Controlled Drought Affects Morphology and Anatomy of *Salvia splendens*

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**Abstract.** Polyethylene glycol 8000 (PEG-8000) was applied to a soilless growing medium at the concentrations of 0, 15, 20, 30, 42, or 50 g·L⁻¹ to impose controlled drought. Salvia (*Salvia splendens* F. Sellow. ex Roem & Shult.) seeds were planted in the growing medium to determine if controlled drought affects morphology and anatomy of salvia. Polyethylene glycol decreased emergence percentage and delayed emergence up to 5 days. Stem elongation of salvia treated with the five lowest concentrations was reduced up to 35% (21 days after seeding), and salvia were a maximum of 53% shorter and the canopy was 20% more narrow compared to nontreated seedlings 70 days after seeding. These morphological changes were attributed to PEG-8000 mediated reduction in leaf water potential (Ψᵪ). The growing medium Ψᵪ ranged from −0.29 to −0.85 MPa in PEG-8000 treated plants, and plant height was positively correlated with Ψᵪ 21 days after seeding. Stem diameter of PEG-treated seedlings was reduced up to 0.4 mm mainly due to reductions in vascular cross-sectional area. Xylem cross-sectional area decreased more than stem and phloem cross-sectional area. Polyethylene glycol 8000 reduced vessel element number, but not diameter.

Drought is commonly used to reduce elongation (or growth) of greenhouse ornamentals. Plants grown in substrates that have a low water potential (Ψᵪ) typically have low leaf Ψᵢᵥ and turgor potential (Ψᵠ). Turgor potential, in excess of the minimum “yield” threshold, is the driving force for cell elongation (Carpita and McCann, 2000; Cosgrove, 1997; French, 1997; van Volkenburgh 1999). Thus, commercial greenhouse growers use drought stress to slow elongation of plants such as vegetable transplants (Latimer, 1992; Latimer and Severson, 1997).

The disadvantage of using drought to reduce ornamental seedling growth is that it can cause damage, including leaf abscission and reduced germination (Mohr and Schopfer, 1995). Even minor damage, such as marginal leaf scorching, would decrease seedling quality. Further complicating the matter, seedlings are grown in small substrate volumes (8 mL for trays with 288 seedlings) that dry out quickly between irrigations. It would be desirable for growers to have a method of controlling drought stress so that seedlings are shorter, but not damaged.

Osmotic compounds, such as polyethylene glycol (PEG) can be used to impose controlled drought. Polyethylene glycol forms hydrogen bonds with water and decreases the matric potential of substrates (Kjellander and Florin, 1981; Steuter et al., 1981). Postgermination PEG drenches reduced elongation of salvia and french marigold (*Tagetes patula* L.) (Burnett et al., 2004). Other plants that exhibited less shoot or root growth when exposed to PEG compounds of varying molecular weight include rice (*Oryza sativa* L.), monterey pine (*Pinus radiata* L.), maize (*Zea mays* L.), and sorghum (*Sorghum bicolor* (L.) Moench (S. vulgare Pers.)) (Choi et al., 2000; Gill et al., 2001; Lawlor, 1970; Zou et al., 2000).

Annual salvia was chosen as the model crop candidate for height control using controlled drought for several reasons. Firstly, salvia grows rapidly, and chemical growth retardants are usually recommended for commercial greenhouse production (Nau, 1998). Secondly, previous research reported that drought-stressed salvia (leaf Ψᵪ = −1.1 to −1.4 MPa) were shorter and more compact than nonstressed salvia (Eakes et al., 1991). Morphological changes such as reduced stem and leaf elongation frequently reflect anatomical changes. It would be of interest to correlate morphological information with anatomical data, in order to describe the manifested macroscopic changes. For this reason, the two objectives of this experiment were to confirm that PEG reduces Ψᵪ, Ψᵠ, solute potential (Ψₛ), and shoot elongation of a commercially important summer annual bedding plant, salvia, and to determine the mechanism by which plant morphology is modified. This research builds upon the foundation of previous research to determine whether drought stress may be used to control elongation of commercial seedlings (Eakes et al., 1991). In addition, previous research did not determine how salvia anatomy was affected by reduced elongation.

**Materials and Methods**

Polyethylene glycol 8000 (Fisher Scientific, Fairlawn, N.J.) was mixed with water and added to a commercial peat-based growing medium specifically formulated for germinating seedlings [a mixture of sphagnum peat, perlite, and vermiculite (Germinating mix; Fafard, Anderson, S.C.) resulting in the following concentrations in the growing medium: 0, 15, 20, 30, 42, and 50 g·L⁻¹]. Each PEG-8000 and growing medium combination was shaken vigorously in a large plastic container for 10 min to produce a homogenous mix. Treated growing medium was placed in 6x6 cell sections cut from 288-trays (cell volume = 8.5 mL). Each 6x6 section was one experimental unit. Substrate Ψᵪ of three samples from each treatment was measured using a vapor pressure osmometer (model 5520 Vapro; Wescor, Logan, Utah). The volumetric water content of the growing medium during these measurements was approximately 29%, which was the target water content for the duration of the experiment.

Two salvia ‘Bonfire’ seeds (Ball Seed Co., West Chicago, Ill.) were planted in each of the 36 cells in an experimental unit on 6 June 2003. Salvia seeds were grown until emergence in a growth
chamber (model E-15; Conviron, Winnipeg, Canada) (light levels ≈300 μmol·m⁻²·s⁻¹, temperature ≈24 °C). While in the growth chamber, plants were misted overhead as needed to maintain a constant volumetric water content (29% ± 3%). Fourteen days after seeding, plants were transferred to a mist bench in a glass greenhouse (average temperature = 25 ± 4.4 °C) and irrigated 20 s every 20 min from 0600 until 1800 hr 15 d after seeding. The mist timing was changed to 20 s every 30 min from 0600 until 1800 hr 19 d after seeding, to maintain appropriate irrigation levels. The smaller seedling was removed from cells containing more than one plant 27 d after seeding. All seedlings were hand-misted overhead twice weekly for 20 s with a 20N–8.7P–16.6K fertilizer (20–20–20 General Purpose; Scotts Co., Marysville, Ohio) solution with a N concentration of 200 mg·L⁻¹, beginning when the first true leaves were visible (23 d after seeding). When plugs were fertilized, they were removed from the mist bench.

Experimental unit weight was recorded twice daily throughout the experiment and irrigation was adjusted as necessary to maintain constant water contents (29% ± 3%). Since PEG is water soluble, this also prevented leaching which would have resulted in loss of PEG and thus change treatment levels. Empty tray weights were noted at the beginning of the experiment. After emergence, one representative seedling from the outermost row was harvested bi-weekly. The weight of this seedling was multiplied by the number of plants in each tray to account for changes in plant weight throughout the experiment. Each tray contained ≈307 mL of growing medium, and the same volume of excess treated growing mix was dried in a drying oven at approximately 80 °C.

The amount of water in each tray was calculated by subtracting the tray, seedling, and dry growing medium weight from the experimental unit weight. The drying oven did not completely remove all the water bound to PEG-8000 from the growing medium. Thus, the estimated dry weight of the growing medium included some water, which was bound to the PEG-8000 at temperatures up to 80 °C and therefore not available for plant uptake.

Data collected include number of days to emergence (cotyledons perpendicular to hypocotyl) and emergence percentage. Plants in the outermost rows of the 6x6 cell sections were not measured to prevent edge effects. Height from the top of the growing medium and width between the widest two leaf tips was measured 21 and 70 d after seeding. At harvest (70 d after seeding) leaf area was measured using a leaf area meter (LI-3100; LI-COR, Lincoln, Nebr.). Roots were washed to remove the growing medium, then the longest root length was recorded. Shoot (stems and leaves) and root tissues were dried in an oven at 80 °C for at least 3 d; then dry weights were measured. Compactness (leaf area/height at harvest) and stem density (stem dry weight/stem length) were calculated from these data. All living seedlings except those from the border were harvested for shoot data, but, for roots, only four representative plants were harvested.

Leaf water relations. Midday leaf $\Psi_m$, $\Psi_s$, and $\Psi_p$ of the second acropetal pair of leaves from representative plants from three blocks was measured 21, 35, and 56 d after seeding using individually calibrated leaf-cutter thermocouple psychrometers (J.R.D. Merrill Specialty Equipment, Logan, Utah). Leaf samples enclosed in the psychrometer chambers were equilibrated in a water bath at 25 °C for 4 h before measurement. Water potential of intact leaves was measured for 1 s using a microvoltmeter. Then, leaf samples were frozen to disrupt cell membranes and remove $\Psi_p$. Samples were then re-equilibrated as described above and $\Psi_s$ was measured. Finally, $\Psi_p$ was calculated by subtracting $\Psi_s$ from $\Psi_m$.

Results and Discussion

Salvia emergence percentages decreased with increasing PEG-8000 rates ($P$ value = 0.0020; Burnett, 2004). Emergence percentage decreased from 81% in controls to 61% for seedlings treated with 50 g·L⁻¹ of PEG-8000. Seed germination is typically reduced by water stress (Mohr and Schopfer, 1995). PEG-8000 reduced the matric potential of substrates (Steuter et al., 1981). In this experiment, the growing medium water potential decreased quadratically with increasing PEG-8000 concentration across a broad range [-0.21 (0 g·L⁻¹) to -0.85 MPa (50 g·L⁻¹); Burnett et al., 2004]. PEG delayed emergence up to 5 d (from 11 d for control plants to 16 d for seeds exposed to 50 g·L⁻¹; days to emergence = 11 + 3.32 × 10⁻² x + 1.36 × 10⁻⁴ x²; $R^2 = 0.75, P = 0.0001$, $x$ = PEG-8000 concentration in the growing medium). In previous experiments, PEG-8000 reduced and delayed germination of *Tagetes patula* (Burnett, 2004). All salvias emerged within 1 d of the normal germination time of 12 to 15 d (Nau, 1998).

Salvia stem elongation decreased quadratically with increasing PEG-8000 concentrations shortly after germination (21 d after seeding) and at harvest (Fig. 1 A and C). Seedlings treated with 50
Leaf area was reduced less than shoot dry weight, but height was not measured (Eakes et al., 1991). In contrast, van Iersel and Nemali (2004) found that drought-stressed marigolds were shorter but less compact than nonstressed marigolds. PEG-treated marigolds were less compact than nontreated marigolds (Burnett et al., 2004). The relationship between height, compactness, and drought stress may be species specific. Salvia treated with PEG had less dense stems when treated increasing PEG concentrations. Stem density is another measure of compactness, and it was 0.5 g·m⁻¹ for nontreated salvia compared to 0.24 g·m⁻¹ for salvia treated with 50 g·L⁻¹ of PEG-8000 (Fig. 2B). PEG-treated salvia probably had lower stem densities because they had thinner stems than nontreated salvia (Fig. 3A).

At harvest, salvia had smaller leaf areas with increasing rates of PEG-8000 (Fig. 4A). Salvia treated with 50 g·L⁻¹ of PEG-8000 had 31% less leaf area than control plants. Shoot dry weight also decreased quadratically for plants grown in more PEG-8000 (Fig. 4B). There was a large difference in shoot dry weight (40 mg) between nontreated plants and salvia treated with the lowest concentration (15 g·L⁻¹) of PEG-8000. PEG-8000 affected shoot dry weight more than leaf area (Fig. 4A–B). Osório et al. (1998) reported that leaf area and shoot dry weight of *Eucalyptus globulus* Labill. are equivalently affected by drought stress, while Eakes et al. (1991) found that shoot dry weight of annual salvia is affected by drought stress to a larger degree than leaf area. Similar to compactness, this relationship is probably species specific and deserves more attention in the future.

Root length and dry weight decreased linearly (dry weight) or quadratically (length) for seedlings treated with more PEG-8000 (Fig. 4B–C). Seedlings treated with 50 g·L⁻¹ of PEG-8000 had roots that were ≥70 mm shorter and weighed 47% less than root of nontreated salvias (Fig. 4B–C). Similarly, drought-stressed pepper (*Capsicum annuum*, L.) seedlings had shorter and lighter roots than nonstressed peppers (Lescovar and Cantliffe, 1992; Watts et al., 1981). Roots are often less affected by drought stress than shoots, and drought-stressed plants often have higher root to shoot ratios than nonstressed plants (Hsiao and Jing, 1987; Sharp and Davies, 1979). However, root to shoot ratios were not significantly different in this experiment (data not shown).

**Leaf water relations.** Early in the experiment (21 d after seeding), leaf $\Psi_0$ decreased quadratically from −0.6 MPa in control plants to a minimum of −1.5 MPa in seedlings treated with 42 g·L⁻¹ of PEG-8000 (Fig. 5A). Osmotic potential and $\Psi_0$ decreased linearly as PEG concentration in the growing medium increased (Fig. 5A). PEG reduced $\Psi_0$ from −0.8 in nontreated salvia to −1.3 MPa in salvia treated with 42 g·L⁻¹ of PEG-8000. Leaf $\Psi_0$ of all PEG-treated seedlings was below zero 21 d after seeding. Since $\Psi_0$ was negative and decreased with the addition of PEG-8000, cell elongation was probably reduced by PEG-8000, because $\Psi_p$ is the driving force for cell elongation (Carpita and McCann, 2004).
For controls, 50 g·L–1 PEG-8000. Polyethylene glycol 8000 did not significantly affect more 21 d after seeding than 35 and 56 d after seeding. Vessels treated with 30–50 g·L–1 of PEG-8000 all had \( \Psi_p \) close to zero. However, \( \Psi_p \) of seedlings decreased linearly with increasing PEG rate, but \( \Psi_s \) was not affected by PEG-8000 treatments (Fig. 5C). For controls, \( \Psi_p \) was 0.33 MPa, and the lowest \( \Psi_p \) (–0.04 MPa) was measured in salvia treated with 42 g·L–1 of PEG-8000. Salvia were harvested 70 d after leaf area/height at harvest) and stem density (B: stem dry weight/stem length × 0.48–9.39 \( \times 10^{-4} \) (PEG). By comparison, drought-stressed apple cactus had narrow vessel diameter (Cereus peruvianus L.) and apple \( \Psi_p \) was 0.33 MPa, and the lowest \( \Psi_p \) (–0.04 MPa) was measured in salvia treated with 42 g·L–1 of PEG-8000. Salvia were harvested 70 d after leaf area/height at harvest) and stem density (B: stem dry weight/stem length × 0.48–9.39 \( \times 10^{-4} \) (PEG). By comparison, drought-stressed apple cactus had narrow vessel diameter (Cereus peruvianus L.) and apple

\[ \Delta P = \rho g \frac{1}{4} A \Delta l \]
decrease with increasing PEG-8000 concentration in the growing medium (data not shown). However, this trend was not significant, and PEG-8000 did not affect the pith cross-sectional area in roots either. Root, stele, and cortex diameter of soybean \[Glycine max\] (L.) Merr. and peach \[Prunus persica\] (L.) Batsch. also were not affected by drought stress (Rieger and Litvin, 1999). With regard to elongation, roots are less sensitive to drought stress than shoots (Frensch, 1997). In this experiment, drought stress did not affect the anatomical root features examined. Controlled drought stress also did not affect whole leaf, palisade, or spongy mesophyll thicknesses (data not shown).

**Conclusions**

Additions of 15–50 g·L$^{-1}$ of PEG-8000 to the growing medium reduced and delayed salvia germination. Drought-stressed salvia had reduced shoot and root elongation. Leaf $\Psi_w$ and $\Psi_p$ were lower for seedlings treated with PEG-8000, and reductions in leaf $\Psi_w$ were significantly correlated with height reductions. Turgor potential is the driving force for cell elongation, so reduced $\Psi_w$ and $\Psi_p$ contributed to reduced elongation of annual salvia. Salvia also exhibited typical anatomical changes associated with mild drought stress. Seedlings treated with PEG-8000 had narrower stems due to an overall reduction in vascular tissues. Pith and cortex tissues were not significantly affected by controlled drought. Stem diameter could have simply been smaller because treated seedlings were smaller. However, xylem tissue appeared to decrease because of controlled drought, not only because salvia seedlings grown in PEG-8000 were smaller than nontreated salvia. Xylem tissue was reduced because there were fewer xylem elements, not smaller xylem element diameter in treated salvia as compared to nontreated salvia. It appears that PEG-8000 may be used as a replacement or supplement for chemical growth retardants in the commercial production of annual salvia. PEG appeared to reduce elongation mainly by reducing $\Psi_w$ and $\Psi_p$. However, reduced xylem development likely contributed to the observed morphological changes.

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Fig. 4. The effects of PEG-8000 in the growing medium on leaf area [A: leaf area = 13.3 – 0.0654(PEG)], shoot and root dry weights [B: shoot dry weight = 85.4 – 2(PEG)+0.0237(PEG)²; root dry weight = 40 – 0.386(PEG)], and root length [C: root length = 180 – 2.7(PEG)+0.0274(PEG)²] per plant for salvia treated with PEG-8000 as measured at harvest (70 d after seeding). Data points are the mean of six replications with bars representing standard error, and curves show significant linear or quadratic effects.

Fig. 5. Water, osmotic, and turgor potential of salvia leaves treated with varying concentrations of PEG-8000. Second acropetal pair of salvia leaves were measured midday. Data points are the mean of three replications with bars representing standard error, and curves show significant linear or quadratic effects.

$A: \Psi_w, \Psi_s, and \Psi_p, 21$ d after seeding $[\Psi_w = -0.626 - 0.0247(PEG) + 0.00018(PEG)^2; \Psi_s = -0.903 - 0.00507(PEG); \Psi_p = 0.134 - 0.00861(PEG)]; B: \Psi_w, \Psi_s, and \Psi_p, 35$ d after seeding $[\Psi_w = -0.986 - 0.00439(PEG)]; C: \Psi_w, \Psi_s, and \Psi_p, 56$ d after seeding $[\Psi_w = -0.49 - 0.0107(PEG); \Psi_s = 0.43 - 0.00674(PEG)].
Fig. 6. Stem cross sections of entire stems from annual salvia treated with 0, 15, 30, or 50 g·L⁻¹ of PEG-8000. Figures are labeled as follows: P = pith, X = xylem, Ph = phloem, and C = cortex; A: 0 g·L⁻¹; B: 15 g·L⁻¹; C: 30 g·L⁻¹; D: 50 g·L⁻¹ of PEG-8000.

Fig. 7. Close-ups of stem cross sections from annual salvia treated with 0, 15, 30, or 50 g·L⁻¹ of PEG-8000. Figures are labeled as follows: P = pith, X = xylem, and Ph = phloem; A: 0 g·L⁻¹; B: 15 g·L⁻¹; C: 30 g·L⁻¹; D: 50 g·L⁻¹ of PEG-8000.