To Mom and Dad for all their love and support.
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WHOLE PLANT RESPONSE OF CHRYSANTHEMUM TO PACLOBUTRAZOL, CHLORMEQUAT CHLORIDE, AND (S)-ABSCISIC ACID AS A FUNCTION OF EXPOSURE TIME USING A SPLIT-ROOT SYSTEM

By

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Paclobutrazol and chlormequat chloride inhibit production of gibberellins and internode elongation whereas (S)-abscisic acid is used as an anti-transpirant to increase postharvest longevity while maintaining aesthetic quality of plants exposed to drought. Little is understood of the timing of chemical movement into the plant following drench applications to peat based media. The split-root system was developed to evaluate chemical uptake in a whole plant system, similar to commercial application situations. The objective of this study was to evaluate uptake of paclobutrazol, chlormequat chloride, and (S)-abscisic acid applied as a media drench and determine the critical uptake period.

Roots of chrysanthemum (Dendranthema x grandiflora) ‘Snowmass’ were separated and grown in two adjoining compartments of a cell pack. The chemical being evaluated was applied to one-half of the root system, which was excised at prescribed time intervals to terminate the plant’s exposure to the chemical. Uptake was determined as a function of plant response.

Paclobutrazol uptake was slow and continual, reaching a plateau at 15.85 days with elongation 44% of the control. Chlormequat chloride uptake was rapid, reaching a plateau at 6.78 hours with elongation 55% of the control. (S)-abscisic acid uptake was rapid as all
exposure intervals between 7.5 min and 4 h had transpiration of 0.4 microgram/sq. cm/sec, compared to 9.7 microgram/sq. cm/sec for the uncut water control, measured at 4 h after treatment (HAT). At 24 and 48 HAT, transpiration of the uncut control was 14.0 and 13.1, the 7.5 min exposure interval was 4.4 and 5.5, and the 1 h exposure interval was 2.5 and 3.6 microgram/sq. cm/sec, respectively. Differences in efficacy between exposure intervals and across time were probably due to metabolism.

Uptake differences were observed with the chemicals in this study. Paclobutrazol uptake is slow, probably due to adsorption and desorption reactions between the chemical and organic matter in the media. Chlormequat chloride and (S)-ABA are taken up rapidly and remain in solution. Uptake of these chemicals is by mass flow with the transpiration stream and could possibly be affected by factors affecting transpiration, such as light, temperature, or humidity, at or shortly after application.
CHAPTER 1
INTRODUCTION

Introduction

Plant growth regulators (PGRs) are organic compounds, which in small amounts, are applied to promote, inhibit, or otherwise modify any physiological plant process (Tukey et al., 1953). This general class can be further divided into plant hormones, or phytohormones, which are naturally occurring compounds produced by a plant that move from a site of synthesis to a site of action (Tukey et al., 1953), and plant growth retardants, which are synthetic compounds applied to control plant size without obvious phytotoxicity (Davis and Curry, 1991). Plant growth retardants have been widely researched, developed, and used commercially for the past 40 to 50 years to manipulate plant shape, form, and overall crop quality for agricultural and horticultural purposes. For ornamental crops, growth regulators are important for producing compact or appropriately-sized plants, maintaining quality prior to sale, promoting shelf-life, and improving aesthetic quality (Arteca, 1996). To be commercially desirable, growth regulators must provide consistent results across a reasonable range of environmental and cultural conditions that could occur during production (Davis and Curry, 1991).

Typical application methods for plant growth regulators are foliar sprays or media drenches, but also include media sprays, bulb and seed soaks, and cutting and liner dips. Each method has advantages and disadvantages that must be considered so the proper application technique is used. Sprays typically are easier and quicker to apply, but often have less uniformity due to transport resistance across the leaf surface or reduced coverage from overlapping plant canopies, especially in multi-plant containers. Higher concentrations are applied and phytotoxicity is more likely to occur. Drenches are more labor intensive than sprays, but produce longer-lasting and more uniform results. Another advantage is greater control on
typically insensitive crops or crops requiring greater size reduction, even though lower concentrations are applied (Gent and McAvoy, 2000). Newer techniques gaining popularity due to uniform, early growth control are bulb soaks and liner dips.

Plant growth regulators are extremely important to the horticulture industry, both during production and at retail. Even though research has been conducted to determine uptake, translocation, metabolism, and mode of action, basic and applied research questions still remain. It is known that a threshold concentration of a plant growth regulator must be present for promotion or inhibition of intended biological activity (Birecka, 1967; Lever, 1986) and that plant growth regulators are passively taken up by the root system from media drenches or submersion in solution (Lever, 1986). However, it is unknown how much chemical exposure time is required to achieve maximum efficacy and which portion of the uptake period is most critical for efficacy. Research investigating the uptake pattern of growth regulators relative to efficacy could provide information to answer applied questions, including the importance of environmental conditions at the time of application, differences in plant response to application method, and the recommendation that media applications should be applied in the morning to actively growing plants to avoid potential problems with phytotoxicity (Armitage, 1994).

A split-root system using chrysanthemum (Dendranthema x grandiflorum) ‘Snowmass’ was developed to determine the uptake of media-applied plant growth regulators as a function of plant response. Three growth regulators were investigated – two plant growth retardants and one plant growth hormone. Chlormequat chloride and paclobutrazol are important to the horticulture industry and commonly used as media drenches to control plant size. Abscisic acid is currently under development for commercial use to regulate water loss of horticulture crops at retail and subsequently improve aesthetic quality and increase shelf-life. The following sections provide
information about the discovery, chemistry, transport, accumulation, metabolism, and importance of these three plant growth regulators.

**Paclobutrazol**

Paclobutrazol ([2RS, 3RS]-1-[4-chlorophenyl]-4, 4-dimethyl-2-[1, 2, 4-triazol-1-yl]pentan-3-ol) is an important plant growth retardant that reduces plant growth and increases the commercial and aesthetic value of many floriculture and ornamental crops. Triazole growth regulator activity was discovered during screening programs for fungicidal activity (Davis et al., 1988) and paclobutrazol was discovered in 1980 by ICI Plant Protection Division (Goulston and Shearing, 1985). The molecule has a ring structure containing three nitrogen atoms (Davis and Curry, 1991) and consists of two enantiomers. The (2R, 3R) enantiomer provides fungicidal activity while the (2S, 3S) enantiomer provides growth regulating activity (Sugavanam, 1984). It was identified in initial research as PP333 and is commercially available under the trade names of Bonzi, Downsize, Paczol, Piccolo, Clipper, Cultar, and Parlay (Lever, 1986; Barrett, 2006).

Paclobutrazol is a potent regulator of gibberellin biosynthesis and inhibits the oxidation of kaurene to kaurenoic acid. Specifically, it interacts with kaurene oxidase, a cytochrome P-450 oxidase, and inhibits the microsomal oxidation of kaurene, kaurenal, and kaurenol (Hedden and Graebe, 1985). Reduced levels of gibberellins lead to a decrease in cell division and elongation at the apical meristem of the shoot, but has little effect on the production of leaves or root growth (Giafagna, 1995). Paclobutrazol must be translocated to the meristematic region and maintain a threshold concentration for efficacy (Lever, 1986). It is often referred to as an “anti-gibberellin” because physiological treatment effects can be reversed by the application of GA (Cox, 1991). However, this term is misleading because paclobutrazol does not block the activity of existing endogenous or applied exogenous GA₃ (Lever, 1986) by competing for the same active site. Instead, paclobutrazol should be referred to as a “gibberellin inhibitor.”
Transport of paclobutrazol occurs passively in the xylem (Barrett and Bartuska, 1982; Wang et al., 1986; Early and Martin, 1988), with little to no movement in the phloem (Richardson and Quinlan, 1986). It was proposed that transpiration was required to draw the chemical through the xylem to the meristematic regions (Lever, 1986) and confirmed by Early and Martin (1988), who demonstrated using $^{14}$C-paclobutrazol that the pattern of radioactivity followed the pathway of normal water movement. A previous study indicated that paclobutrazol uptake from solution and movement within tissue was rapid, with significant levels of labeled material detected within 12 hours of treatment (Early, 1986). Movement of paclobutrazol in the plant is acropetal, with no movement out of mature leaves (Richardson and Quinlan, 1986; Early and Martin, 1988). Accumulation within a plant is primarily in root and leaf tissue, with one study determining ~80% of labeled material accumulated in basal and midsection leaves (Early and Martin 1988). Only a small portion of applied paclobutrazol actually reaches the site of action.

Metabolism is generally thought to occur very slowly within plant tissue, but there have been differing reports. Early and Martin (1988) reported that breakdown of paclobutrazol ranged from 32 to 58.5%, with most degradation occurring in leaf tissue, as only 7.8 to 12.2% of $^{14}$C activity remained as paclobutrazol nine days after treatment. However, Sterrett (1985) reported in apple that 85% of $^{14}$C activity in shoot tissue remained as paclobutrazol 27 days after injection.

Degradation of paclobutrazol occurs in soil under aerobic conditions and mesophilic temperatures due to microbial activity (Jackson et al., 1996). Eight Pseudomonas and one Alcaligenes have been identified as having the capacity to degrade paclobutrazol. Degradation is temperature dependent, with a test in aqueous suspensions determining that it takes 4.5 years at
25°C and 2.5 years at 60°C. With the addition of *Pseudomonas*, degradation was very rapid and took 13 days (Jackson, Line and Hasan 1996). In field situations, paclobutrazol has a half-life ranging from 3 to 12 months (Lever 1986) or 12 to 18 months, although some have reported persistence as long as 3 years (Jacyna and Dodds, 1995). Some commercial greenhouse operations have had issues dealing with chemical residues.

Paclobutrazol is a wide spectrum growth retardant that shows a response over many species, including some that were insensitive to other classes of plant growth retardants. Plants treated with paclobutrazol typically have shorter internodes and thicker green leaves. Response is dependent on many factors, including species and/or cultivar sensitivity, cultural and environmental conditions, media composition, irrigation method, application method, and application dose.

Paclobutrazol has a low water solubility of 30 to 35 ppm and is a non-polar molecule. Binding to soil components is related to organic matter content, clay content, and cation exchange capacity (Davis, 1988) while efficacy in commercial potting media is related to bark percent and composition (Million et al., 1998). A hydrophobic attraction exists between the non-polar portion of the paclobutrazol molecules and the waxy layers of the bark, creating a reversible adsorption reaction (Barrett, 1982). These binding reactions are thought to occur very rapidly because no loss of efficacy was observed due to leaching during application (Million et al., 1999) or irrigation as soon as 1 hour after application (Barrett et al., 1987). Over time, paclobutrazol desorbs from the bark and is available for uptake by the roots (Barrett, 1982).

Adsorption reactions generally take place in the upper levels of the soil and media, above the root zone (Barrett, 1982), and were confirmed using a bioassay. Over time, there was a slow re-distribution to the middle and lower layers of the media (Million et al., 1999). Uptake and
efficacy of paclobutrazol is dependent on root proximity (Davis, 1988). It has been proposed in certain situations that paclobutrazol could effectively work as a slow-release growth retardant due to its slow desorption from soil components and prolonged efficacy (Jacyna and Dodds, 1995). Paclobutrazol does not pose a leaching hazard, but moves slowly through the soil and media profile over time.

Chemical activity in commercial media is influenced by the amount and properties of bark present. Bark particle size is important, with activity reduced more in the fine (<2 mm) fraction of fresh and composted bark samples than in medium (2-4 mm) or coarse (>4 mm) fractions (Million et al., 1998). Type of bark also influences efficacy, with a 4-, 5-, and 10-fold higher concentration of paclobutrazol required for old composted bark, fresh pine bark, and composted pine bark to achieve similar height reduction as Sphagnum peat or coir based media (Million et al., 1998). The reduction in chemical efficacy has been proposed to be related to component surface area and density (Million et al., 1998).

**Chlormequat Chloride**

Chlormequat chloride was discovered during a screening program of quaternary ammonium compounds for growth retardant activity and first described by Tolbert (1960a). It has been used extensively since the 1960s to prevent lodging in grain and cereal crops and for growth control of potted greenhouse crops, especially poinsettia (*Euphorbia pulcherrima*), chrysanthemum (*Dendranthema x grandiflora*), azalea (*Rhododendron* sp.), geranium (*Pelargonium hortorum*), and hibiscus (*Hibiscus rosa-sinense*). Chlormequat chloride is commercially available under the trade name Cycocel.

Chlormequat chloride, also known as chlorocholine chloride, is an onium growth retardant containing a quaternary ammonium group (Gent and McAvoy, 2000). It has a chemical name of (2-chloroethyl) trimethylammonium chloride and a chemical formula of $C_7H_{13}Cl_2N$ arranged as
Cl\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}N(CH\textsubscript{3})\textsubscript{3} (Tolbert, 1960a). It is a choline derivative containing a substituted Cl for a hydroxyl group (Tolbert, 1961; Cathey, 1964). Chlormequat chloride inhibits the cyclization of geranylgeranyl pyrophosphate to copallyl pyrophosphate in the gibberellin biosynthesis pathway (Rademacher, 2000).

Chlormequat chloride is highly mobile in both xylem and phloem tissue (Lord, 1981; Kust, 1986) and rapidly absorbed and translocated. It is highly water soluble (Cathey and Stuart, 1961) and passively absorbed by all plant tissues, allowing it to be effectively applied as a spray or drench (Tolbert, 1960b). However, studies with winter barley indicate that applications were more effectively taken up by the roots than the leaves (Belzile et al., 1972). In one study with wheat (Dekhuijzen and Vonk, 1974), uptake during the first six hours by the roots resulted in 20% growth inhibition two weeks later. Chlormequat chloride preferentially accumulates in the meristematic regions (Belzile et al., 1972), newly expanding leaves (Intrieri and Ryugo, 1974), and young tissues and organs (Birecka, 1967) with some re-distribution to root tissue (Intrieri and Ryugo, 1974). Studies have estimated that 25 to 33% of applied chlormequat chloride in plant tissue is present in the upper portion of the plant (Birecka, 1967; Dekhuijzen and Vonk, 1972).

Metabolism of chlormequat chloride is thought to occur rapidly, but differing results have been reported. Early reports suggested chlormequat chloride was not broken down in plant tissues (Birecka, 1967; Blinn, 1967), contributing to the persistence of growth retardation (Cathey, 1964). However, further studies showed that chlormequat chloride was broken down to choline by substitution of the chlorine ion with a hydroxyl group (Belzile et al., 1972; El-Fouly and Jung, 1969; Schneider, 1967). One study hypothesized that reduced chlormequat chloride efficacy and duration of control compared to other plant growth retardants discovered around the
same time was due to rapid metabolism to choline, a compound with little growth regulator activity (Schneider, 1967). Further metabolism results in the subsequent conversion of choline to betaine, glycine, and serine, which are incorporated into the protein fraction of the plant (Stephan and Schutte, 1970; Dekhuijzen and Vonk, 1974). Metabolism of chlormequat chloride is rapid, with Jung and El-Fouly (1966) reporting complete breakdown to choline within 10 days. Other researchers determined the biological half-life to be 13 days (Mooney and Pasarela, 1967) and 25 days (Bier and Dedek, 1970) and the effective growth retarding period to be approximately 20 days (Intrieri and Ryugo, 1974). Chlormequat chloride is also rapidly metabolized by soil microorganisms (Blinn, 1967; El-Fouly and Jung, 1969) or broken down by steam sterilization (Cathey, 1964) and does not persist from one crop to the next (Cathey and Stuart, 1961).

Application of chlormequat chloride to crops results in plants with shorter internodes and thicker, darker green leaves (Tolbert, 1960a, 1960b). A threshold concentration is required for growth inhibition (Birecka, 1967), with reports of low concentrations actually promoting stem elongation (Tolbert, 1961; Halevy and Wittwer, 1965). Improper application or excessively high concentrations result in severe marginal leaf chlorosis (Armitage, 1994) or chlorotic spotting.

Abscisic Acid

Introduction

Abscisic acid was first identified as a substance controlling plant physiological processes in the 1960s by three research groups. A group at the University of California, Davis with Carns and Addicott investigated substances controlling abscission in cotton (Gossypium hirsutum). An initial substance was identified in the 1950s and named “abscisin”, but it was never isolated or quantified (Addicott and Lyon, 1969). A second and more active compound isolated from young cotton bolls shown to promote leaf abscission was named “abscisin II”, characterized (Ohkuma
et al., 1963), structure determined (Ohkuma et al., 1965), and confirmed by synthesis (Cornforth et al., 1965). A second group at Milstead Laboratory, University of Wales with Wareing investigated a compound isolated from sycamore leaves (Acer pseudoplatanus) that induced resting bud formation (Robinson and Wareing, 1964) and called it “dormin”. Isolation of the compound (Cornforth et al., 1965) led to the discovery that “abscisin II” and “dormin” was the same compound and confirmed the chemical structure. A third research group at Wye College, University of London with Rothwell and Wain (1964) continued preliminary studies of Van Steveninck with a compound present in the fruit of lupin (Lupinus luteus var. Weiko II) that induced flower drop. It was identified as being identical to “abscisin II” and “dormin”.

To reduce confusion associated with multiple names for the same compound, it was renamed abscisic acid, abbreviated ABA, by the involved researchers and presented at the Sixth International Conference in Plant Growth Substances (Ottowa, July 24-28, 1967) (Addicott et al., 1968). Reasons for the proposed name included: (a) indication of the compound’s chemical nature, (b) facilitated naming of derivatives, and (c) close enough to the original name to avoid confusion. Confirmation of the name change was published in 1968 (Addicott et al.).

Abscisic acid has a chemical formula of C$_{15}$H$_{20}$O$_{4}$, a molecular weight of 264 mass units (Ohkuma et al., 1963), and a chemical name of 3-methyl-5-(1’-hydroxy-4´-oxo-2´,6´,6´-trimethyl-2´-cyclohexen-1´-yl)-cis, trans-2,4-pentadienoic acid (Addicott et al., 1969). Synthesis of ABA results in two isomers (or enantiomorphs), designated as (±)-ABA or (RS)-ABA, with (+)-ABA or (S)-ABA as the naturally occurring form. ABA can also isomerize to a trans configuration as a 1:1 equilibrium mixture when exposed to light, but the compound is biologically inactive (Mousseron-Canet, 1966, cited by Milborrow, 1970).
Early research indicated both (+)- and (-)-ABA have biological activity, making it the first optically active plant hormone (Milborrow, 1970). Cornforth et al. (1965) reported synthetic racemic ABA had one-half the inhibitory activity of the natural form as determined by bioassay, but Milborrow reported both forms of ABA were equally active in inhibiting coleoptile growth of dissected wheat embryos. Sondheimer et al. (1971) determined that germination, shoot growth, and root growth of barley (Hordeum vulgare) responded to both forms of ABA, but (S)-ABA was more effective. However, research soon demonstrated that only (+)-ABA was capable of controlling stomatal function. Kriedemann et al. (1972) showed that (±)-ABA was approximately one-half as effective at the same concentration as (+)-ABA, but doubling the concentration of (±)-ABA provided results equal to initial (+)-ABA concentration. Results were confirmed by Cummins and Sondheimer (1973).

Abscisic acid regulates many plant physiological processes, including abscission, dormancy, germination, growth, root geotropism, and stomatal function. It is now classified as a naturally occurring plant hormone based on meeting the following requirements: (a) it occurs in numerous plant species and plant tissues, (b) it is present endogenously in small quantities, and (c) it moves from the site of synthesis to the site of action (Addicott and Lyon, 1969; Wareing, 1978). When initially discovered, multiple patents were issued for agriculture use (Addicott and Lyon, 1969) but ABA is not widely used due to rapid metabolism in plant tissue, high cost of production due to expensive starting materials and low yield potential, and instability in UV light (Addicott and Lyon, 1969; Milborrow, 1969; Gianfagna, 1995). Much research has focused on the role of ABA in regulation of plant water status in response to environmental stress, mainly drought stress, and the possibility of use as an anti-transpirant for efficient water usage and crop protection.
Abscisic acid synthesis has been studied extensively, but the complete pathway is not fully understood. It is a sesquiterpenoid C$_{15}$ compound composed of three isoprene residues and can be synthesized by two pathways, either directly from a C$_{15}$ precursor or indirectly from the cleavage of a C$_{40}$ carotenoid (Milborrow, 1969). Both pathways have two compounds in common, starting with mevalonic acid (MVA) which is converted to farnesyl pyrophosphate (FPP) (Walton and Li, 1995). At this point, the direct pathway leads to abscisic acid while the indirect pathway continues synthesis to violaxanthin, a C$_{40}$ carotenoid compound, with ABA formed from enzymatic cleavage. Studies using labeled ABA were inconclusive due to poor incorporation of radioactive precursors, such as MVA and CO$_2$ (Walton and Li, 1995).

Further research has identified the primary synthesis pathway as originating from a C$_{40}$ carotenoid. Work with several corn (Zea mays) mutants that lack the ability to synthesize carotenoids and application of carotenoid synthesis inhibitors produced plants that had a diminished ability to accumulate ABA (Walton and Li, 1995). Additional strong evidence for the direct pathway arose from a feeding study by Creelman and Zeevaart (1984) using labeled $^{18}$O. The proposed pathway of synthesis is trans violaxanthin, 9'-cis-neoxanthin, xanthoxin, ABA aldehyde, and finally ABA (Li and Walton, 1990).

Abscisic acid synthesis occurs in response to environmental stress conditions, especially water stress. ABA concentration in leaf tissue has been shown to increase 10 to 50 fold within 4 to 8 hours of water stress, with the dramatic rise in ABA levels primarily due to increased rate of biosynthesis (Walton and Li, 1995). This rapid response protects the plant from both immediate and long term damage due to excessive water loss under adverse conditions. As soon as plant water status returns to normal, ABA is rapidly metabolized so normal functioning resumes. It
has been proposed that the mechanism for sensing plant water status and regulating the increase in ABA synthesis or metabolism is leaf turgor pressure (Pierce and Raschke, 1980).

Early research was successful in identifying the main substances produced as ABA was metabolized; however, rate of metabolism and metabolites produced differs between ABA enantiomers. Researchers conclude that almost any change in ABA configuration results in inactivation and limited to no biological activity. Studies with isolated metabolites from bean axes (*Phaseolus vulgaris*), designated as M-1 and M-2 (Sondheimer et al., 1971), indicated one metabolite was converted to the other (Walton and Sondheimer, 1971), later identified as phaseic acid (PA) and dihydrophaseic acid (DPA) (Milborrow, 1974). Site of ABA metabolism was hypothesized to occur in the cytoplasm (Milborrow, 1979) and confirmed by experimentation in spinach leaves (*Spinacia oleracea*) (Hartung et al., 1980).

Abscisic acid metabolism is rapid, especially under stress conditions, but reported rates vary extensively between hours and days. Two main classes of studies have been conducted, with one group using exogenously applied labeled ABA and one group observing endogenous ABA and metabolite levels during stress cycles and recovery periods. In spinach (*Spinacia oleracea*), 32% and 64% of leaf-applied $^{14}$C-ABA was metabolized after 8 and 24 hours, respectively (Hartung et al., 1980). In grape vine (*Vitis vinifera*) leaf tissue, the half-life was approximately 3 hours and 95% of labeled ABA was metabolized after 24 hours (Loveys, 1984). The fastest metabolism of exogenously applied ABA was reported using feeding studies to petioles of cherry leaves (*Prunus* sp.), with a half-life of $36.2 \pm 5.1$ minutes (Gowing et al., 1993).

One hypothesis for the faster rate of metabolism reported when ABA was fed to the petiole versus applied to leaf tissue is the location of ABA relative to the degradative enzymes. In the
absence of stress, leaf-applied ABA is sequestered in the chloroplasts due to high stromal pH while xylem-derived ABA arrives in the apoplastic space and moves into the cytoplasm, the cellular location of the degradative enzymes. Studies observing ABA metabolism in response to water stress cycles have reported a lag time of one and a half hours for ABA levels to start decreasing (Zeevaart 1980). The recovery period required for ABA levels to return to pre-stress levels vary from hours to days: three to four hours for Xanthium (Zeevaart, 1980); one day for grapevine (Vitis vinifera) (Loveys and Kriedemann, 1973) and sorghum (Sorghum bicolor) (Beardsell and Cohen, 1975); and two days for Brussels Sprouts (Brassica oleracea) (Wright, 1978), maize (Zea mays) (Beardsell and Cohen, 1975), and pea (Pisum sativum) (Dorffling et al., 1974).

**Movement**

To understand how abscisic acid functions as a plant hormone, it is important to understand how it moves from the site of synthesis to the site of action. Abscisic acid can be synthesized in both leaf and root tissue and has been shown to accumulate in all plant parts (Shindy et al., 1973). Long distance movement occurs in both xylem and phloem while short distance movement occurs in the apoplastic space and the cytoplasm.

Early investigations studying ABA applied exogenously either by spray or stem/petiole introduction reported differential plant response between application methods. It was hypothesized that the differences were due to lack of sufficient penetration of leaf tissue, rate of movement through the tissue, or rapid compartmentalization or metabolism during transport. ABA is capable of penetrating the leaf cuticle and remains biologically active, but the rate is slowed relative to the thickness of the waxy layers (Blumenfeld and Bukovac, 1971, 1972). Differences in response were most likely attributed to a combination of factors including slower
movement across the cuticle, amount of ABA compartmentalized in the leaf tissue, and amount of ABA actually reaching the active site.

Movement of ABA in plant tissue was initially investigated by Dorffling and Bottger (1968) who reported a rate of 24 to 36 mm/hr in Coleus petiole segments. Ingersoll and Smith confirmed this result in cotton (Gossypium hirsutum) and reported a rate of 20 to 30 mm/hr in their initial study (1970) and a revised rate of 22.4 mm/hr, which was independent of petiole length or applied ABA concentration, in a second study (1971). In contrast, rate of movement in vascular tissue can be 7 to 10 cm/hr. ABA moves through the plant by vascular and non-vascular pathways, and actual rate of ABA movement through the plant depends on the pathway or combination of pathways used.

**Signaling Sequence**

Abscisic acid is produced in both leaf and root tissue in response to water stress through a combination of hydraulic and chemical signals. In leaf tissue, the initial signal has been identified as zero turgor pressure (Pierce and Raschke, 1980). In roots, drying soil surrounding the root tips is “sensed” and ABA is synthesized, which is translocated to the shoots (Tardieu and Davies, 1993). During stress situations, re-distribution of foliar ABA, up-regulation of synthesis in leaves and roots, and/or translocation from the roots significantly increase ABA concentration by 30-fold surrounding the guard cells (Pei and Kuchitsu, 2005).

Under normal water status, 80 to 90% of leaf ABA is contained in mesophyll chloroplasts (Loveys, 1977). Synthesis occurs in the cytoplasm of the leaf mesophyll (Hartung, et al., 1981) and distribution is determined by pH gradients. ABA as an un-disassociated molecule is capable of crossing membranes and moves into the chloplast, where it disassociates to ABA- and is trapped due to high stromal pH. Under water stress, stromal pH decreases and releases ABA (Hartung, 1980, 1981; Cowan, 1982), although the signaling mechanism is not known. ABA
accumulates in the apoplast, even after leaves begin synthesis (Cornish, 1985), and is available for use by the guard cells.

The site of action for ABA is the outer plasmalemma of the guard cells (Hartung, 1983). The plasma membrane is impermeable to ABA in its anion form (ABA-) but ABA-binding proteins in the membrane have their active sites facing towards the apoplastic space (Hornberg, 1984), enabling them to respond quickly to changes in apoplastic ABA levels. Binding of ABA to the proteins results in an efflux of up to 85% of K⁺ from the guard cells (Outlaw, 1983), resulting in decreased turgor and stomatal closure. Some secondary messengers that may also play a role in stomatal closure are calcium and pH (Pei and Kuchitsu, 2005).

Reports have indicated lag periods exist both between initial stomatal closure and an increase in ABA levels and between removal of water stress and stomatal re-opening. Redistribution of ABA from the chloroplast to the apoplast allows ABA to be present at the site of action before any synthesis occurs. If the water stress continues over a period of time, synthesis occurs to replenish available ABA supply. After water stress has been lifted, continued uptake of ABA occurs from the apoplastic pool even after synthesis has stopped (Raschke, 1975; Ackerson, 1980) and continues until ABA has been sequestered in the chloroplasts or metabolized. Stomata will not re-open until the ABA concentration has fallen to pre-stress levels (Cornish, 1985).

Role in Stomatal Closure

Abscisic acid has been shown to play an important role in regulating plant water status under stress conditions by controlling stomatal aperture and plant transpiration rate. The first report was by Little and Eidt (1968) who were investigating the effects of ABA on dormancy of coniferous and deciduous trees and observed a simultaneous inhibition of bud break and transpiration. Mittelheuser and Van Steveninck (1969) reported application of exogenous (RS)-
ABA inhibited transpiration in wheat and observed stomatal closure in wheat and barley. They also showed stomatal response to ABA was rapid and reversible, with closure within ten to fifteen minutes of application and re-opening as the ABA solution was diluted (1971). Results from Jones and Mansfield (1970) indicated that stomatal closure was due to effects on guard cell functioning and not due to CO₂ concentration. Further studies investigating stomatal functioning using exogenously applied ABA are listed in Table 1-1.

Conclusive evidence of the relationship between abscisic acid and stomatal functioning was obtained using the ABA-deficient tomato mutant *flacca*. Compared to the normal phenotype of ‘Rhinelands Ruhm’, *flacca* contained a ten-fold lower concentration of ABA and wilted rapidly because of abnormal stomatal functioning (Tal and Imber, 1970). Applications of ABA to *flacca* resulted in normal stomatal functioning and normal phenotypic appearance, but plants reverted to the wilty phenotype within a few days after applications ceased (Imber and Tal, 1970).

In addition to studies investigating the effects of abscisic acid applied exogenously, research has also demonstrated that plants produce and/or accumulate endogenous abscisic acid in response to water stress. Wright and Hiron (1969) observed in excised wheat leaves a forty-fold increase in leaf ABA content after a few hours of wilting. In Brussels sprouts (*Brassica oleracea*), Wright and Hiron (1972) observed an increase in the ABA level and leaf resistance as the plants wilted. After plants were re-watered, stomata returned to normal functioning as the ABA level decreased over a few days to the pre-stress level.

**Abscisic Acid as a Chemical Signal**

Initial observations indicated that ABA accumulation in leaf tissue rose as the turgor pressure of leaf cells reached zero (Pierce and Raschke, 1980). Many researchers noted that the rapid and significant increase in leaf ABA content occurred faster than it could be synthesized.
It was hypothesized that ABA could be 1) released from storage in the chloroplasts or 2) translocated from other parts of the plant, such as the roots. Both theories are correct, but serve different functions. In response to rapid leaf dehydration, changes in stromal pH releases ABA from the chloroplasts, making it available for the guard cells (Hartung et al., 1980).

Translocation of ABA from root tissue acts as a signal of impending water deficit and acts to close stomata before any significant change in leaf water status occurs to optimize water use (Zeevaart and Creelman, 1988).

Abscisic acid had been hypothesized to be the chemical signal translocated from the roots to the shoots under decreasing water availability due to the relationship between xylem [ABA], stomatal conductance, and transpiration rate. Many studies attempted to demonstrate causation and not merely correlation between xylem [ABA] levels and observed plant response. One of the first requirements was to show that a plant has the capacity to differentiate between leaf- and root-derived ABA (Davies and Zhang, 1991). When leaf tissue is not stressed, leaf-derived ABA is usually sequestered in the chloroplasts due to a pH gradient. Root-derived ABA arriving via the transpiration stream is unloaded at the site of evaporation, the cell walls adjacent to guard cells (Meidner, 1975), and close to the site of action, which are the guard cells (Hartung, 1983).

It is also important to demonstrate that enough ABA moves in the transpiration stream to account for the observed plant response (Davies et al., 1994). ABA moves in the xylem with the transpiration stream and preventing transpiration by covering leaves with tinfoil resulted in no increase in ABA levels in leaf tissue (Zhang and Davies, 1987). Feeding studies in maize (Zhang and Davies, 1990) reported that increased xylem [ABA] correlated with decreased leaf conductance. Similar results occurred when ABA was injected into the stem (Tardieu and
Davies, 1993). When ABA was removed from collected xylem sap and fed back to plants, all anti-transpirant activity disappeared (Zhang and Davies, 1991).

The final step in proving the hypothesis was to demonstrate causation between xylem [ABA] and reduced stomatal conductance, which was reported using two split-root system experiments. Zhang and Davies (1989) kept one column well watered and one allowed to air dry. Stomatal conductance for partially-watered plants was reduced after six days, but leaf turgor was never less than the well watered plants. A split-root system using apple (Gowing, et al., 1990) finally demonstrated the role of a positive signal from the roots controlling leaf conductance. Roots were set up in three split-root treatments: well-watered, partially-watered with re-watering after 24 days, and partially-watered with drying roots removed after 24 days. During the drying cycle, leaf turgor of partially-watered plants was never lower than the well-watered plants. After re-watering and root removal, both groups of plants recovered and responded similarly to well-watered plants. Re-watering restored the water status of the roots and removed the need to produce a root-sourced signal and root excision removed the site of synthesis.

**Agricultural Importance**

With the discovery of abscisic acid as a plant hormone responsible for controlling stomatal function relative to plant water relations, there was much discussion of ABA as the ideal anti-transpirant for agriculture use. An ideal agent should be: (a) non-toxic for use on ornamentals and food crops, (b) not permanently damage the stomatal mechanism, (c) act specifically and selectively on guard cells, and (d) persist for an extended period of time (Jones and Mansfield, 1972). ABA meets most of the listed criteria, but there has been difficulty in obtaining a commercially available supply due to structural inactivation in light, inactivity of the (R)
enantiomer, a high cost of production, and difficulty obtaining sufficient quantities naturally or synthetically (Addicott and Lyon, 1969; Milborrow, 1969; Gianfagna, 1995).

Work with ABA analogs has been promising as anti-transpirants on marigold (*Tagetes petula*) and tomato (*Lycopersicon esculentum*) (Sharma et al., 2005, 2006). Comparison with (RS)-ABA demonstrated that both the analogs and synthetic ABA were effective in reducing plant water usage. However, when compared to the analogs, (RS)-ABA had a shorter efficacy period (Sharma et al., 2006) and negative effects on plant aesthetics, including superficial wilting of tomato under normal water status (Sharma et al., 2006), flower drop on impatiens (*Impatiens walleriana*) (Gibson and Crowley, 2006) and bedding plants (Blanchard et al., 2007), and yellowing of older, lower leaves (Barrett and Campbell, 2006).

A formulation of (S)-ABA is currently in testing by Valent BioSciences for use as an anti-transpirant and holding agent for floriculture crops. Initial studies show that (S)-ABA applied to bedding plants reduced transpiration and increased postharvest longevity (Stamps and Chandler, 2005; Barrett and Campbell, 2006; Gibson and Crowley, 2006; Blanchard et al., 2007).
<table>
<thead>
<tr>
<th>Source</th>
<th>Crop</th>
<th>Application method</th>
<th>ABA concentration</th>
<th>Lag Time</th>
<th>Time to complete closure</th>
<th>Time to recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jones and Mansfield (1970)</td>
<td><em>Xanthium pennsylvanicum</em></td>
<td>Leaf surface</td>
<td>$10^{-4}$ M (0.02 μg/cm²)</td>
<td>2-9 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cummins et al. (1971)</td>
<td>Barley (<em>Hordeum vulgare</em>)</td>
<td>Petiole</td>
<td>$10^{-7}$ M</td>
<td>5 min</td>
<td></td>
<td>5 min after removal</td>
</tr>
<tr>
<td>Loveys (1984)</td>
<td>Grape (<em>Vitis vinifera</em>)</td>
<td>Petiole</td>
<td>2 to 8 x $10^{-11}$ M/cm²</td>
<td>30 min</td>
<td></td>
<td>4 to 5 h</td>
</tr>
<tr>
<td>Horton (1971)</td>
<td><em>Vicia faba</em></td>
<td>Isolated epidermal strips</td>
<td>$1.9 \times 10^{-5}$ M (+)-ABA (10 mg/L)</td>
<td>8 min</td>
<td>30 min</td>
<td></td>
</tr>
<tr>
<td>Kriedemann et al. (1972)</td>
<td>Bean (<em>Phaseolus vulgaris</em>)</td>
<td>Petiole</td>
<td></td>
<td>8 min</td>
<td>30 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Broadleaf Dock (<em>Rumex obtusifolia</em>)</td>
<td>Petiole</td>
<td></td>
<td>8 min</td>
<td>35 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Beet (<em>Beta vulgaris</em>)</td>
<td>Petiole</td>
<td></td>
<td>9 min</td>
<td>52 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Corn (<em>Zea mays</em>)</td>
<td>Petiole</td>
<td></td>
<td>3 min</td>
<td>105 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Rosa</em> spp.</td>
<td>Petiole</td>
<td></td>
<td>32 min</td>
<td>108 min</td>
<td>9 min</td>
</tr>
<tr>
<td>Itai et al. (1978)</td>
<td>Fed to trans. stream</td>
<td>Petiole</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER 2
WHOLE PLANT RESPONSE OF CHRYSANTHEMUM TO PACLOBUTRAZOL AND CHLORMEQUAT CHLORIDE AS A FUNCTION OF EXPOSURE TIME USING A SPLIT-ROOT SYSTEM

Introduction

Plant growth regulators (PGRs) which inhibit production of gibberellins and inhibit internode elongation are important in the production of many floriculture crops. Paclobutrazol and chlormequat chloride are two of these chemicals and are often applied either as a foliar spray or as a substrate drench. Media applications have become increasingly popular due to ease of application, uniformity of effect, and duration of efficacy. However, little is understood of the timing of movement into the plant following drench applications to peat-based media commonly used in container production. Paclobutrazol has relatively low solubility in water and is only xylem mobile. It is known that paclobutrazol binds to organic media components following drench applications. Chlormequat chloride is highly water soluble and moves in both the xylem and phloem.

The split-root system used in this study was developed to evaluate chemical uptake in a practical and efficient whole plant system, similar to commercial application situations. The objective of this study was to evaluate uptake of paclobutrazol and chlormequat chloride applied as a media drench and determine when uptake occurred.

Materials and Methods

Bare-root cuttings of *Dendranthema x grandiflora* ‘Snowmass’ were obtained from commercial sources (Yoder Brothers, Barbenton, Ohio). Roots of each cutting were cleaned and loosely separated into two equal parts. Cuttings were planted in a 2-cell pack with each half of the roots in separate, adjoining cells. Planting medium was Fafard 2 (Apopka, Florida) consisting of 65 peat: 20 perlite: 15 vermiculite. Newly planted cuttings were placed in a mist
house for one week to establish and then were moved to a fan and pad greenhouse. Plants were pinched to three nodes two weeks after planting and all lateral shoots except the top lateral were removed two weeks after pinch. Plants were grown under a non-inductive photoperiod with night interruption lighting from 2200 HR to 0400 HR. Plants were fertilized at each irrigation with a 20N-4.4P-16.6K (Peter’s fertilizer) with N at 300 mg•L⁻¹. Treatments began once plants were approximately 12 cm tall.

The chemical being studied was applied to one half of the root system in 20 mL of solution. The treated half of the root system was then excised at prescribed time intervals to terminate the plant’s exposure to the chemical. At each prescribed excision time another set of control plants, which had been drenched with only water, was included. Roots of plants for the longest time interval were not excised. Following root excision of the last treatment, plants were planted into 11.5 cm pots using Fafard 2 medium and returned to the greenhouse for the duration of the experiment. This allowed all plants to have the same soil volume for the duration of the experiment and to maintain adequate water availability as the plants grew.

Chemical uptake during the exposure interval was determined as a function of plant response. Plant height (cm) was recorded on the day of application and the last day of the experiment. Stem elongation was calculated as the difference between initial and final plant height. To standardize for possible growth reduction caused by root removal, stem elongation of each treated plant during an exposure interval was compared to the corresponding control block average.Results are given as stem elongation as a percent of the control. Data were analyzed using ANOVA and Tukey’s mean separation. Regression analysis was conducted as a NLIN procedure to calculate a linear-plateau model (SAS 9.1, Cary, North Carolina).
Paclobutrazol was applied at a concentration of 6 mg•L$^{-1}$ so each plant received 0.12 mg active ingredient (a.i.). Plants exposed to paclobutrazol were separated into six treatments consisting of exposure times of 1, 2, 4, 8, 12, or 24 days. Exp. 1 was initiated on 9 Sept. and repeated on 4 Nov. 2006. Results from each trial were very similar and data was pooled. Plants were set up in a randomized complete block design, with three blocks and three replicates per treatment.

Chlormequat chloride was applied at a concentration of 15,000 mg•L$^{-1}$ for Exp. 2, with each plant receiving 300 mg a.i., and 20,000 mg•L$^{-1}$ for Exp. 3, with each plant receiving 400 mg a.i.. More chemical was used to compensate for faster growth under higher temperatures. Exp. 2, initiated on 31 May 2007, had 6 treatments consisting of exposure times of 1, 2, 4, 6, 8, or 16 days. Plants were set up in a randomized complete block design, with four blocks and three replicates per treatment. Because exposure of one day or longer showed maximum response in Exp. 2, Exp. 3, initiated on 20 July, had 7 treatments consisting of exposure times of 15 min, 30 min, 1 h, 2 h, 4 h, 1 day, and 16 days. Plants were set up in a randomized complete block design, with three blocks and three replicates per treatment.

**Results and Discussion**

The split-root system was an effective model to evaluate chemical uptake on a whole plant level. Chrysanthemum was an effective test plant because plants grew rapidly, remained vegetative under photoperiod manipulation, were responsive to paclobutrazol and chlormequat chloride, and had a uniform response across each treatment. Plants were not significantly affected by root excision (N.S. at $\alpha \leq 0.05$), with the 1 and 24 day controls from the second paclobutrazol experiment having stem elongation of 20.1 cm and 21.5 cm, respectively, a difference of 1.4 cm (Table 2-1). This small difference could be explained by the difference in root system size and available water for maximum growth.
Paclobutrazol was taken up slowly from the media, with longer exposure resulting in greater inhibition of stem elongation (Figure 2-1.). Data for the two experiments were similar and pooled together for analysis. Data were regressed and fit to a linear-plateau model with \( y = \% \) stem elongation and \( x = \) paclobutrazol exposure (days). The amount of stem elongation was reduced as exposure time following the drench application increased and reached a plateau at 15.85 days (±0.90) with elongation 44% of the control.

In contrast, chlormequat chloride was taken up rapidly from the media over a relatively short period. Plants were exposed to chlormequat chloride for 1 to 16 days in Exp. 2 and stem elongation was reduced by all chlormequat chloride treatments (Table 2-2). However, there was little difference in stem elongation (between 51 and 55% of the control and N.S. at \( \alpha \leq 0.05 \)) for the different exposure intervals, indicating that amount of chlormequat chloride needed for maximum efficacy was taken up by the plants within 24 hr of application. Exp. 2 was conducted to evaluate the importance of uptake during shorter exposure times (Figure 2-2). Data was regressed and fit to a linear-plateau model with \( y = \% \) stem elongation and \( x = \) chlormequat chloride exposure (hours). Uptake was rapid and reached a plateau at 6.78 hours (±1.91) with elongation 55% of the control. The results of Exp. 3 agree with observations in Exp. 2 that chlormequat chloride taken up during the first day of exposure had greater efficacy than chemical taken up between days 1 and 16.

These studies show a difference in the pattern of uptake following drench applications of paclobutrazol and chlormequat chloride. Paclobutrazol is xylem active and a strong inhibitor of stem elongation and commonly used as a spray, drench, or liner dip for growth control. Results from this study indicating slow uptake explain some of the findings of others and grower observations. This study has demonstrated that environmental conditions at the time of
application, such as temperature, humidity, and light level probably do not influence chemical uptake and efficacy. Efficacy of media applied paclobutrazol depends on media composition, amount of chemical (a.i.) applied, irrigation method, and species and/or cultivar sensitivity.

Paclobutrazol has a low water solubility of 35 ppm (Lever, 1986) and there is a hydrophobic attraction between non-polar portions of the growth retardant and waxes of the bark particles (Barrett, 1982). Paclobutrazol is partitioned out of solution to the waxy layers of the bark, but can still be obtained, absorbed, and utilized by the plant. These binding reactions are thought to occur very rapidly because no loss of efficacy was observed due to leaching during application (Million et al., 1999) or irrigation as soon as one hour after application (Barrett et al., 1987). Adsorption reactions generally take place in the upper levels of the soil and media, above the root zone (Barrett, 1982). Over time, there was a slow re-distribution to the middle and lower layers of the media (Million et al., 1999). Paclobutrazol is not rapidly broken down and has a relatively long half-life, resulting in residue issues in certain commercial situations.

Efficacy is reduced in media containing pine bark and influenced by type and particle size. Old composted bark, fresh pine bark, and composted pine bark required a 4-, 5-, and 10-fold higher concentration of paclobutrazol to achieve similar height reduction as Sphagnum peat- or coir-based media. Finer bark particles reduce efficacy more than medium or coarse bark particles, presumably due to the larger surface area available for adsorption (Million et al., 1998).

In contrast to paclobutrazol, chlormequat chloride is a weaker inhibitor of stem elongation when applied as a media drench, requiring 400 mg a.i. vs. 0.12 mg a.i. Chlormequat chloride is highly water soluble (Cathey and Stuart, 1961) and xylem and phloem mobile. It can be applied as a spray or drench, with drenches having greater uptake and efficacy (Belzile and Vonk, 1972).
Chlormequat chloride is rapidly absorbed by plants in experiments when it is added to the nutrient solution. In one study, wheat plants were exposed to nutrient solution plus chlormequat chloride for six hours and then returned to normal nutrient solution. After two weeks, 20% growth inhibition was reported (Dekhuijzen and Vonk, 1974). Because of its complete water solubility (MSDS label, 2008), chlormequat chloride should remain in solution and not readily partition to media components, allowing it to be taken up by mass flow with the transpiration stream.

Efficacy of chlormequat chloride is dependent on amount of active ingredient applied and species and/or cultivar sensitivity. The chemical has a high affinity for remaining in solution and should remain available for uptake as long as it is not washed out of the medium. Based on the results of this study, chlormequat chloride has the potential to be influenced by environmental conditions at time of application. Factors that could influence transpiration, such as light, temperature, and humidity, could affect the rate of uptake as the chemical moves into the plant by mass flow. These factors were not evaluated and could be a potential for future research.

In summary, plant response to paclobutrazol indicates slow uptake, which is due to rapid partitioning to media components and slow desorption in an active state until the chemical pool is sufficiently depleted. Efficacy is dependent on the amount of active ingredient reaching the root zone of the plant and related to the percent, type, and particle size of bark in the medium. Response to chlormequat chloride indicates rapid uptake due to the chemical remaining in solution and not interacting with media components. Uptake occurs by mass flow due to transpiration and could be influenced by environmental conditions at time of application that regulate plant water status, including light, temperature, and humidity.
Figure 2-1. Chrysanthemum elongation response to exposure time following paclobutrazol media drench to one half of root system. Exposure time was time from application to excision of treated roots. Elongation is expressed as percent of control plants with roots excised at same time. The best-fit regression was a linear plateau model $y = 0.96 - 0.033x$ and the join point at 15.85 days ($n = 9$).
Figure 2-2. Chrysanthemum elongation response to exposure time following chlormequat chloride media drench to one half of root system. Exposure time was time from application to excision of treated roots. Elongation is expressed as percent of control plants with roots excised at same time. The best-fit regression was a linear plateau model \( y = 0.75 - 0.029x \) and the join point at 6.78 hours (\( n = 9 \)).
Table 2-1. Plant elongation response following application of water to one half of root system. Treatment was exposure time from application to excision of roots. Data were analyzed using ANOVA and differences were N.S. at $\alpha \leq 0.05$ (n = 9).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control elongation (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>20.1</td>
</tr>
<tr>
<td>2 days</td>
<td>20.7</td>
</tr>
<tr>
<td>4 days</td>
<td>22.6</td>
</tr>
<tr>
<td>8 days</td>
<td>21.8</td>
</tr>
<tr>
<td>12 days</td>
<td>21.6</td>
</tr>
<tr>
<td>24 days</td>
<td>21.5</td>
</tr>
</tbody>
</table>

Table 2-2. Plant elongation response to exposure time following chlormequat chloride media drench to one half of root system. Exposure time was time from application to excision of treated roots. Elongation is expressed as percent of control plants with roots excised at same time. Data were analyzed using ANOVA and determined to be N.S. at $\alpha \leq 0.05$ (n = 12) (Exp. 2).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Elongation (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>51</td>
</tr>
<tr>
<td>2 days</td>
<td>52</td>
</tr>
<tr>
<td>4 days</td>
<td>55</td>
</tr>
<tr>
<td>6 days</td>
<td>51</td>
</tr>
<tr>
<td>8 days</td>
<td>51</td>
</tr>
<tr>
<td>16 days</td>
<td>53</td>
</tr>
</tbody>
</table>
CHAPTER 3
WHOLE PLANT RESPONSE OF CHRYSANTHEMUM TO (S)-ABSCISIC ACID AS A FUNCTION OF EXPOSURE TIME USING A SPLIT-ROOT SYSTEM

Introduction

Abscisic acid (ABA) is responsible for controlling stomatal function. Under water stress, increased concentration of ABA accumulates at the guard cell, due to re-distribution from chloroplasts, increased synthesis by leaf tissue, or transport from root tissue (Pei, 2005). Exogenous applications of ABA to detached leaves and whole plants with normal water status have resulted in stomatal closure and decreased transpiration (Mittelheuser and Van Steveninck, 1969). It has been proposed that ABA would be an ideal anti-transpirant for agriculture use under field conditions to reduce water usage and irrigation requirements (Jones and Mansfield, 1972), but this has not occurred due to the high cost of synthesis, production of a racemic mixture, and difficulty obtaining sufficient quantities (Addicott and Lyon, 1969; Gianfagna, 1995). (RS)-ABA is effective in controlling other plant physiological processes, but stomata respond only to (S)-ABA (Kriedemann et al., 1972).

Development of commercially available (S)-ABA has created interest among the horticulture industry as a holding agent to increase postharvest longevity of ornamentals. Many high-value potted floriculture crops are subjected to water stress during shipping and display at retail, decreasing their quality and value. Recent studies on hibiscus (*Hibiscus rosa-sinensis*), impatiens (*Impatiens walleriana*), and bedding plants indicate that treatment with (S)-ABA increases postharvest longevity. Because of the nature of the hormone, care must be taken when applying to avoid flower drop or yellowing of older, lower leaves (Stamps and Chandler, 2005; Barrett and Campbell, 2006; Gibson and Crowley, 2006; Blanchard et al., 2007).
Most studies evaluating ABA uptake have used labeled material and results were obtained from radiography or isolation and extraction procedures. The split-root system used in this study was developed to evaluate ABA uptake in a practical and efficient whole plant system, similar to commercial application situations. The objective of this study was to evaluate the uptake of ABA applied as a media drench and determine how fast uptake from the media occurs.

Materials and Methods

Bare-root cuttings of *Dendranthema x grandiflora* ‘Snowmass’ were obtained from commercial sources (Yoder Brothers, Barberton, Ohio) and grown according to procedures in Ch. 2. Plants were not exposed to water stress during the growth period and treatments began when plants were approximately 15 cm tall. (S)-abscisic acid was applied to one half of the root system at a concentration of 2,000 mg•L\(^{-1}\) (determined during an initial rate screen) in a volume of 20 mL so each plant received 40 mg of active ingredient (a.i.). The treated half of the root system was excised at different time intervals to stop the plant’s exposure to the chemical. Plants exposed to (S)-ABA were separated into 7 treatments consisting of exposure times of 7.5 min, 15 min, 30 min, 1 h, 2 h, 4 h, and an uncut plant. Plants in two control treatments of water only were included, one with half the root system removed and one with a complete root system. Abscisic acid was applied at 1100 HR to well-watered plants in a high light environment and water was withheld for the duration of the experiment. ABA efficacy was determined by measuring transpiration rates using a LI-1600 steady-state porometer (LI-COR, Lincoln, Nebraska).

The first experiment was conducted on 7 Sept. 2007 and data were collected four hours after (S)-ABA application. Light level at time of transpiration measurement was 950 \(\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}\). The second experiment was initiated on 5 Oct. 2007 and data were collected 24, 48, and
72 hr after (S)-ABA application and light levels at time of measurement were 250, 600, and 700
μmol•m⁻²•s⁻¹, respectively. The third experiment was initiated on 2 Jan. 2008 and data were
collected at 4, 24, and 48 hours after (S)-ABA application and light levels at time of
measurement were 600, 700, and 600 μmol•m⁻²•s⁻¹, respectively. Experiments were set up in a
randomized complete block design, with two blocks and six plants per treatment. Data were
analyzed using GLM and Waller-Duncan for mean separation (SAS 9.1, Cary, North Carolina).

Results and Discussion

Transpiration rates for control plants without and with root excision were 15.4 and 10.9
μg•cm⁻²•s⁻¹, respectively, in Exp. 1 (Figure 3-1). There was a difference in transpiration that can
be attributed to root excision and could possibly be due to a decrease in available water or a
direct response to the root excision. However, transpiration of plants with excised roots was
considerably greater than transpiration of plants given (S)-ABA. In Exp. 2 and 3 there was a
relatively smaller difference in transpiration for the plants in the control treatments (Figures 3-2
and 3-3).

For the first experiment (Figure 3-1), data were collected 4 h after application of (S)-
abscisic acid. All plants treated with (S)-ABA had decreased transpiration rates compared to the
controls. (S)-ABA uncut plants had leaf transpiration of 0.89 μg•cm⁻²•s⁻¹ and plants in (S)-ABA
treatments with exposure intervals between 7.5 min and 4 h were between 0.7 and 2.68 μg•cm⁻²•s⁻¹.
Although the exposure interval of 7.5 min reduced transpiration compared to the control,
transpiration in these plants was greater than in the other ABA treatments. (S)-ABA was taken
up by the roots from the medium and moved into the xylem or stem tissue very rapidly over a
period of minutes. Results from this experiment indicate that the plants took up sufficient (S)-
ABA in a short exposure period to close stomata within 4 h of application, making this an
effective model system to test (S)-ABA uptake. However, it was unknown if the amount of (S)-ABA taken up during shorter exposure intervals was sufficient to provide the same duration of stomatal closure compared to longer exposure intervals.

Exp. 2 and 3 were conducted to answer the above question. Transpiration for Exp. 2 recorded at 24, 48, and 72 h after (S)-ABA application. The time from ABA application to excision of roots did not affect transpiration rate measured at 24 h, but there were differences at 48 and 72 h (Fig. 3-2). At 24 h, lower transpiration rates for all (S)-ABA exposure intervals were observed, compared to both controls, and were between 1.14 and 1.72 μg•cm⁻²•s⁻¹. At 48 h after application, transpiration rates for exposure intervals of 7.5 and 15 min were 5.35 and 6.56 μg•cm⁻²•s⁻¹, respectively, which were greater than transpiration rates between 2.09 and 3.26 μg•cm⁻²•s⁻¹ for all intervals between 30 min and 4 h. At 72 h after application, transpiration rates for all (S)-ABA exposure intervals were reduced compared to the uncut control. The cut control was not included because plants were wilted. Although all cut (S)-ABA treatments were not significantly different from the uncut (S)-ABA treatment, there was a general trend of decreasing transpiration as exposure interval increased.

Data from Exp. 3 confirms findings from Exp. 1 and 2 (Fig. 3-3). At 4 h after application, transpiration rates of all (S)-ABA exposure intervals were less than for water controls. The only difference between experiments was the reduced efficacy of the 7.5 min exposure interval compared to all other (S)-ABA exposure intervals in Exp. 1. At 24 h after application, transpiration rate of the 7.5 min exposure interval was 4.43 μg•cm⁻²•s⁻¹, which was greater than transpiration rates between 1.97 and 2.51 μg•cm⁻²•s⁻¹ for exposure intervals between 30 min and 4 h. At 48 h after application, transpiration rates for exposure intervals of 7.5 min, 30 min, and 2
were 5.49, 4.18, and 3.53 μg•cm²•s⁻¹, respectively, indicating that longer exposure intervals were needed to achieve maximum stomatal closure over the duration of the effect.

Efficacy of a chemical is dependent on the amount present at the active site. The concentration of ABA surrounding the guard cells is in continual flux because of movement from the roots, distribution between cellular compartments, synthesis of new ABA, and metabolism of existing ABA (Pei and Kuchitsu, 2005). In the experimental system, our simplified model assumes: 1) only xylem transport of (S)-ABA absorbed from the medium, 2) metabolism of endogenous (RS)- and exogenous (S)-ABA during the duration of the experiment, and 3) ignores synthesis and re-distribution because plants are not subjected to water stress or pressure dehydration. Because uptake is stopped with root excision, a fixed amount of (S)-ABA is translocated to the guard cells and available to regulate stomatal function.

Experimental results indicate that sufficient (S)-ABA was applied to close stomata and decrease leaf transpiration rate. (S)-ABA was taken up by the roots from the media and moved into the xylem or stem tissue very rapidly over a period of minutes. This experiment did not measure rate of movement through the plant and/or stomata reaction time, but 4 h was sufficient for (S)-ABA to move through the plant to the guard cells and cause stomatal closure. It is unknown how rapid ABA movement is into the plant from soil application. However, early studies observed rates of stomatal closure by applying exogenous ABA to detached leaves by placing the petiole in a solution containing ABA. Complete stomatal closure was fastest for bean (*Phaseolus vulgaris*), requiring 30 min. and slowest for rose (*Rosa* spp.), requiring 108 min. (Kriedemann, 1972). Other studies looking at movement of ABA through petiole segments reported rates of 24 to 36 mm/hr in *Coleus* (Dorffling and Bottger, 1968) and 22.4 mm/hr in
cotton (*Gossypium hirsutum*) (Ingersoll and Smith, 1971), but the rate of movement in vascular tissue can be 7 to 10 cm/hr.

Increased leaf transpiration rates of all (S)-ABA exposure intervals over the efficacy period (Figs. 3-2 and 3-3) can possibly be explained by metabolism. Studies observing rate of metabolism have reported that 32% and 64% of leaf applied $^{14}$C-ABA was metabolized after 8 and 24 hours, respectively (Hartung et al., 1980). The half-life in grape (*Vitis vinifera*) leaf-tissue was approximately 3 hours and 95% of labeled ABA was metabolized after 24 hours (Loveys, 1984). Recent studies investigating media applied ABA reported that water use was suppressed for three days in tomato plants (*Lycopersicon esculentum*) (Sharma et al., 2006). Also, the trend of increasing leaf transpiration as exposure interval decreases can be explained as a smaller concentration of (S)-ABA taken up and available to elicit maximum stomatal response.

This research indicates that (S)-ABA applied as a drench to peat-based media is taken up very rapidly and causes stomatal closure within 4 h. This is in contrast to results obtained for paclobutrazol where uptake was slow, with longer exposure resulting in greater inhibition of stem elongation (Ch. 2). Regression analysis was significant as a linear plateau model, reaching a plateau at 15.85 days ($\pm 0.90$) with elongation 44% of the control. Media composition, amount of chemical applied, and species/cultivar sensitivity can influence paclobutrazol efficacy.

Even though (S)-ABA is taken up rapidly, exposure intervals of one hour or greater are needed to achieve maximum reduction in leaf transpiration over the efficacy period of 48-72 h. Stomatal response is dependent on the amount of ABA present at the guard cells (Kriedemann et al., 1972). The minimum exposure interval needed to achieve maximum stomatal response could differ depending on factors influencing uptake. Possible factors to investigate are the concentration of (S)-ABA applied and the environmental conditions at application.
application concentration was determined by an initial rate screen, but it is possible that if the concentration was increased, an increase of (S)-ABA could be absorbed in the same time interval, resulting in a shorter exposure interval needed to achieve maximum stomatal response at 48-72 h. Also, absorbed (S)-ABA is translocated from roots to shoots in the transpiration stream. If transpiration is reduced due to light level or other environmental factors at and shortly after application, it is possible that uptake could be reduced, resulting in a longer time interval needed for maximum stomatal response. Another unknown factor is media moisture level at the time of application, an important parameter for efficacy of liner dips. Plants with reduced moisture level may already be conserving water by stomatal closure and reduced transpiration, potentially slowing the uptake of (S)-ABA with the transpiration stream.
Figure 3-1. Effects of (S)-ABA exposure interval on transpiration rate of *Dendranthema x grandiflora* ‘Snowmass’ (Exp. 1). Treatment time was time from application to excision of treated roots. Data was recorded at 4 hours after application. Data were analyzed using ANOVA and Waller-Duncan mean separation. Letters signify differences between treatments at $\alpha \leq 0.05$ (n = 6).
Figure 3-2. Effects of (S)-ABA exposure interval on transpiration rate of *Dendranthema x grandiflora* ‘Snowmass’ (Exp. 2). Treatment time was time from application to excision of treated roots. Data for cut control was not recorded at 72 h after application due to plants wilting. Data were analyzed using ANOVA and Waller-Duncan mean separation. Letters signify differences between treatments at $\alpha \leq 0.05$ and are for comparison within each time observation (n = 6). A) 24 h after application, B) 48 h after application, C) 72 h after application.
Figure 3-3. Effects of (S)-ABA exposure interval on transpiration rate of *Dendranthema x grandiflora* ‘Snowmass’ (Exp. 3). Treatment time was time from application to excision of treated roots. Data for cut control was not recorded at 48 h after application due to plants wilting. Data were analyzed using ANOVA and Waller-Duncan mean separation. Letters signify differences between treatments at $\alpha \leq 0.05$ and are for comparison within each time observation ($n = 6$). A) 4 h after application, B) 24 h after application, C) 48 h after application.
CHAPTER 4
CONCLUSION

Plant growth regulators are important to the horticulture industry and research has been conducted to determine uptake, translocation, metabolism, and mode of action. Most research has been conducted using labeled material and results determined using radiography or isolation and extraction procedures. Little to no work has been published on the uptake rate of media-applied plant growth regulators, an important factor in determining how much chemical exposure time is required to achieve maximum efficacy and which portion of the uptake period is most critical for efficacy. Results from this type of study could be used to answer applied research questions concerning the importance of environmental conditions at the time of application, differences in plant response relative to type of media application (drench vs. sub-irrigation), and the recommendation that drenches be applied in the morning. The objectives of this study were to develop a whole-plant system to evaluate uptake of media-applied plant growth regulators and to use the system to determine uptake of paclobutrazol, chlormequat chloride, and (S)-abscisic acid.

A split-root system using *Dendranthema x grandiflora* ‘Snowmass’ was developed as a model system to determine uptake as a function of plant response. Roots were separated into two compartments of a cell pack. The chemical under evaluation was applied to one-half of the root system, which was excised at prescribed time intervals to terminate the plant’s exposure to the chemical. Uptake was determined as a function of plant response. The system was effective because root excision had little to no effect on the plant. Stem elongation for the 1 and 24 day controls from the second paclobutrazol experiment was 20.1 cm and 21.5 cm, respectively, a difference of 1.4 cm and statistically non-significant. Transpiration rates for the non-cut and cut controls from the third (S)-ABA experiment were 9.67 and 8.73 μg•cm⁻²•s⁻¹ at 4 hours after
application (HAT) and 14.04 and 12.35 μg·cm⁻²·s⁻¹ at 24 hours after application. Observed differences were due to exposure interval and theoretically amount of chemical taken up, although this parameter was not determined.

Paclobutrazol uptake was determined to be slow, with longer exposure resulting in greater inhibition of stem elongation. These results explain the findings and observations of other research studies. Paclobutrazol has low water solubility (Lever 1986) and is not readily leached out of the media by significant amounts of water as soon as one hour following application (Barrett et al., 1987). The chemical, as determined by bioassay, is generally located in the upper levels of the media, with gradual re-distribution to lower layers over time (Million et al., 1999). There is a hydrophobic attraction between the non-polar portions of the molecule and waxes of bark particles (Barrett, 1982). Data from this study propose that the binding reaction occurs rapidly because little inhibition of stem elongation was observed from one day of exposure. The reaction is reversible and paclobutrazol can still be obtained, absorbed, and utilized by the plant until the chemical pool has been depleted, which follows the observed linear-plateau model.

In contrast to paclobutrazol, chlormequat chloride uptake was determined to be rapid. Uptake pattern also followed a linear-plateau function and reached a plateau at 6.78 hours, in contrast to 15.85 days for paclobutrazol. Chlormequat chloride is rapidly absorbed by wheat plants when added to nutrient solution, with exposure of six hours resulting in 20% growth inhibition (Dekhuijzen and Vonk, 1974). Chlormequat chloride is highly water soluble (Cathey and Stuart, 1961) and translocation of media-applied chemical occurs in the xylem with the transpiration stream. Results from this study suggest that the chemical remains in solution, with little interaction with media components, and is readily absorbed by mass flow. Under this assumption, uptake of chlormequat chloride is dependent on transpiration flux and could
potentially be influenced by factors affecting plant transpiration, such as light, temperature, and humidity.

Uptake of (S)-abscisic acid was also determined to be rapid. Sufficient chemical was absorbed during exposure intervals of 7.5 min to 4 hours to close stomata and significantly reduce transpiration rate at 4 hours after application. Since (S)-ABA is rapidly metabolized and effects are generally short-lived, experiments were conducted to determine if uptake during shorter exposure intervals was sufficient to provide the same duration of stomatal closure compared to longer exposure intervals. A general trend observed was shorter exposure intervals having reduced efficacy at 24, 48, and 72 hours after application. Differences were more pronounced at the later data observations, with exposure of at least one hour required for maximum efficacy. (S)-ABA efficacy is dependent on the amount present at the active site and, in this model system, is a function of uptake and metabolism. Abscisic acid is rapidly metabolized in leaf tissue (Loveys and Kriedemann, 1973; Hartung et al., 1980,). Uptake could possibly be influenced by application concentration and environmental factors that affect transpiration rate. Efficacy of (S)-ABA is commercially important and factors during and shortly after application that influence uptake are important to investigate.

The split-root system model was used in this study to effectively evaluate the uptake of three plant growth regulators – paclobutrazol, chlormequat chloride, and (S)-ABA. This system answered the initial question of how much chemical exposure time was required for maximum efficacy under normal growing conditions. Questions that possible future research could answer is the importance of environmental or other factors during or shortly after application on chemical uptake, especially for chemicals that remain in solution and do not partition to media components. In addition, this system has the potential to be used to answer uptake questions for
other chemical pesticide classes applied as media drenches, including herbicides, fungicides, and insecticides.
WORKS CITED


BIOGRAPHICAL SKETCH

Jessica Boldt was raised in Melbourne, Florida and became interested in horticulture through working in the family greenhouse operation. She attended the University of Florida and completed two internships during her degree program, a six-month internship at White’s Nursery and Greenhouse in Chesapeake, VA, and a three-month internship at Pleasant View Gardens in Loudon, NH. She graduated in 2005 with a B.S in environmental horticulture (landscape and nursery management specialization) and a B.A. in business administration.

Jessica was a graduate research assistant during her graduate program at the University of Florida and conducted variety trialing on pre-release plant material while assisting with the public trial garden. She graduated in 2008 with a M.S. in horticultural science (environmental horticulture specialization).