Shoot Wilt of *Petunia* sp. Following Drenching with Exogenous ABA[1]

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Abstract

A new agricultural product containing the physiologically active *cis* form of abscisic acid (C-ABA) is scheduled to become available for ornamental production in 2012. This plant growth regulator generally prolongs irrigation intervals of ornamental plants. During pre-registration testing, the product greatly reduced whole plant transpiration on a variety of species, extending the time between irrigations whether applied as a drench or a foliar spray. However in some instances, drench application resulted in whole plant wilting of some annual species after one to three days. This occurred most often with certain cultivars of coleus, tomato and petunia. Investigations reported here associate wilting of petunia with low xylem water potential and near complete stomatal closure caused by prevention of water movement into root stele. Evidence is presented implicating that the exogenous C-ABA induced closure of root aquaporins led to shoot wilt.

Index words: root aquaporin, wilt syndrome, annual plants, transpiration, petunia, PGR.

Species used in this study: *Petunia* × *hybrida*.

Significance to the Nursery Industry

Temporarily reducing plant transpiration chemically prior to shipping has many advantages for producers, retailers and landscape installers. It could greatly reduce losses resulting from dehydration during shipping and/or storage. Shelf life of these plants would be extended without additional watering, or watering could be delayed until the end of the day, such that in the case of retailers, wet floors and hoses would not be a safety concern. Additionally, reduced transpiration would extend the holding time prior to transplanting during landscape installation. In 2012, Valent BioSciences Corporation is scheduled to market such a chemical as Contego™. Its active ingredient is the *cis* form of abscisic acid, the natural plant growth regulator that induces stomata closure. In preliminary trials, most species responded favorably by closing stomata independent of soil water availability. However under usually cool conditions, plants of a few annual species unexpectedly wilted. This wilt is shown here to be due the inability of roots to absorb water from the soil. This inconsistent response of bedding plants to application of this product re-affirms the tried-and-true rule to test chemicals on a sample of plant materials prior to application to an entire crop for all species and cultivars and in various environmental conditions.

Introduction

In 2003 Valent BioSciences Corporation began evaluating applications of the physiologically active *cis* form of abscisic acid (Contego™; C-ABA) produced by a patented fermentation process. This new production process is much more efficient than previous methods, permitting economical application to large acreages. During preliminary tests, crude formulations were found to induce stomatal closure on a wide range of species. It was active both as a foliar spray and as a substrate drench (C. Campbell, 2010, pers. comm.).

In 2006, commercial significance of C-ABA was recognized by the environmental horticulture industry (1). Similar positive effects of extended shelf-life of annual plants from ABA analogs were reported (9). Thereafter, research of C-ABA on ornamental crops increased. Of nine ornamental plant species sprayed with C-ABA, five benefitted from treatments with delayed wilt when water was withheld. Two species showed no benefit, while the other two displayed phytotoxic symptoms, principally leaf yellowing (2). To increase the crop list for C-ABA, much research focused on woody ornamental crops and young transplants, both of which could benefit from improved drought tolerance (C. Campbell, 2010, pers. comm.). Research by van Iersel (13), and others (unpublished results) demonstrated that spray or drench treatments of C-ABA improved short-term drought tolerance of perennial and woody species of hydrangea (*Hydrangea macrophylla* (Thumb.) Ser.), sweet viburnum (*Viburnum odoratissimum* Ker Gawl) and Japanese ligustrum (*Ligustrum japonicum* Thumb.), to name a few. Application of C-ABA also increased drought tolerance and overall market quality of bedding plant species such as *Catharanthus roseus* (L.) G. Don., *Impatiens walleriana* Hook, *Pelargonium* ×*hortorum* L.H. Bailey, *Petunia* ×*hybrida* Vilm., and *Verbena* ×*hybrida* L. (2, 15).

With improved formulations and increased research, anomalies began to be noted. For some species, particularly certain cultivars of tomato (*Solanum lycopersicum* L), coleus (*Solenstemon* sp.) and petunia (*Petunia* ×*hybrida* Vilm.), an unexplained whole plant wilt occurred within one to three days after drenching (C. Campbell, 2010, pers. comm.). The wilting syndrome never occurred if C-ABA was applied as a foliar spray. Circumstantial evidence associated this syndrome with low light levels and environments conducive to low evaporative demand (C. Campbell, 2010, pers. comm.). After conducting preliminary research with C-ABA on woody shrubs in the fall of 2007, this anomaly was intriguing, so experiments were initiated to elucidate possible causes of

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this reaction. The objective of this research was to investigate water relations of petunia plants treated with C-ABA to rule out possible causes of the severe wilt syndrome.

Materials and Methods

Two experiments were conducted in December 2007 at the Mid-Florida Research and Education Center — Apopka, FL. In the first experiment, 18 multi-stem petunias growing in 11 cm (4.25 in) diameter square black polyethylene containers (730 cm^3; 44.6 in^3), in a mostly sphagnum peat moss substrate were obtained from Pure Beauty Farms (Miami, FL) on November 26, 2007. Plants were placed on wire benches in a high-light (full sun, 1510 $mmol \cdot m^{-2} \cdot sec^{-1}$ (7550 ft-candle) maximum PAR), 12 × 33 m (40 × 100 ft) inflated clear double polyethylene hoop-style greenhouse and irrigated daily by hand until treatments began. At treatment initiation, plants were about 9 cm (4 in) tall.

On December 3 plants were relocated to an adjacent structurally identical, but low-light (310 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ (1550 ft-candle) maximum PAR) greenhouse covered with inflated double-layer white polyethylene. Plants were assigned to three treatments in a completely randomized design. Treatments consisted of an untreated control, a 0.946 mM (250 ppm) and a 3.783 mM (1000 ppm) C-ABA drench with 60 mL (2 oz) of volume per container. C-ABA was obtained from Valent BioSciences Corp. (Libertyville, IL) with dilutions made using deionized water just prior to treatment. The control treatment received the same volume of deionized water without C-ABA. Drenches were applied at 1430 h. Both greenhouses were heated with natural gas when temperature dropped below 22C (72F) and cooled by evaporative cooling pads above 26.7C (80F).

The day after treatment (DAT) each plant was given 60 mL (2 oz) of tap water at around 0800 h, resulting in some drainage from all containers. Stomata conductance (gs) was measured on one leaf of each plant with a porometer (LI-Cor 1600, LI-Cor, Lincoln, NE). Measurements began at 0845, 1035, 1235, 1435 and 1655 h, and usually required 20 to 25 min each time. The order in which plants were measured was randomized each time. Immediately after the last gs measurement (1715 h), a stem was removed from each plant for determination of xylem water potential (Ψ_T) using a pressure chamber (Model 3000, SoilMoisture Equipment Inc., Santa Barbara, CA). Pressure was increased at a rate of 20 $kPa \cdot sec^{-1}$ (0.20 $bar \cdot sec^{-1}$) using compressed nitrogen gas. Immediately after balance pressure was obtained, the stem was removed and placed in a 50 mL (1.7 oz) Erlenmeyer flask of de-ionized water filtered through a 0.45 micron filter. Each clean flask was rinsed with the filtered water before filling. These were left on the greenhouse bench overnight.

The second DAT plants were irrigated by hand to container capacity around 0800 h. Beginning at 1250 h, gs was measured as described above first on whole plants, and then the stems placed in the flasks the previous afternoon. After gs measurements, most of the substrate was removed from the roots of each plant by gently massaging root balls in 17 liters (4 gal) of water in one of two 19 liter (5 gal) polyethylene buckets. Plant order for root washing was random and water in buckets was not changed throughout the root washing. Once washed, lower ends of roots were quickly severed using scissors to expose root xylem, without standardization of remaining root quantity or length. Roots remaining on plants were immediately submerged in a 250 mL (8 oz) beaker containing about 100 mL (3.5 oz) of a paper-filtered 0.5% Acid Fuchsin (Sigma-Aldrich, Milwaukee, WI) dye solution. This was to determine if, and where, xylem blockage may have occurred. At 1540 h, dye absorbance was rated on a 0 to 4 scale, where 0 indicated no dye uptake, 1 was a slightly noticeable coloration in leaves, 2 was ca. 1/3 red, 3 was 2/3 red, while 4 indicated plants were mostly reddish due to very high concentrations of dye uptake. Dye uptake rating was determined again at 0800 h the third DAT. Evidence of wilt, principally substantial drooping of leaves, was noted at all measurements.

The second experiment was conducted similar to the first, except the treatments were replicated in both the low-light greenhouse and high-light greenhouses described in Exp. 1. For Exp. 2, 44 petunias of the same cultivar were again obtained from Pure Beauty Farms on December 14, 2007, and placed outdoors in full sun on black polyethylene ground cover with overhead irrigation (7.5 mm; 0.3 in, daily). Plants were in the same container size as in Exp. 1 and plant canopy sizes were similar to those used in Exp. 1. On the morning of December 18th 36 uniform plants were selected and then randomly divided into two groups, with each group placed on wire benches in either the high- or low-light greenhouse. Plants were irrigated to container capacity. That afternoon, solutions of C-ABA were diluted and applied as described for Exp. 1. Treatments were applied first in the high-light greenhouse, then the low-light one. Applications were complete by 1515 h.

The next morning, all plants in the high-light house received 60 mL (2.0 oz) of potable water before 0800 h, with 50 mL (1.7 oz) applied to the plants in the low-light house around 0810 h, resulting in some drainage from all containers. Stomata conductance measurements began at 0840 h, first in the high-light house, then in the low-light one. This order was maintained the rest of the day. After the fifth set of gs measurements, beginning at 1640 h, Ψ_T was measured as in Exp. 1, with excised stems placed in flasks of water. The second DAT measurement of gs of whole plants began at 1315 h, with assessment of dye infiltration in the leaves beginning at 1500 h. In the low-light house, measurements of gs were continued a second day (2 DAT) before stems were sampled for water potential measurements in late afternoon, and placed in Erlenmeyer flasks to rehydrate overnight described above.

Data were analyzed separately for each experiment using a completely randomized design, with three treatments and six single-plant replicates per treatment. In Exp. 2, data were analyzed separately by greenhouse. Diurnal stomata conductance was analyzed as repeated measures using a split plot approach, with treatment as the main plot and time as the subplot independently for each day. All data were analyzed using SAS ver. 9.1 (SAS Institute, Cary, NC).

Results and Discussion

Experiment 1. It was partly cloudy with above-average temperatures outdoors the day treatments were applied (Table 1). The following two days of gs measurements, climatic conditions were nearly identical. The sky was clear and outside temperatures were close to seasonal normal. Light levels in the low-light greenhouse during gs measurements were about one-fifth those under full sun outside. PAR in the greenhouse ranged from 150 to 310 $\mu mol \cdot m^{-2} \cdot sec^{-1}$ (750 to 1550 ft-candle) between 1030 h and 1500 h each day. Some

Table 1. Solar radiation and exterior temperature (at 2 m; 6.5 ft) summary for the dates the two C-ABA drench experiments were conducted. Average minimum temperate for December is 9.9C, while average maximum temperature was 21.9C. (Assessed 11-09-2011; http://www.idcide.com/weather/fl/apopka.htm). Daily temperatures are from the Florida Automated Weather Network — Apopka tower, located about 46 m (150 ft) east of the greenhouse location.

Date	Mean solar radiation ($W \cdot m^{-2}$)	Minimum measured temperature (C)	Maximum measured temperature (C)
3 Dec	144.0	16.9	25.9
4 Dec	183.8	5.0	20.8
5 Dec	164.5	6.0	20.8
18 Dec	118.2	4.1	19.7
19 Dec	202.1	8.7	22.1
20 Dec	—	12.0	24.2
21 Dec	168.7	14.4	21.9

treated plants exhibited wilt by the 1235 h measurement 1 DAT. At that time three of the six plants treated with 250 ppm C-ABA were wilted, compared to 2 of 6 plants in the 1000 ppm C-ABA treatment. Wilted plants remained wilted the rest of the day and were still wilted at 1250 h the following day. Three additional plants were wilted the second day in the 1000 ppm C-ABA treatment at 1250 h. No control plants exhibited wilt.

Differences in gs among treatments depended on time of measurement. There were no differences at the first or last measurement 1 DAT (Fig. 1A). However during midday, gs were higher in control plants than either of the C-ABA treatments, with no differences between C-ABA treatments.

Stem water potential (Ψ_T) near sunset (1728 h, UNSO 2008) followed the opposite pattern of midday gs. Control plants had higher (less negative) Ψ_T than either C-ABA treatment, with no differences between C-ABA treatments (Table 2). Higher Ψ_T in control plants is opposite of what was expected based on gs. Closed stomata of C-ABA treated plants should have resulted in higher Ψ_T than control plants since there was adequate soil moisture and minimum water loss from transpiration. However more negative Ψ_T data corresponded to visual observations of plant wilt.

Two DAT, differences in gs of whole plants were comparable to that measured at the same time the first day after treatment (Table 2). In contrast, gs of leaves of stems in flasks were higher than those of leaves of whole plants for both C-ABA treatments, but not for the control. Among stems in flasks, there were no differences in gs between control and 250 ppm C-ABA treated plants, but both were higher than that of plants in the 1000 ppm treatment. Examination of individual values found this difference was due to low gs (18, 35 and 69 $\mu mol \cdot m^{-2} \cdot sec^{-1}$) for three of the stems from plants treated with 1000 ppm C-ABA. The other three stems had gs similar to those of the 250 ppm treatment (99, 104 and 119 $\mu mol \cdot m^{-2} \cdot sec^{-1}$). All stems in flasks were turgid. It appears some C-ABA from the 1000 ppm treatment may have been translocated to leaves before stem removal for Ψ_T measurements. Similar gs among leaves from flasks indicate low gs of most of the treated whole plants 1 DAT was not due to C-ABA translocation into shoots.

Visual dye ratings two hours after initiation were also similar among treatments (Table 2). Only one plant, from the 1000 ppm treatment, did not have visual evidence of dye uptake. It was also the only plant of four plants, all C-ABA treated, which were still visibly wilted after about 3 hr in the dye solution without evidence of dye uptake. Three plants from the 250 ppm and five plants from the 1000 ppm ABA

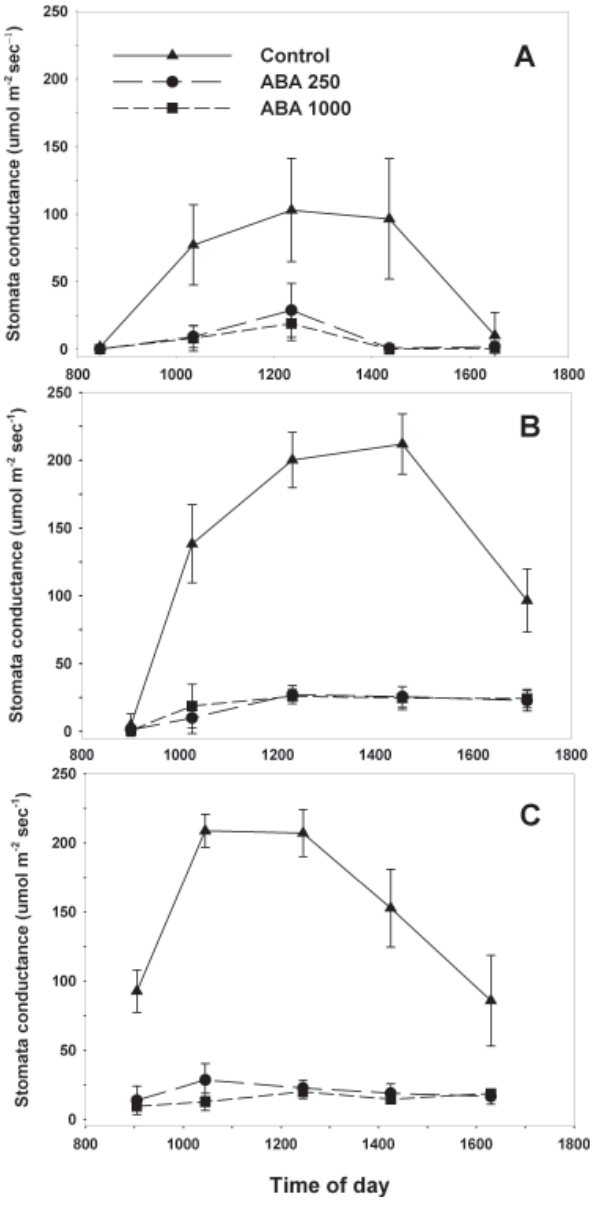

Fig. 1. Stomatal conductance measured from petunias in the low-light greenhouse (A) one day after drenching with C-ABA treatments during Exp. 1, (B) one day after drenching with C-ABA treatments during Exp. 2, and (C) two days after treatment in Exp. 2. Control plants were drenched with the same volume of tap water. Each point is the mean of six plant replicates. Bars indicate the one standard deviation.

Table 2. Comparison of means from data collected on the first and second day after containers of *Petunia* × *hybrida* plants were drenched with *cis*-abscisic acid (C-ABA; Valent BioSciences Corp.) in the low-light greenhouse in Experiment 1. Research was conducted in a temperature-controlled, inflated white polyethylene-covered hoop house at the Mid-Florida Research and Education Center — Apopka, FL.

C-ABA treatment	Ψ_T [z] (MPa)	gs whole[y] plants 1 DAT[x] (μmol·m^{-2}·s^{-1})	gs whole plants 2 DAT (μmol·m^{-2}·s^{-1})	gs stems[w] in flask 2 DAT (μmol·m^{-2}·s^{-1})	Dye rating[v]
Control	–0.31a[u]	103a	126a	140a	3.2a
250 mg·liter^{-1}	–0.82b	29b	16b	116a	2.0a
1000 mg·liter^{-1}	–0.75b	19b	15b	74b	2.0a

[z]Shoot water potential.

[y]Whole plants — undisturbed plants in containers still attached to the roots in substrate.

[x]DAT — days after treatment.

[w]Stems from which water potential was determined, then placed in flask of deionized water.

[v]Visual rating of dye in leaves of whole plants ≈ 2 h after placement in dye solution; where 0 = no dye uptake, 1 = slightly noticeable coloration in leaves, 2 = 1/3 of leaves with visible dye, 3 = 2/3 leaves with dye, and 4 indicated plants mostly reddish.

[u]Means with the same letter are not significantly different (α ≥ 0.05) within column based on F-Protected LSD. Means are based on 6 plant replicates per treatment.

treatments were wilted when placed in the dye solution. The following morning (3 DAT), about 18 h after placement in the dye solution, all plants exhibited dye in the stems and leaves. This indicated there was no significant blockage in the xylem from the exposed stele to minor veins in leaves. All plants were turgid except the one plant in the 1000 ppm C-ABA treatment.

Experiment 2. High-light greenhouse. As in Exp. 1, it was cloudy the day of treatment, but colder, with a 6C (11F) lower maximum outside temperature. The following days, outdoor conditions were similar to those during Exp. 1 (Table 1). One DAT there were differences in gs between control and both sets of treated plants with the first measurement. These differences continued for the rest of the day (Fig. 2). As in Exp. 1, there were no differences in gs between C-ABA treatments. By late afternoon, most plants in both C-ABA treatments exhibited wilt while there was no wilt in control plants. As in Exp. 1, Ψ_T was more negative for both C-ABA treatments than control (Table 3).

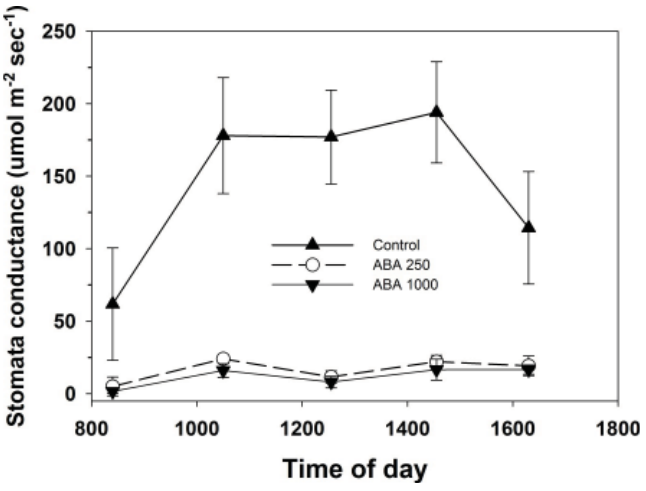

Fig. 2. Stomatal conductance measured from petunias one day after drenching with C-ABA treatments in the high light greenhouse during Exp. 2. Control plants were drenched with the same volume of tap water. Each point is the mean of six plant replicates. Bars indicate the one standard deviation.

Two DAT, gs of treated whole plants was still less than control plants (Table 3). There remained no differences in gs between C-ABA treatments. Compared to the same time 1 DAT, gs were higher 2 DAT for all treatments, although mean solar radiation was lower (Table 1). PAR was not recorded during measurements. As in Exp. 1, gs of leaves of C-ABA treated stems in flasks were higher than the gs of leaves from whole plants. While wilt was still evident for C-ABA treated whole plants, all stems in flasks were fully turgid. Among stems in flasks, there were substantial differences in gs between C-ABA treatments, with a gradient of decreasing gs as C-ABA concentration increased (Table 3). Lower gs of stems in flasks from treated plants compared to control plants suggest some translocation of C-ABA to leaves before stems were severed. Differences in gs between the two concentrations suggest greater translocation from the higher concentration. Despite evidence that some C-ABA was translocated to leaves, sizeable differences in gs between stems in flask and whole plants supports the concept that something other than C-ABA induced stomata closure and caused wilting of whole plants. Dye was very obvious in all plants except two of the 1000 ppm C-ABA treated plants two h after immersion of roots into the dye. Light traces of dye were found in both these plants the following morning. For the most part, the measured water relations response of these petunias to C-ABA drench verifies the results found in Exp. 1. Exceptions were lower gs of flask stems compared the control, and the differential effect on gs of the two C-ABA concentrations.

Low-light greenhouse. As found during Exp. 1 differences in gs between control and C-ABA treatments 1 DAT depended on the time of day (Fig. 1B). There were no differences among treatments the first measurement. Values for gs in the low-light house at first measurement were much lower for control plants than those in the high-light greenhouse (Fig. 2). However for the remainder of the first DAT, gs of control plants were always greater than either C-ABA treatment at each measurement time. After the midmorning measurement, gs values were alike within treatments between greenhouses (Fig. 1B and Fig. 2).

Contrary to Exp. 1, no plant exhibited wilt 1 DAT, thus diurnal gs measurement were continued a second day (2 DAT). During the second DAT, only four of 12 C-ABA treated plants exhibited wilt symptoms, and then only in late

Table 3. Comparison of means from data collected on the first and second day after containers of *Petunia* × *hybrida* plants were drenched with *cis*-abscisic acid (C-ABA; Valent BioSciences Corp.) in the high light greenhouse of Experiment 2. Research was conducted in temperature-controlled, inflated clear polyethylene-covered hoop houses that the Mid-Florida Research and Education Center — Apopka, FL.

C-ABA treatment	Ψ_T[z] (bar)	gs whole[y] plants 1 DAT[x] (μmol·m^{-2}·s^{-1})	gs whole plants 2 DAT (μmol·m^{-2}·s^{-1})	gs stems[w] in flask 2 DAT (μmol·m^{-2}·s^{-1})	Wilt rating[v]
Control	–3.1a[u]	177a	220a	259a	0a
250 mg·liter^{-1}	–7.7b	11b	25b	144b	3b
1000 mg·liter^{-1}	–8.2b	8b	20b	65c	4b

[z]Shoot water potential.

[y]Whole plants — undisturbed plants in containers still attached to the roots in substrate.

[x]DAT — days after treatment.

[w]Stems from which water potential was determined, then placed in flask of deionized water.

[v]Mean visual rated degree of wilt one DAT. Ranking ranged from 0 = no wilt to 4 = complete leaf and stem wilt in 25% increments.

[u]Means with the same letter are not significantly different ($\alpha \geq 0.05$) within column based on F-Protected LSD. Means are based on 6 plant replicates per treatment.

afternoon. Of these, three were in the 250 ppm treatment. Due to limited wilt, there were no differences in wilt rating among treatments (Table 4). Stomata conductance during the second DAT was very similar to that measured 1 DAT (Fig. 1B and 1C). Mean gs of control plants remained above 200 μmol·m^{-2}·sec^{-1} through midday, while gs of C-ABA treated plants were similar between concentrations but about one-eighth lower than controls. Water potentials measured at the end of the second DAT were also similar to those measured in Exp. 1 (Table 2), and those of plants in the high-light greenhouse (Table 3). The Ψ_T of C-ABA treated plants was similar between treatments, with both being more negative than that of control plants (Table 4).

On the third DAT, midday gs of C-ABA treated whole plants remained similar (Table 4). Values of gs for both C-ABA treatments were less than those of control plants, as were values for stems in flask of C-ABA treated plants compared to stems from control plants. As in the high-light house, gs of stems in flasks differed among treatments, with a gradient of decreasing gs with increasing C-ABA concentration (Table 3). As noted for the high-light greenhouse, this indicates some C-ABA accumulated in leaves, with the quantity proportional to the concentration level.

Even though there was little wilt the second day after C-ABA drenches, Ψ_T of the C-ABA treated plants were more negative near sunset than control plants and gs were much less. Dye was found in all plants two h after they were put in the dye solution 3 DAT, again verifying no blockage of xylem in cut roots.

Although effects of C-ABA drenches on shoot wilt in Exp. 2 were not fully repeated in the low-light greenhouse, the same physiological responses were recorded. In the high-light greenhouse, responses to C-ABA drenches were closer to those from Exp. 1 under low-light conditions. This, in addition to the much slower response in the low-light greenhouse during Exp. 2, calls into question whether light level is a critical factor for the wilt syndrome. The principal exception in responses to C-ABA drenches between experiments was development of partial stomata closure in stems in flasks in Exp. 2. This partial closure is consistent with expected responses from C-ABA concentrations observed in previously published results (2, 13, 15). This indicates more C-ABA was absorbed by roots during Exp. 2 in late December than in early December.

To explain this difference in gs between experiments, potential environmental differences were examined that may have occurred in the low-light greenhouse. In early December, mean hourly temperatures inside declined from 30.8 to 25.4C (87 to 78F) from application through sunset (1728 h), while in late December, mean temperatures inside declined from 27.3 to 21.4C (81 to 70F). Mean solar radiation was similar both days, but outside temperatures were warmer

Table 4. Comparison of means from data collected on the second and third day after containers of *Petunia* × *hybrida* plants were drenched with *cis*- abscisic acid (C-ABA; Valent BioSciences Corp.) in the low-light greenhouse in Experiment 2. Research was conducted in a temperature-controlled, inflated white polyethylene-covered hoop house at the Mid-Florida Research and Education Center — Apopka, FL.

C-ABA treatment	Ψ_T[z] (bar)	gs whole[y] plants 3 DAT[x] (μmol·m^{-2}·s^{-1})	gs stems[w] in flask 3 DAT (μmol·m^{-2}·s^{-1})	Wilt rating 2 DAT[v]
Control	–2.6a[u]	253a	219a	0a
250 mg·liter^{-1}	–6.0b	28b	145b	2b
1000 mg·liter^{-1}	–7.2b	28b	97c	2b

[z]Shoot water potential.

[y]Whole plants — undisturbed plants in containers still attached to the roots in substrate.

[x]DAT — days after treatment.

[w]Stems from which water potential was determined, then placed in flask of deionized water.

[v]Visual rated degree of wilt at the first DAT. Ranking ranged from 0 = no wilt to 4 = complete leave and stem wilt in 25% increments.

[u]Means with the same letter are not significantly different ($\alpha \geq 0.05$) within column based on F-Protected LSD. Means are based on 6 plant replicates per treatment.

in early December (Table 1). These differences resulted in somewhat higher and prolonged water vapor pressure deficits (vpd) after drenching in early than late December (Exp. 1 vs. Exp. 2, data not shown). A scatter plot of gs of control plants versus mean vpd suggest petunia stomata were not sensitive to vpds below 3.5 kPa (data not shown). Thus greater transpiration and uptake of substrate water likely would have occurred in Exp. 1 compared to Exp. 2. The only other difference between experiments was that inside air temperatures stayed above 20C (68F) through midnight after drenching in Exp. 1, but had dropped below 16C (61F) by 2200 h in Exp. 2 (data not shown). Whether these differences had any effect is undetermined.

These experiments prove that petunia wilt observed on occasion from a C-ABA drench was not due to excessive stomata aperture, blockage for water movement in stems or blockage within root stele. Indeed, stomata of wilted plants were closed, suggesting the closure that reduced transpiration did not moderate internal water potential. This closure was not related to C-ABA induced stomata closure since it was rapidly reversed by immersing cut stems into clean water, even for most of the heavily wilted plants. Stomata closure and wilt were both induced by high water stress, indicated by Ψ_T measurements and the rapid return of turgidity and high gs of wilted stems in the flasks reported in Exp. 1. Dye absorption from cut roots was rapid for most plants, observable within 20 minutes for some. While it cannot be justifiably said for the entire root system, there was no major blockage to water movement in roots proximal and some distance from the crown. Thus, cause of shoot wilt was independent of whole plants hydraulic architecture and C-ABA induced effects on stomata closure. Similar physiological conditions may have occurred in tomato and coleus cultivars (C. Campbell, 2010 pers. comm.) where the wilt syndrome was observed. The data presented here point to an inability of water to enter the stele of roots for transport up to leaves. A plausible explanation is induced closure of root aquaporins.

Aquaporins are special channels within cellular membranes that specifically enhance water movement between cells and appear to be ubiquitous in plants (4). They are instrumental in movement of water from roots to leaves (11) and from soil into roots (6). Aquaporins are gated structures, meaning they can be reversibly opened or closed though various physiological mechanisms. As the understanding of aquaporin membrane channels improves, it becomes increasingly clear that they are structures that operate in a coordinated manner (3).

Aquaporin opening has been mediated by free Ca^{2+} (6) and exogenous ABA in very low concentrations, from 0.100 to 4 µM (0.026 to 1.0 ppm) (5, 12, 14). However effects generally lasted less than 3 h. In contrast, much higher concentrations of exogenous (+/–) ABA, from 50 to 200 µM (13 to 53 ppm) ABA were shown to decrease hydraulic conductivity of intact soybean roots, with the rate of decrease proportional to increasing concentration (7). Here, C-ABA concentrations were 5 to 77 times higher than the mixed isomer solution used by Markhart et al. (7) and close to the range of propionic acid used by Tournaire-Roux et al. (10) to close aquaporin channels *in situ* in intact *Arabidopsis* roots. While plant wilt syndrome described here was not directly linked to aquaporin closure, results support a hypothesis that aquaporin closure was the cause. Why this combination of microclimate and cultivar would cause aquaporin closure sporadically is unknown.

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