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# Inoculation of Almond Rootstock with Symbiotic Arbuscular Mycorrhizal Fungi

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**Objectives:**

1. Determine if there is value in adding AM fungi inoculum, particularly at planting of bare root (field grown) and the potted-plant nursery stock.
2. Determine if pre-plant fumigation impacts the extent and nature of mycorrhizal populations in the soil and is this of consequence?
3. Characterize the mycorrhizal fungi populations present on field grown nursery stock vs. potted plants at the time of planting and during the first season after planting as well as resulting tree performance.

**Interpretive Summary:**

Soil borne arbuscular mycorrhizal (AM) fungus forms a symbiotic (mutualistic) relationship with most plants. The fungus colonizes the root and grows out into the soil. Hyphae net work, the part of the fungus that's in the soil acts as an extension of the root system. Recent reviews suggest a multifunctional nature of AMF association with roots. The beneficial effects include better nutrient uptake, mineralization of organic nutrients, improving host plant's resistance to drought, seedling establishment, pathogen resistance, increased herbivore tolerance, increased pollination, heavy metal tolerance and increased soil stability. Research shows that AM fungal functioning may be more complex than previously thought. A diversified population of the symbiotic AM fungi in the soil is important factor contributing to these beneficial effects.

The status of AM fungal population in California almond orchard is not well documented. The purpose of this study is to determine if specific practices associated with planting almonds (e.g., pre-plant fumigation, inoculation with AM fungus, or other factors like choice of field grown vs. potted nursery stock) have an impact on AM fungal populations to the extent subsequent tree performance is affected.

A field trial was initiated in early 2008 to examine the effects of Arbuscular Mycorrhizal (AM) fungi on almond tree growth. The trial was planted on 7 February 2008 at the San Joaquin Valley Agricultural Sciences Center. Trees used in the study were either traditional bare root (1/2" caliper) Nonpareil/Nemaguard or 3/8" caliper 'potted' Nonpareil/Nemaguard trees. Three AM treatments were imposed on the bare root trees (control, field cultured AM and commercial cultured AM) and potted trees were utilized as either controls, or field cultured AM (five total tree treatments). Tree growth, soil nutrient status, arbuscular mycorrhizal population and root colonization will be monitored for several years. The results of this study will provide scientific information to growers on utilization of arbuscular mycorrhizal symbiosis in almond orchards.

Almond trees planted for this project are now completing their second year of growth in fumigated and non-fumigated soils. During the second year, trunk caliper measurements were taken on three dates (390, 491 and 609 days after planting). While no trees have died in the test, several trees in non-fumigated plots now appear weak and unhealthy.

Trees in the test plots have demonstrated consistent trends in trunk caliper increase throughout the second year of growth. At 609 days after planting (October 8), highly significant differences ( $p < 0.001$ ) are present between average trunk caliper of trees in fumigated plots (85.2 mm) and non-fumigated plots (48.2 mm). Comparing bare root versus potted trees at this same date, significant differences ( $p = 0.048$ ) in trunk caliper were also noted. Trunk caliper of bare root trees averaged 68.2 mm, whereas potted trees averaged 64.5 mm. No significant differences were identified in growth comparisons of arbuscular mycorrhizal (AM) treated trees (average trunk caliper = 66.1 mm) versus non-AM treated trees (average trunk caliper = 67.6 mm) at 609 days after planting. Progression of tree growth as represented by trunk caliper increase since project inception in these main effect comparisons are presented in **Figure 1**.

Performance evaluation will be continued for several years. Colonization by AM fungi causes a slight decrease of plant growth initially according to some published literatures. AM fungi derive most of their carbon from the host plant. Estimates vary, but plants have been shown to direct 4% to 20% more photoassimilate to mycorrhizal root systems. Therefore mycorrhizal colonization may lead to carbon enrichment in the root zone and increase in soil microbial population and diversity. Further research is needed to investigate these effects.

Soil samples were randomly selected between almond young trees in November 2008. Soil phosphate concentration levels were determined. The values are expressed in ppm ranging from 25 to 30 ppm. The result indicates that there is sufficient phosphate in the soil to support the growth of young almond tree. The phosphate level is high and

may prevent good mycorrhizal colonization. In some cases, mycorrhizal colonization can occur but it will not show stimulation in plant growth. Other beneficial effect of symbiosis may still function such as improvement of water stress and soil structures.

Roots of Sudan grass from trap cultures were analyzed for AM fungal colonization in fumigated and non-fumigated soils collected in 2007 at Firebaugh, CA. Sudan grass plants were grown under limiting phosphate nutrient conditions for boosting colonization and inoculum production. Results from PCR and gel electrophoresis analysis indicate that *Glomus Mosseae*, *Glomus 3*, *Gigasproa rosea*, *Glomus intraradices* were present in the soil and in colonized roots of Sudan grass.

Molecular techniques for identification of AM mycorrhizal fungal species were developed and performed at Albany lab. The procedure includes cloning and DNA sequencing. Several hundred clones have been obtained for DNA sequence analysis. The current data were used to establish phylogenetic relationship of almond AM fungi to other species of AM mycorrhizal fungi. Two almond AM fungal strains can be clearly identified as *Glomus intraradices*. Another almond AM fungal strain is closely related to *Glomus clarum*. Population analysis of AM fungal species in almond orchards is in progress. For detailed data, please see the 2008 – 2009 Final Reports on CD included with the Proceedings (08-HORT9-Hua).

The study of arbuscular mycorrhizal (AM) fungi has fundamental and practical importance. First because in most environments "root biology" is actually "mycorrhizal biology", and second because of the practical importance of AM in fields as diverse as sustainable agriculture, horticulture, reforestation, and ecosystem management. In the last few decades, interest in AM fungi has increased. The symbiosis has the potential for sustainable production of important crops and reducing the use of chemical fertilizers and pesticides. For successful application of AM fungi with economically profitable returns, the soil conditions must be suitable for AM colonization. Plant genotypes also influence the symbiosis.

**Figure 1.** Growth of almond trees from February 2008 to October 2009.

