

Whole-plant gas exchange measurements of mycorrhizal ‘Iceberg’ roses exposed to cyclic drought

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Abstract

Nursery stock is purported to benefit from arbuscular mycorrhizal (AM) symbioses when subjected to drought, low fertility, or transplant stress. Yet these benefits have not been well defined. Whole-plant gas exchange measurements describe plant performance under environmental strain more reliably than individual leaf measurements. Understanding the whole-plant response to drought stress will yield decision-making tools for ornamental plant producers about benefits from mycorrhizal symbiosis. Container-grown *Rosa x hybrida* ‘Iceberg’ rose plants were subjected to repeated drought episodes intended to simulate missed irrigation cycles during commercial production or retail sales periods. Whole-plant gas exchange parameters of mycorrhizal, low phosphorus (ML) and non-mycorrhizal, low (NML) and high (NMH) phosphorus treated roses were compared using 14-day continuous measurements. The NMH plants, which were provided supplemental KH_2PO_4 fertilization, had larger plant canopies and initially had higher whole-plant photosynthesis (P_{net}) rates than similar-sized NML and ML plants. Gas exchange, carbon, and water use efficiencies of ‘Iceberg’ roses were not significantly improved by colonization with the AM fungus *Glomus intraradices*. All plants had similar water and carbon use efficiencies at the end of the third drought episode. Photosynthetic capacity decreased after ‘Iceberg’ roses were rewatered, following water deficit stress, regardless of mycorrhizal status. During the second drought cycle, maximum P_{net} approximated 70% of pre-drought levels and continued to decline. Improved shoot hydration, and thus aesthetic appearance during drought strain episodes, was not achieved by *G. intraradices* colonization of ‘Iceberg’ roses.

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1. Introduction

The benefits of arbuscular mycorrhizal (AM) fungi on plant performance are not well defined for ornamental production systems where plants receive regular fertilization and irrigation. Mycorrhizal associations have been reduced in many host plants when fertilizers,

particularly phosphate, are heavily applied (Abbott and Robson, 1991; Brundrett, 1991; Collins-Johnson and Pflieger, 1992; Jakobsen, 1986; Miller et al., 1995). However, a survey of fertilized nursery field soils in Tennessee found indigenous, unidentified AM fungi associated with the roots of red maple (*Acer rubrum* L.), cherry (*Prunus serrulata* L.) and flowering dogwood (*Cornus florida* L.) cultivars, regardless of soil type, pH, or fertility (Klingeman et al., 2002). An unidentified AM fungus was recovered from the roots of *C. florida* liners grown in nursery containers with a fertilizer-amended,

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composted pine bark medium (W.E.K., unpublished data). *Glomus intraradices* Schenk and Smith also has been found to extensively colonize heavily fertilized rose bushes grown in a commercial greenhouse (Augé et al., 1986a).

The consistency of host plant responses to AM symbiosis is often confounded by variability in experimental or environmental conditions as well as differing efficacy among host plant species (Augé, 2001). For instance, when experiments are not specifically designed to produce similar-sized AM and non-AM plants, endomycorrhizal symbiosis often results in growth rate increases in amply watered and droughted plants by influencing nutrient acquisition and possibly hydration (Augé, 2001). However, when compared with similar, but non-mycorrhizal plants subjected to drought stress, the influence of AM symbiosis on host plant water relations can range in extent from subtle, momentary responses to sustained, quantifiable improvements in plant performance (Augé, 2001).

Leaf photosynthesis can increase among AM colonized plants as a function of phosphorus (P) nutrition and stomatal conductance, compared with non-mycorrhizal hosts (Davies et al., 1993; Koide, 1993). However, these and other studies rely upon brief but repeated gas exchange measurements taken from individual leaves (Augé, 2001). These studies present momentary glimpses of leaf or plant gas exchange, rather than data integrated through time, and rarely incorporate root respiration (Klingeman et al., 2000). In comparison with individual-leaf measurements, whole-plant gas exchange data have demonstrated less variability following repeated measurements among insecticide-treated azaleas (Klingeman et al., 2000). Moreover, short-term leaf photosynthesis data are often complicated by a poor relationship of dry matter production to yield (Evans, 1993). That these data are not closely associated may be attributed to: (1) gas exchange measurements taken from a leaf section that is not representative of the whole (e.g. herbaceous) plant, (2) a leaf selection with gas exchange not characteristic of the plant canopy, (3) an inability to account for diurnal variation in canopy photosynthesis and respiration, and (4) alterations in CO₂ exchange that occur as leaves mature (van Iersel and Bugbee, 2000).

Maintaining irrigated, healthy nursery stock is a routine challenge for retail marketplaces that are faced with unreliable and unskilled labor. Retail space is also limited. Parking lots that are converted into seasonal plant showcases may not include ready access to water. Moreover, consumer's shopping during the hottest part of the day can interrupt the timing of adequate irrigation. AM fungi have been shown to limit plant stress during water deficits (Nelson, 1987). As a result, emphasis on mitigation of drought injury has been a convincing sales tool for marketers recommending

inoculation of ornamental plants with mycorrhizal fungi. In fact, commercial mycorrhizal products are being widely promoted for many nursery and retail applications.

Our research objectives were undertaken with the advantage of continuous, whole-plant gas exchange measurements to investigate the influence of AM symbiosis on roses subjected to missed irrigation cycles that commonly occur during commercial production and retail sales periods. These objectives address two specific questions: can colonization by *G. intraradices* increase the ability of container-grown *Rosa x hybrida* 'Iceberg' roses to manage net carbon exchange rates (CER) more optimally than non-mycorrhizal roses when exposed to episodes drought strain? Do roses colonized by *G. intraradices* recover more readily from episodes of drought?

2. Materials and methods

2.1. Plant material establishment

In September 2000, rooted cuttings were taken from 3 year-old container-grown *Rosa x hybrida* 'Iceberg' stock plants. New plants were transplanted into 16.5 cm × 15.2 cm azalea pots (Dillen Products, Middlefield, OH) in a 4:1 (v/v) mixture of calcined montmorillonite clay (=Turface) (Turface Industrial Materials Corp., Deerfield, IL) and pasteurized silica sand. Plants were arranged in randomized complete blocks on greenhouse benches and maintained for 8 weeks under a 16-h photoperiod, which was achieved by supplementing natural light with sodium vapor lamps suspended approximately 60 cm above the rose canopy. Measured at noon on a clear day, the photosynthetic photon flux density at plant canopy height averaged $745 \pm 222 \mu\text{mol m}^{-2} \text{s}^{-1}$ across the greenhouse bench. The greenhouse was maintained at 80% RH with daytime temperatures of $25 \pm 3^\circ\text{C}$ and the nighttime temperature of $21 \pm 3^\circ\text{C}$.

2.2. AM fungal isolate, 'Iceberg' rose inoculation and fertility treatments

G. intraradices Schenk and Smith isolate UT143 (INVAM, Morgantown, WV), which was cultured on *Sorghum bicolor* 'DeKalb DK 40Y' grown in Turface: sand was used as the experimental AM treatment. Roses were inoculated during transplanting by removing sorghum shoots at the crown, cutting colonized sorghum roots into pieces less than 5 cm long, and thoroughly mixing the cut roots and growth medium with the Turface: sand mixture at a ratio of 4 parts sterile medium to 1 part colonized medium. Thus, the

inoculum was comprised of extraradicular and radicular hyphae and spores of *G. intraradices* UT143.

Inoculated roses were provided low phosphorus levels (ML), and non-inoculated roses received either low phosphorus levels (NML) or high phosphorus levels (NMH). Each treatment included 12 plants in paired experimental units. Fertility treatments were established in the greenhouse during an 8-week period while watering roses in 3-day long fertigation cycles. Within a cycle, all plants received a 13N-0.9P-10.8K fertilizer (Champion 13-2-13 Plug Plus soluble fertilizer, Chilean Nitrate Corp., Norfolk, VA.), which provided 21.42 mM N, 0.66 mM P, and 6.38 mM K during the first two fertigations. On the third irrigation, NMH plants received 0.66 mM P and 0.66 mM K from the supplementation of technical grade KH_2PO_4 (Fisher Scientific, Springfield, NJ), while NML and ML plants received only water. Low and high phosphorus levels were included for both non-mycorrhizal treatments to assure that suitable non-mycorrhizal control plants were produced that were similar in size to AM-colonized plants. None of the roses received any additional fertilization once gas exchange measurements were initiated, which was 9 weeks after roses were inoculated.

2.3. Growth chamber design

Responses of inoculated and non-inoculated roses to three drought cycles were investigated using a whole-plant gas exchange system, which has been previously described (van Iersel and Bugbee, 2000). The open-flow gas exchange system consisted of ten of 5 mm thick acrylic chambers that were 0.61 m high, \times 0.36 m wide and \times 0.47 m long. Groups of four individual chambers were maintained within two separate Conviron E-15 growth chambers (Conviron, Winnipeg, CAN). Two additional empty acrylic chambers were held outside the controlled-climate units. Empty chambers corrected for variations in ambient CO_2 levels while buffering the temperature of the incoming airflow. The system allowed continuous measurements of CER and incorporated both above- and below-ground gas exchanges of experimental units. Net photosynthesis during the photoperiod (P_{net}), dark period respiration (R_{dark}), growth or daily carbon gain (DCG), and carbon use efficiency (CUE, the net amount of carbon incorporated into plant material divided by the total amount of carbon fixed in gross photosynthesis) were calculated (Klingeman et al., 2000; van Iersel and Bugbee, 2000). Within the system, CO_2 levels were maintained at approximately $375 \pm 11 \mu\text{mol mol}^{-1}$. A 16-h photoperiod was maintained at $480 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux, which was measured at plant canopy height. Temperatures in the chambers were held at $25 \pm 1^\circ\text{C}$ ($77 \pm 2^\circ\text{C}$) and $85 \pm 5\%$ relative humidity.

2.4. Whole-plant gas exchange measurements

Gas exchange was recorded continuously for 14 days. The CER data were collected as measurements cycled between chambers housing two experimental blocks. Within each block, the experimental unit was two similar-sized rose plants from ML, NML, and NMH treatments. Measurements were taken within each chamber on 7-min intervals. Since sufficient chamber numbers were available to continuously measure only two randomly chosen experimental blocks at a time, the experiment was repeated three times to yield six total replicates.

2.5. Rose exposure to cyclic irrigation “failure”

Once gas exchange measurements were initiated, roses were subjected to three drought strain cycles intended to simulate missed irrigation episodes. Preliminary observations on an extra block of similarly treated roses revealed no apparent difference in extent or rate of wilting among treatments. Thus, during each drought episode, water was withheld from roses until foliage of 75% of plants in paired experimental blocks became flaccid and wilted. In the first two drought episodes, water was withheld for 4 days. At the conclusion of these drought episodes (at the end of the fourth and eighth photoperiod), pots and their contents were weighed before all plants were rewatered. After rewatering, pots were allowed to gravity drain to field capacity, and reweighed. Within each drought cycle, the difference in mass between unwatered (drought strained) pots and watered pots was used to calculate water use for each treatment. On day 8, rose foliage was pruned to similar size among treatments to prolong the drying time. The third and final drying cycle lasted 6 days. Water use efficiency (WUE) during each drying cycle was calculated as the net amount of carbon incorporated into the plants divided by their water use during that period.

2.6. Destructive plant sampling

At the conclusion of the study, leaf area, and leaf, stem, and root dry weight data were collected from each experimental unit. Dry weight and area of the leaves pruned following the second drying cycle were measured, and leaf area during the first two drying cycles was estimated as the final leaf area plus the pruned leaf area. Leaf tissue of recently matured, fully expanded leaves was dried and 30 g from each experimental unit was submitted to quantify macro- and micronutrient content. Total N was determined using the Dumas method and S by infrared spectrometry (Mills and Jones, 1996) using a CNS 2000 analyzer (LECO Corp., St. Joseph, Mich.), while phosphorus (P) and potassium (K) and micronutrients were determined after dry ashing using inductively coupled

plasma spectrophotometry (Jarrell-Ash ICAP 9000, Thermo Jarrell Ash Corp., Franklin, MA). At the conclusion of the study, 15-week post-inoculation, young, thin roots were cut from four sections collected equidistantly around the container circumference. Fine root samples were pooled within pot, washed and cut into 1 cm sections. Root pieces were cleared with 10% KOH in an autoclave at 121 °C for 15 min, stained with trypan blue for 1 h, then stored in lactophenol destaining solution (Augé et al., 2001). Root sections were mounted in glycerine on 2 to 3 slide subsamples, which were treated as a single experimental unit. Root colonization (%) by AM fungi was quantified using counts of hyphae, vesicles, and arbuscules tallied among 100 microscopic fields-of-view per experimental unit (McGonigle et al., 1990).

2.7. Data analysis

Repeated gas exchange measurements, which yielded P_{net} and R_{dark} data, were averaged within each light and dark period. Data were calculated both on a whole plant basis and per unit leaf area. Daily gas exchange and CUE data were analyzed statistically by analysis of variance (SAS Institute, 1988). WUE values were analyzed within each drought strain period. Finally, at the conclusion of the study, calculated mean average plant parameters, including root and shoot dry weights, leaf area, leaf nutrient content, and water use parameters, were analyzed using analysis of variance. Treatment means were separated using Fisher's protected least significant difference ($P \leq 0.05$).

3. Results

In response to supplemental fertilizations, NMH roses yielded greater shoot dry weight ($P < 0.01$) and leaf area ($P < 0.01$) than ML and NML plants, but similar root dry weights ($P > 0.08$) (Table 1). In contrast, ML roses and NML controls were similar sized ($P < 0.48$ to 0.07) (Table 1). Because of their similar sizes, ML and NML plants

were compared to determine AM influences on rose growth, gas exchange, and water use parameters. Microscopic counts of fungal structures among fine root tissues revealed hyphae, vesicles, and arbuscules among ML roses. NML and NMH roots contained small numbers of unidentifiable hyphae. Arbuscules were not observed in NML and NMH roses (Table 1).

At the conclusion of this study, foliar concentrations of copper, zinc, manganese, sulfur, aluminum, boron, iron, and sodium did not differ among treatments ($P < 0.72$ to 0.08) (Table 2). Significantly higher concentrations of molybdenum ($P = 0.02$) were found in ML roses than in NMH plants. NMH roses had significantly higher P ($P < 0.0001$), K ($P = 0.0003$), and Mg ($P < 0.0001$) concentrations than either ML or NML roses. Greater concentrations of calcium ($P < 0.0001$) were also recovered from ML and NMH leaf tissues than from NML roses (Table 2).

Expressed on a whole-plant basis, the CER of well-watered 'Iceberg' roses was greater in the larger NMH roses than in NML plants during both daytime (P_{net}) ($P = 0.02$) (Fig. 1A) and nighttime (R_{dark}) ($P = 0.04$) (Fig. 2A) measurements at the beginning of this study. The initial high whole-plant P_{net} of NMH roses was related to their relatively large leaf area, since there were no differences in the leaf P_{net} among treatments on day 1 (Fig. 1B). P_{net} was mostly similar among treatments throughout the experiment, during both dry-down and recovery. Once drought was initiated, P_{net} rates declined among all rose treatments and increased subsequent to each irrigation (indicated by arrows; Figs. 1 and 2), although not to pre-strain levels.

The soilless media dried quickly during the first and second cycles simulating missed irrigation. Once the second watering cycle had been completed, plants in all treatments were pruned to reduce the leaf areas of the roses. A longer soilless media drying time extended observations of whole-plant gas exchange and was expected to better demonstrate plant response.

Following pruning, whole-plant R_{dark} of ML roses was significantly higher than that of NML and NMH

Table 1

Mean growth parameters and mycorrhizal colonization, quantified among 'Iceberg' rose experimental treatments, at the conclusion of 14-day, continuous gas exchange observations

Treatment	Shoot	Root	Canopy	Hyphae ^a	Vesicles	Arbuscules
	Dry weight (g)	Dry weight (g)	Leaf area(cm ²)	(%)		
Mycorrhizal, low P (ML)	2.8	3.9	515	30	22	4
Non-mycorrhizal, low P (NML)	2.5	3.9	490	2	0	0
Non-mycorrhizal, high P (NMH)	4.0	3.2	647	1	0	0
LSD _{0.05}	0.8	0.7	96	4	11	2
Significance ^b	**	NS	**	***	**	***

Dry weight and leaf area values are representative of unpruned roses, as measured during the first two drought cycles.

^aVAM fungal structures are reported as the percent of affirmative observations (\pm SE) tallied among 100 microscopic fields-of-view.

^bNon-significant (NS) or significantly different at $P < 0.05$ (*), $P < 0.01$ (**), or $P < 0.001$ (***)

Table 2

Major and minor elemental composition of leaf tissue samples analyzed among 'Iceberg' rose experimental treatments at the conclusion of 14-day, whole-plant gas exchange observations

Treatment	Major elements (mg g ⁻¹)							
	C	N	P	K	Ca	Mg	S	
Mycorrhizal, low P (ML)	434.0	17.4	1.20	18.7	17.2	2.2	1.3	
Non-mycorrhizal, low P (NML)	437.9	17.8	1.15	17.8	15.7	2.1	1.1	
Non-mycorrhizal, high P (NMH)	422.6	17.6	1.72	21.0	17.5	2.8	1.1	
LSD _{0.05}	12.1	1.4	0.16	1.2	0.6	0.1	0.3	
Significance ^a	*	NS	***	***	***	***	NS	
	Minor elements (µg g ⁻¹)							
	Al	B	Cu	Fe	Mn	Mo	Na	Zn
Mycorrhizal, low P (ML)	35.8	105.1	5.6	52.1	38.8	16.0	182.9	11.3
Non-mycorrhizal, low P (NML)	30.6	102.3	5.1	48.8	29.9	15.7	203.1	10.3
Non-mycorrhizal, high P (NMH)	51.9	98.9	5.6	88.1	28.6	14.5	244.6	10.4
LSD _{0.05}	36.5	8.8	0.8	43.2	11.0	1.0	60.4	2.5
Significance ^a	NS	NS	NS	NS	NS	*	NS	NS

^aElemental means, within columns, are non-significant (NS) or significantly different at $P \leq 0.05$ (*), 0.01 (**), 0.001 (***).

roses ($P = 0.002$ to 0.02) (Fig. 2A). The low whole-plant CER during the third drought cycle was primarily due to the pruning of the plants. Since plants were pruned after the second drought cycle, comparisons of whole-plant photosynthesis from the first two drying cycles with those taken during the third cycle are not meaningful (Fig. 1A). When the P_{net} data are expressed per unit leaf area, the highest P_{net} during the second and third drought cycle were only about 70% of pre-stress rates (Fig. 1B).

Trends for whole plant CER among treatments were comparable for both P_{net} and R_{dark} throughout the study (Fig. 1A and 2A). Whole plant R_{dark} was higher in NMH roses than in ML and NML treatments, both initially ($P < 0.04$) and during the fourth dark period ($P < 0.007$) (Fig. 2A). These differences were attributed to the larger shoots of NMH roses (Table 1). When calculated per unit leaf area, R_{dark} was higher among ML roses than among NMH roses during the second ($P < 0.001$), fifth ($P < 0.02$), and seventh ($P < 0.01$) dark periods, while nighttime respiration of NMH roses was higher than that of NML and ML roses during the fourth ($P < 0.03$) and eighth ($P < 0.03$) dark periods (Fig. 2B).

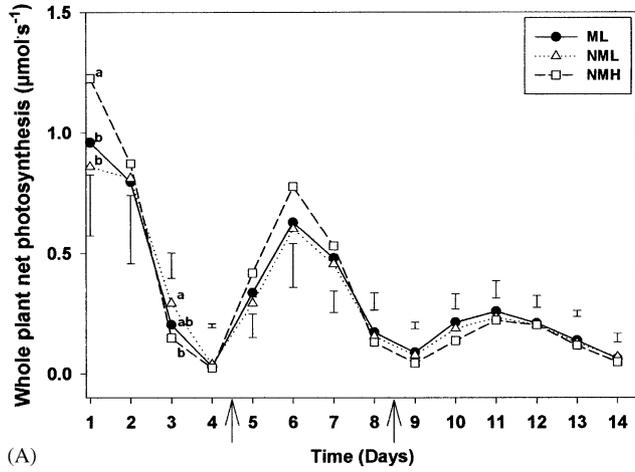
Analysis of dry weight data taken at the conclusion of the study indicated that, although the leaf area remaining on pruned roses and total rose dry weights were statistically similar among treatments ($P = 0.10$ and 0.08 , respectively), ML roses had a slightly larger leaf area than NMH and NML roses. It is likely that treatment differences in R_{dark} during the third drying cycle are attributable to disparities in remaining canopy leaf area of ML roses, rather than the influence of *G. intraradices*. This is consistent with our finding that

R_{dark} rates per unit leaf area did not differ among treatments during the third drying cycle ($P = 0.41$ to 0.72) (Fig. 2B).

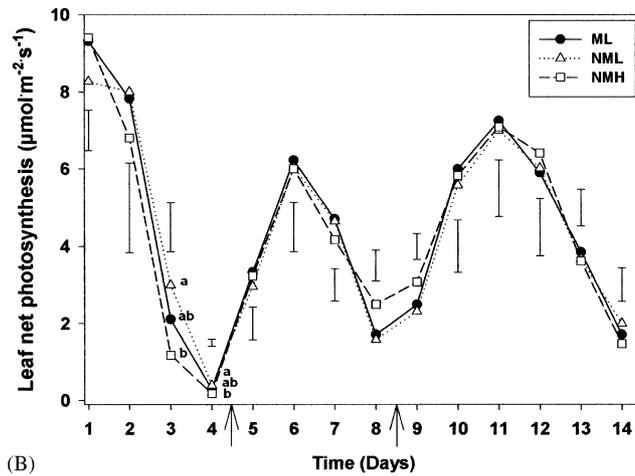
Water use efficiency (WUE) is the ratio of carbon fixed into dry weight to the total amount of water lost by evapotranspiration. NMH roses had higher WUE than either ML or NML roses ($P = 0.03$) only during the initial drought strain period (Fig. 3). The higher WUE efficiency of the NMH plants during the first drying period may have been an indirect effect of their larger leaf area, as compared to the other two treatments (Table 1).

Since WUE was calculated as the ratio between the net amount of carbon fixed and the total amount of water lost from the pots and plants, these values depend on both transpiration and evaporation from the growth medium. Since a plant cannot use water lost by evaporation for growth or transpiration, a high ratio of transpiration to evaporation is expected to result in higher WUE. Plants with a large leaf area would be expected to transpire more than plants with a smaller leaf area and would have a higher ratio of transpiration to evaporation. As a result, plants with a larger leaf area would be expected to have a higher WUE.

Carbon use efficiency (CUE) is a performance measure that quantifies the ability of a plant to convert photosynthate into dry matter. CUE is expressed in mol carbon fixed into tissue dry matter per mol carbon fixed in gross photosynthesis (van Iersel and Bugbee, 2000). CUE demonstrated trends similar to daytime CER measurements (Fig. 4) and differed among treatments on day 1 ($P = 0.03$) and day 3 ($P = 0.02$). CUE did not differ among treatments, in response to repeated drying, following day 3 ($P = 0.08$ to 0.97). The low P_{net}



(A)

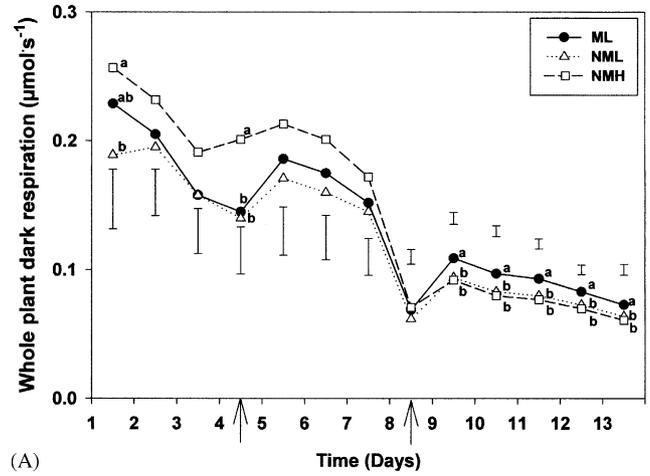


(B)

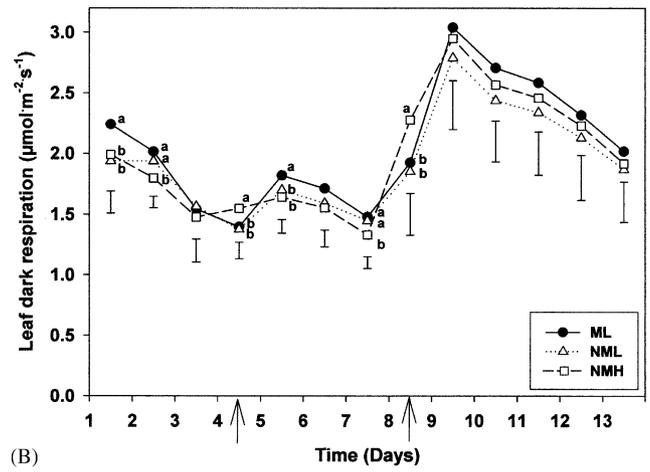
Fig. 1. (A) Whole-plant net photosynthesis (P_{net}) of ‘Iceberg’ roses subjected to the following treatments: ML, NML, and NMH. (B) P_{net} of ‘Iceberg’ roses per unit leaf area. Values represent the mean P_{net} of two-plant experimental units. Arrows indicate irrigation events. Error bars represent the least-significant differences among means separated using Fisher’s LSD test ($\alpha = 0.05$). Means within day having the same letter are not significantly different from each other.

(Fig. 1A) in NMH roses can explain the low CUE of these plants on day 3. On day 4, CUE in all treatments was negative, indicating that plants respired more carbohydrates than those fixed in gross photosynthesis. Under the conditions of these drought episodes, these plants therefore were losing dry weight on day 4.

During the repeated gas exchange measurements of ‘Iceberg’ roses, oscillations in P_{net} were occasionally observed during the photoperiod (Fig. 5). These fluctuations in carbon uptake occurred in approximately 40-min, peak-to-peak cycles. Oscillations were not consistent among chambers at any given time and were short-lived. Oscillations did not occur frequently and were only observed during the second day of the initial drought cycle. P_{net} decreased gradually during the oscillations, suggesting that stomatal opening and closing may have responded to increasingly severe drought stress.



(A)



(B)

Fig. 2. (A) Nighttime respiration (R_{dark}) of ‘Iceberg’ roses subjected to the following treatments: ML, NML, and NMH. (B) R_{dark} of ‘Iceberg’ roses per unit leaf area. Values represent a mean R_{dark} of two-plant experimental units. Arrows indicate irrigation events. Error bars represent the least-significant differences among means separated using Fisher’s LSD test ($\alpha = 0.05$). Means within day having the same letter are not significantly different from each other.

4. Discussion

In field and laboratory experiments, AM-colonized ornamental and crop plants, including roses, have demonstrated more drought tolerance than non-mycorrhizal plants (Augé et al., 1986b, 1992, 1995; Duan et al., 1996). During experimentally induced droughts, in which stomatal conductance and transpiration were quantified, *G. intraradices* has demonstrated an ability to influence water relations in cultivars of hybrid roses (*Rosa x hybrida* L.) grown in a calcined montmorillonite clay medium (Augé et al., 1986b, 1987). Similarly, droughted ‘Samantha’ hybrid roses that were colonized by *G. deserticola* and an unidentified strain of *G. intraradices* had lower leaf water potential at stomatal closure than similar sized, non-mycorrhizal roses (Augé et al., 1986b). Delays in stomatal closure were also

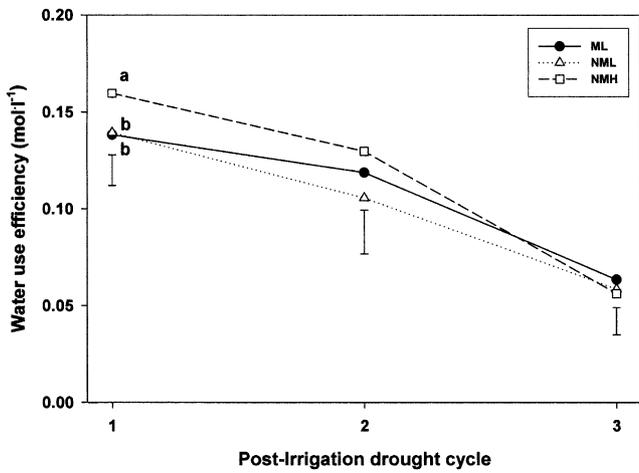


Fig. 3. Water use efficiency of roses during three consecutive drying cycles. The treatments are ML, NML, and NMH. Cycle 1 indicates ‘Iceberg’ rose WUE from days 1 to 4, cycle 2 from days 4 to 8, and cycle 3 from days 8 to 14. Error bars represent the least-significant differences among means separated using Fisher’s LSD test ($\alpha = 0.05$). Means within day having the same letter are not significantly different from each other.

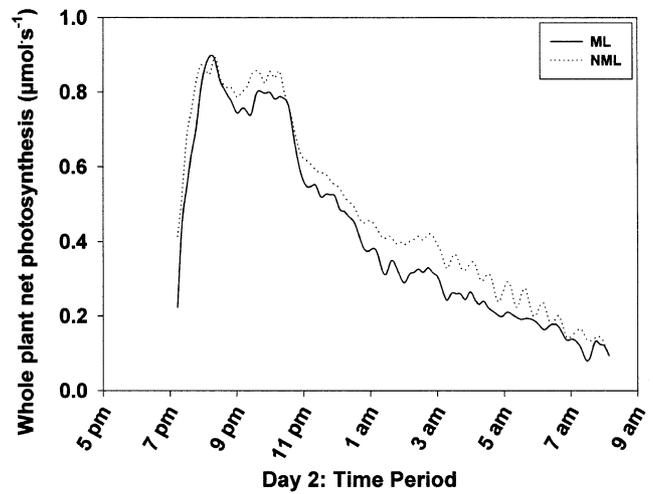


Fig. 5. Cyclic oscillations in net photosynthesis in ‘Iceberg’ roses were occasionally noted during continuous gas exchange measurements. Data are shown for roses in mycorrhizal, low phosphorus (ML = solid line) and non-mycorrhizal, low phosphorus (NML = dotted line) treatments. Oscillations occurred in approximately 40-min cycles, peak-to-peak and were observed in chambers with a treatment-pair of plants that comprised a single experimental unit.

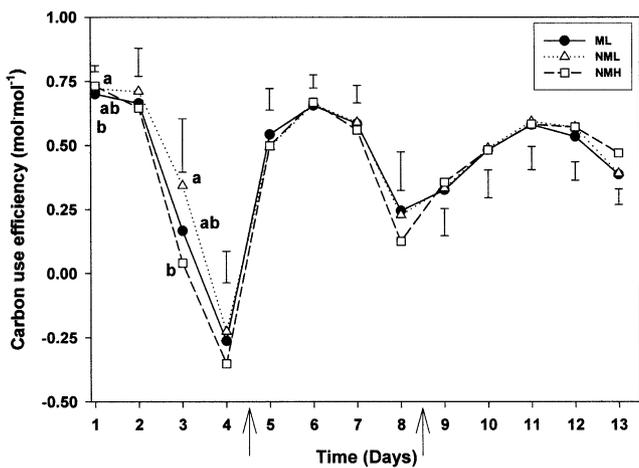


Fig. 4. Carbon use efficiency of roses in the following treatments: ML, NML, and NMH. Arrows indicate irrigation events. Error bars represent the least-significant differences among means separated using Fisher’s LSD test ($\alpha = 0.05$). Means within day having the same letter are not significantly different from each other.

observed among *G. intraradices* colonized cowpeas (*Vigna uncinula* (L.) Walp.) in drying soils (Duan et al., 1996). The results of these studies were based on infrequent measurements on single leaves or groups of leaves.

Yet, based on whole-plant measurements, colonization of ‘Iceberg’ roses by *G. intraradices* isolate UT-143 neither enhanced net carbon exchange rates nor aided post-strain recovery of roses, compared with non-mycorrhizal roses, following exposures to episodic environmental stress characteristic of missed irrigation

cycles. At the conclusion of the 14-day study, all treatments had similar WUE and CUE values. Leaf P_{net} levels at peak performance during the second and third drought cycles were approximately 70% of pre-drought capacity. This indicates limited potential for photosynthetic recovery among ‘Iceberg’ roses exposed to stress from periodic water deficits, regardless of mycorrhizal status, and reinforces the necessity of adequate irrigation when aesthetics motivate consumer purchase decisions. Contrary to expectations, which were based on previous rose research, it is probable that the *G. intraradices* isolate selected for this study presented a neutral, rather than a mutual effect, in its symbiosis with ‘Iceberg’ rose. Still, additional research is needed to determine if *G. intraradices* UT-143 can confer benefits to roses challenged by periodic water stress provided time, in addition to the 15 weeks of root colonization chosen for this study, or colonization of rose roots to a greater extent than was experimentally observed.

Host plants often differ in response to AM symbiosis when compared among different AM genera, species, and even species isolates (Augé, 2001; Fidelibus et al., 2001; Newman and Davies, 1987). Within the genus *Glomus*, species influences on host plant gas exchange are imperfectly understood. *G. fasciculatum* and *G. deserticola* have usually resulted in increased stomatal opening among colonized plants, and *G. intraradices*, *G. etunicatum*, and *G. mosseae* usually increased both stomatal conductance and transpiration. The extent to which colonized host plants have responded has differed among plant species, however (Augé, 2001). Further,

some Glomalean fungi, including *G. intraradices*, may have the ability to alter soil moisture retention properties. This is accomplished when mycorrhization alters the production of the soil-binding glycoprotein glomalin or water stable aggregates that affect soil structure and increase matric potential (Augé et al., 2001; Wright and Upadhyaya, 1998). These abilities have not been quantified among the various isolates.

That neither leaf nor soil water potential were measured in this study presents a drawback to comparisons with studies that characterize physiological drought. Yet the investigation of temporal drought, as evident from the overall aesthetic deterioration of 'Iceberg' rose and their performance observed during each dry-down cycle, is directly applicable to production and retail scenarios. The plants responded similarly and foliage wilted, becoming flaccid regardless of mycorrhizal status, before rewatering.

Early attribution of the influence of AM symbiosis on water relations to enhanced P nutrition has been supported by numerous studies (Safir et al., 1971; Augé, 2001). Although soil P becomes less available to plants as soils dry (Viets, 1972), enhanced plant growth during drought has been related in several studies to increased P nutrition among AM-colonized hosts (Augé, 2001). In this study, *G. intraradices* was expected to enhance P nutrition, yielding greater plant growth. This potential bias was limited by establishing experimental ML and NML treatments that had similar sizes and nutrient contents without additional KH_2PO_4 . Non-mycorrhizal plants with supplemental KH_2PO_4 (NMH treatment) did yield larger plants. While increased P and K levels among NMH roses are attributed to the supplemental KH_2PO_4 fertilization, a physiological rationale for higher molybdenum and magnesium levels that were observed in ML and NMH treatments, respectively, cannot be readily ascribed. A mycorrhizal role for increased calcium levels, which was higher for ML and NMH treatments than NML roses, is not supported.

Cyclic oscillations in stomatal aperture, transpiration, and leaf water potential have been reported for several plant species (Barrs, 1971), including roses (Rose and Rose, 1994; Rose et al., 1994) and sunflowers (Laisk et al., 1991). These oscillations can be induced by water potential imbalances both between guard and subsidiary cells and between plant canopies and root systems (Rose et al., 1994). Oscillatory transpiration is unpredictable and transitory (Barrs, 1971; Rose and Rose, 1994). Several plants adjacent to oscillating 'Fire 'N Ice' roses, which were maintained under identical conditions, failed to exhibit oscillations. When oscillations did occur, they were synchronous among stomata on an individual plant (Rose and Rose, 1994). Observations of cyclic oscillation in 'Iceberg' rose, although unpredictable, support the value of repeated or continuous measure-

ments for reporting leaf gas exchange (Rose and Rose, 1994).

There is no agreement in the literature about the importance of extraradical hyphae for direct water uptake (see Augé, 2001 for discussion). This study did not include an assessment of extraradical hyphae. Therefore, we cannot speculate on their involvement in nutrient and water uptake. Roots of AM plants were extensively colonized by hyphae, which is typically associated with colonization of soil. Regardless of extent of intraradical and extraradical AM colonization of roses and soil, AM symbiosis did not affect gas exchange under the conditions imposed by this study.

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