton, International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM), West Virginia University, Morgantown, West Virginia.

Equal numbers of seed of each tree species were placed into substrate with and without inoculum in a randomized complete design with three multiseeded containers as replications per treatment. Containers were placed in a heated greenhouse, initially under intermittent mist and then hand watered as needed. No fertilizer or chemical pesticides were applied at any time during the germination period because research has shown that high levels of nitrogen and/or phosphorus (Johnson et al., 1980; Koide et al., 1999; Ruehle and Wells, 1984; Rupp and Mudge, 1985), or application of certain fungicides (Fontanet et al, 1998; Smith and Read, 1997; Trappe et al., 1984), can adversely affect colonization.

After 8 weeks, all germinated oak seedlings were sent to Dr. Orson Miller, Curator of Fungi, Virginia Tech, Blacksburg, Virginia, and all germinated sweetgum seedlings were sent to Dr. Morton. Seeds of the flowering dogwood, American sycamore, and lacebark elm failed to germinate in sufficient numbers for analysis. Roots were examined for colonization and substrate for mycelium production.

RESULTS AND DISCUSSION

No root colonization was found for any of the test species. In addition, no mycelium growth was found in any of the substrate samples. As a result several unreplicated substrate trials, using soilless and soil-based substrates, were quickly conducted — again with no colonization. Lack of colonization has also occurred in similar substrate trials recently conducted by other university researchers (pers. commun.).

Two additional experiments were therefore designed, and are currently ongoing. One involves the use of a product reported to aid in VAM colonization, while the other involves the use of six substrates (various percent blends of tulip tree bark and pine bark versus a commercial peatlite substrate) with and without mycorrhizal inoculation. Both involve the use of endomycorrhizal species, red maple and sweet gum, respectively. These experiments will be harvested and analyzed in November 2000.

Failure to achieve colonization may be due to one or more factors. Where pine bark was used possible phenolic phytotoxicity may have occurred. Water management is extremely critical in soilless substrates. Although containers in the main experiment were hand watered when shoots began to appear in order to prevent the substrate from being too wet, excess moisture may have jeopardized colonization. The quick substrate screenings that were conducted under intermittent mist might also have been too wet.

Though the inoculum in the main experiment was certified for viable spores, the viability of a few commercially available inoculants has been questioned (pers. commun.). More research is needed, therefore, to address the complex blend of species, propagation substrates, inoculant products, and cultural practices involved in the propagation of trees from seed. It cannot be assumed that because inoculum is introduced during propagation that successful colonization has occurred. While in some instances it is possible to examine the roots of ectomycorrhizal trees and observe mycorrhizae, endomycorrhizal colonization must be microscopically confirmed. Nurseries advertising that they have inoculated during propagation should periodically confirm that colonization has occurred.

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