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Effect of Nursery-Produced Endomycorrhizal Inoculum on Growth of Redwood Seedlings in Fumigated Soil

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Inoculation of fumigated nursery bed soil with spores of Glomus mosseae *(Nicolson & Gerdemann) Gerdemann & Trappe greatly increased growth of redwood (*Sequoia sempervirens *(D. Don) Endl.) seedlings over non-inoculated seedlings. Nursery production and use of endomycorrhizal inoculum are discussed.* Tree Planters' Notes 40(3): 7 -11; 1990.

Mycorrhizae form an important symbiotic relationship with their hosts' roots; neither host nor fungus are likely to survive alone. Endomycorrhizal fungi, also called **vesicular-arbuscular mycorrhizae** (VAM), are ubiquitous fungi found throughout the world on more than 1,000 genera of higher plants *(19)*, including grasses *(13)*, hardwoods *(8, 18)*, and conifers *(7, 9)*.

Endomycorrhizae occur naturally on roots of redwood (*Sequoia sempervirens* (D. Don) Endl.) and giant sequoia (*Sequoiadendron giganteum* (Lindl.) Buchh.) in California forests *(9)*. *Glomus mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe occurs naturally in the soil of Ben Lomond State Forest Nursery, located at Ben Lomond, CA.

Because the common nursery practice *(10)* of soil fumigation greatly reduces VAM populations in nursery beds, reintroduction of VAM before seeding is imperative for achieving acceptable levels of seedling survival and growth *(8, 18)*. Inoculation of fumigated beds with VAM or planting VAM-colonized seedlings in fumigated soil results in normal growth of hardwood seedlings *(8, 10, 18)*. Hardwood seedling inoculation has been accomplished by transferring inoculum-bearing soil from forest sites to the nursery *(18)*, by transferring inoculum-bearing soil grown on an intermediate crop in the greenhouse *(11)*, and by growing colonized cover crops in the seedbed the year before hardwood seeds are sown *(5, 8)*.

Early colonization of young seedling roots occurs via the applied inoculum. Later, more extensive root colonization occurs as a result of inoculation from nearby earlier colonized roots *(17)*. No attention has been given to developing procedures for large-scale inoculation of conifers dependent upon VAM.

Powell *(16)* reviewed field inoculation procedures for many annual and perennial crop plants. Kough *et al. (7)* demonstrated an early growth response of endomycorrhizae-inoculated redwoods and other members of the Taxodiaceae and Cupressaceae in plastic growth tubes. Menge *(12)* comprehensively reviewed inoculum production and storage technology for endomycorrhizal pot cultures. Sudan grass (*Sorghum sudanense* (Piper) Stapf) was found to support endomycorrhizal fungi, including *G. mosseae (1, 3, 4)*.

Bareroot redwood seedlings grown on fumigated, non-inoculated soil at Ben Lomond State Nursery were nonmycorrhizal, stunted, and off-color, and frequently failed to survive. This report investigates the utility of sudan grass and 2+0 redwood for production of *G. mosseae* inoculum to inoculate fumigated nursery beds and describes an efficient and practical inoculation procedure. The study was carried out at the Ben Lomond State Forest Nursery near Santa Cruz on the central California coast.

Methods

Laboratory. *Spore extraction from soil.* To ensure cost-efficient use of valuable nursery bed space

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and labor, it was necessary to determine spore numbers of *G. mosseae* in the inoculum-soil. The assay procedure involved two steps: extracting spores from a sample of the inoculum-soil and counting spores in that sample. All assays were replicated three times for each soil.

Ten fifty-milliliter samples of soil were collected from redwood production beds (RPB) and sudan grass beds (SGB) and combined for each inoculum source. The number of *G. mosseae* spores per milliliter of soil was determined by using a firmly packed 25-ml soil subsample taken from each of the combined soil collections. Using the sieving method of Gerdemann and Nicolson *(6)*, each soil sample was gently washed through soil sieves stacked from the top down in the following order: mesh openings of 250 μm (for collection and removal of debris) and of 150, 106, and 53 μm respectively (for spore collection). Although the majority of the spores were collected on the 106-μm sieve, small quantities of spores were also collected on the 150- and 53-μm sieves. These three collections containing spores and soil were combined by washing onto the screen of a small-diameter, 25-μm soil sieve.

Spore numbers assay. Spores of *G. mosseae* are not readily decanted free of similar sized soil particles because the spores sink rather than float in water. For this reason the usual method of decanting *(6)* could not be used and the following spore recovery method was devised. Two-hundred and fifty milliliters of a 35% sucrose solution was carefully layered beneath 150 ml of distilled water in a 500-ml graduated glass cylinder. Congo red stain was previously added to the sucrose solution to make the sucrose-water interface visible. The combined sieved soil collection was gently rinsed with distilled water down the inner surface of the graduated cylinder and allowed to settle for 20 minutes. Spores of *G. mosseae* were collected by pipette from the upper region of the sucrose solution.

Collections were prepared for spore counting by using vacuum filtration. The filtration system consisted of a 0.45-μm, 47-mm Gelman Gn-6 gridded filter membrane seated in a 115-ml Nalgene disposable analytical filter unit, to which a vacuum pump was attached. While a vacuum of less than 250 mm Hg was applied, the spore suspension in the pipette was slowly dispersed over the filter membrane. Spore collection from the graduated cylinder was repeated as necessary to recover all spores. Spores of *G. mosseae* were identified and counted with a microscope at 20 to 40× power. Assays were replicated three times to estimate the number of spores per milliliter contained in the inoculum-soil.

Field. *Inoculum collection and preparation.* Inoculum from the redwood production beds came from recently harvested 2+0 beds, and inoculum from sudan grass beds came from beds seeded 1 year earlier. Both RPB and SGB soils containing spores of *G. mosseae* were collected 8 weeks before use (immediately after harvest of the 2+0 RPB beds) from the top 6 inches of the bed. These soils were air dried (< 20 °C) on heavy paper, sieved (1/8-inch mesh) to remove debris, and stored at temperatures below 20 °C in 4×4×3-foot wooden boxes in an enclosed area.

Seed bed preparation, inoculation, sowing, and care. Test beds were fumigated 2 weeks before inoculation with 175 pounds per acre methyl bromide (57%) and chloropicrin (43%). Bed plots were laid out according to a randomized block design. Four 4 × 50-foot replicates were used for each of the three treatments: non-inoculated control, inoculum from RPB, and inoculum from SGB. Inoculum-soil containing spores of *G. mosseae* was used to inoculate the test beds according to the experimental design and rates: 0 spores/ft^2 = controls and 100 spores/ft^2 = inoculated.

Soil samples from RPB and SGB beds containing spores of *G. mosseae* were incorporated into the prepared beds with a fertilizer drill set to place the inoculum at a depth of 0.5 to 2.0 inches (12 to 50 mm). Drill spacing was set to approximate the seed drill spacing of eight rows within a 4-foot-wide bed. Inoculation rates were calibrated into the fertilizer drill to

provide about 50 spores/ft^2 for each inoculum source. To ensure good dispersal of spore inoculum at a final rate of 100 spores/ft^2, the inoculum was drilled into the bed in two passes in opposite directions.

Inoculum drilling was followed immediately by seed drilling. Upgraded seed from the L.A. Moran Reforestation Center (lot 4750, Humboldt County source, 65% lab germination rate) was drilled into the top 4 to 6 mm of the bed with a Love seed drill, set to achieve a seedling density of 25/ft^2. All seed beds were irrigated immediately after sowing to a depth beyond inoculum placement. Beds were irrigated thereafter as needed to maintain moisture in the root area.

Data collection. Seedling height and caliper were measured at the end of the first and second growing seasons, except that height measurements were not taken on seedlings of treatment groups requiring top pruning during the second year. Caliper was measured immediately beneath the basal bud whorl at the top of the hypocotyl. Roots of seedlings from each treatment were examined microscopically (cleared and stained) at the end of both growing seasons for mycorrhizae *(2, 14).*

Results and Discussion

Adequate inoculum levels were reflected in the uniform growth of seedlings grown in inoculated beds. Inoculum source bed spore numbers varied with the host: RPB soil yielded about 1 spore/ml, SGB soil about 0.2 spores/ml. These numbers may seem low when compared with spore counts from soil of other endomycorrhizal fungi. However, spores of *G. mosseae* are large and have a high nutrient reserve that potentially allows several germinations and produces hyphae that can "explore" soil area for host roots (John Menge, personal communication).

Seedlings from the non-inoculated beds did not meet nursery standards at the end of the second year and were culled. All seedlings from the inoculated beds were top-pruned after the first year to keep height within nursery standards. There were no differences in plant growth between the inoculum sources (RPB and SGB) (table 1). Non-inoculated seedlings were significantly shorter in height and smaller in caliper than inoculated seedlings at the end of both the first and second growing seasons. Nearly all seedlings in the inoculated beds were mycorrhizal at the end of the first year. As a result of natural dispersal of VAM, a few seedlings in non-inoculated beds became mycorrhizal by the end of the first year, and a few more were mycorrhizal by the end of the second year.

Natural reintroduction of endomycorrhizal fungi into fumigated soil at Ben Lomond State Nursery occurs over time. We observed that seedlings growing along small surface watercourses in fumigated but non-inoculated beds were noticeably larger than nearby seedlings, in the same bed, that were not associated with these drainages. Evidently, VAM inoculum (spores and possibly hyphal fragments) from non-fumigated adjacent beds was carried to the fumigated beds during periods of heavy rain or irrigation. Very likely, inoculum moves along with soil during ripping, discing, and leveling operations. Spores also are dispersed naturally by wind *(21)* and by animals, which ingest spores (in sporocarps) and pass them through their digestive tracts *(15, 20, 21).*

Spores of *G. mosseae* appear to be particularly susceptible to damage under conditions of desiccation or excessive heat (Adams, unpublished data). Because seed beds are dry in preparation for sowing, conditions in these beds may not be

Table 1—*Mean caliper and height of redwood seedlings inoculated with* Glomus mosseae *from two sources*

Measurement and growth year	Inoculum source		Non-inoculated control
	RPB	SGB	
Caliper (mm)*			
1	3.8 a	3.7 a	1.5 b
2	6.5 a	6.9 a	3.2 b
Stem height (cm)			
1	27.8 a	29.1 a	6.5 b
2	NA	NA	16.3

*Measurements of hypocotyl diameter were taken immediately below basal bud whorl.

Means within a row (year) followed by the same letter do not differ at P = 0.01 (Duncan's multiple range test). RPB = redwood production bed, SGB = Sudan grass bed. NA = not available; RPB and SGB seedlings were topped during the second growing season.

favorable for spore survival. Therefore, it is imperative that the inoculated soil be irrigated immediately after sowing to avoid any loss of inoculum due to desiccation or excessively high temperatures.

Giant sequoia and incense-cedar (*Libocedrus decurrens* Torr.), both endomycorrhizal hosts, were inoculated at the same time as the redwood. The response of these hosts to inoculation closely paralleled that of redwood.

Sudan grass was to be grown each year specifically as a host crop for spore production and would be inoculated with RPB soil. However, although spores were produced, their relatively low numbers (compared to the RPB source) necessitated far too much soil collection and handling to be cost efficient. To meet time schedules and availability of personnel at the Ben Lomond State Nursery, soil inoculation methods must be easily performed and offer cost-effective benefits. Handling of inoculum (harvest, storage, preparation, and soil inoculation) must be manageable by nursery personnel and equipment without undue expenditure of time and equipment. The procedure described here meets the nursery's needs for dependable growth of quality seedlings. It is also noteworthy to point out that no disease problems have occurred over the subsequent years that RPB soil has been used.

Summary

Glomus mosseae is a very useful mycorrhizal fungus for colonization of redwood seedlings in fumigated nursery beds and appears to be equally useful with seedlings of giant sequoia and incense-cedar. Inoculum is readily and easily produced in "working" nursery beds. However, care must be taken to protect the inoculum from drying or heating at any time from collection through seedbed irrigation. Disease development must be closely monitored.

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