

THE EFFECTS OF EXOGENOUS ABSCISIC ACID ON EVAPOTRANSPIRATION AND
ROOT HYDRAULIC CONDUCTANCE OF TOMATO

by

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(Under the Direction of Marc W. van Iersel)

ABSTRACT

Research completed from 2009 to 2011 evaluated the effects of exogenous abscisic acid (ABA) on the evapotranspiration, leaf gas exchange, and root conductance of tomato (*Solanum lycopersicum*). In the evapotranspiration study, tomatoes drenched with six rates (0-1000 mg·L⁻¹) of ABA exhibited a rate-dependent reduction in cumulative evapotranspiration and extensions of shelf life of unwatered plants by up to 6 d. Research on the short-term effects of ABA reported reduced stomatal conductance, photosynthesis, and transpiration within 60 min. One side effect of concentrated ABA drenches was an ABA-induced wilt occurring within 24 h after ABA application that was not related to low substrate water contents. The root conductance of tomato was evaluated following ABA drenches at six rates using decapitated root systems. ABA caused rate-dependent reductions in water flux through the roots. These results suggest that ABA reduces root conductance, limiting water transport to the leaves and causing the wilt.

INDEX WORDS: Abscisic acid, tomato, evapotranspiration, leaf gas exchange, root conductance

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DEDICATION

To my family and friends, who have supported me and kept me sane over the last 2 years as I furthered my academic prowess. I would particularly like to thank my parents Joseph and Ada, who have always encouraged me to keep striving for my personal best in all my endeavors.

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CHAPTER 1: INTRODUCTION

It is common for plants in the retail market to receive irregular watering and lose aesthetic value within a short period of time. Inadequate watering rapidly diminishes a plant's salability and drastically shortens its shelf life (Armitage, 1983). In addition, the widespread adoption of "pay-by-scan" systems has shifted much of the post-production care from retailers to growers, and growers are only paid for the number of plants that are sold (Naab, 2007). These changes to the retail sector have made it more important for growers to maintain a salable crop during retail.

Drought stress is a powerful environmental stress for plants that is evident through wilting and can lead to irreversible damage to leaves and roots if not promptly addressed. Abscisic acid (ABA) can mitigate the effects of drought stress by reducing transpiration, preventing partial reduction of photosynthesis rates, and increasing the plant's water use efficiency (Rajasekaran and Blake, 1999). ABA naturally regulates plant growth in a myriad of ways, but use in the commercial horticultural sector has been limited by cost and lack of species-specific studies. Synthetic analogs of ABA offer a longer period of stability than natural ABA by making minor adjustments to the molecule, but cost is still a prohibitive factor (Churchill et al., 1998; Flores and Dorffling, 1990; Petracek et al., 2005). It has also been observed that ABA analogs limit water loss in tomatoes more effectively than natural ABA, but they persist longer in the soil and may have undesirable long-term effects on plant growth (Sharma et al., 2006).

The hormone ABA was initially thought to be solely responsible for leaf abscission, but since its identification in the early 1960s, studies show that it plays a part in dormancy and

acclimation to environmental stresses, such as drought and cold (Giraudat et al., 1994; Wilen et al., 1994; Willmer and Fricker, 1996). It also functions during seed development to prevent premature seed germination (Kays and Paull, 2004). The effects of ABA and its analogs on stomatal aperture and conductance have been studied to a great extent. ABA facilitates the closure of stomata by accumulating in the leaves, which causes the guard cells to respond by either closing stomata or preventing them from opening (Jiang and Hartung, 2008; Tallman, 2004). Because of this anti-transpirant activity, ABA has been shown to extend the shelf life of both *Salvia splendens* and *Hydrangea macrophylla* for up to 5 and 11 days, respectively (Kim and van Iersel, 2011; van Iersel et al., 2009).

Comparatively little work has been done to evaluate the effects of ABA on the roots of plants. It is known that ABA can be produced in the roots of many plants and is transferred through the xylem to the leaves when the plant is exposed to low soil moisture conditions (Jiang and Hartung, 2008), but other studies also show that ABA can be produced in the chloroplasts and other plastids when plants are subjected to water stress (Mitsunori et al., 2000). ABA synthesized in the roots serves local root cell inhibitory functions (Torrey, 1976). The possibility of physiological changes occurring in the roots when treated with exogenous ABA applications has not been addressed in depth. One such change could be the cause of an unexplained wilt that has been seen when tomatoes are treated with high doses of ABA. The plants wilt rapidly although the substrate still contains ample water, and the cause of this is not understood.

Two main studies were conducted looking at the effects of ABA on different physiological parameters. The objectives of the first study were to evaluate the effects of ABA on the evapotranspiration, stomatal closure and shelf life of tomato plants. For the second study, the objectives were to determine the mechanism of ABA-induced wilting, and to quantify how

ABA affects root and stem hydraulic conductance. Tomato (*Solanum lycopersicum*) was used in these studies because it is sensitive to ABA applications (Sharma et al., 2006) and wilts rapidly when exposed to high ABA rates, even when ample water is present in the soil.

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CHAPTER 2: LITERATURE REVIEW

Abscisic acid (ABA) is a plant hormone with a variety of influences on plant growth. In 1963, ABA was first identified as the most potent component of the β -inhibitory complex, a number of substances removed by ether extraction from the roots of *Vicia faba*, *Zea mays*, and *Pisum sativum* that inhibit cell division and elongation (Torrey, 1976). It was initially thought the only role of ABA was in fruit and leaf abscission (Ohkuma et al., 1963). Since that time, studies have found that ABA has a wide range of activities. These roles range from responses to environmental stress and stomatal aperture control to seed development.

In the development of seeds, ABA acts in a variety of functions. Perhaps its most important role is in conferring and maintaining seed dormancy to prevent premature germination (Millar et al., 2006). The ABA levels in the seed remain high until the seed is imbibed, after which the concentration of ABA drops rapidly. In both barley (*Hordeum vulgare*, a monocot) and *Arabidopsis* (a dicot), studies have shown that ripened, non-dormant seeds show a remarkable drop in the ABA levels once they become imbibed (Millar et al., 2006). The drop occurs within the first six hours following imbibition in both non-dormant and dormant seeds, and after 12 hours, non-dormant seeds had half the ABA content of dormant seeds (Millar et al., 2006). Similar drops in ABA were seen in barley and *Arabidopsis*, despite barley having much larger seeds (Millar et al., 2006). ABA8'hydroxylase is an integral enzyme in lowering the ABA content in seeds and acts independently of other dormancy factors, such as seed coat effects (Millar et al., 2006). Interestingly, other work has found that exogenous applications of ABA can re-impose dormancy on seeds. Apple (*Malus sp.*) and *Taxus baccata* embryos that had

broken dormancy and then treated with ABA were unable to germinate unless a cold treatment or gibberellins were applied, respectively (Walton, 1980).

In addition to the work done on the influence of ABA on seed germination, much research has looked at the role of ABA in cold acclimation of plants. Plants transiently increase their ABA content during periods of cold stress, and this acclimation is halted in the presence of cycloheximide, a protein biosynthesis inhibitor, indicating that new proteins must be produced for the acclimation process to occur (Zeevaert and Creelman, 1988). Wilen et al. (1994) reported that an additional 2 °C in freeze tolerance was achieved using a variety of ABA analogs in *Brassica napus* and *B. campestris*, compared to the control plants. A freezing tolerance of -10 °C was measured in potato treated with 20 mg·L⁻¹ ABA, as compared to -4 °C for controls, and tests showed that the endogenous level of ABA also increased, although higher doses of ABA instituted little to no additional cold tolerance than 20 mg·L⁻¹ ABA treatments (Chen et al., 1982). These studies and many others clearly show that ABA is involved in initiating cold hardiness in plants, likely by regulating genes that control the synthesis of necessary proteins.

With regards to ABA's effect when a plant experiences an injury, there is still much debate. Peña-Cortéz et al. (1989) discussed that ABA was thought to be largely responsible for activating proteinase-inhibitor I and II genes in potato and tomato, and stated that wounded and non-wounded leaves in wild type plants had increased endogenous ABA levels when compared to ABA-deficient mutants of potato (droopy) and tomato (*sit*). However, later studies have reported contradicting results. Tomato treated with 100 µM ABA did show an increased expression of the ABA-responsive *le4* gene, but was responsible for inducing low amounts of proteinase-inhibitor proteins or mRNAs when compared to jasmonic acid or systemin, suggesting that ABA is not necessary for the wound-inducible signal pathway for defense gene

activation (Birkenmeier and Ryan, 1998). Increases in leaf ABA concentration were noted in wounded leaves about 0.5 cm from the site of injury, which may be due to localized dehydration that follows wounding (Birkenmeier and Ryan, 1998). More research will need to be conducted to fully elucidate ABA's role in plant wounding response.

ABA's influence on stomata has been studied to great extent. Generally, ABA is produced by plants in response to drought conditions, causing the rapid closure of stomata and a reduction in transpiration (Kim and van Iersel, 2011). More specifically, ABA functions at the outer surface of the plasmalemma of guard cells, where it prevents stomatal opening by blocking K^+ and H^+ influx to the stomata while facilitating the flux of K^+ , Cl^- and malate out of the stomata, which results in loss of turgor pressure within the cells and subsequent stomatal closure (Zeevaart and Creelman, 1988). The rate at which ABA is translocated into the leaves has been found to be proportional to the degree of drought stress imposed upon the plant. Studies have shown that up to a 20-fold increase in ABA can be seen in the leaves of tomatoes after exposure to severe drought stress, although a 2-fold increase was all that was needed to elicit a response from the stomata (Kriedemann et al., 1972). In wheat, up to a 40-fold increase in ABA was noted in the leaves after the plants were subjected to severe drought stress (Kriedemann et al., 1972).

However, water stress is not the only factor that determines ABA accumulation, as studies have reported that nutrient deficiencies can work alongside drought stress in regulating ABA synthesis. One such experiment evaluated the effects of nitrogen nutrition and water stress on stomatal conductance and found that leaves with low N levels began to accumulate ABA at a higher water potential (-0.84 MPa) than leaves with high N levels (-1.5 MPa), suggesting that drought stress and N-deficiency together influence leaf accumulation of ABA (Radin and

Ackerson, 1981). In addition to nutrient deficiencies, water potential itself can affect how the stomata respond to ABA. When grown under soil water potential conditions ranging from -0.3 to -1.5 MPa, the stomata of epidermal sections of *Commelina communis* exposed to the lower water potentials were more sensitive to ABA being introduced into the xylem and the mesophyll chloroplasts near the stomata (Tardieu and Davies, 1992).

Studies have also examined the effect of an artificially high level of ABA on leaf physiology. Plants grown while exposed to elevated ABA levels for long periods of time vary from control plants in that the density of stomata in the leaves is higher, but less than half the size of normally developed stomata (Franks and Farquhar, 2001). With regards to ABA and its effect on stomatal aperture, it can be stated that as ABA levels in the leaf decline, the stomates begin to reopen and function normally. Studies have found that the stomata begin to open once the ABA concentrations in the transpirational stream decreased, but also reported that there were still high levels of unmetabolized ABA in the leaves (Walton, 1980). In addition, other work has found that once moisture was added to the soil and turgor was restored, the catabolism of ABA into phaseic acid (PA) was rapidly accelerated (Zeevaart and Creelman, 1988). In tomato, the half life for ABA was about 7 hours, and ABA catabolism seems to be more active during dark conditions (Zeevaart and Creelman, 1988).

Studies in plant cell signal transduction have been able to shed some light on how ABA causes the stomates to close. It has been known that ABA employs a Ca^{2+} pathway to regulate stomatal turgor, and ABA also uses cyclic adenosine 5'-diphosphoribose (cADPR) in the process (Leckie et al., 1998). Microinjections of cADPR into stomates resulted in lower turgor and more free Ca^{2+} in the cytosol (Leckie et al., 1998). Others have found that ABA uses nitrate reductase (NR) to synthesize nitric oxide (NO), a key signaling molecule for stomatal closure, in

Arabidopsis thaliana (Desikan et al., 2002). These findings demonstrate a small portion of the myriad of molecules necessary for ABA-induced stomatal closure to function.

As for ABA's impact on photosynthetic capacity, the answer is not as clear. Models predicting the effects of ABA-induced stomatal closure and its effect on photosynthesis and quantum yield suggest that groups of closed stomates fully account for losses in total photosynthesis (Downton et al., 1988). In addition, obstruction of translocation of photoassimilates led to increased levels of ABA in the leaves not related to water stress conditions, which caused the closure of the stomates and reduced photosynthetic capacity and CO₂ exchange rate (Setter et al., 1980). However, other work has concluded that the effects of ABA on photosynthetic capacity and its potential reduction were independent of ABA's effect on the stomates. ABA controls the flux of solutes such as Cl⁻ out of the stomates, and the presence of these anions in the cytoplasm reduces the activity of ribulose 1,5-bisphosphate carboxylase (Rubisco), which in turn reduces the leaf's capacity for photosynthesis and carbon assimilation (Seemann and Sharkey, 1987). Conversely, an experiment where leaves were treated with exogenous ABA demonstrated no significant decrease in electron transport or Rubisco activity. The same study also found that as CO₂ levels were increased from 340 to 1000 mg·L⁻¹, stomates began to reopen in unstressed leaves, although this reopening was diminished or completely inhibited in leaves that had been previously water-stressed or treated with ABA, respectively (Robinson et al., 1988). That same study concluded that stomatal closure mediated by ABA caused a decline in quantum yield, or the efficiency of the yield of photochemical products per total quanta absorbed (and thus photosynthesis), and that stomates were closing in patches rather than uniformly, explaining why non-stomatal inhibition of photosynthesis has not been observed in leaf slices or individual cells (Robinson et al., 1988). Further research has

found that ABA's effect on transpiration led to lower carbon fixation (Ward and Bunce, 1987), whereas other work has looked at the effect of ABA on mesophyll cells and found no evidence of reduced photosynthetic capacity following ABA treatment (Bradford et al., 1983). Due to the number of contradicting theories and the growing concern over fresh water supply, this aspect of ABA's impact on plant biology will likely receive more attention in the coming years as the need for efficient, drought-tolerant crops becomes more imperative.

The question of how ABA affects root function is one that has recently been receiving attention. However, the findings of these research projects are often contradictory. This is likely due to the difficulty of fully examining and understanding a complex transport system such as the roots. ABA had a transient effect on root hydraulic conductance and cellular conductance of excised maize root systems, increasing the former by 3-4 fold and the latter by 7-27 fold when treated with concentrations ranging from 0.1-1 mg·L⁻¹ ABA (Hose et al., 2000). A study performed on *Phaseolus vulgaris* found that an ABA solution injected into the pressure chambers containing the roots at a rate of 7.8×10^{-5} g·cm⁻² leaf area impacted the plants in four ways: it increased volume flux, stimulated a pulsed release of solutes into the xylem, led to a gradual increase in solute flux, and it decreased overall root conductance after several hours passed (Fiscus, 1981). Earlier research also concluded that one of ABA's major roles is to protect the membranes from stress damage, such as drought, salt or cold stress, by limiting root hydraulic conductance and hardening of fragile membrane tissues (Markhart et al., 1979). One common trend in the studies which concluded that ABA increased root conductance is that the effects were transient, lasting only for a few hours, after which ABA is presumably acting to prevent membrane damage.

Research with complete tobacco plants has demonstrated that ABA may have a role in aquaporin regulation, and that ABA increased root hydraulic conductance (Mahdiah and Mostajeran, 2009). In addition, the plants exposed to exogenous ABA expressed more genes that control the development of PIP (plasma membrane intrinsic proteins)-type aquaporins, suggesting that ABA regulates the production of these water channels (Mahdiah and Mostajeran, 2009). Currently, evidence suggests that endogenous ABA interacts with these channels during times of drought stress, and that root water flux may be coordinated with stomatal conductance via aquaporin activity (Tyerman et al., 2002). Recently, Wan et al. (2004) concluded that ABA could potentially increase transcellular water flow by gating these aquaporin channels and preventing them from collapsing. This agrees with the suggestion posited by Hose et al. (2000) that ABA may be responsible for the transient water flux increases seen in their study by regulating aquaporins. This area of study requires further research to fully understand the signaling pathway between ABA and aquaporin channels and how much of an effect this interaction has on overall root water flux.

As for anatomical changes to the roots, a recent study found that ABA caused an increase in cambium layers, a more prominent endodermis, and other plastic changes to anatomical structures within the roots (Ünyayar et al., 2004). Some studies also support the notion that ABA is acting to prevent stress damage in the plant. Zhang et al. (1995) found that ABA temporarily increases water uptake before limiting it by causing morphological changes to the roots (i.e., suberization) as a means of protecting the plant from drought stress. In addition, Zhang et al. (1995) attempted to bring the drought-stressed plants back to their pre-stressed hydraulic conditions and were able to do so within a few days, which they attributed to a flush of new root growth aiding with conductance. This study is consistent with the idea that stresses

lead to ABA-induced changes in the cell membranes, such as hardening, as a means to survive adverse conditions.

Another area of research that is only recently receiving attention focuses on the effects of ABA within the xylem. ABA which is synthesized in the roots must be transported through the xylem in order to reach the leaves and initiate stomatal closure. However, few studies have evaluated whether ABA has an effect on the overall flow of the xylem. Research has suggested that ABA in the xylem (ABA_{xyl}) is decreased when ABA transport is diverted to the leaves (Jeschke and Hartung, 2000). In addition, Sauter and Hartung (2002) found that ABA in the xylem can be increased during a number of events. Their experiment, which examined perfused bean internodes, exposed the internodes to natural levels of ABA and conjugated ABA (predominantly ABA-glucose ester) and concluded that xylem ABA concentration could be increased when ABA_{xyl} is low, when ABA in the stem parenchyma is high, and when the pH in the xylem increases. It can also be argued that an indirect function of ABA may be to maintain stem hydraulic conductance during stress conditions. Xylem cavitation occurs when plants are exposed to drought stress and air bubbles form in the xylem, rendering that section of the xylem unable to conduct water. Upon sensing drought conditions in the roots, the ABA produced will close the stomates, which in turn prevents the xylem from developing tensions that would cause cavitation. This notion is consistent with the findings of Cochard (2002) that stomatal closure prevented xylem embolism and may confer greater drought resistance in maize.

Presently, research centered on the effects of ABA on the roots is still controversial and future studies will need to be conducted to fully elucidate the impact that ABA has on water transport channels in the cell membranes and overall root conductance. Conditions of the plants will need to be evaluated in both the short and long term to quantify how quickly ABA is

affecting the plants, why it increases conductance temporarily, the signaling pathway it takes to regulate these water channels, how long its effects on the cell membranes and water channels last, and whether or not exogenous applications can supplement this without causing permanent harm to the plants.

A recent development with ABA concerns its use as a commercial growth regulator. Past research depended on using costly formulations of ABA that were not economically feasible for commercial applications, but new processes have yielded a cheaper *s*-ABA chemical that will be available to commercial growers (Barrett and Campbell, 2006). More specifically, Valent BioSciences Corp. has found a way to produce *s*-ABA, the active ingredient in natural ABA, by microbial fermentation and plans to register it as ConTego Pro for commercial applications to bedding annuals (Runkle, 2009). In recent years, studies have examined the effects of this chemical with promising results. Blanchard et al. (2007) reported an average increase in shelf life of 5 days across a variety of annual bedding plants using sprench (a heavy overhead spray application that results in the top of the substrate being moistened) applications of 125 and 250 mg·L⁻¹ ABA, with minimal differences in the plants' responses between the application rates. A similar study was conducted using a range of ABA concentrations from 250 to 2000 mg·L⁻¹ on *Salvia splendens* and reported an extension of shelf life of 3 to 5 days over the controls, depending on dose (Kim and van Iersel, 2011). Sharma et al. (2006) examined the effects of an ABA analog on tomato, snapdragon, and nasturtium and found that ABA effectively reduced total water use for all three crops and showed potential as a holding agent.

Along with extension of shelf life, these and other studies have found that the application method is important in determining efficacy across a range of crops. In *Hydrangea macrophylla*, drenches of ABA ranging from 250 to 1000 mg·L⁻¹ were more effective at limiting stomatal

conductance (g_s) and extending shelf life than sprays, possibly due to a lack of an adjuvant in the spray applications (van Iersel et al., 2009). Another study looked at both spray and drench applications of $500 \text{ mg}\cdot\text{L}^{-1}$ ABA on a variety of popular bedding plants and found a delayed time to wilting from 3 days for marigolds to 4 days for petunia (Waterland et al., 2010a). Waterland et al. (2010a) also reported that pansies maintained higher turgor when receiving ABA as a spray, whereas other crops including petunia and impatiens were more receptive to drench applications. Sharma et al. (2005b) reported that tomatoes and marigolds retained more ABA analog in leaves and roots when it was applied as a root dip rather than a foliar spray, although tomato was more receptive to ABA analogs than marigold in general. Similarly, root dips were more effective than foliar sprays in another study by Sharma et al. (2005a) that evaluated using ABA to reduce transplant shock and slow moisture loss in tomatoes, without imparting long-lasting detrimental effects.

A commonly reported detriment to using highly concentrated doses of ABA is that plants may develop lower leaf chlorosis and/or abscission (Kim and van Iersel, 2011; van Iersel et al., 2009; Waterland et al., 2010a). Waterland et al. (2010b) recently looked at the possibility of using common forms of cytokinins (BA) and gibberellins (GA_{4+7}) to prevent leaf chlorosis caused by ABA applications and concluded that PGR applications with both BA and GA_{4+7} were the most effective at reducing chlorosis caused by $1000 \text{ mg}\cdot\text{L}^{-1}$ ABA. However, the easiest way to reduce chlorosis may be to find the lowest dose of ABA that will effectively control stomates and transpiration and not impart negative side effects. Species-specific studies will need to be conducted to determine the lowest effective ABA application rate and method in order to maximize the potential of this new commercial tool.

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CHAPTER 3

DETERMINING THE EFFECTS OF ABSCISIC ACID DRENCHES ON EVAPOTRANSPIRATION AND LEAF GAS EXCHANGE OF TOMATO¹

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Abstract

It is common for plants in the retail market to receive inadequate water and lose aesthetic value within a short period of time. The plant hormone abscisic acid (ABA) is naturally produced in response to drought conditions and reduces transpiration by closing the stomata. Thus, ABA may lengthen shelf life of retail plants by reducing water loss. Two studies were conducted to look at long- and short-term effects of ABA on plant water use, shelf life, and leaf gas exchange. In the long-term study, ABA was applied to tomatoes (*Solanum lycopersicum*) as a 100 mL drench at concentrations ranging from 0 to 1000 mgL⁻¹ and ABA effects on water use and time to wilting were quantified. Half of the plants were not watered following ABA application, and the other plants were rewatered as needed. In general, higher ABA concentrations resulted in less water consumption by both rewatered and unwatered plants. ABA delayed wilting of plants that did not get rewatered by 2 to 5 d as compared to control plants when treated with 62.5 – 125 mgL⁻¹ or 250 – 1000 mgL⁻¹ ABA, respectively. In rewatered plants, ABA reduced daily water loss for 5 d, after which there were no further treatment effects. Negative side effects of the ABA application were rate-dependent chlorosis of the lower leaves, followed by leaf abscission. The objective of the short-term study was to determine the effects of 100 mL drenches of 250 mgL⁻¹ ABA solution on leaf gas exchange. ABA drenches reduced stomatal conductance (g_s), transpiration (E), and photosynthetic rate (P_n) within 60 minutes. After two hours, E , g_s , and P_n were reduced by 66, 72, and 55 % respectively, compared to the control plants. These studies demonstrate that ABA rapidly closes stomata, limits transpirational water loss and can extend the shelf life of retail plants by up to 5 d, which exemplifies its potential as a commercially applied plant growth regulator.

Introduction

Inadequate watering during retail diminishes the salability and shelf life of plants (Armitage, 1983). In addition, the adoption of “pay-by-scan” systems has shifted much of the post-production care from retailers to growers, since growers are only paid by the number of plants that are sold (Naab, 2007). One way to reduce the water use and extend the shelf life of plants in retail settings is the application of exogenous ABA, although the duration of the effects is dependent on species, application rate, and application method (Blanchard et al., 2007; Kim and van Iersel, 2011; Sharma et al., 2006; van Iersel et al., 2009). The effects of ABA can be profound, and have commercial implications. Until recently, use of ABA was cost-prohibitive for horticultural applications and relegated to research. However, breakthroughs in microbiology have made it possible to produce ABA at much lower costs (Petracek et al., 2005). As such, there is potential for the use of ABA as a commercially applied plant growth regulator, although there are still questions of possible phytotoxic responses and deleterious long-term effects (Abrams et al., 1997; Kim and van Iersel, 2011; Waterland et al., 2010b).

The phytohormone abscisic acid (ABA) was first identified in the mid 1960s (Ohkuma et al., 1965), and has since been shown to affect a myriad of plant processes, including acclimation to cold and drought stress (Bravo et al., 1998; Wilen et al., 1994). Studies have also examined the effect of artificially high levels of ABA on leaf physiology. Plants grown under high ABA conditions exhibited smaller stomates in higher density than control plants (Franks and Farquhar, 2001). One of the most scrutinized ABA effects is that on g_s . ABA is produced in response to drought conditions, accumulating in the leaves and causing the guard cells to respond by either closing stomata or preventing them from opening (Jiang and Hartung, 2008; Tallman, 2004; Willmer and Fricker, 1996), thus reducing transpiration (Mahdieh and Mostarejan, 2009;

Milborrow, 1974). Specifically, when a plant is exposed to decreased soil moisture, tissues in the root tips, cortex and stele begin to synthesize ABA (Hartung et al., 2002). From there, the ABA is translocated through the xylem to the leaves (Hartung et al., 2002). The guard cells take up the ABA quickly, within 30 minutes in some cases (Walton, 1980). Once inside the guard cells, ABA regulates movement of ions and solutes, such as H^+ , K^+ , Cl^- , and malate, in and out of the stomates, causing the guard cells to lose turgor and the stomata to close (Walton, 1980). These findings support the idea that ABA produced in the roots is the primary hormone responsible for closing stomata and preventing water loss, resulting in plant acclimation to drought conditions (Davies et al., 1987). However, not all ABA is produced in the roots, since Christmann et al. (2007) showed that arabidopsis (*Arabidopsis thaliana*) leaves are capable of *de novo* synthesis of ABA. Once leaves are rehydrated, the ABA was rapidly catabolized and concentrations decreased to pre-stress levels (Zeevaart and Creelman, 1988).

The potential of ABA to extend the retail shelf life of bedding plants has recently been examined with encouraging results. ABA ($250 \text{ mg}\cdot\text{L}^{-1}$) was applied as a drench (spraying the leaves such that the top few centimeters of the substrate are moist) to a variety of bedding plants and the shelf life of New Guinea impatiens (*Impatiens hawkeri*) was prolonged by almost 6 d, although the same rate extended the shelf life of verbena (*Verbena \times hybrida*) by only 1 d (Blanchard et al., 2007). Other studies have found that drench applications of ABA increased the shelf life of hydrangea (*Hydrangea macrophylla*) by up to 11 d (van Iersel et al., 2009) and of salvia (*Salvia splendens*) by up to 5 d (Kim and van Iersel, 2011), depending on the rate. Work on six different annual bedding plants found that marigold (*Tageta patula*) was more responsive to a $500 \text{ mg}\cdot\text{L}^{-1}$ ABA drench than a foliar spray of the same rate and shelf life was increased by 3 d, whereas pansy (*Viola \times wittrockiana*) achieved a 3 d shelf life extension with a spray rather

than a drench application, demonstrating that application method is as important a consideration as rate (Waterland et al., 2010a). However, there are still questions about exogenous ABA on plant water use, such as its effects on evapotranspiration (ET) and how quickly it affects leaf gas exchange. In addition, the plants in the studies mentioned above were all subjected to drought stress. Little work has been done comparing the effects of ABA on drought stressed versus well-watered plants.

We conducted two studies to quantify the effects of ABA drenches on tomato. Tomatoes were used as the model crop because they are responsive to ABA applications (Sharma et al., 2006). The objectives of the first study were to evaluate the effects of exogenous ABA on the evapotranspiration of drought stressed and rewatered tomatoes and to quantify how ABA affects the time to wilting, in an attempt to increase species-specific information for the commercial use of ABA. The objectives of the second study were to examine the short-term effects of exogenous ABA on g_s , E , and P_n of tomato.

Materials and Methods

Long-Term Evapotranspiration and Shelf Life

Tomatoes ‘Supersweet 100’ were seeded in 72-cell trays and grown on an ebb and flow bench providing a nutrient solution containing $100 \text{ mg}\cdot\text{L}^{-1}$ N (15-5-15 Cal-Mag, Scotts, Marysville, OH; 15N-2.2P-12.45K) for 20 d. Seedlings were transplanted on 10 Sept. 2009 into 10 cm round pots filled with soilless substrate (Fafard 2P, Conrad Fafard Inc., Agawam, MA) and grown on the load cell system (see below) in a glass greenhouse. The tomatoes were fertilized with 2.6 g/plant of controlled release fertilizer (Osmocote 14-14-14, Scotts; 14N-6.16P-11.62K) as a top dressing and watered by hand daily. A datalogger (CR10, Campbell Scientific

Inc., Logan, UT) recorded environmental conditions using a quantum sensor (QSO-sun, Apogee Instruments, Logan, UT) and a temperature and humidity sensor (HMP50, Vaisala, San Jose, CA). Daily minimum and maximum temperatures were 18.9 ± 2.7 and 26.0 ± 5.2 °C, minimum and maximum vapor pressure deficit were 0.168 ± 0.09 and 0.953 ± 0.858 kPa, and daily light integral was 11.8 ± 4.3 mol·m⁻²·d⁻¹ (mean ± s.d.) in the period before the ABA treatments (Fig. 3.1).

ABA stock solution (10% w/v s-ABA, the biologically-active form of ABA, VBC-30101, Valent BioSciences, Long Grove, IL) was diluted with deionized water to yield concentrations of 0, 62.5, 125, 250, 500, and 1000 mg·L⁻¹. This range of concentrations was chosen based on prior research that showed high efficacy at concentrations of 250 to 2000 mg·L⁻¹, although rates higher than 1000 mg·L⁻¹ resulted in significant leaf abscission in annual salvia (*Salvia splendens*) (Kim and van Iersel, 2011). ABA applications were made on 13 Oct. 2009, approximately one month after transplanting. The tomatoes were watered to runoff to assure that all pots were at container capacity, after which each pot was drenched with 100 mL of ABA solution applied to the substrate surface. After the ABA applications, water was withheld from half of the plants (unwatered), while the other half were rewatered as needed.

Visual observations of wilting, chlorosis, and lower leaf abscission were taken daily for the duration of the study. In addition, pot weight was measured with individually-calibrated 1 and 2 kg load cells (LSP-1 and LSP-2, Transducer Techniques, Temecula, CA) mounted on level sections of a metal frame and connected to a multiplexer (AM416, Campbell Scientific, Logan, UT) and data logger (CR10, Campbell Scientific). Weight measurements were taken every 10 s, and these measurements were averaged and recorded every 10 min. Cumulative weight loss over the course of the study was calculated as a measure of cumulative evapotranspiration. For

rewatered plants, data were corrected for the amount of water applied at each irrigation. Daily water use was determined as the change in pot weight in 24 h, after correcting for irrigation when needed. Environmental conditions during the measurement period are shown in Fig. 3.1.

The experiment was designed as a randomized complete block with a split plot, with ABA concentrations as the main blocking factor and the rewatered/unwatered factor as the split. There were two blocks and the experimental unit was an individual plant. A total of 24 plants were used (6 ABA treatments \times 2 water treatments \times 2 blocks). ABA treatments were analyzed separately for the rewatered versus the unwatered treatments using regression analysis. Since hormonal effects on plant physiology are generally not directly proportional to the hormonal concentration, ABA concentrations were transformed using $\log([ABA]+50)$ before testing for linear and quadratic effects of ABA. The data were analyzed separately for each day. Time to wilting of unwatered plants was also analyzed using regression.

Short-Term Gas Exchange Responses

Tomatoes 'Supersweet 100' were seeded in 72-cell trays in a glass greenhouse. Following germination, seedlings were grown to size on an ebb and flow bench providing a $100 \text{ mg}\cdot\text{L}^{-1}$ N fertilizer solution (15-5-15 Cal-Mag; 15N-2.2P-12.45K). The seedlings were transplanted into 10 cm round pots filled with soilless substrate (Fafard 1P, Conrad Fafard) on 21 Jan., 2011 and grown under an overhead irrigation system until the plants reached the adult leaf stage. Each plant received 2.6 g/plant of controlled release fertilizer (Osmocote 14-14-14, Scotts; 14N-6.16P-11.62K). ABA stock solution (VBC-30101, Valent BioSciences) was diluted with deionized water to $250 \text{ mg}\cdot\text{L}^{-1}$. This concentration was chosen based on the results from the long-term evapotranspiration study, that it would not impart negative side effects of lower leaf abscission and chlorosis, while being effective in reducing water loss.

The plants were then transferred to two growth chambers (E-15, Conviron, Winnipeg, Manitoba, Canada) on 6 Feb., 2011 and given a minimum of 2 d to acclimate to the conditions within. The growth chambers were set to 12 h days at 25 °C with a photosynthetic photon flux of 515 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and 12 h nights at 21 °C. While in the growth chamber, plants were watered by hand daily until the start of data collection.

To collect gas exchange data, the cuvette of a portable leaf photosynthesis system (Ciras-2, PP Systems, Amesbury, MA) was clamped onto the uppermost, fully-expanded leaf of a plant prior to treatment and leaf gas exchange was recorded every 5 minutes. Once stable readings were achieved, treatments were applied and leaf gas exchange data were collected for approximately 3 h. Plants were drenched with 100 mL of deionized water or 250 $\text{mg}\cdot\text{L}^{-1}$ ABA solution. Two plants (one ABA treatment and one control) were treated per day over the course of 11 days (8 Feb. through 18 Feb., 2011). A total of 5 replications (2 treatments \times 5 replications) were used, although stable readings were not achieved for one ABA treatment, resulting in only four usable replications for this treatment. To determine instantaneous water use efficiency (WUE), P_n was divided by E. Leaf gas exchange data were normalized by expressing them as a percentage of the pre-treatment rate and analyzed by ANOVA (SAS 9.2, SAS Institute, Cary, NC).

Results and Discussion

Long-Term Evapotranspiration and Shelf Life

Cumulative evapotranspiration (ET) increased over time in a stepwise manner, because the plants used more water during the day than at night, when the stomata are closed and the vapor pressure deficit is smaller (Fig. 3.2). ABA-treated plants had lower ET than control plants,

indicating that ABA caused stomatal closure, limiting transpirational water loss. A reduction in the cumulative ET of the unwatered, ABA-treated plants was apparent within 1.5 h ($P = 0.007$, Fig. 3.2, bottom), and similar differences were seen in the rewatered plants starting 4.5 h after ABA application ($P = 0.03$, Fig. 3.2, top). ABA effects on cumulative ET remained significant throughout the remainder of the study, both in the unwatered and rewatered treatments ($P < 0.001$). By the end of the second day following ABA application, unwatered control plants had lost 82 mL of water, plants treated with $62.5 \text{ mg}\cdot\text{L}^{-1}$ ABA had lost 66 mL, and plants treated with $125\text{--}1000 \text{ mg}\cdot\text{L}^{-1}$ ABA had lost only 49 to 41 mL (Fig. 3.2, bottom). By day 8, the control plants had used most of the available water (301 mL) and had begun to wilt, while the ABA-treated plants were still transpiring due to remaining water availability in the substrate (Fig. 3.2, bottom). This signifies that ABA successfully limited water consumption in the treated plants, extending their shelf life, whereas the control plants had little available water left. Compared to the unwatered plants, cumulative ET after 13 d was much higher in the rewatered control plants (567 mL) than in the unwatered controls (335 mL, 41% less) (Fig. 3.2). Differences in cumulative ET between unwatered and rewatered plants were smaller for ABA treated plants (365 mL versus 293 mL for the rewatered and unwatered plants treated with $1000 \text{ mg}\cdot\text{L}^{-1}$, Fig. 3.2).

The reduced water use of the ABA-treated plants shows that ABA effectively closed stomata, limiting transpirational water loss. This is consistent with previous reports (Jiang and Hartung, 2008; Kim and van Iersel, 2011; Walton, 1980), which indicated that ABA limits g_s . Walton (1980) reported that ABA levels in leaves were inversely related to stomatal aperture, but concluded that ABA levels alone are not a great indicator of stomatal conductance, because only a portion of ABA in the leaves is involved with regulating stomata (Walton, 1980). We also

found that ABA drenches reduced water use quickly (within 1.5 h, Fig. 3.2, bottom). Because a clear relationship between cumulative evapotranspiration and ABA concentration is present for both the unwatered and rewatered treatments, our results suggest that high ABA levels are correlated with stomatal closure. This inverse relationship between cumulative ET and ABA concentration was to be expected, because of the core idea that ABA is largely responsible for limiting stomatal conductance by controlling ion flux in and out of guard cells (Walton, 1980). Because we applied ABA as a drench, these results also indirectly corroborate the conclusions of Jiang and Hartung (2008) that ABA is transported by the roots up the xylem to the leaf tissue, fulfilling the requirement of a hormone being a long-distance signal when necessary.

The ABA concentration also affected daily ET, with the unwatered control plants using 35 mL on the first full day following ABA application, plants in the 62.5 mg·L⁻¹ ABA treatment using only 25 mL, and higher ABA concentrations reducing daily ET even more, to 15-18 mL (Fig. 3.3, bottom). On day 3, the daily ET of the unwatered control plants was 45 mL, whereas the ABA treated plants used 27 to 28 mL. Unwatered control plants had higher daily ET than unwatered, ABA-treated plants during the first 5 days, but on day 6, daily ET of the unwatered control plants dropped sharply and the plants started to wilt. Daily ET started to decrease on day 7 for the unwatered 62.5 mg·L⁻¹ treatment, and on day 8 for the unwatered 125 and 250 mg·L⁻¹ treatments (Fig. 3.3, bottom), indicating the onset of drought stress. From day 9 to 12, daily ET was highest with the highest ABA concentrations (500-1000 mg·L⁻¹ ABA). These findings are similar to those reported by Kim and van Iersel (2011), who found that ABA drenches delayed water loss from the substrate, with the substrate volumetric water content of the control treatments dropping to 0.1 m³·m⁻³ by day 2, while the 1000 and 2000 mg·L⁻¹ plants reached 0.1 m³·m⁻³ on days 8 and 9, respectively. In addition, van Iersel et al. (2009) reported that

hydrangeas not treated with ABA used 50% of the available water by day 7, whereas the substrate of plants treated with 500 and 1000 mg·L⁻¹ ABA still had 80 and > 90% of the plant available water left, respectively, at this time.

In the rewatered treatments, ABA reduced daily ET in a dose-dependent manner through day 5. Water use was similar in all treatments after day 5, suggesting that the ABA might have lost its effectiveness. This is likely due to exogenous ABA in the plants being catabolized. The half life of ABA in tomato was reported to be only 7 h (Zeevaart and Creelman, 1988).

Generally, ABA drenches delayed wilting in unwatered plants longer as ABA concentration increased ($P < 0.0001$). Control plants wilted after 6-7 d, while the plants treated with 62.5 mg·L⁻¹ ABA wilted after 9 d, and plants treated with 500 and 1000 mg·L⁻¹ ABA after 13 d (Fig. 3.4). The time to wilting was prolonged by overcast weather on days 2, 3, and 5, shortly after the ABA applications (Fig. 3.1). Wilting was only noted in the unwatered plants; rewatered plants did not wilt. Similar results of shelf life extension were also described for salvia and hydrangea following ABA applications (Kim and van Iersel, 2011; van Iersel et al., 2009), as well as a variety of annual bedding plants (Blanchard et al., 2007; Sharma et al., 2006). These and other studies have found that exogenous ABA can extend the shelf life of common bedding plants from 1 d in the case of verbena (Blanchard et al., 2007), to 4.3 d for petunia (Waterland et al., 2010a), and up to 11 d for hydrangea (van Iersel et al., 2009). In addition, van Iersel et al. (2009) reported that ABA drenches reduced stomatal conductance of unwatered hydrangea for nine days. We found similar results with tomato, in particular with the rewatered plants where ABA reduced daily ET for 5 d, after which ABA lost its efficacy (Fig. 3.3, top). Past work has shown that ABA has a half life of 7 h in turgid tomato shoots (Zeevaart and

Creelman, 1988), and that ABA catabolism into phaeic acid occurs faster in dark conditions (Zeevaart, 1983).

Foliar chlorosis, followed by abscission, was a negative side effect of the ABA treatments (results not shown). Chlorosis of lower leaves of plants treated with 250-1000 mg·L⁻¹ was first noted 2 d following ABA applications, and leaf abscission occurred after 4 or 5 d, after which abscission tapered off. Chlorosis has been frequently mentioned as a side effect of high doses of ABA (Blanchard et al., 2007; Kim and van Iersel, 2011; van Iersel et al., 2009; Waterland et al., 2010b). Symptoms were more severe with higher ABA concentrations, which was also reported when salvia and hydrangea were treated with high levels of exogenous ABA (Kim and van Iersel, 2011; van Iersel et al., 2009).

Short-Term Gas Exchange Responses

ABA drenches quickly and effectively closed stomata and limited transpirational water loss. Significant reductions in E , g_s , and P_n were seen 60 min following ABA application ($P < 0.05$; Fig. 3.5). After two hours, ABA reduced E , g_s , and P_n by 66, 72, and 55 % respectively, compared to the control plants (Fig. 3.5). These reductions continued until the end of the 3 h study, when ABA reduced E , g_s , and P_n by 71, 77, and 61 %, respectively, compared to control plants (Fig. 3.5). A rapid physiological response to ABA is consistent with the findings that ABA drenches (250 – 2000 mg·L⁻¹) reduced g_s and E of salvia within 3 h (Kim and van Iersel, 2011), and that g_s of hydrangea was negatively correlated with ABA concentration within 1 d after treatment (van Iersel et al., 2009). Walton (1980) reported that there was a 50 % increase in ABA within the stomata within 30 min after water stress was initiated, indicating that endogenous ABA levels can fluctuate quickly in response to stress.

ABA tended to increase water use efficiency (WUE), with the ABA-treated plants showing a 28 % higher WUE than control plants, although the difference was never significant ($P > 0.086$; Fig. 3.5). However, this does suggest that stomatal aperture has a direct effect on WUE of the plant, a well-known correlation. Mild water deficits can induce stomata to close, and endogenous ABA has a role in stomatal regulation. ABA can be manipulated by implementing partial root zone drying, which involves exposing part of the root system to dry soil, thus stimulating these roots to produce endogenous ABA, inducing stomatal closure and increasing WUE (Davies et al., 2002).

The effect of the ABA applications on the transpiration rates and stomatal conductance are consistent with previous reports that ABA is largely responsible for controlling stomatal conductance, which in turn greatly affects transpiration rate (Kim 2011; Walton, 1980) and photosynthesis (Franks and Farquhar, 2001; Liang et al., 1997). Reductions in g_s due to drought stress can in turn lead to reduced photosynthetic rates (Farquhar and Sharkey, 1982). Liang et al. (1997) reported that drought stress-induced ABA increases led to the reduction of both g_s and P_n of two tropical tree species over a period of 4 d, and that recovery to pre-stress rates following re-watering is species-dependent, as only one of the tree species was able to fully recover. Furthermore, other research examined the effect of injecting ABA into the petioles of attached soybean (*Glycine max*) leaves and found that quantum yield and photosynthetic capacity were significantly reduced (Ward and Bunce, 1987). Conversely, Franks and Farquhar (2001) treated half of the *Tradescantia virginiana* plants in the study with $793 \text{ mg}\cdot\text{L}^{-1}$ ABA solution for 14 d and reported that photosynthetic capacity was not significantly affected, although the ABA-treated plants had lower g_s and higher WUE. They concluded that studies where photosynthesis appears to be diminished could be due to patchy distribution of stomatal regulation by ABA, and

that many studies that have reported lower P_n rates following ABA treatment were done in the short-term, whereas their experiment was conducted over the long-term (Franks and Farquhar, 2001). These conclusions are supported by Bradford et al. (1983), who found that long-term treatment with 2.6 and 7.9 $\text{mg}\cdot\text{L}^{-1}$ ABA solution applied as a spray had marginal effects on carbon assimilation rates of tomato. Our findings indicate that ABA has a clear limiting effect on g_s and P_n , although future studies will be necessary to fully elucidate the role of ABA in limiting P_n as findings are still controversial.

Past studies have reported negative side effects of excessive exogenous ABA application to include chlorosis and lower leaf abscission (Kim and van Iersel, 2011; van Iersel et al., 2009), but neither was noticed on the plants for the duration of the study, at least partly due to the short-term nature of this study.

Conclusions

The use of ABA as a commercial means to extend shelf life of retail plants shows promise. ABA drenches quickly and effectively closed stomata, thus limiting transpirational water loss. This effect lasted 5 d in rewatered plants, while ABA delayed wilting and extended the shelf life of unwatered plants by up to 7 d. This effect would be beneficial in extending the salability of retail plants. Negative side effects of the ABA drenches, chlorosis and leaf abscission, were noted and generally became more pronounced as ABA concentration increased. The lowest effective rate of ABA should be applied to extend shelf life without imposing side effects.

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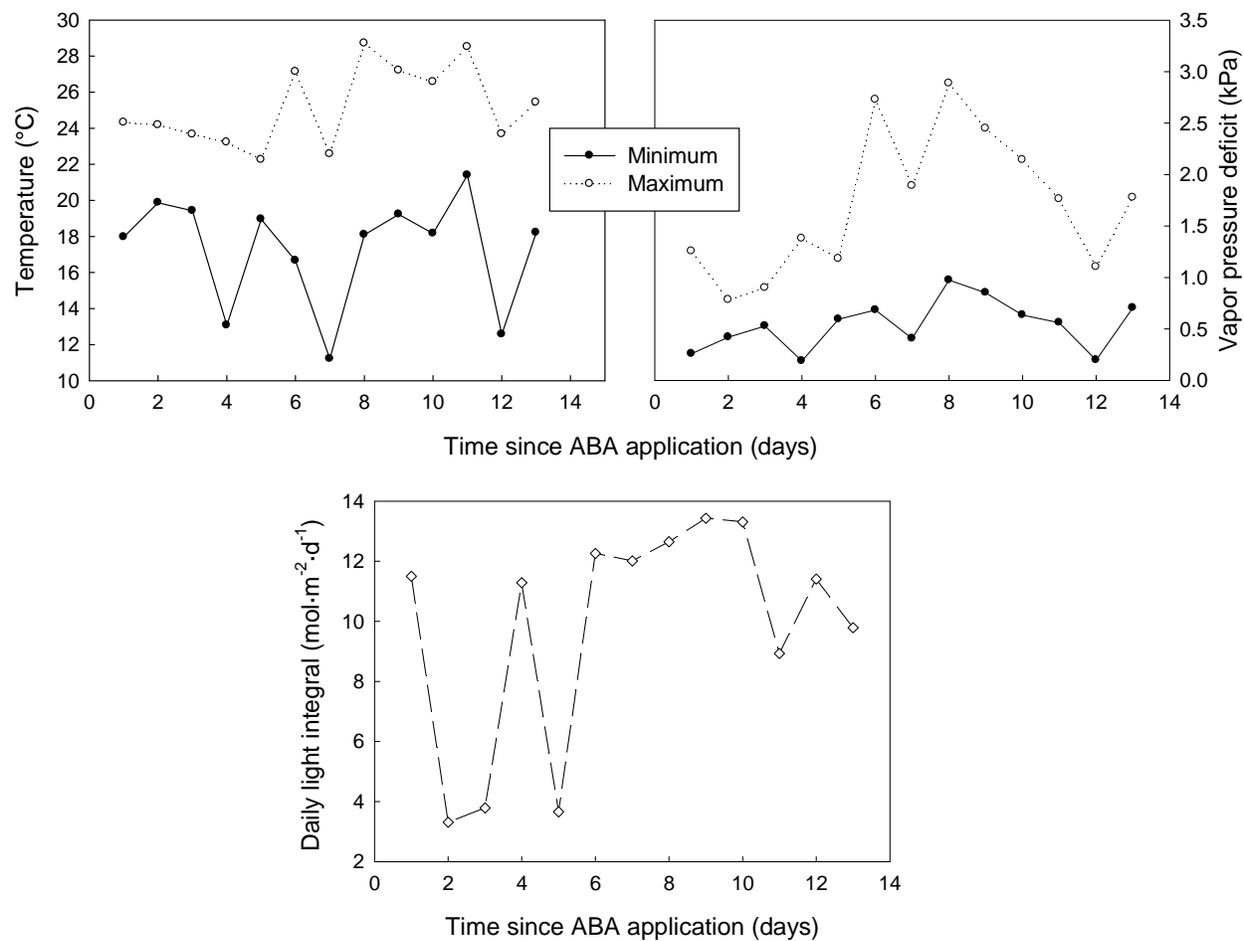


Figure 3.1. Environmental conditions inside the greenhouse for the 13 d duration of the evapotranspiration study. Minimum and maximum daily values for temperature and vapor pressure deficit are given, as well as the daily light integral.

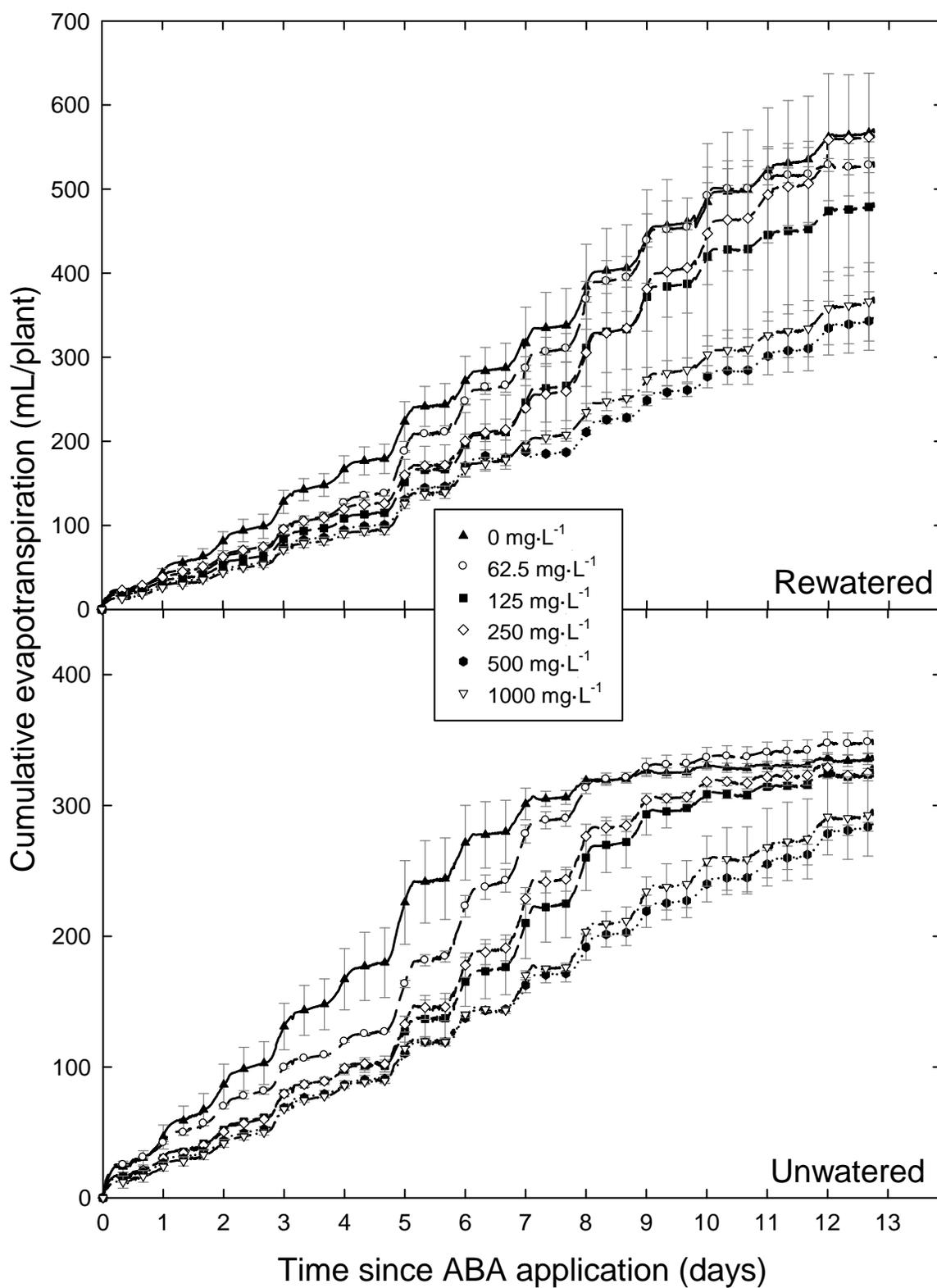


Figure 3.2. Cumulative evapotranspiration of tomato during a 13 d period as affected by drenches with different concentrations of ABA. Plants were either rewatered (top) or unwatered (bottom) following ABA application. Significant effects of ABA treatments were first noted 4.5 hours after ABA application in the rewatered plants ($P < 0.03$) and 1.5 hours after ABA application in the unwatered plants ($P < 0.007$) and persisted throughout the remainder of the study. Data represent the mean \pm standard error (n=2).

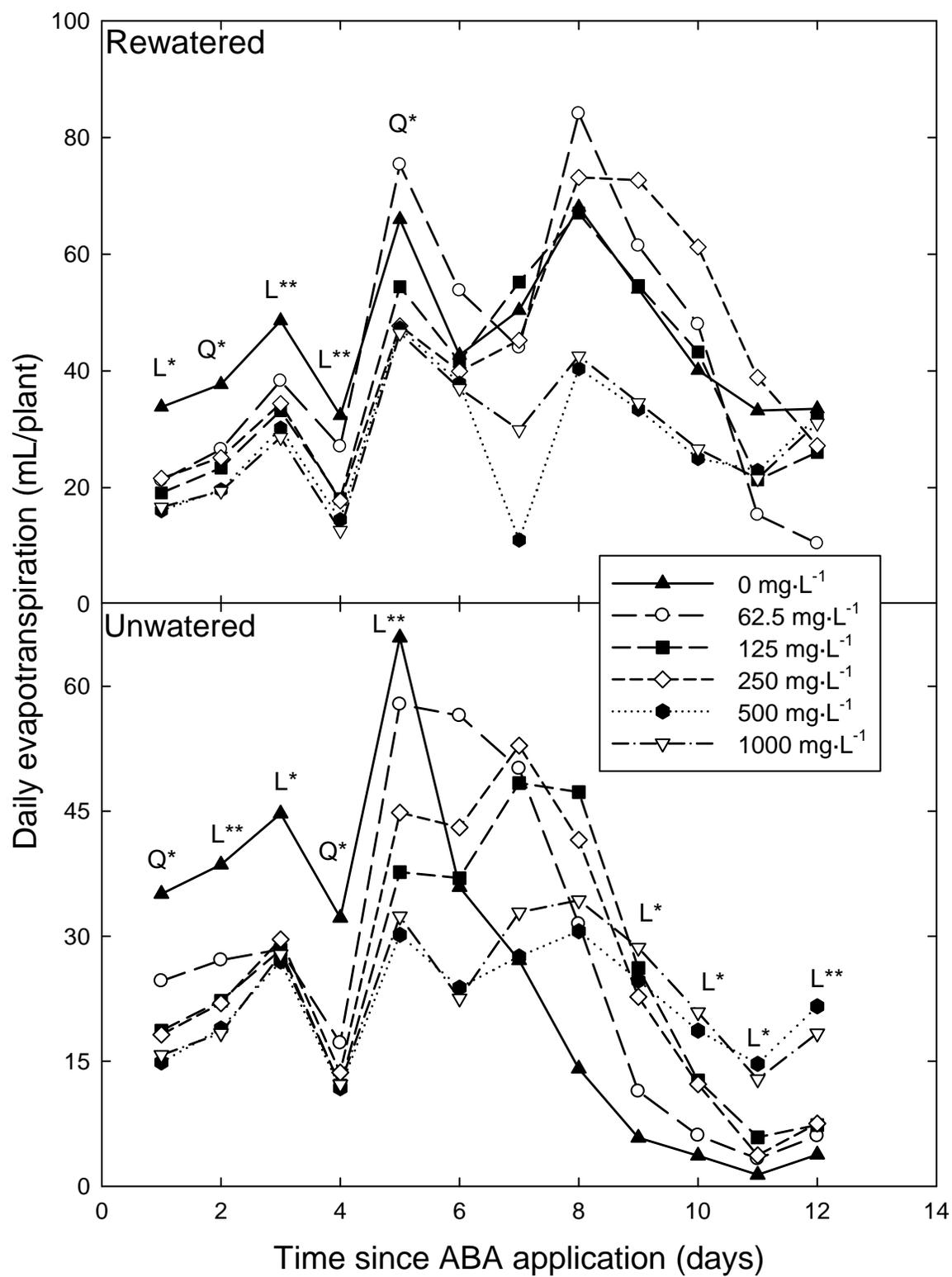


Figure 3.3. Daily water use of tomato during a 13 d period as affected by drenches with solutions with different concentrations of ABA. Plants were either rewatered (top) or unwatered (bottom) following ABA application. Significant effects of ABA were first noted 1 d after ABA application (* = $P < 0.05$), (** = $P < 0.01$); L = linear, Q = quadratic effects of $\log([\text{ABA}] + 50)$.

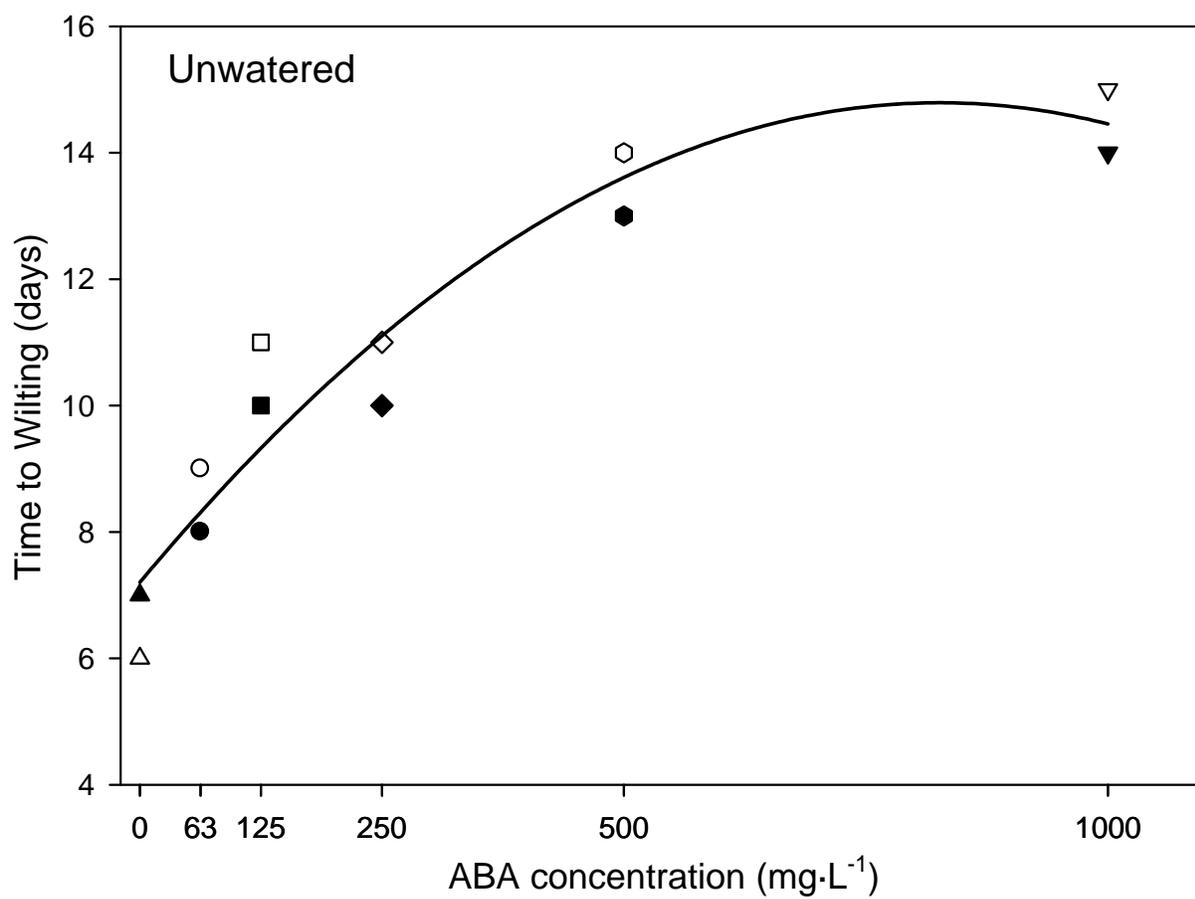


Figure 3.4. The effect of drenches with ABA solutions of different concentrations on time to wilting of tomato plants ($R^2 = 0.90$, $P < 0.0001$). Plants were not rewatered after the initial ABA applications.

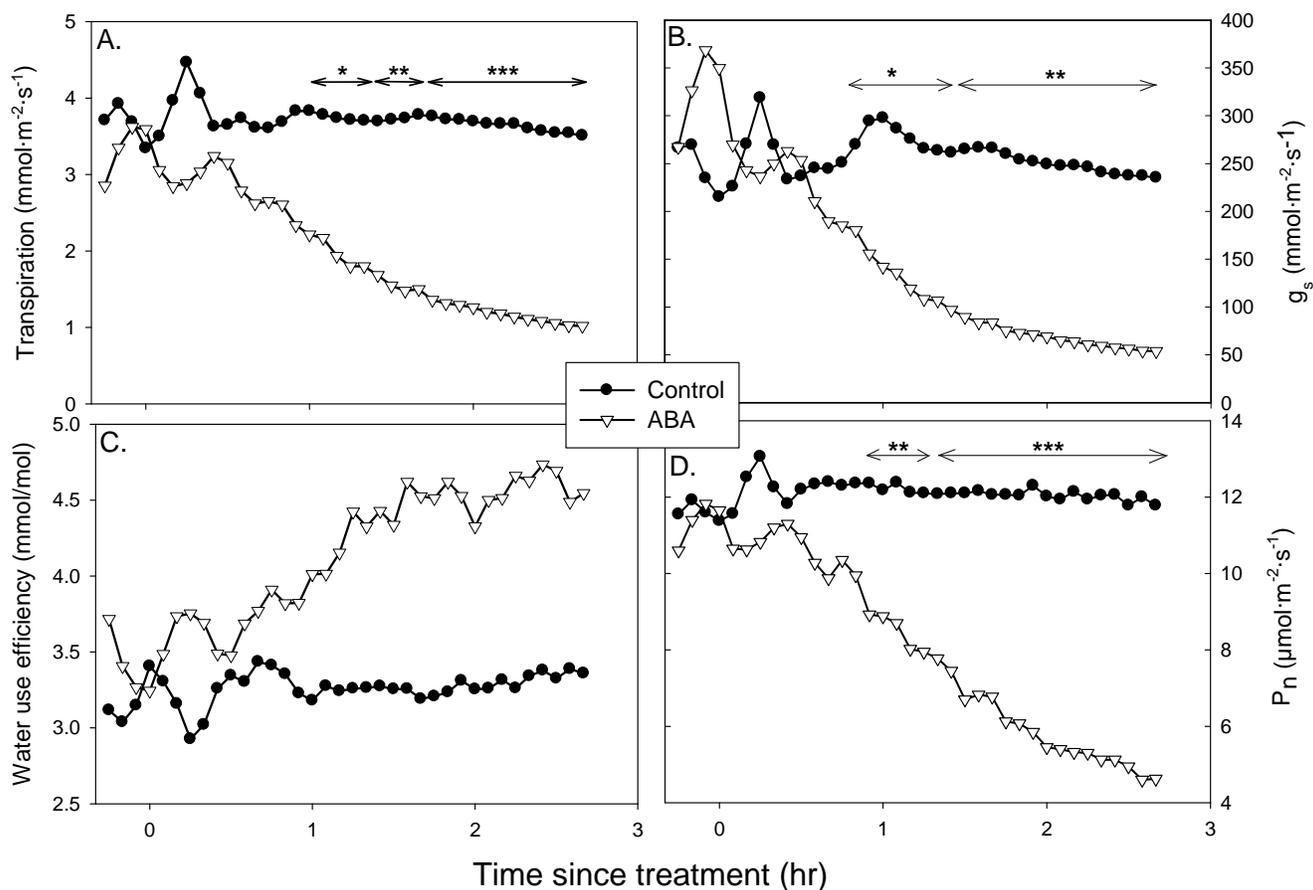


Figure 3.5. Transpiration (A), stomatal conductance (g_s , B), water use efficiency (C), and photosynthesis (P_n , D) of tomatoes drenched with 100 mL of either deionized water (control, $n=5$) or 250 mg·L⁻¹ ABA solution ($n=4$) (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, no significant effects on water use efficiency). Plants were treated at time “0”.

CHAPTER 4

**CONCENTRATED EXOGENOUS ABA DRENCHES REDUCE ROOT HYDRAULIC
CONDUCTANCE AND CAUSE WILTING IN TOMATO (*Solanum lycopersicum*)²**

²Astacio, M.G. and M.W. van Iersel. To be submitted to HortScience.

Abstract

Previous work has shown that exogenous ABA applications can greatly reduce transpiration and extend the shelf life of unwatered plants. Paradoxically, we have seen that concentrated ABA drenches may actually induce wilting. These wilting symptoms occur despite the presence of ample water in the substrate, suggesting that ABA may interfere with the ability of roots to take up water. Our objective was to develop a better understanding of this wilting using tomato (*Solanum lycopersicum*) as a model. In our first study, ABA applied as a drench reduced transpiration and water consumption, yet the relative water content (RWC) of the leaves of ABA-treated plants dropped. Control plants had a leaf RWC of 97%, whereas plants treated with 2000 mg·L⁻¹ ABA had a RWC of 60%, which resulted in wilting. Leaf ABA concentrations ranged from 2.6 (control) to 62.6 nmol·g⁻¹ fresh weight (FW) in the 1000 mg·L⁻¹ ABA treatment, indicating effective transport of ABA from the roots to the leaves. The reduced leaf RWC suggests that ABA drenches are limiting water transport through the roots to the leaves. The effects of ABA on the hydraulic conductance of the roots and stems of tomatoes were quantified to determine if ABA drenches limit water transport through the roots. The volume of water conducted by the root systems during a four-day period ranged from 36.7 mL in the control treatments to 8.1 mL in roots systems drenched with 1000 mg·L⁻¹ ABA, a reduction of 78%. When the conductance study was repeated using decapitated roots and excised stems, root water flux was again affected by ABA, but water flux through internodal stem sections did not show an ABA effect. Results suggest that ABA-induced wilting is caused by a reduction in root conductance and we hypothesize that ABA affects aquaporins in the roots, limiting water uptake.

Introduction

A common problem in garden centers is that the plants may not receive enough water. Inadequate watering rapidly diminishes a plant's salability and drastically shortens its shelf life (Armitage, 1983). Researchers have been looking for ways to reduce transpiration and potentially increase the shelf life of plants. Due to a breakthrough in microfermentation production, abscisic acid (ABA) has recently become a commercially feasible growth regulator to help plants maintain their salability longer (Runkle, 2009). ABA increases the shelf life of salvia (*Salvia splendens*) by up to 5 d (Kim and van Iersel, 2011) and of hydrangea (*Hydrangea macrophylla*) by up to 11 d (van Iersel et al., 2009).

Although named for its supposed role in fruit and leaf abscission, ABA has since been found to be a part of a myriad of plant developmental and physiological processes, including acclimation to drought and other stresses. ABA is produced in response to drought, and its concentration in the leaves is negatively correlated with stomatal aperture (Mahdieh and Mostajeran, 2009; Walton, 1980), thus limiting water lost due to transpiration (Franks and Farquhar, 2001; Jiang and Hartung, 2008). In addition, once a plant is exposed to well-watered conditions, the leaf concentration of ABA rapidly decreases and stomatal conductance returns to its pre-stress state (Franks and Farquhar, 2001).

ABA effectively extends the shelf life of plants by limiting water consumption. ABA extended the shelf life of various bedding plant species by 1 d (*Verbena ×hybrida*) to almost 6 d (*Impatiens hawkeri*) (Blanchard et al., 2007). However, side effects may occur when using high concentrations of ABA, such as chlorosis and leaf abscission (Kim and van Iersel, 2011). We have also noticed that ABA drenches may induce wilting, even though the substrate is still moist. The cause of this ABA-induced wilting is not understood. However, similar symptoms have

been described in tomato when it is exposed to flooding, which decreased both leaf and root hydraulic conductance, with root water flux decreasing to 50% of the pre-stress level within 8 h (Bradford and Hsiao, 1982). Bradford and Hsiao (1982) concluded that stomatal closure, along with petiole epinasty, is a flooding response used to maintain leaf water potential. They also concluded that ABA may be responsible for closing stomata, and other work reported that leaves of flooded tomato plants experienced a 6-8 fold increase in ABA content (Hiron and Wright, 1973). However, it was not certain how ABA was accumulating in leaves without a loss of turgor and anaerobic root conditions were thought to be the cause of this accumulation (Bradford and Hsiao, 1982).

Exogenous ABA injection applications ($13 - 53 \text{ mg}\cdot\text{L}^{-1}$) into a pressure chamber diminished conductance of decapitated soybean (*Glycine max* L.) root systems over the course of 3-4 hours (Markhart et al., 1979). However, other findings support the idea that ABA increases root conductance. Ion and volume exudation rates of excised sunflower (*Helianthus annuus* L.) root systems were increased when the roots were transferred to jars containing $1 \text{ mg}\cdot\text{L}^{-1}$ ABA solution and the increased solute flux into the roots elevated the osmotic flow of water (Glinka, 1980).

The conclusions of past studies on root conductance and ABA are sometimes contradictory. Hose et al. (2000) examined excised root cells of maize (*Zea mays* L.) with pressure probes and concluded that $0.01 - 1 \text{ mg}\cdot\text{L}^{-1}$ ABA transiently increased hydraulic conductance of root cells for a few hours by stimulating the opening of water channels (aquaporins) in the cell membranes. Conversely, $20 \text{ mg}\cdot\text{L}^{-1}$ ABA impacted the root systems of common bean (*Phaseolus vulgaris*) seedlings in four ways; it increased volume flux, stimulated a pulsed release of solutes into the xylem, which led to a gradual increase in solute flux, and there

was a decline in root conductance after several hours had passed (Fiscus, 1981). This corroborates the findings of Markhart et al. (1979), who determined that ABA limits root hydraulic conductance as a means of protecting the membranes from cold stress damage.

More recent studies have again produced contradictory findings: tobacco (*Nicotiana tabacum*) plants exposed to $0.26 \text{ mg}\cdot\text{L}^{-1}$ ABA applied as a hydroponic nutrient solution had increased root and cell conductance compared to control plants within 24 h and expressed more genes involved in the development of plasma membrane intrinsic protein (PIP)-type aquaporins, suggesting that ABA activates and regulates the production of aquaporin water channels (Mahdih and Mostajeran, 2009). One common factor among the studies that concluded that ABA increased root conductance is that the increase was transient, lasting a few hours. It is possible that ABA temporarily increases water uptake before limiting it by causing morphological changes to the roots (i.e., suberization) as a means of protecting the plant from drought stress. Zhang et al. (1995) found that maize seedlings (*Zea mays*) long exposed to drought conditions were able to return to pre-drought hydraulic conditions within a few days after rewatering and that a flush of new root growth aided in increasing conductance. A short-term increase in root conductance was also noted on both briefly and mildly droughted maize seedlings (Zhang et al., 1995), which may be due to ABA temporarily increasing conductance before limiting it. Presently, research centered on the effects of ABA on the roots is still controversial and future studies will need to be conducted to fully elucidate the impact that ABA has on water transport channels in the cell membranes and overall root conductance, both in the short- and long-term.

The objectives of our studies were to examine the effects of ABA drenches on the leaf physiology of tomato and determine the effects of ABA on root and stem conductance, in order

to better understand the ABA-induced wilting. Tomatoes were used as the model crop because they are sensitive to ABA applications (Sharma et al., 2006) and wilt rapidly when exposed to high ABA concentrations, although ample water is present in the substrate.

Materials and Methods

Leaf Physiological Responses

Tomatoes ‘Supersweet 100’ were seeded in 72-cell trays in a glass greenhouse and were grown on an ebb and flow bench providing a $100 \text{ mg}\cdot\text{L}^{-1}$ N fertilizer solution (15-5-15 Cal-Mag, Scotts, Marysville, OH; 15N-2.2P-12.45K) for 14 d following germination. Seedlings were transplanted on 28 Feb. 2011 into 15 cm diameter round pots filled with soilless substrate (Fafard 1P, Conrad Fafard Inc., Agawam, MA) and then grown under an overhead irrigation system until the plants reached a mature leaf stage. The plants each received 11.7 grams of controlled release fertilizer (14-14-14 Osmocote, Scotts, Marysville, OH; 14N-6.2P-11.6K). The tomatoes were transferred to a growth chamber (E-15, Conviron, Winnipeg, Manitoba, Canada) on 10 Mar. 2011 and given 3 d to acclimate to the conditions within, during which time they were hand watered. The growth chamber was set to 12 h days at $25 \text{ }^{\circ}\text{C}$ with an average photosynthetic photon flux of $405 \text{ }\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, and 12 h nights at $21 \text{ }^{\circ}\text{C}$. At the commencement of the experiment, the pots were placed inside plastic bags sealed around the stems of the plants to prevent evaporation. The weights of the plants were measured with load cells (LSP-1 and LSP-2, Transducer Techniques, Temecula, CA) connected to a data logger (CR10, Campbell Scientific, Logan, UT) to determine cumulative transpiration.

An ABA stock solution (10% w/v s-ABA, the biologically-active form of ABA, VBC-30101, Valent BioSciences, Long Grove, IL) was diluted with deionized water to yield

concentrations of 0, 125, 250, 500, 1000, and 2000 mg·L⁻¹ ABA. Drench applications of ABA were made on 13 Mar., 2011 and symptoms of the ABA-induced wilt were noted within 24 h of application. At this time, measurements of leaf net photosynthesis (P_n), transpiration (E), and stomatal conductance (g_s) were taken with a portable gas exchange system (CIRAS-2, PP Systems, Amesbury, MA) on the uppermost fully-expanded leaf of each plant. Substrate water content (SWC) was measured using a soil moisture sensor (Theta Probe ML2x, Delta-T Devices, Burwell, Cambridge, UK). Relative water content (RWC) data were taken by sampling 12 leaf discs from each of the treatment groups (3 discs × 4 replications per ABA treatment rate). Leaf discs were weighed initially to determine fresh weight (FW). They were then placed in a Petri dish with deionized water overnight to establish a turgid weight (TW). Then the discs were dried in an oven at 75 °C for one day to determine dry weight (DW). Once the fresh, turgid and dry weights for the leaf discs were determined, RWC was calculated using the following equation:

$$\text{RWC} = ((\text{FW}-\text{DW})/(\text{TW}-\text{DW})) \times 100\%.$$

Fresh leaf samples were collected from each plant and immediately frozen in liquid nitrogen to be used for ABA quantification using an enzyme-linked immunosorbent assay (ELISA) kit (PDK 09347, Agdia, Elkhart, IN). Leaf samples were kept at -80 °C until the assay was completed. The ELISA assay was done according to the protocol from Serrano et al. (1995) with minor adjustments. The samples were ground into a powder using liquid nitrogen and 0.75 g of ground leaf tissue was mixed with 15 mL of extraction buffer (80% methanol). Samples were then incubated for 16 h on a shaker at 4 °C. Samples were then centrifuged and supernatant was collected and stored at -20 °C to be used in the assay. Because of the high ABA concentrations, the samples were further diluted with tris(hydroxymethyl)aminomethane-buffered saline solution to 1:100, 1:1000, and 1:10000 ratios. Controls were diluted to a ratio of

1:100. ABA concentrations were then quantified using ELISA following the manufacturer's instructions. Leaf ABA concentrations are expressed on a fresh weight (FW) basis.

The experimental design was a randomized complete block with 4 replications and six concentrations of ABA. The experimental unit was an individual plant. The load cell data were analyzed using regression (SAS 9.2, SAS Institute, Cary, NC). Since hormonal effects on plant physiology are generally not directly proportional to the hormonal concentration, ABA concentrations were transformed using $\log([ABA]+50)$ before testing for linear and quadratic effects of ABA, and significance was analyzed for 10 min intervals of data separately. Data for E , g_s , P_n , SWC and RWC were analyzed using ANOVA followed by mean separation using Fisher's protected LSD. Leaf ABA concentrations were \log_{10} transformed to meet the assumption of equal variance, before fitting a hyperbolic equation.

Root and Stem Hydraulic Conductance

Two studies were done to look at conductance. For the first study, tomatoes 'Supersweet 100' were seeded in 72-cell trays and grown in a glass greenhouse. Following germination, plants were transplanted into 10 cm diameter round pots filled with soilless substrate (Fafard 2P, Conrad Fafard, Agawam, MA). At the time of transplanting, each plant received 3.1 g controlled release fertilizer (14-14-14 Osmocote, Scotts, Marysville, OH; 14N-6.2P-11.6K) and the plants were watered daily. The ABA stock solution (10% w/v s-ABA, VBC-30101, Valent BioSciences) was diluted with deionized water to yield concentrations of 0, 62.5, 125, 250, 500, and 1000 $\text{mg}\cdot\text{L}^{-1}$. This range of concentrations was chosen based on prior knowledge that high ABA concentrations induced wilting in tomatoes.

At the commencement of the experiment (17 Feb., 2010), the shoots were cut off below the first node and the remaining portions of the root/shoot junction were inserted into plastic

tubing (Bev-a-Line IV, ThermoPlastic Processes, Georgetown, DE; internal diameter 6.3 mm). The stems were sealed to the inside of the tubes with a silicone gel (3140 RTV coating, Dow Corning, Midland, MI) and the stem/tube connection was sealed with Parafilm to prevent leaks. All tubes were connected to a manifold, which was connected to a vacuum pump (MOA-P122-AA, Gast Mfg. Corp., Benton Harbor, MI) to simulate transpirational pull, with an approximate suction of 14 kPa (Fig. 4.1). Water levels in the tubes were marked daily for the duration of the study, and these readings and the tubing diameter were used to calculate the water flow through the root systems. Once the vacuum pump was turned on, initial readings of water flux were taken prior to ABA application for 3 h to determine an unstressed conductance baseline. Applications were made as a 50 mL drench of ABA solution.

The experiment was designed as a randomized complete block with four replications and six ABA concentrations. The experimental unit was an individual plant. The study was terminated 9 d following initiation, at which time root conductance in all treatments was minimal. ABA concentrations were transformed using $\log([\text{ABA}] + 50)$ before testing for linear and quadratic effects of ABA on root hydraulic conductance. The data were analyzed using linear and quadratic regression in the SAS program (SAS 9.2, SAS Institute, Cary, NC).

For the second study, tomatoes ‘Supersweet 100’ were seeded in 72-cell trays and grown in a glass greenhouse. Following germination, plants were transplanted into 10 cm diameter round pots filled with soilless substrate (Fafard 2P, Conrad Fafard) and grown under an overhead irrigation system for 21 d. The plants each received 2.6 g controlled release fertilizer (14-14-14 Osmocote, Scotts; 14N-6.2P-11.6K). At the commencement of the experiment (6 Jul., 2010), the pots were well-watered. Shoots were cut off approximately 5-6 cm above the substrate surface, and about 4 cm long, internodal stem sections with a diameter of about 0.75 cm wide

were cut off from the remaining shoots. The stem sections and root systems were then sealed into plastic tubes as described above and the tubes were connected to a vacuum pump (Fig. 4.1). The bases of the stem sections were placed in beakers with 100 mL of deionized water (Fig. 4.2).

Once the pump was turned on (providing average suction of 14 kPa), initial readings of water movement through the root systems and stem sections were taken prior to ABA application for 3 h to determine an untreated conductance baseline. ABA solutions were prepared as described above. All treatment applications to the root systems were made as a 100 mL drench of either ABA solution or deionized water (control). For the stem sections, the deionized water in the beakers was replaced with either 100 mL of ABA solution or fresh deionized water (control). The water levels in the tubes were marked at various times, and these marks were used to determine the cumulative water flow through the roots or stems. At the end of the study, the root systems were washed off and visually examined for any detrimental side effects.

The experiment was a randomized complete block design with a split plot with two replications. The main treatment factor was the ABA concentration (6 rates) and the split consisted of root systems versus stem sections. Individual root systems or stem sections were the experimental unit. The study was conducted twice, and both trials returned similar results. Data were analyzed using regression analysis (SAS 9.2, SAS Institute).

Results and Discussion

Leaf Physiological Responses

After one day, the plants treated with the highest doses of ABA (500-2000 mg·L⁻¹) were observed to have wilt symptoms, whereas control plants and plants treated with lower concentrations of ABA (125-250 mg·L⁻¹) appeared turgid. Cumulative transpiration data show

that transpiration rates were similar prior to the application of ABA (results not shown). Following ABA treatment, differences in transpiration became apparent. Cumulative transpiration decreased with increasing ABA concentration. At 60 min following ABA application, cumulative transpiration was highest in the lowest ABA concentration (control treatment, $P < 0.01$, Fig. 4.3), and this continued for the remainder of the study. At 28 hr post-ABA application, the control plants maintained the highest transpiration, culminating in about 317 mL cumulative transpiration since treatments were applied. By this time, the plants treated with $125 \text{ mg}\cdot\text{L}^{-1}$ ABA had transpired about 95.1 mL, a reduction of 70% when compared to the control treatments (Fig. 4.3), and those treated with $2000 \text{ mg}\cdot\text{L}^{-1}$ ABA treatment had transpired 40 mL, a reduction of 87% compared to the control plants (Fig. 4.3).

The whole plant transpiration results were consistent with our leaf gas exchange measurements. At the end of the experiment, control plants had a g_s of $127 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, compared to $18 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for the $2000 \text{ mg}\cdot\text{L}^{-1}$ ABA treatments, and effects on E were similar (Table 4.1). Thus, ABA effectively limited transpirational water loss. As a result, the substrate water content tended to increase with increasing ABA concentration, but this was not significant (Table 4.1). These substrate water data show that the ABA-induced wilt is not related to a lack of water in the substrate since the substrate water contents were all similar, but only plants treated with $500\text{-}2000 \text{ mg}\cdot\text{L}^{-1}$ ABA were wilting. These data also show that the plants were not exposed to extreme flooding conditions, and that our reductions in flow were not related to a flooding response, as was concluded by Bradford and Hsiao (1982). All ABA concentrations also reduced P_n . Control plants had a P_n of $4.26 \text{ }\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, compared to $-2.13 \text{ }\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for the $2000 \text{ mg}\cdot\text{L}^{-1}$ ABA treatment (Table 4.1). Relative water contents of the leaf discs showed

significant differences between the ABA-treated plants and control plants. Control leaves had a RWC of 97%, whereas 2000 mg·L⁻¹ ABA treatments had a RWC of 60% (Table 4.1).

ABA concentrations in the leaves increased as the ABA dose increased (Fig. 4.4). Control plants had an ABA concentration of 2.57 nmol·g⁻¹ FW. This concentration is slightly higher than previously reported endogenous ABA levels for unstressed tomatoes, which range from 0.49 to 2.4 nmol·g⁻¹ FW (Neill and Horgan, 1985; Sharp et al., 2000; Thompson et al., 2007). The ABA concentrations of ABA-treated plants ranged from 12.5 (125 mg·L⁻¹ ABA) to 62.6 nmol·g⁻¹ FW (2000 mg·L⁻¹ ABA), 24× higher than that in the control plants (Fig. 4.4). The higher concentration ABA treatments resulted in leaf ABA concentrations that are higher than most previously reported, stress-induced endogenous levels. Water-stressed tomatoes have shown increases of anywhere from 2-22× the unstressed ABA levels, although typically reported increases fall within increases in the 10-15 fold range (Bray, 1988; Neill and Horgan, 1985; Thompson et al., 2007). Thompson et al. (2007) reported a 2× increase in ABA concentration to 3.6 nmol·g⁻¹ FW in drought-stressed tomato, whereas Neill and Horgan (1985) reported an ABA concentration of 15 nmol·g⁻¹ FW, or a 10× increase, in their drought-stressed tomato. The 10-15× increases would roughly compare to concentrations seen in our 250 and 500 mg·L⁻¹ ABA treatments (Fig. 4.4). Increases of ABA in tomatoes due to other stresses vary, with endogenous ABA concentrations increasing 1.5 to 3× in response to cold stress (Daie and Campbell, 1981) and salt stress eliciting a 2× increase of ABA concentrations (Chen and Plant, 1999).

We found that ABA quickly and effectively closed stomata and limited transpirational water loss. Despite the plants all having similar substrate water contents and ABA-treated plants exhibiting decreases in transpiration, we also determined that the RWC of leaves from ABA-treated plants was lower than in control plants. In combination with reduced transpiration, low

RWC in the leaves suggests an impairment of water transport through the roots to the leaves, which could be a possible explanation for the ABA-induced wilt.

Root and Stem Hydraulic Conductance

Pre-treatment measurements of water flow through the root systems showed no differences among the plants (Fig. 4.5). ABA drenches quickly affected water flow through the root systems. Roughly 2-3 hours post-ABA application, a decrease in flow rate through the roots was evident as ABA concentration increased ($P < 0.05$, Fig. 4.5, bottom). This translated into a decrease in cumulative root flow for ABA-treated plants within one day following ABA application ($P < 0.001$, Fig. 4.5, top). These differences persisted for both flow rate and cumulative flow for the remainder of the study. Root flow rates decreased in all treatments during the first 3 d, and this was most pronounced in ABA-treated plants (Fig. 4.5). By the termination of the experiment, control plants had a flow rate of $1.7 \mu\text{L}/\text{min}$, whereas plants treated with $1000 \text{ mg}\cdot\text{L}^{-1}$ had a flow rate of $0.25 \mu\text{L}/\text{min}$, a reduction of 85% (Fig. 4.5). No sloughing or necrosis of the roots was visible at the termination of the study.

The results are consistent with our hypothesis that reductions in the hydraulic conductance of the roots in response to ABA may be the cause for the ABA-induced wilting. Our results corroborate earlier findings that root hydraulic conductance was reduced in the long-term by ABA treatment (Davies et al., 1982; Fiscus, 1981; Markhart et al., 1979). Other studies have reported contradictory results that ABA increases root hydraulic conductance. Glinka (1977) found that treating decapitated root systems of sunflower with $1 \text{ mg}\cdot\text{L}^{-1}$ ABA resulted in a higher exudation rate and proposed that ABA is affecting the water permeability of the endodermal layer. Glinka (1980) conducted further work with ABA on sunflower root systems and reported that treating the roots with $1 \text{ mg}\cdot\text{L}^{-1}$ ABA solution increased flux of K^+ and NO_3^-

ions into the xylem as well as increasing the hydraulic conductance of the roots. Cornish and Zeevaart (1985) conducted studies exposing tomato roots to drought stress and found that endogenous ABA was progressively produced and accumulated in the roots, and it was concluded that the plant may accumulate ABA to modify its water use prior to the leaves experiencing wilting. Secondary hardening of roots in response to drought stress was noted but was not substantial, although this study was measuring endogenous ABA and did not employ concentrated exogenous ABA applications as we did (Cornish and Zeevaart, 1985). In addition, the role of ABA during cold acclimation seems to affect root conductance. When soybean roots were treated with $13 \text{ mg}\cdot\text{L}^{-1}$ ABA and subsequently exposed to $10 \text{ }^{\circ}\text{C}$, root hydraulic conductance was higher in ABA-treated plants than control plants and ABA helped to mitigate cold stress damage (Markhart, 1984). This increase in root hydraulic conductance in response to ABA treatment and cold stress was rapid (within 4 hr) and similar to the transient increases seen in conductance of wheat roots when chilled and treated with ABA (Davies et al., 1982). An important point to consider is that these past studies either worked with endogenous ABA or applied it at a much lower concentration than the rates used in our current study, and that contradictory results may be due to differences in methodology.

ABA also affects symplastic transport pathways, such as aquaporins, to regulate transpiration (Mahdieh and Mostajeran, 2009; Hose et al., 2000). ABA had a role in aquaporin regulation in intact tobacco plants and increased root hydraulic conductance by stimulating the expression of genes that control the development of PIP (plasma membrane intrinsic protein)-type aquaporins, suggesting that ABA regulates the production of these water channels (Mahdieh and Mostajeran, 2009). Endogenous ABA may interact with these channels during periods of drought stress, and root water flux may be coordinated with stomatal conductance via aquaporin

activity (Tyerman et al., 2002). Wan et al. (2004) concluded that ABA could potentially increase transcellular water flow by gating aquaporin channels and preventing them from collapsing. This agrees with the suggestion that ABA may be responsible for the transient water flux increases through maize root cells by regulating aquaporins (Hose et al., 2000). Aquaporin involvement may also explain that ABA was altering membrane permeability during periods of cold stress to increase water flux (Markhart, 1984), a finding reported well before the first detailed description of aquaporins in 1993 (Agre et al., 1993). This area of study requires further research to fully understand the signaling pathway between ABA and aquaporin channels and how much of an effect aquaporins can have on overall root water flux.

In the root system and stem section study, as ABA concentration increased, cumulative water flow through the roots decreased (Fig. 4.6), indicating a decrease in root conductance. Treatment effects were obvious within 12 hours. At the termination of the experiment, the cumulative flow through roots of the control plants was 27.4 mL, whereas the cumulative flow of plants treated with $1000 \text{ mg}\cdot\text{L}^{-1}$ ABA was only 4.8 mL, a reduction of 83% (Fig. 4.6). There were no visible symptoms of ABA-induced side effects on the appearance of the roots. Results were similar to those of the study looking at root conductance only (Figs. 4.5, 4.6).

Unlike the root systems, water flow through the stem sections was unaffected by ABA, but the flow rate through the stems decreased rapidly after the first day (Figs. 4.6, 4.7). Cumulative water flow through the stem sections was between 29.6 and 36.5 mL, with no trend of an ABA-related effect. The rapid decrease in water flow through the stem sections suggests that the deionized water may have had detrimental effects on the functionality of the stem xylem. When considering rate of flow through the stem and root systems, a similar trend as described above is apparent. ABA reduced the rate of flow through the root systems. The control root

systems averaged a rate of 5.4 $\mu\text{L}/\text{min}$ a few hours following ABA application, whereas the 1000 $\text{mg}\cdot\text{L}^{-1}$ treatment had a rate of 2.6 $\mu\text{L}/\text{min}$, a reduction of 52% (Fig. 4.7). Conversely, the stem sections all had similar rates of flow throughout the study, irrespective of ABA treatment (Fig. 4.7). Thus, ABA did not have an apparent effect on stem cumulative water flow or flow rate, whereas our root systems displayed similar results to the study with root systems discussed above following treatment with ABA. These results are also consistent with other studies that have reported a decrease of root hydraulic conductance in the long term due to ABA (Davies et al., 1982; Fiscus, 1981; Markhart, III et al., 1979).

Conclusions

All exogenous ABA drenches reduced transpiration quickly. However, despite reduced transpiration, the leaves of ABA-treated plants (500-2000 $\text{mg}\cdot\text{L}^{-1}$) wilted. This is supported by the reduction in RWC for all ABA treatments. We also demonstrated that ABA levels in the leaves rose dramatically following drenches with ABA. These results suggest that the ABA-induced wilt may be an effect of unnaturally high ABA concentrations applied as drenches to the roots. Root systems quickly showed a significant ABA-induced reduction in water flux. This suggests that ABA causes reductions in root conductance, which hinders water transport to the shoots and could be responsible for the drops in leaf relative water content and the ABA-induced wilt.

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Table 4.1. Substrate water content and leaf physiological parameters of tomatoes in response to drenches with ABA solutions. Readings were taken 24 hr after ABA treatment application and were analyzed. Means followed by the same letter are not significantly different from each other (Leaf gas exchange: n = 4); (RWC data: n = 2).

ABA concentration	Substrate water content	Photosynthesis	Transpiration	Stomatal conductance	Relative water content
(mg·L ⁻¹)	(m ³ ·m ⁻³)	(μmol·m ⁻² ·s ⁻¹)	(mmol·m ⁻² ·s ⁻¹)	(mmol·m ⁻² ·s ⁻¹)	(%)
0	0.27 a	4.26 a	1.62 a	127 a	97 a
125	0.25 a	0.68 b	0.63 b	57 b	57 b
250	0.32 a	0.75 b	0.46 b	37 b	60 b
500	0.28 a	-2.38 c	0.64 b	51 b	58 b
1000	0.30 a	-0.71 bc	0.22 b	17 b	62 b
2000	0.31 a	-2.13 bc	0.23 b	18 b	60 b

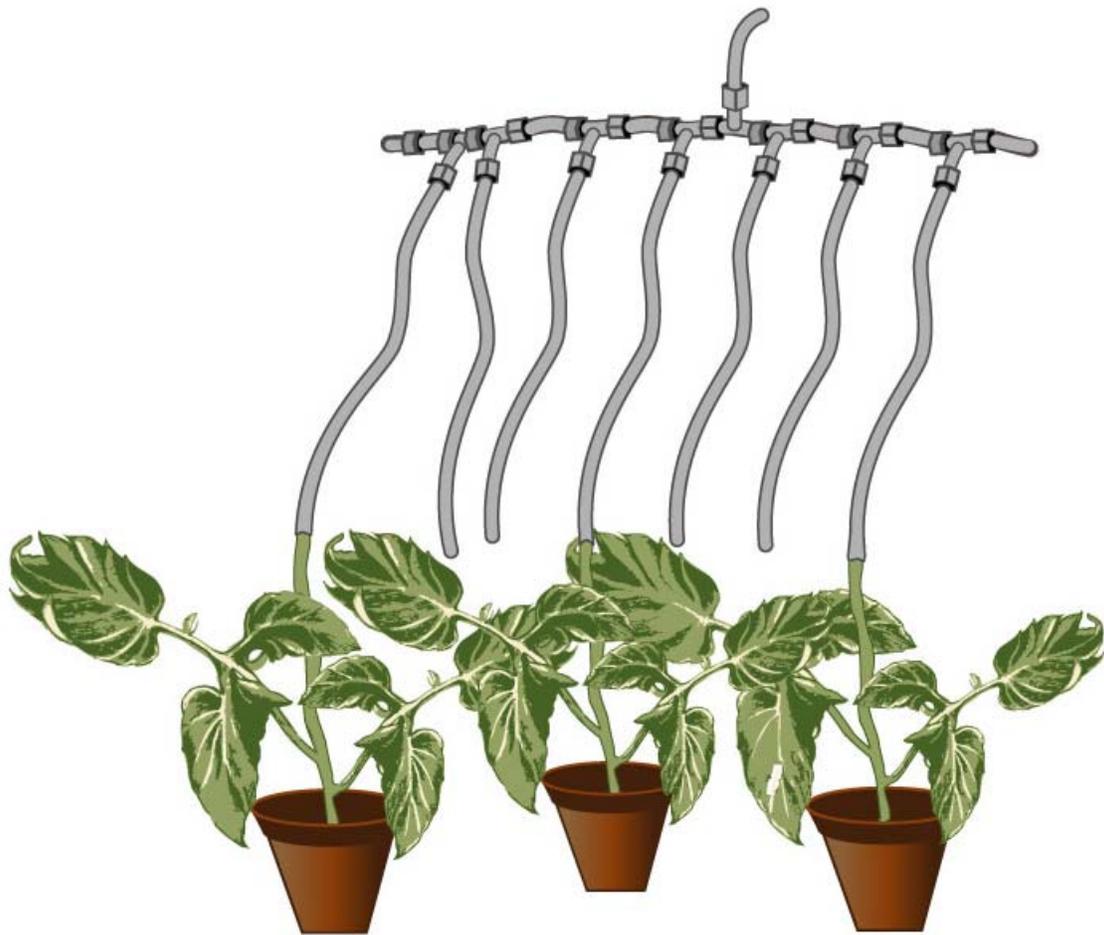


Figure 4.1. A diagram of the tube and vacuum system used to simulate the effects of transpirational pull on stem sections and root systems. Note: all leaves were removed for the experiments, roots were attached to the tubes below the first node, and the vacuum pump is not shown.



Figure 4.2. An image of how root systems and stem sections were sealed to the tubing for conductance measurements.

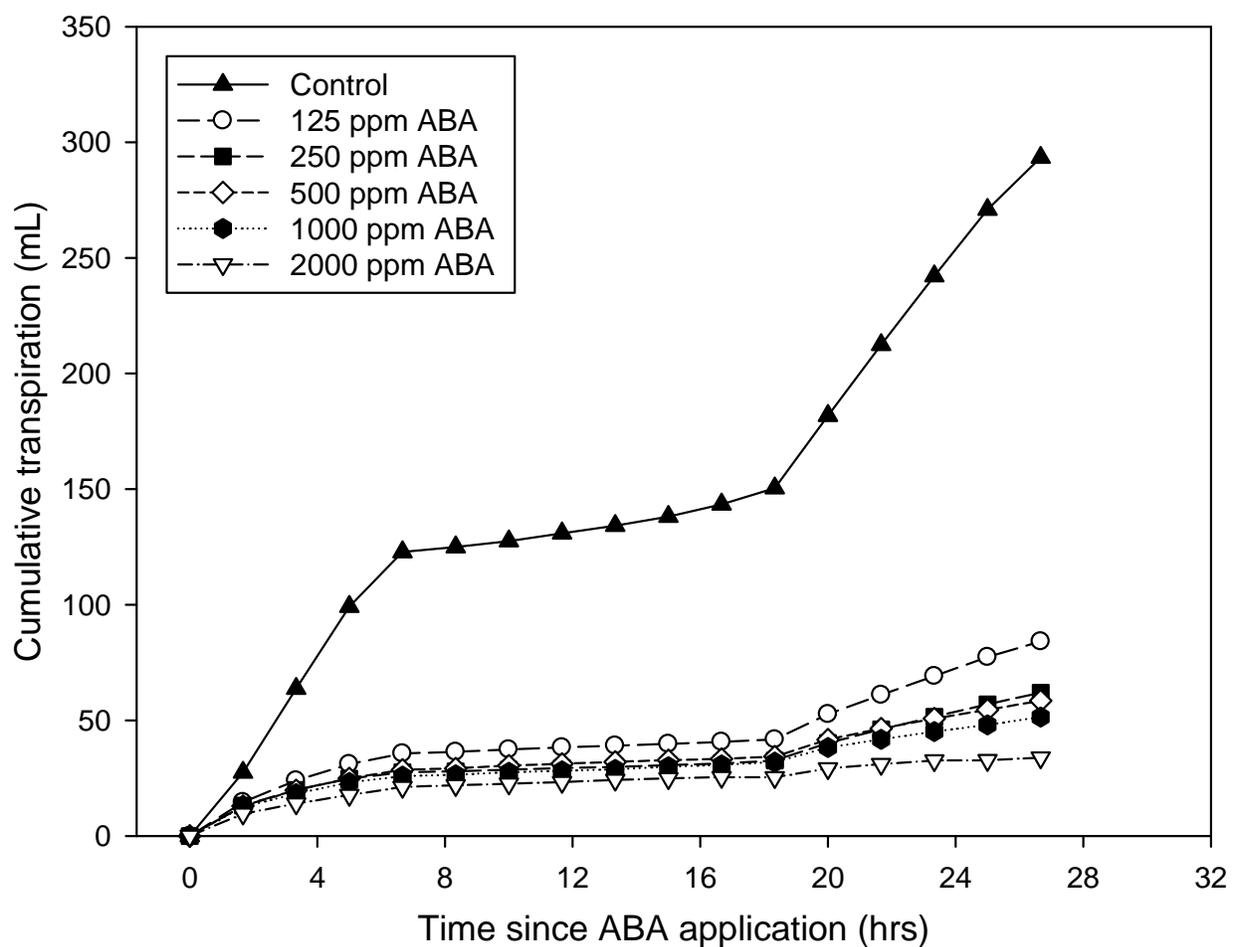


Figure 4.3. Cumulative transpiration of tomato plants over a 28 h period following treatment with ABA drenches. ABA was applied at time “0”. Significant effects of ABA were noted starting 60 min after the ABA application and lasted the duration of the study ($P < 0.01$, linear effects after 60 min; $P < 0.05$, quadratic effects after 70 min and beyond).

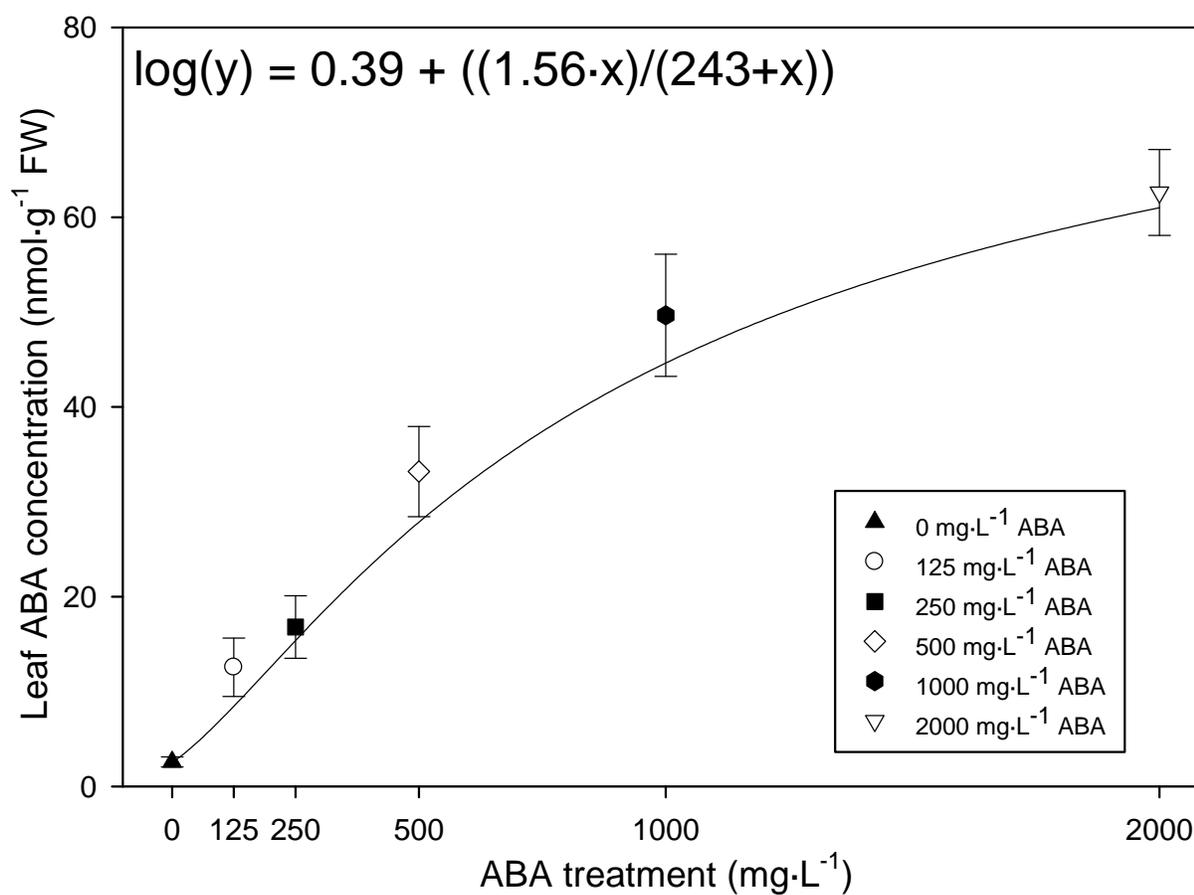


Figure 4.4. ABA concentration in leaves of tomatoes treated with ABA drenches, expressed per unit fresh weight (FW). ABA treatments were made 28 hr prior to leaf sampling. Data represent the averages of 4 repetitions \pm standard error ($R^2 = 0.81$, $P < 0.0001$).

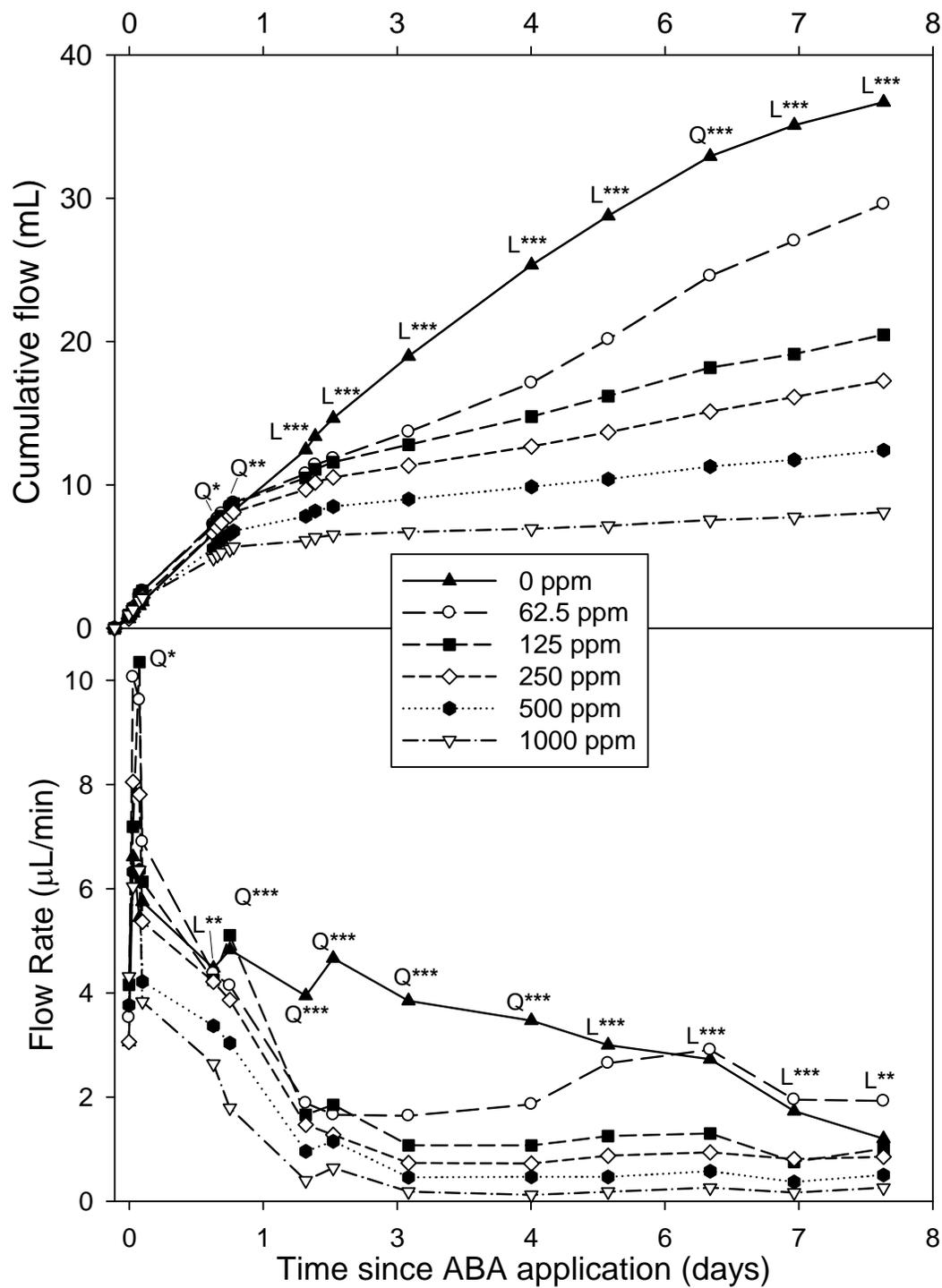


Figure 4.5. Rate of flow and cumulative flow of water through decapitated root systems of tomato. Cumulative flow and rate of flow were both reduced following ABA applications. ABA was applied at time 0 (* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$); L = linear, Q = quadratic effects of $\log([ABA]+50)$.

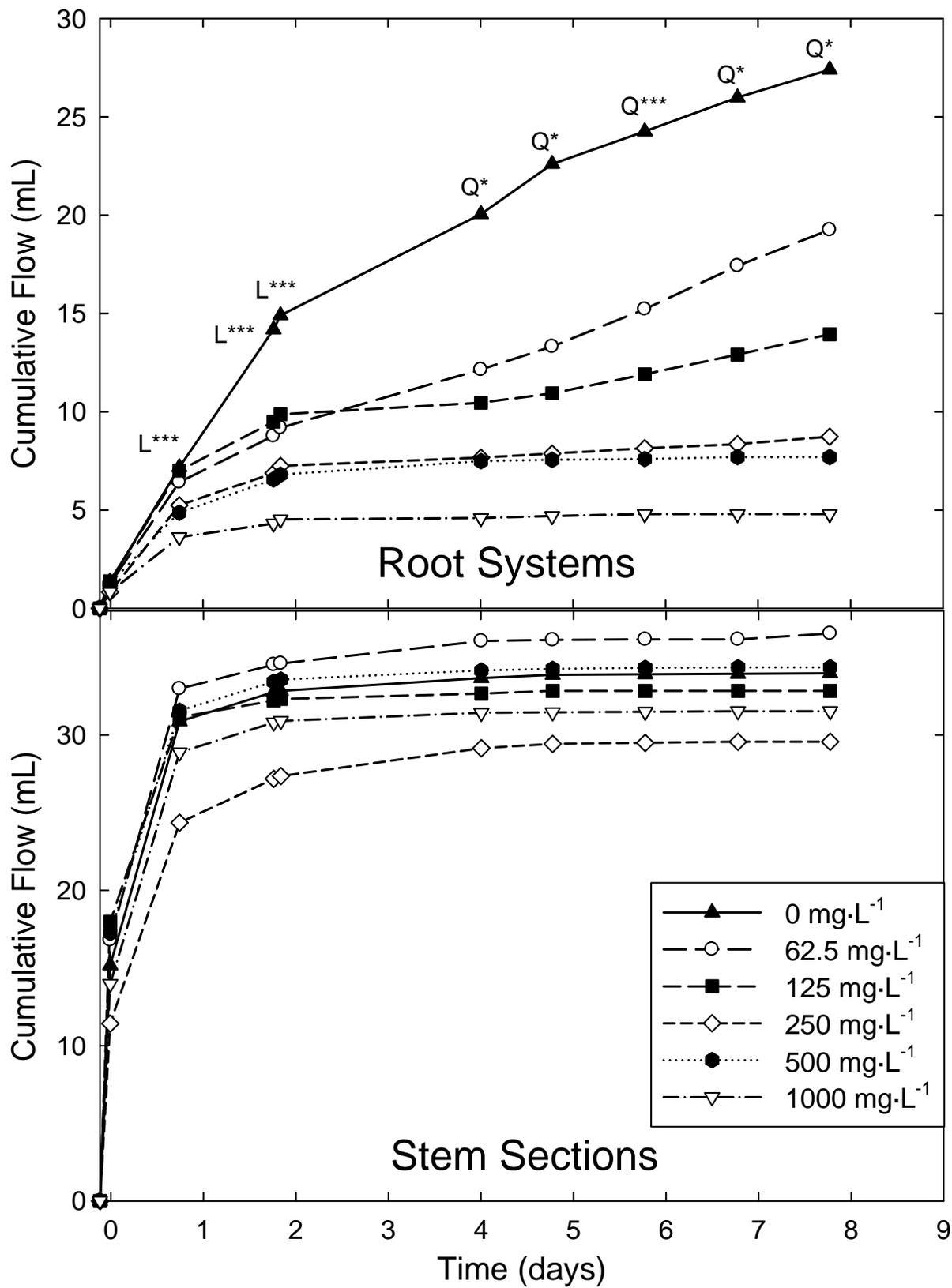


Figure 4.6. Cumulative water flow through decapitated root systems (top) and stem sections (bottom) during an eight day period following drenches with various ABA concentrations. Flow was reduced in roots by ABA. * = ($P < 0.05$), ** = ($P < 0.01$), *** = ($P < 0.001$); L = linear, Q = quadratic effects of $\log([\text{ABA}] + 50)$. No significant effect was observed in the stems ($P > 0.64$).

Figure 4.7. Rate of flow for root systems and stem sections over an 8 d period following ABA application. * = ($P < 0.05$), ** = ($P < 0.01$), *** = ($P < 0.001$); L = linear, Q = quadratic effects of $\log([ABA]+50)$. No significant effects were noted in stem sections ($P > 0.22$).

CHAPTER 5: SUMMARY AND CONCLUSIONS

The use of ABA as a commercial means to extend the shelf life of retail plants shows promise. We have shown that ABA drenches quickly and effectively closed stomates in tomato, thus limiting transpirational water loss. Our short-term study showed that ABA reduced transpiration and stomatal conductance within 60 min following application. This effect translated into a reduction of water use in rewatered plants for a 5 d period, while the shelf life of unwatered plants was extended by 6 d. Shelf life extension would be beneficial during shipping and in lengthening the salable period of retail plants. Negative side effects of the ABA drenches, including chlorosis and leaf abscission, were noted and generally became more pronounced as ABA concentration increased. The lowest effective rate of ABA should be applied to extend shelf life without imposing side effects.

One other side effect that plants drenched with concentrated ABA exhibit is wilting, although the stomata were tightly closed. We found that highly concentrated exogenous ABA drenches closed stomata and reduced transpiration quickly. However, despite reduced transpiration, the leaves of ABA-treated plants still demonstrated wilt. This is supported by our relative water content data, which show a reduction in leaf water content for all ABA treated plants. We have also demonstrated that ABA drenches increase the ABA concentration in the leaves dramatically. These results suggest that the ABA-induced wilt may be an effect of unnaturally high ABA concentrations applied as drenches to the roots, which causes a decrease in water transport to the leaves. In Chapter 4, we show that root systems quickly show a significant ABA-induced reduction in water flux. This research suggests that ABA causes

reductions in root conductance, which hinders water transport to the shoots and could be responsible for the drops in leaf relative water content and wilting symptoms.

Because of the ABA-induced wilt and other detrimental side effects associated with applying ABA as a concentrated drench, future research may wish to explore the effectiveness of foliar applications exclusively. Foliar sprays of ABA require a surfactant to facilitate the uptake of the chemical by the plant, and studies have shown that ABA sprays with surfactant are effective on some bedding plants. Further work will need to be done on a wide array of bedding plants using a range of ABA rates to determine if foliar applications can be used and still successfully control transpiration and lengthen shelf life consistently.