Exogenous Abscisic Acid Application Effects on Stomatal Closure, Water Use, and Shelf Life of Hydrangea (*Hydrangea macrophylla*)[1]

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Abstract

A lack of adequate watering reduces the shelf life of many ornamental plants during retail. Our goals were to determine whether sprays or drenches with abscisic acid (ABA) can reduce transpiration and extend the shelf life of hydrangea (*Hydrangea macrophylla*). During the first 5 days after treatment, ABA drenches of 125 to 1000 ppm reduced stomatal conductance (g_s) by 50 to 80% as compared to water. ABA-induced stomatal closure reduced plant water uptake from the substrate; control plants took up half of the plant-available water during the first 7 days after treatment, while it took 14 days for plants drenched with 1000 ppm to take up half of the available water. Control plants wilted after 12 days and time to wilting of drenched plants increased with increasing ABA concentrations, up to 23 days in the 1000 ppm treatment. Spray treatments had little effect on g_s and no detectable effect on water uptake or time to wilting. Some yellowing of older leaves was seen with ABA drenches of 500 or 1000 ppm. Despite this side effect, ABA drenches have potential to extend the shelf life of hydrangeas in retail environments.

Index words: ABA, stomatal conductance, substrate water content, transpiration.

Species used in this study: French hydrangea (*Hydrangea macrophylla* (Thunb.) Ser.).

Chemicals used in this study: abscisic acid (ABA).

Significance to the Nursery Industry

Plant care in retail environments is often poor, and plants may experience severe drought stress and become unsalable. The plant hormone ABA has long been known to close stomates and reduce plant water use. This study shows that drenches with ABA solution are an effective way to close stomates, reduce transpiration, and extend the shelf life of hydrangeas. The effect of ABA is strongly dose-dependent. While shelf life was extended by about 11 days at high ABA concentrations (500 or 1000 ppm), these drench concentrations resulted in some yellowing of the lower leaves. Since there is variability in ABA response among species, and possibly cultivars, further study is needed to determine optimal ABA use in *Hydrangea macrophylla* and related species. ABA sprays were not effective, possibly because they lacked an adjuvant in the spray solutions.

Introduction

Poor care of ornamental plants in retail environments can lead to substantial loss of quality. Adoption of 'pay-by-scan' systems in the retail industry has shifted much of the financial risks associated with quality loss from retailers to growers. With pay-by-scan, growers often provide post-production care and are paid only for those plants sold by the retailer. The adoption of such third-party systems has gained popularity recently as the number of mass merchandisers has increased, and with it, the number of horticultural specialists working for these retailers diminishes (9).

Inadequate watering is one of the most common of problems affecting plant quality during retail. Drought stress during the retail period can negatively affect plant health and aesthetic quality, decreasing the plants' salability and shortening shelf life, resulting in shrinkage (1, 8). Holding agents, which can slow growth and reduce water use, may lengthen the shelf life of containerized plants (8, 13, 14). Exogenously-applied abscisic acid (ABA) lately has received increasing interest as a potential holding agent that, unlike plant growth regulators such as paclobutrazol and uniconazole, does not stunt plant growth in the long term (11, 13).

The role of ABA in plants was first identified in the 1960s. Originally thought to be mainly involved in leaf abscission, ABA is now known to be involved in several developmental processes, such as seed development and germination as well as acclimation to environmental stresses such as cold, salt, and drought (6, 17, 18). ABA is produced in plant roots and transported to leaves through the xylem when low soil moisture is detected (7). ABA accumulation in leaves reduces stomatal conductance due to guard-cell mediated changes in stomatal aperture and can both inhibit opening of stomates and cause open stomata to close (7, 15). The role of ABA in CO_2 and water exchange is therefore of great interest in water conservation in horticultural, agricultural, and natural settings (11, 13). However, commercial use of ABA has long been cost-prohibitive, but a recent breakthrough in ABA production has made it much less expensive (10), making it potentially a cost-effective choice for growers (2).

Exogenously-applied ABA rapidly deactivates due to both photoisomerization and metabolism, and such low stability decreases its practicality of use for some purposes. ABA analogs are chemically similar to natural ABA, but minor changes made to the molecular structure help increase their stability and uptake (4, 5), which can increase their efficacy. For example, Sharma et al. (14) found that ABA analogs decreased water loss from tomato (*Solanum lycopersicon*) seedlings more effectively than regular ABA. However, ABA analogs may cause phytotoxicity (12) and their longevity in plants raises the potential for long term growth reductions.

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Thus, the long-term effect of ABA analogs on plant growth needs to be evaluated before they can be recommended for commercial use (14).

The effectiveness of exogenous ABA varies with concentration, mode of application, and plant species (3, 4, 14). Higher rates of ABA reduce plant water loss more effectively than low rates (8, 12, 14). ABA can be applied as a foliar spray or substrate drench, and the root-drench method appears to be more effective. Working with an ABA analog, Sharma et al. (13) showed that root dip treatments (placing cell packs in solution) resulted in higher tissue concentrations of ABA than foliar treatments.

Research has not yet elucidated the apparent differences in ABA efficacy among different species. Sharma et al. (14) illustrated varying interspecific responses to ABA analogs applied by root-dip to tomato, nasturtium (*Tropelaum majus*), and snapdragon (*Antirrhinum majus*), which had different levels of water use reductions, ranging from 25% (nasturtium) to 62% (in tomato). Earlier, Sharma et al. (13) found that use of analog ABA on tomatoes reduced water use by 62%, while marigolds (*Tagetes patula*) tested in the same study showed only a 9% decrease in water use. Blanchard et al. (3) found that nine bedding plant species showed widely varying responses to ABA applications. New Guinea impatiens (*Impatiens hawkeri*) and petunia (*Petuni*a ×*hybrida*) had increased shelf life following ABA applications, while impatiens (*I. walleriana*) showed no effect.

While exogenous applications of ABA can reduce water loss and extend shelf life, commercial use of ABA is hindered by a lack of knowledge about species-specific application rates and mode of application. The objectives of this study were to determine the optimal rate and method of application for ABA when applied to hydrangea, to determine ABA effects on stomatal conductance, and to quantify how ABA affects the amount of plant available water in the substrate. Hydrangea was selected as the model species, because it wilts readily, both during production and in the landscape.

Materials and Methods

Plant Material and treatments. Fifty salable, uniform flowering hydrangeas ('Mini Penny') in #2 (6.8 liter) containers filled with an aged pine bark-based substrate were obtained from a commercial nursery (McCorkle Nurseries, Dearing, GA). The plants were kept well-watered using overhead irrigation until the start of the experiment, when they were moved into a greenhouse. All pots were watered to runoff just prior to ABA applications.

ABA stock solution (10% w/v s-ABA, the biologically-active form of ABA, 63-022-VB, Valent BioSciences, Libertyville, IL) was diluted with deionized water to yield concentrations of 125, 250, 500, and 1000 ppm. This concentration range was chosen based on previous research that showed good efficacy of ABA at concentrations of 250 to 2000 ppm, although concentrations of 1000 or 2000 ppm induced significant leaf abscission in annual salvia (*Salvia splendens*) (8). A control treatment with 0 ppm ABA (deionized water) was included as well. In the drench treatments, 250 mL of ABA solution was applied directly to the substrate, because this volume could be applied with little or no leaching. Foliar spray-treated plants were sprayed with hand-held spray bottles until the foliage was uniformly wetted. No adjuvant was included in the spray solutions. After ABA application, water was withheld for the duration of the study, which was terminated once all plants were wilted. ABA applications were made on September 10, 2008.

Data collection. Visual observations of wilting were made daily during the three week duration of the study. Measurements of substrate volumetric water content (VWC) were taken with dielectric soil moisture sensors (10HS, Decagon Devices, Pullman, WA) connected to dataloggers (EM50R, Decagon) in three of five replicate blocks of drench treatments, and two blocks of the spray treatments. A substrate-specific calibration was developed to convert the datalogger signal to VWC (r^2 = 0.94). After thoroughly watering the containers prior to the ABA applications, the substrate had a VWC of 0.41 $m^3 \cdot m^{-3}$, while there was no plant-available water (PAW) left at a VWC of 0.09 $m^3 \cdot m^{-3}$ (*i.e.* plants were severely wilted at this VWC and the substrate did not dry out any further). VWC data are expressed as plant-available water, where 100 and 0% plant available water equal the amount of water at the beginning and end of the experiment for each pot (approximate VWCs of 0.41 and 0.09 $m^3 \cdot m^{-3}$, respectively). There were no significant differences in PAW between the spray and drench treatments immediately following the ABA applications. Plant-available water was determined every 10 minutes over the duration of the study. Leaf stomatal conductance (g_s) and transpiration were measured approximately every three days at mid-day using a steady-state porometer (Li-1600, LiCor, Lincoln, NE) on the uppermost fully-expanded leaf.

Experimental design and data analysis. The experimental design was a randomized complete block with a split plot, with ABA application method as the split. A randomized complete block was used to account for environmental gradients within the greenhouse, while the split plot was used to facilitate comparisons between spray and drench treatments. There were five blocks, and the experimental unit was a single plant. ABA effects on g_s were analyzed separately for the spray versus the drench treatments and for each day, using regression analysis. ABA concentrations were transformed using log([ABA]+1) before testing for linear and quadratic effects of ABA on g_s, because hormonal effects are generally not directly proportional to the applied dose (16). Based on the observed ABA effects on time to wilting, ABA concentrations were not transformed before that analysis.

Results and Discussion

Stomatal conductance. Drench applications of ABA affected g_s for the first nine days after application (Fig. 1). For example, drenches with 125 ppm ABA decreased g_s by over 60% as compared to the control treatment from 1 to 4 days after the application, while higher ABA concentrations reduced g_s even more. At 1, 4, 5, or 8 days after application, g_s was negatively correlated with ABA concentration. On day 9, only the 1000 ppm treatment still caused a clear reduction in g_s. Spray applications were less effective than drench applications. Effects of ABA sprays on g_s were seen only on days 4 and 5, when plants treated with 1000 ppm had approximately 55% lower g_s than untreated control plants. Treatment effects of ABA on transpiration (not shown) were similar to those on g_s. Previous research has indicated similar effects of ABA on stomatal conductance and transpiration (8, 10), which is to be expected because of ABA's effects on stomatal aperture (18).

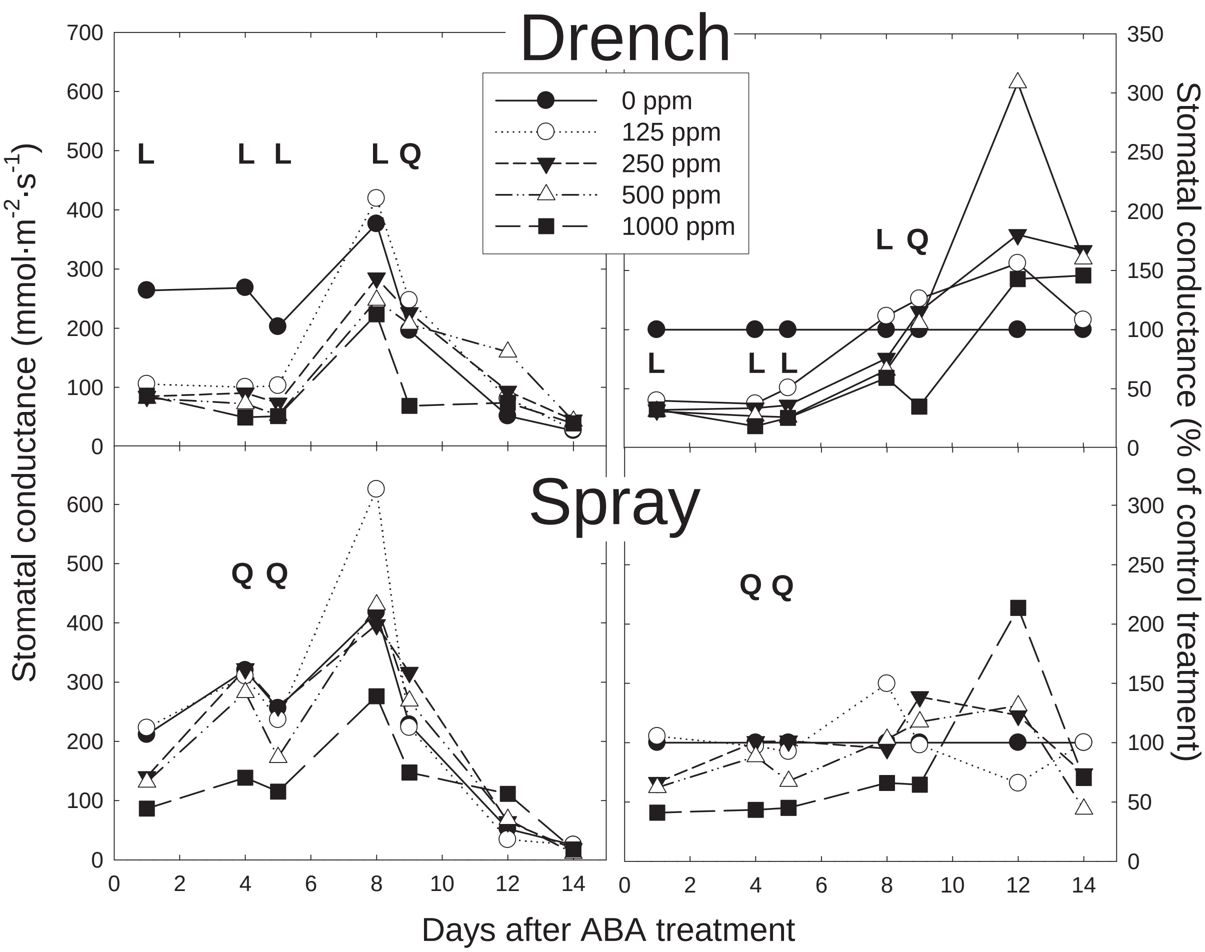

Fig.1. The effect of ABA drenches (top) and sprays (bottom) on stomatal conductance of hydrangea during a two-week period after application. Plants were not watered during this period. Graphs on the left show the actual conductance values, while graphs on the right show the same data as a percentage of conductance of control plants. 'L' and 'Q' indicate linear and quadratic effects of ABA concentration {transformed using log([ABA]+1)} on a specific day (*P* = 0.05).

Although there were no significant effects of ABA on g_s beyond the first nine days of the study, this lack of treatment differences during the latter part of the study does not necessarily mean that ABA no longer reduced g_s of treated plants. Stomatal conductance of control plants (both spray and drench) was high (> 200 $mmol{\cdot}m^{-2}{\cdot}s^{-1}$) during the first 9 days after application (Fig. 1). The high g_s and transpiration rates (results not shown) of untreated control plants during this period hastened depletion of plant-available water in the substrate (Fig. 2), leading to drought stress and low g_s in the control treatment at 12 and 14 days (< 50 $mmol{\cdot}m^{-2}{\cdot}s^{-1}$). The drought-induced stomatal closure of the control plants, which were wilting at this stage, resulted in a lack of differences in g_s at 12 and 14 days.

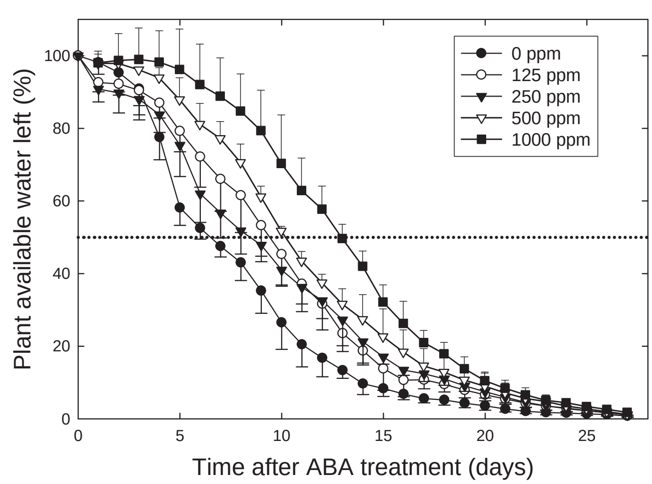

Fig. 2. The decrease in plant available water in the substrate of hydrangeas following drenches with different concentrations of ABA. 100% plant available water refers to the amount of water in the substrate immediately following the drench application, while 0% refers to the amount of water left at the end of the study (volumetric substrate water contents of approximately 0.41 and 0.09 $m^3{\cdot}m^{-3}$, respectively). Plants were no longer watered after the drenches. Error bars indicate the standard deviation (n = 3).

Plant-available water and extension of shelf life. The ABA effects on g_s and transpiration were reflected in plant-available water in the substrate. The spray treatments, which had little effect on g_s, had no effect on plant-available water (results not shown). However, the drench treatments, which had consistent effects on g_s, also affected plant-available water. Plant-available water in the control treatment decreased to 50% after 7 days. At this time, plant-available water was > 90% in the 1000 ppm and 80% in the 500 ppm treatment,

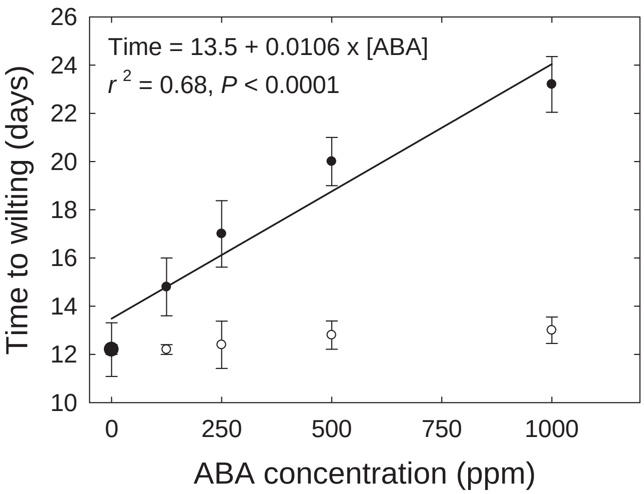

Fig. 3. The effect of ABA drenches (●) and sprays (○) on the time to wilting of hydrangeas. Plants were no longer watered after the drenches. The regression line and equation indicate the effect of ABA drenches on time to wilting. ABA sprays did not affect time to wilting. Data shown are the mean ± standard error (n = 5).

indicating that ABA was highly effective in reducing evapotranspiration. It took 11 and 14 days for the plant-available water to drop to 50% in the 500 and 1000 ppm treatments, respectively (Fig. 2). The effect of ABA on plant-available water depended on the ABA concentration: plant available water decreased slower with higher ABA concentrations.

The slower decrease in plant-available water after ABA drenches resulted in prolonged shelf life of ABA-treated plants. There was a concentration-dependent delay in time to wilting of drenched plants, but no effect on the time to wilting of sprayed plants, which all wilted after 12 to 13 days (Fig. 3). Among the drenched plants, control plants wilted after 12 days and there was a linear increase in the time to wilting with increasing ABA concentrations. Wilting was delayed by an additional 11 days (to 23 days) in plants drenched with 1000 ppm ABA (Fig. 3).

The amount of plant-available water left in the substrate at the time of wilting was strongly correlated with the ABA concentration (PAW = 13.8 – 0.0105 × [ABA], where PAW = plant-available water; $r^2 = 0.98$, $P = 0.003$), and ranged from 14.7% in the control treatment to 3.8% in the 1000 ppm ABA treatment.

Drench treatments were more effective in reducing water use and prolonging shelf life than spray treatments. This finding is consistent with previous reports of ABA (or ABA analogs) effects on physiology. For example, Sharma et al. (12) found that root-applied ABA analogs were more effective than sprays in reducing transplant shock of tomato seedlings. They suggested that this difference in efficacy may be due to the greater quantity of ABA applied in a root dip as compared to a spray. Blanchard et al. (3) compared effectiveness of ABA using sprench (spray until runoff) versus spray (spray until wetted) treatments and found that the mode of application that delivered more chemical to the plant (sprench) had greater effects. Churchill et al. (4) used drenches and sprays of both analogs and natural ABA and found that freezing tolerance of winter rye was increased by 7.5C (13.5F) in response to ABA drenches, but only by 2 or 3C (3.6 or 5.4F) after foliar application. ABA efficacy depends on how much is taken up by the plant, and how easily the chemical is transported, stored, and metabolized within the plant (4) and it is thus not surprising that application methods that supply more ABA to the plant are more effective. Sharma et al. (13) compared the uptake of ABA analogs following root dip and spray applications and found that the root dip treatment resulted in higher foliar concentrations of ABA analogs than the spray treatment. Although the volume of spray solution that was applied to the plants was not quantified in our study, it was much less than the 250 mL applied in the drench treatments. Furthermore, it is important to note that we did not use a spray adjuvant, and ABA efficacy may be substantially increased if an adjuvant is added to the solution (C. Campbell, personal communication).

Mild chlorosis occurred on older leaves of hydrangeas drenched with high concentrations of ABA (500 and 1000 ppm) in this study. No symptoms were seen on the inflorescences of any of the plants. Blanchard et al. (3) reported phytotoxicity following ABA applications to pansy (*Viola ×wittrockiana*) (necrosis) and lobelia (*Sutera cordata*) (leaf yellowing). Leaf abscission of salvia (*Salvia splendens*) increased with increasing ABA concentrations (8).

In summary, drench applications were more effective in reducing plant water use and delaying wilting than foliar sprays, which had little or no effect. ABA drenches reduced stomatal conductance rates and transpiration, decreased water uptake from the substrate, and prolonged shelf life in a dose dependent manner from 12 to up to 23 days. The potential use of ABA to prolong shelf life is promising. Further studies are needed to elucidate possible species-specific effects to optimize delivery method and concentration. One consideration for determining the optimal rate and method of application is that the response to ABA may be species-specific. For example, Blanchard et al. (3) tested efficacy of ABA on several bedding plant species and found that sprenches with 250 ppm ABA extended shelf life of *Impatiens hawkeri* by 6 d, that of verbena (*Verbena × hybrid*) by 3 d, and had no effect on impatiens (*Impatiens walleriana*) or bacopa (*Sutera cordata*). Sharma et al. (13) found that ABA analogs had much more pronounced effects on tomato than on marigolds. Species differences in responses to ABA may be due to differences in retention and accumulation of ABA in the roots. Thus, guidelines developed for one species should not be used for others.

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