Do Mycorrhizae Influence the Drought Tolerance of Citrus?[1]

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Abstract

The benefits of vesicular-arbuscular mycorrhizal (VAM) fungi for increasing drought tolerance have been demonstrated under nutrient-limiting conditions, particularly in low phosphorus (P) soils. Horticultural plants grown in soilless media, under greenhouse fertilizer regimes, are usually non-mycorrhizal, but have optimum P and the desired size and nutritional characteristics available when transplanted. Since plant nutrition can influence responses to environmental stress, potential benefits of VAM fungi for reducing transplant stress, such as drought, should be evaluated where growth and nutrition of mycorrhizal and non-mycorrhizal plants are similar at time of outplanting. In this way, nutritional and any unique non-nutritional effects of mycorrhizae on stress tolerance of plants can be identified. Non-nutritional effects of VAM fungi on drought tolerance of woody horticultural plants have not yet been clearly demonstrated.

Index words: Root hydraulic conductivity, transpiration, photosynthesis, greenhouse inoculation, phosphorus nutrition

Evaluation of the Influence of VAM on Drought Stress

The potential for vesicular-arbuscular mycorrhizal (VAM) fungi to improve stress tolerance of plants has been cited often without regard for whether the improvements are unique mycorrhizal effects on the host or an indirect result of increased absorption of nutrients by mycorrhizal roots (3). The perception that VAM fungi *per se* have the capability to substantially alter the physiology of plants is based upon the misleading experimental approach of using non-mycorrhizal controls that are nutritionally inferior. What have been identified are the well-known effects of increased P uptake by mycorrhizae on plants: greater water uptake, photosynthesis, transpiration, nitrogen fixation, salt tolerance and disease resistance. However, potential non-nutritional effects of mycorrhizae on drought, such as increased uptake and transport of water by VAM hyphae, have not been clearly demonstrated.

Gerdemann (2) first noted that, "In studies on the effect of VAM on disease, it should be determined whether changes in resistance are caused by increased nutrient absorption or if the effect is more direct." At that time, studies on effects of VAM fungi on plant stresses other than soilborne disease were just underway. However, the pioneering approach to testing the hypothesis "do mycorrhizae increase water uptake" had been published. In 1971 and 1972, Safir *et al.* (15, 16) reported that hydraulic conductivity of soybean roots was dramatically improved by the mycorrhizal fungus *Glomus mosseae* compared to nutrient deficient non-mycorrhizal plants. However, when the nutritional needs of the non-mycorrhizal plants were met, the difference in conductivity was not apparent. They discounted the possibility that hyphae were providing low-resistance pathways for water transport because a soil drench with a fungicide toxic to hyphae did not alter the conductivity of mycorrhizal soybeans.

The use of nutritionally comparable controls was established as an approach to studying effects of mycorrhizae on plant water relations (16). Some time later, Nelsen and Safir (13, 14) confirmed that mycorrhizae increased hydraulic conductivity of onions compared to P-deficient non-mycorrhizal plants, and that the difference could be eliminated by P fertilization under well-watered conditions. There were complications in comparing mycorrhizal and P-fertilized non-mycorrhizal plants under prolonged drought cycles, however (14). Despite fertilization with high levels of superphosphate, non-mycorrhizal plants were deficient in P because of a lack of hydraulic conductivity in dry soil. Nelsen and Safir (14) concluded that the ability of mycorrhizal hyphae to maintain P delivery to the root at low soil moisture was the basis for improved drought tolerance.

When P in the soil solution around the root is depleted, hyphal uptake and translocation of P to the root at low soil water potential is an extremely important beneficial effect of VAM. Improved P status of the mycorrhizal plant primarily results in greater hydraulic conductivity of roots, which enhances soil water uptake and increases drought tolerance (1, 8, 14). Nevertheless, the hypothesis that mycorrhizal hyphae *per se* increase water uptake under drought stress cannot be tested under these circumstances.

Levy and Krikun (10) and Levy *et al.* (11) studied water relations of nutritionally comparable VAM and non-my-

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corrhizal citrus rootstock seedlings under well-watered and drought conditions. Unfortunately, in the first study plant growth and P status data were not reported, so the basis for their finding that mycorrhizal plants had greater transpiration, stomatal conductance, and photosynthesis during recovery from drought cannot be evaluated. In the second study, plant growth and P parameters were reported but, in this case, the root systems of mycorrhizal plants were larger in size. They questioned whether the differences in transpiration and root conductivity were due to rooting volume limitations in pots and greater stress experienced by mycorrhizal plants.

Graham and Syvertsen (5, 6, 7) attempted to clarify these questions by growing citrus plants of equal size, growth rate, and P status in a non-limiting soil volume. In their first study (5), non-mycorrhizal plants were of equal size but were P-deficient compared to VAM plants. Root systems of mycorrhizal sour orange (*Citrus aurantium*) and Carrizo citrange (*Poncirus trifoliata* × *C. sinensis*) were twice as conductive as non-mycorrhizal root systems of similar size under well-watered conditions. Transpiration rates of VAM plants were higher due to the greater conductivity of the roots (5, 17). The basis for the lower water uptake by non-mycorrhizal roots of P-deficient citrus could not be explained, but served to reemphasize the profound effect P nutrition alone can have on water uptake by roots. When the magnitude of increase in hydraulic conductance of the mycorrhizal roots was compared to that of the non-mycorrhizal roots, it was impossible for all the additional water uptake by mycorrhizal roots to be via hyphal entry points. The primary influence of P nutrition on root conductivity was confirmed in a second experiment, wherein mycorrhizae had no effect on root conductivity when P content and size of non-mycorrhizal plants were similar. However, additional P fertilization of the mycorrhizal, as well as the non-mycorrhizal plants, reduced colonization of the inoculated plants and may have minimized the influence of mycorrhizae on root conductivity.

In a second study (6), mycorrhizal seedlings of 5 different rootstocks were grown under well-watered conditions in a P-deficient sandy soil and only the non-mycorrhizal plants were amended with soluble P. In this way, interaction between P-fertilization and the mycorrhizal colonization process was avoided. This yielded non-mycorrhizal plants of equal size, growth rate, and slightly greater P content than mycorrhizal plants. Plants inoculated with *G. intraradices* had at least half of their root systems colonized and consistently had higher root-shoot ratios, even though root length was comparable to that of non-mycorrhizal plants. This difference in root mass was due to the greater dry weight per length of mycorrhizal roots. Apparently mycorrhizal roots containing lipids in vesicles and chlamydospores (12) were denser than non-mycorrhizal roots.

Several physiological parameters were measured on these 2 sets of plants. No differences in photosynthesis, transpiration, and root hydraulic conductivity could be detected under well-watered conditions. These results confirmed those of Nelson and Safir (13), who used a similar experimental approach and found that hydraulic conductivity and transpiration of P-amended, non-mycorrhizal onions were similar to those of mycorrhizal plants under well-watered conditions.

Under drought-stress conditions, the maintenance of comparable mycorrhizal and non-mycorrhizal plants is more difficult (14). Because the delivery of P to the non-mycorrhizal root is reduced when hydraulic conductivity of the soil and root is low, it is necessary to study responses during soil water deficits of short duration and to use a soluble form of P to insure that P availability to non-mycorrhizal roots is sustained.

When P was supplied in soluble form before stress cycles, there was still a tendency for P content of non-mycorrhizal sour orange and Carrizo citrange seedlings to decrease during drought compared to well-watered plants (7). Nevertheless, mycorrhizal and non-mycorrhizal plants were comparable in P status after 2 drought cycles within 21 days. Seedlings colonized by *G. intraradices* did not respond differently than non-mycorrhizal plants to drought stress with respect to transpiration and leaf water potential. Root hydraulic conductivity of mycorrhizal and non-mycorrhizal plants was decreased by drought compared to well-watered plants, but the reduction was greater for mycorrhizal seedlings. Thus, in the absence of a P-nutritional effect, mycorrhizae did not appear to increase water uptake of citrus under drought stress as a result of hyphal uptake and transport. To the contrary, mycorrhizae decreased root conductivity when measurements were made soon after water-stress cycles.

Horticultural Implications

The utilization of mycorrhizae in horticulture hinges on their ability to reduce transplant stress (3). Considering the metabolic cost of maintaining the mycorrhizal symbiosis, as high as 10% of carbon allocation for citrus (9), the benefit of mycorrhizae in water-uptake and efficiency of production of horticultural plants needs to be reassessed. If mycorrhizae increase the carbon-demand for root growth and effectively reduce root conductivity under drought stress to some degree, then the net effect of VAM colonization may be a decrease in water-uptake efficiency compared to non-mycorrhizal plants under non-nutrient-stressed conditions.

Mycorrhizal fungi will enhance stress tolerance if the plant is transplanted into an environment where there is chronic nutrient and water stress. This has enormous implications for native woody plants that are highly dependent on mycorrhizae for nutrient uptake and are often outplanted into degraded soils where VAM fungal populations are deficient (18). Where populations of indigenous VAM fungi are too low to rapidly colonize the roots, there may be an obligate requirement for pre-inoculation of the plant for revegetation, since subsequent fertilizer and water inputs are not economically feasible.

In the case of fruit and woody ornamental crops transplanted into orchard or landscape situations, the considerations can be quite different. Although these crops also are highly dependent on VAM for P and micronutrient uptake, the additional cost of fertilization after transplant is relatively low compared to the value of the crop. Furthermore, the outplanting site may have more than adequate populations of indigenous VAM fungi present. The experience with citrus is that non-mycorrhizal roots require 9 to 12 months to become significantly colonized by indigenous mycorrhizal fungi under moderate P-fertility conditions (J.H. Graham, unpublished). Preliminary results from outplanting studies in Florida indicate that, if the non-mycorrhizal tree receives adequate P and micronutrient fertilization before transplant, it performs as well as the mycorrhizal plant of similar size and nutrition under irrigation and moderate P availability (J.H. Graham and L.W. Timmer, unpublished data). It appears that, under normal orchard conditions in Florida, non-mycorrhizal citrus with adequate P relations when outplanted performs well enough until VAM fungi are acquired in the field site.

Containerized plants grown in soilless media under greenhouse fertilizer regimes are non-mycorrhizal when transplanted (3). However, inoculation with VAM fungi may not be justified if mycorrhizae do not substantially increase transplant performance. Moreover, the current lack of a commercial VAM inoculant of high quality precludes application of mycorrhizae because of the risk of introduction of unwanted pests into clean semi-sterile media used in container production (3). Furthermore, the expectation that VAM plants have the capability to grow more quickly than under a soluble fertilizer regime has not been demonstrated, even for a crop highly dependent on mycorrhizae like citrus (4). Any benefits in terms of improved nutrient balance and uniformity of mycorrhizal plants may not outweigh the cost of inoculation compared to that of fertilizer application.

Significance to the Nursery Industry

The capability of VAM fungi to reduce plant stresses such as drought needs to be critically evaluated when considering the need for inoculation of nursery stock for transplant. This means that non-mycorrhizal plants with optimum nutrition and size characteristics should be the standard for comparison, because such plants can be economically produced in containers with soluble fertilizers. Furthermore, the outplanting situation needs to be evaluated from the standpoints of the indigenous VAM fungi available to colonize the non-mycorrhizal plant, and the fertilizer and water inputs that the plant will receive until mycorrhizae develop.

Currently, considering the unavailability of high quality VAM inoculum and the lack of demonstrable benefits for transplanted citrus under Florida conditions, mycorrhizal inoculation of woody horticultural plants cannot be recommended without qualifications.

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