Moisture Stress Conditioning Effects on *Salvia splendens* 'Bonfire'

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Abstract. We determined the influence of moisture stress conditioning (MSC) (exposing plants to four nonlethal dry-down cycles) on gas exchange and water loss of *Salvia splendens* F. Sellow 'Bonfire'. During day 1 following final irrigation, no differences in leaf water potentials (ψ_L) were observed due to MSC. However, MSC plants had lower midday net photosynthesis (Pn), transpiration (E), and leaf conductance (g_L) than controls. Stomatal inhibition of photosynthesis (SI) of MSC plants was greater than that of controls. Further, the lack of differences in mesophyll resistance to CO_2 (r_m) due to MSC indicate gas exchange differences during day 1 were stomatal in nature. During day 2, MSC plants exhibited greater Pn, E, and g_L , while SI and r_m were greater for controls. MSC plants maintained positive Pn rates and turgor and lower ψ_L than control plants during day 2. Higher water-use efficiency estimates were observed for MSC plants than for controls.

Materials and Methods

Bedding plant growers are becoming more aware of the need for improved postproduction performance (Armitage, 1986; Hammer, 1988). Current recommendations for improving postproduction performance include reducing irrigation frequency, fertilization, and greenhouse temperatures during the final few weeks of production (Armitage, 1987; Seeley, 1985). Such hardening-off steps prepare plants for suboptimal conditions often encountered during shipping, in the retail marketplace, and/or during landscape establishment.

Moisture stress is one of the major factors in quality loss following wholesale production (Nelson and Carlson, 1987). Armitage and Kowalski (1983) have demonstrated the positive influence of low-frequency irrigation regimes on postproduction plant quality of *Petunia × hybrida* seedlings. However, plants were not exposed to moisture stress following production.

MSC, the controlled exposure of plants to moisture deficits during production, has been shown to improve physiological processes directly related to moisture stress tolerance of several agronomic (Ackerson and Hebert, 1981; Brown et al., 1976; Gupta and Berkowitz, 1987; Matthews and Boyer, 1984) and woody (Osonubi and Davies, 1981; Seiler, 1985; Seiler and Johnson, 1988) plant species. These physiological acclimations following MSC include the maintenance of greater stomatal conductance and photosynthesis when compared to controls during potentially detrimental internal moisture levels. Armitage et al. (1983) reported that photosynthesis in hybrid geranium declined at a slower rate as soil moisture levels dropped if the plants were previously exposed to a single moisture stress cycle. Despite these reports, more information is needed about the influence of MSC on essential physiological processes required to facilitate the maintenance of postproduction bedding plant quality. Therefore, the objective of this study was to determine the influence of MSC on gas exchange and water loss of salvia.

Seed of 'Bonfire' salvia were sown on 11 Jan. 1988 and transplanted 14 days later into 500-ml plastic pots containing a 1 peat : 1 perlite (v/v) medium amended with 1 kg Micromax (Sierra Chemical Co., Milpitas, Calif.) and 3 kg dolomitic limestone/m³. Plants were grown in a glasshouse under long-day conditions [night interruption from 2200 to 0200 HR using incandescent lights providing $\approx 4 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ photosynthetic photon flux (PPF) at the top of the plant canopies to extend vegetative growth.

Plants were fertilized with 200 mg N/liter as NH_4NO_3 , 25 mg P/liter as phosphoric acid, and 150 mg K/liter as KCl in the irrigation water. Forty-nine days after seeding, half of the plants were subjected to MSC by exposing them to four nonlethal dry-down cycles and by allowing them to reach visible wilt ($\psi_L = -1.1$ to -1.4 MPa) before dehydration. Plant water status was monitored during dry-down cycles by determining ψ_L on a single leaf for three randomly selected MSC plants twice daily using a pressure chamber (Scholander et al., 1965). Control plants were maintained well-watered by applying small amounts of tap water as needed to prevent leaching. Post-dry-down rehydration was accomplished by submersing MSC and control plant containers in the irrigation nutrient solution for ≈ 20 min.

On day 69 after seeding (21 Mar.), plants were irrigated at 0545 HR and placed under supplemental lighting provided with 1000-W high-pressure sodium lamps for gas exchange measurements. The experimental design was a randomized complete block with 10 blocks; one plant per treatment per block (20 plants total). Gas exchange was measured hourly for each plant on the same recently matured leaf during 0900 to 1600 HR on 21 Mar. and 0900 to 1200 HR on 22 Mar. 1988 (days 69 and 70 after seeding, respectively). Pn, E, g_L , gas analyzer leaf chamber CO_2 concentration (CA), and leaf internal CO_2 concentration (CI) were obtained with a LI-COR (Lincoln, Neb.) 6200 photosynthesis system. Levels of PPF were maintained between 600 and 720 $\mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$. Mean gas analyzer leaf chamber relative humidity, temperature, and CO_2 concentra-

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Abbreviations: CA, leaf chamber CO_2 concentration; CI, leaf internal CO_2 concentration; E, transpiration; g_L , leaf conductance; MSC, moisture stress conditioning; Pn, net photosynthesis; r_m , mesophyll resistance to CO_2 ; SI, stomatal inhibition of photosynthesis; WUE, water-use efficiency; ψ_L , leaf water potential.

tions during measurements were $36\% \pm 6\%$, $26 \pm 3\%$, and $361 \pm 26 \text{ mg}\cdot\text{liter}^{-1}$, respectively. Water-use efficiency (WUE) was calculated by dividing simultaneous Pn measurements by E rates. r_m and SI were determined as follows: $r_m = \text{CI}/\text{Pn}$ (Osonubi and Davies, 1980), and $\text{SI} = [(\text{CA} - \text{CI})/\text{CA}] \times 100$ (Jones, 1985).

To prevent significant losses in total leaf area during ψ_L measurements, three blocks were randomly selected for readings at 0630, 1200, and 1630 HR on both days. Water potential was measured on single leaves using a pressure chamber (Scholander et al., 1965). Total leaf area and total shoot dry weight were determined at the end of the experiment. Data were subjected to analysis of variance. Gas exchange and water status data were analyzed within each observation time.

Results

E (Fig. 1A) and g_L (Fig. 1B) during day 1 following final irrigation were lower for MSC than for control plants, except at 1600 HR. Initial E rates for MSC plants on day 2 were similar to those for the end of day 1, but initial E for control plants on day 2 were 54% lower than the previous day. Leaf conductance for MSC plants during day 2 was also higher than for controls and declined over time for both treatments.

Midday Pn was lower for MSC plants than controls during day 1 (Fig. 1C) but, as with E, was higher than for the control throughout day 2. All MSC plants maintained positive Pn rates throughout day 2, with a final mean rate of $2.5 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ at 1630 HR (data not shown). SI was greater for MSC plants than for controls throughout day 1, but during day 2, it had decreased for control plants and was much lower than for MSC plants (Table 1). There were no differences in r_m due to MSC on day 1, but during day 2, r_m for control plants had increased dramatically compared to the previous day and was much higher than for MSC plants. WUE was greater for MSC plants than for controls throughout most of day 1 and was dramatically different throughout day 2 (Fig. 1D).

MSC had no effect on ψ_L during day 1 (Table 2). However, ψ_L for control plants was lower than for MSC plants during the first observation of day 2 (0630 HR). Although this was the extent of ψ_L effects, control plants were visibly wilted at 1200 HR, with a mean ψ_L of -1.28 MPa ; MSC plants showed no visible turgor loss at $\psi_L = -0.98 \text{ MPa}$. MSC plants were still turgid at the end of the experiment (1630 HR), with a mean ψ_L of -1.30 MPa .

MSC reduced shoot dry weight and total leaf area by 42% and 25%, respectively, compared to controls (Table 3).

Fig. 1. Transpiration (A), leaf conductance (B), net photosynthesis (C), and water-use efficiency (D) of *Salvia splendens* 'Bonfire' during the onset of moisture stress as influenced by MSC. Differences between treatment means within an observation time not significant (NS) or significant at $P = 0.05$ (*) or 0.01 (**) by F test.

Table 1. Mesophyll resistance to CO₂ and stomatal inhibition of Pn of *Salvia splendens* 'Bonfire' during the onset of moisture stress as influenced by MSC.

Treatment	Day 1 (HR)								Day 2 (HR)			
	0900	1000	1100	1200	1300	1400	1500	1600	0900	1000	1100	1200
<i>Stomatal inhibition of Pn (%)</i>												
MSC	27	30	36	34	27	25	24	21	21	25	25	25
Control	20	26	30	28	23	22	21	20	5	8	7	7
Significance	**	**	**	**	**	**	**	*	**	**	**	**
<i>Mesophyll resistance to CO₂ (s·cm⁻¹)</i>												
MSC	7.1	7.2	7.6	6.1	7.6	6.7	7.9	11.1	9.7	9.7	12.2	14.6
Control	7.5	7.1	6.6	5.9	6.9	6.6	7.9	10.7	162.9	94.5	118.6	120.4
Significance	NS	NS	NS	NS	NS	NS	NS	NS	**	**	**	**

NS,*,**Not significant and significant at $P = 0.01$ and 0.05 , respectively, by F test.

Table 2. Leaf water potential of *Salvia splendens* 'Bonfire' during the onset of moisture stress as influenced by MSC.

Treatment	Leaf water potential (–MPa) ^z					
	Day 1 (HR)			Day 2 (HR)		
	0630	1200	1630	0630	1200	1630
MSC	0.14	1.06	0.62	0.22	0.98	1.30
Control	0.22	0.77	0.59	0.64	1.28 ^y	---
Significance	NS	NS	NS	*	NS	---

^zMeans at each time are for three randomly selected plants per treatment.

^yAll control plants showing visible wilt.

NS,*,**Not significant and significant at $P = 0.05$, respectively, by F test.

Table 3. Shoot dry weight and total leaf area of *Salvia splendens* 'Bonfire' as influenced by MSC.

Treatment	Shoot dry wt (g)	Total leaf area (cm ²)
MSC	4.7	941
Control	8.1	1267
Significance	**	**

**Significant at $P = 0.01$ by F test.

Discussion

MSC improved tolerance of salvia to moisture stress through reductions in water loss, as indicated by lower E during day 1 compared to control plants. The reductions in E appear to be due to lower g_L for MSC plants compared to controls. Experiments with two tree species have also shown similar responses in E and g_L (Seiler, 1985; Seiler and Johnson, 1988). Low g_L values, as seen in this experiment, have been well correlated with reduced stomatal apertures (Burrows and Milthorpe, 1976), which could account for reduced water loss by MSC plants.

Reductions in stomatal aperture during day 1 could also explain reductions in midday Pn for MSC plants compared to controls. SI rather than non-SI for MSC plants is substantiated by increased SI compared to controls, with no differences in r_m due to MSC. Although midday Pn for MSC plants was lower than that for controls, WUE was higher in MSC plants than in controls, indicating that E was reduced to a greater extent than Pn following MSC. Others, working with tree species, also have reported increased WUE following MSC (Clemens and Jones, 1978; Seiler and Johnson, 1988).

Reductions in transpirational water loss due to less total leaf area and lower E (per unit leaf area) during day 1 for MSC plants delayed the onset of detrimental internal moisture levels

and allowed a higher rate of gas exchange during day 2 than in the controls. Initial E and g_L values for MSC plants on day 2 were similar to those recorded on day 1, while being drastically reduced for control plants. Lower mean ψ_L at 0630 HR on day 2 for control plants suggests that control plants were unable to fully rehydrate overnight, reducing stomatal apertures during day 2. The onset of stomatal closure for annual plants has been reported to occur at ψ_L of -0.7 to -1.6 MPa (Hsiao, 1973). The hypothesis that water was sufficiently limiting for control plants to reduce stomatal opening is supported by reductions in E and g_L during day 2. Although Pn for control plants was also lower during day 2, nonstomatal limitations to Pn appear to be greater than stomatal limitations. Mesophyll resistance to CO₂ increased more than 10 times and SI dropped by 70% to 75% during day 2 compared to day 1, indicating direct disruption of the photosynthetic mechanism rather than reductions of internal CO₂ concentrations due to stomatal closure.

Maintenance of greater Pn rates for MSC plants during day 2 was due not only to reductions in water loss during day 1, but also to the ability of plants to maintain Pn at low ψ_L . By 1630 HR on day 2, MSC plants maintained a mean Pn rate of $2.5 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ with a mean ψ_L of -1.30 MPa. By contrast, at 1200 HR on day 2, control plants had a mean ψ_L of -1.28 MPa and a mean Pn of $0.4 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$. This photosynthetic acclimation of MSC plants to low ψ_L appears to be due to both stomatal and nonstomatal changes with low r_m and greater g_L for MSC plants at similar ψ_L compared to those for control plants. Photosynthetic acclimation to low ψ_L following MSC has also been reported for several agronomic crops (Ackerson and Hebert, 1981; Ashton, 1956; Brown et al., 1976; Gupta and Berkowitz, 1987; Matthews and Boyer, 1984) and woody plant species (Seiler and Johnson, 1988). Despite the lack of agreement attributing these Pn adaptations to stomatal or nonstomatal factors, these authors agree that osmotic adjustment is involved by allowing the plant to maintain turgor during moisture deficits (Brown et al., 1976; Gupta and Berkowitz, 1987; Matthews and Boyer, 1984).

Plant growth was reduced by MSC, a common (Osonubi and Davies, 1978; Seiler, 1985; Seiler and Johnson, 1988), but not universal occurrence (Clemens and Jones, 1978; Osonubi and Davies, 1978). Reduction in plant growth during MSC is probably due to loss of turgor during subsequent dry-down cycles. Turgor loss during moisture stress has been shown to reduce cell division and elongation, as well as essential metabolic processes required for plant growth and development (Hsiao, 1973). Although MSC plants in this study had a mean shoot dry weight that was $\approx 42\%$ less than that for controls, leaf area was reduced to a lesser degree, giving the MSC plants a shorter

but fuller appearance when compared to controls. Despite differences in plant size, floral initiation was evident for both MSC and control plants at termination of the experiment on day 71 following seeding, indicating that MSC should not increase the production time required to produce saleable plants.

Results from this work indicate the use of MSC for salvia could prove important to wholesale growers despite reductions in plant size. Moisture stress conditioning of salvia improved moisture stress tolerance through reductions in transpirational water loss and the ability to maintain P_n to lower ψ_L than in control plants. Improvements in moisture stress tolerance should allow visual quality to be maintained longer and should reduce plant mortality following wholesale production.

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