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The Water Relations of Well-watered, Mycorrhizal, and Non-mycorrhizal Onion Plants[1]

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Abstract. **The water relations of mycorrhizal onions (*Allium cepa* L.) were compared with those of non-mycorrhizal controls grown under low and high soil phosphorus conditions. Mycorrhizal plants had higher leaf water potentials, higher transpiration rates, higher hydraulic conductivities and lower leaf resistances than did non-mycorrhizal plants grown in low soil phosphorus conditions. When controls were grown under high soil phosphorus conditions, all 4 parameters were not different from those of mycorrhizal plants. The magnitude of the effect of mycorrhizal fungi on the water relations of the host may, in part, be a function of phosphorus nutrition. The differences in leaf water potentials, transpiration rates and leaf resistances are considered to be the result of the differences found in hydraulic conductivities.**

Vesicular-arbuscular (VA) mycorrhizal fungi have been shown to improve plant growth by augmenting the phosphorus nutrition of the host plants (8, 12, 13, 17). In addition to changes in growth, changes in the water relations of mycorrhizal plants have been reported (16). In the first report of this type, Safir, Boyer and Gerdemann (14) showed an increase of about 60% in hydraulic conductivity to liquid water flow in soybeans when they were infected with the mycorrhizal fungus *Glomus mosseae*. Later, these same authors (15) demonstrated that the differences in hydraulic conductivity between mycorrhizal and non-mycorrhizal soybeans were eliminated after addition of a complete nutrient solution to the soil.

Levy and Krikun (7) reported differences in the water relations of mycorrhizal and non-mycorrhizal citrus plants upon recovery from a single episode of water stress. Upon rewatering, after 4 days of water withholding, the mycorrhizal plants appeared to recover more quickly than the non-mycorrhizal controls to a condition of higher stomatal conductance and higher photosynthetic

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rate, although the differences were not statistically significant. They did not find differences in hydraulic conductivity and speculated that differences in leaf conductance reflected altered hormonal status. However, since their mycorrhizal and control plants were grown under very high nutritional conditions, their results actually support those of Safir et al. (15) who found that the addition of nutrients eliminated differences in hydraulic conductivity.

In this report we define more fully the effects of mycorrhizal infection on the water relations of the well-watered host plants by examining leaf water potential, transpiration rate, hydraulic conductivity and leaf resistance of mycorrhizal and non-mycorrhizal onions. Furthermore, we report on the effects of soil phosphorus on these same four water relations parameters. In doing so we clarify the different results reported by Safir et al. (14) and Levy and Krikun (7) on the effects of mycorrhizal infection on hydraulic conductivity.

Materials and Methods

'Downing Yellow Globe' onion plants were grown in plastic cups (8.4 cm high × 7 cm diameter) in 200 g of a mix of 1 sand:1 sandy loam soil. The soil mix was sieved through a 2 mm screen and autoclaved for 45 min prior to planting. Soil pH was 7.5 ± 0.1 and soil phosphorus levels were low (10 ppm Bray's P-1 extractable). Plants were grown in a growth chamber with a 14 hr light period, air temperatures of 22°C (day)/16° (night), and relative humidity controlled at either a high (60 ± 5%) or low (40 ± 5%) level.

Pots were randomly divided into 3 groups and arranged in the growth chamber in randomized blocks, with the blocks parallel to the fluorescent lamps. Onions in one group were inoculated just below the seeds with 10 g (600 spores) of soil inoculum from a pot culture containing spores of the mycorrhizal fungus *Glomus etunicatus* (Becker & Gerdemann). Plants in the second group were fertilized with 30 ml of a 1.58 mg/ml solution of KH_2PO_4 to add 50 ppm P to the soil to stimulate growth of the onion plants in a manner similar to that due to mycorrhizal infection. Onions in the third group were uninoculated and unfertilized. Both sets of non-mycorrhizal onions were treated with a soil wash from the mycorrhizal pot culture from which the spores had been sieved, so that other microbial organisms in the pot culture would be present in all three treatments.

Experiments were begun when plants were 8 or 11 weeks, at which time all pots were watered to a common weight, then carefully enclosed in plastic wrap followed by aluminum foil to eliminate evaporation from the soil surface or through the pot. Transpiration was measured by weighing pots twice daily, then dividing the amount of water transpired by the average of the leaf surface area at the start and at the end of each experiment. Control pots, identical to pots containing plants, except containing cylindrically-shaped wood applicators, were also wrapped and weighed to determine the efficiency of reduction in evaporation due to wrapping. Leaf surface area was determined non-destructively using a regression line of actual leaf surface area versus calculated leaf surface area derived from a separate experiment using a range of leaf sizes from plants grown under similar conditions (Fig. 1). Actual leaf area was determined by weighing photocopies of leaves and determining the area by conversion from the weight of each photocopy using the density of the paper. Leaf area was calculated as the surface area of a cylinder equal to π times leaf length times the average of the diameter at 0.5 leaf length and 0.9 leaf length.

Leaf water potential was measured using a Wescor dew point hygrometer and 12 C-52 sample chambers following a previously described method (9). One cm leaf segments were excised from the midpoint of the leaf being sampled, placed into the sample chambers, and the water potential was measured after a 3 hr equilibration period.

The hydraulic conductivity of whole onion plants was calculated using a method discussed by van den Honert (19) and Boyer (2).

$$T = \frac{-(\psi\text{leaf} - \psi\text{soil})}{r_p} \qquad \text{[Eq. 1]}$$

where T = Transpiration rate

ψ = water potential of leaf and soil, respectively

and r_p = plant hydraulic resistance.

Knowing the hydraulic conductivity (L_p) is equal to the inverse of r_p and rearranging equation (1),

$$L_p = -\frac{T}{(\psi\text{leaf} - \psi\text{soil})} \qquad \text{[Eq. 2]}$$

Soil water potential was assumed to be zero bars and the hydraulic resistance of the soil was considered to be negligible because the experiments were performed with the soil at field capacity (4).

Leaf resistance was calculated using an equation described by Kramer (6),

$$T = \frac{.622\,\rho}{P} \times \frac{{}^{e}\text{leaf} - {}^{e}\text{air}}{{}^{r}\text{leaf} + {}^{r}\text{air}} \qquad \text{[Eq. 3]}$$

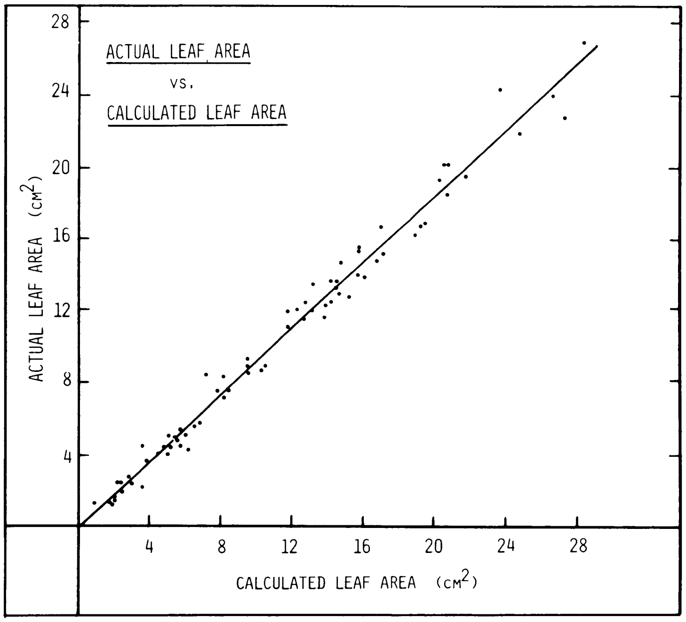

Fig. 1. Regression line of the relationship between actual leaf area and calculated leaf area where the leaf area was calculated as a cylinder equal to π times leaf length times the average of the diameter at 0.5 leaf length and 0.9 leaf length. Regression line = 0.92 × calculated leaf area − 0.24, r^2 = 0.984.

where:

T = Transpiration rate in g cm^{-2} s^{-1}
ρ = density of air in g ml^{-1}
P = atmospheric pressure in mm Hg
e = vapor pressure of leaf and air, respectively in mm Hg
and
r = Leaf and boundary layer resistance to vapor flow respectively in s cm^{-1}.

By rearranging equation 3, leaf resistance plus boundary layer resistance can be determined.

$$r_{leaf} + r_{air} = \frac{.622\,\rho}{P} \times \frac{e_{leaf} - e_{air}}{T} \quad \text{[Eq. 4]}$$

The atmospheric pressure (P) can be measured and the density of air (ρ) can be determined by knowing P and the air temperature, which was measured with a shaded thermocouple held at leaf level. Leaf vapor pressure (e_{leaf}) was determined by measuring leaf temperature with a small thermocouple and assuming the leaf was saturated with water vapor (at 100% relative humidity). Air vapor pressure at leaf level was measured using a dew point hygrometer (Yellow Springs Instrument Co.) equipped with a YSI 9102 probe.

The boundary layer resistance was measured by forming filter paper "onion leaves," wetting them, and placing one end of each in a water source. The weight loss of these "leaves" were then measured with time, and since there was no cuticle, the only resistance to vapor loss is the boundary layer resistance. Therefore, equation 2 becomes,

$$r_{air} = \frac{.622\,\rho}{p} \times \frac{e_{\text{``leaf''}} - e_{air}}{T} \quad \text{[Eq. 5]}$$

Using 4 replicate filter paper leaves, r_{air} was equal to 0.87 s cm^{-1} for the conditions of these experiments.

Finally, the leaf resistance (r_{leaf}) was determined by subtracting the value calculated in equation 5 for boundary layer resistance from the value obtained in equation 4 for leaf plus boundary layer resistance.

At the end of each experiment, mycorrhizal infection was checked to ensure that the mycorrhizal plants were infected and that non-mycorrhizal plants had not been contaminated (11). The experiment was conducted 4 times, 3 times at 60% relative humidity (RH) and one time at 40% RH.

Results

Figure 2 shows leaf water potentials, transpiration rates, hydraulic conductivities, and leaf resistance of 8-week old onion plants from one of the experiments conducted at 60% RH. The mycorrhizal (MYC) onions have significantly higher leaf water potentials than do the non-mycorrhizal, non-phosphorus treated (NM minus P) onion plants (Fig. 2A). Leaf water potentials of non-mycorrhizal plants treated with phosphorus (NM plus P) are not different from that of MYC plants. It is evident that MYC and NM plus P onion plants have a higher (more favorable) water status than do the NM minus P plants.

Transpiration rates exhibit a similar pattern (Fig. 2B). MYC plants have a transpiration rate which is twice that of NM minus P plants on a unit leaf area basis. Again treatment with phosphorus (NM plus P) eliminated the differences between mycorrhizal and non-mycorrhizal plants.

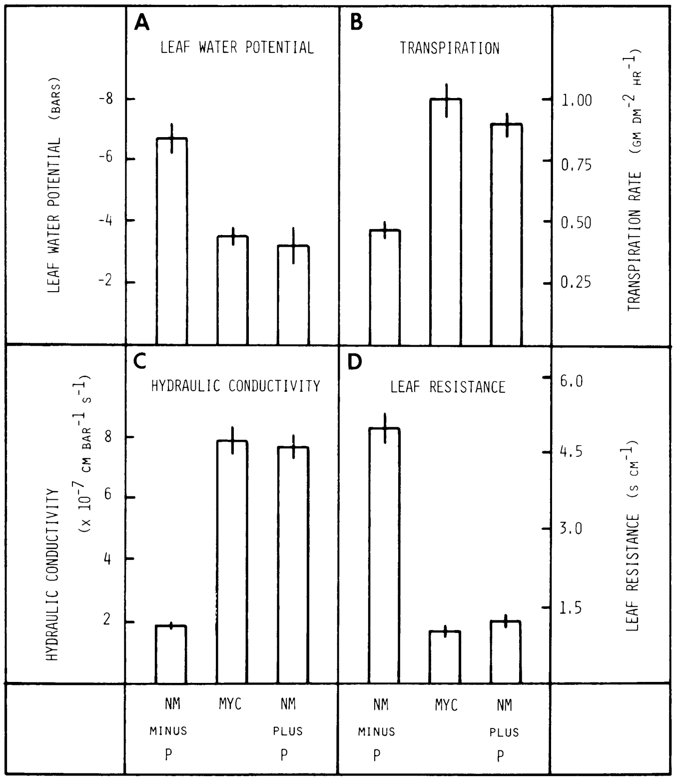

Fig. 2. Measured and calculated water relation parameters of well-watered onion plants from experiment number 1. Values are means of 4 replicates and each bar represents the standard error of the mean. Dry weights of plants were: NM minus P = 14 ± 3 mg, MYC = 173 ± 32 mg, and NM + P = 59 ± 3 mg. The leaf water potentials (A) of NM minus P are significantly lower than the other 2 treatments at the 5% level by Duncan's multiple range test (DMRT). The transpiration rates (B) and hydraulic conductivities (C) of the NM minus P plants are significantly lower and the leaf resistances (D) of the NM minus P plants are significantly higher than the other 2 treatments at the 1% level by DMRT.

Atmospheric vapor pressure determines the ultimate driving gradient for water from the soil, through the plant, and into the atmosphere (19). Because the MYC plants have a higher leaf water potential than do NM minus P controls when exposed to the same driving force, this indicates that it is easier for liquid water to move through the plant to the evaporating surfaces in the leaf. Results of calculations of hydraulic conductivity (a measure of the ease with which liquid water moves through the plant) are shown in Fig. 2C. As expected, MYC plants have a considerably higher hydraulic conductivity than do NM minus P. Since NM plus P plants have leaf water potentials and transpiration rates similar to MYC plants, the hydraulic conductivities of NM plus P plants are also high and not different from MYC plants.

The greater transpiration rates of MYC plants over NM minus P plants when exposed to the same evaporative demand indicate a lower resistance to vapor transfer from inside the leaf to the atmosphere for the MYC plants. Results of calculations of leaf resistance (Fig. 2D) indicate that MYC plants have leaf resistances much lower than do NM minus P plants (1.0 vs. 5.0 sec cm^{-1}).

NM plus P plants have a slightly higher resistance than did the MYC plants, but the differences were not significant.

In every case the results for the other 3 experiments were similar to those reported for experiment 1 (Fig. 2). Leaf water potentials, transpiration rates, and hydraulic conductivities are always higher for the MYC plants when compared to the NM minus P plants; while leaf resistances are always lower for the MYC plants. Treatment of non-mycorrhizal plants with phosphorus (NM plus P plants) always eliminated the differences between mycorrhizal and non-mycorrhizal plants.

Discussion

We have shown that mycorrhizal fungi can alter the water relations of the host plants. The effect is pronounced under conditions of low soil phosphorus as suggested by Safir et al. (15) who eliminated the differences with a complete nutrient solution and as shown in this paper (Fig. 2). The fact that Levy and Krikun (7) did not find a difference in hydraulic conductivity between their mycorrhizal and non-mycorrhizal citrus plants was probably due to the high levels of available phosphorus supplied by the irrigation solution they used (a 0.1% solution of 20 N-20 P-20 K nutrient solution applied daily).

Furthermore, the magnitude of the effect of mycorrhizal infection may also depend on the innate capacity of the host to absorb water and/or nutrients. Baylis (1) showed that plants with more primitive root systems (i.e. Magnolioid types) have a greater growth stimulation when infected by a mycorrhizal fungus than plants with a more advanced, finely divided root system (i.e. grasslike). In comparison to soybeans, onions have a shallow and non-extensive root system (5, 18). This may explain the differences between our present results with onions, and those found under low soil phosphorus conditions by Safir et al. (14, 15) with soybean (400% vs. 60% increase in hydraulic conductivity).

The fact that added phosphorus affects the water relations of the onion in the same way that the mycorrhizal fungus does suggests that the primary cause of the changes is nutritional. A model root system has been hypothesized (3) where changes in membrane permeability (an area where phosphorus would have considerable importance) can have large effects on root resistance to water flow. This may explain the effects of mycorrhizae on hydraulic conductivity.

Finally, the differences found in leaf water potential, transpiration, and leaf resistance (Fig. 2) are probably caused by the differences in hydraulic conductivity. For a given evaporative demand, a decrease in hydraulic conductivity (as in the NM, minus P plants) would lead to a lower leaf water potential (Fig. 2A). A plant can counter this decrease in leaf water potential by increasing leaf resistance (Fig. 2D) by partial or cyclic closing of the stomates, thus allowing partial (or total) recovery of leaf water status. This increase in leaf resistance to vapor transfer would then reduce the transpiration rate (Fig. 2B).

Our results suggest that under conditions of high water and phosphorus availability mycorrhizal infection may not have major effects on plant-water relationships. This is supported by the present work as well as field data (10) that demonstrated that high soil phosphorus levels will prevent infection of onion roots by mycorrhizal fungi, without any yield reduction.

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