ABSTRACT

KRUG, BRIAN ALAN. Physiological and Environmental Factors Affecting Shoot Tissue Boron Concentration of Pansy *(Viola ×wittrockiana)*, Petunia (*Petunia ×hybrida*), and Gerbera (*Gerbera jamesonii*) Plugs. (Under the direction of Brian Earl Whipker).

The overall objectives of these studies were to determine and characterize the causes of an abnormal growth syndrome often observed in pansy (*Viola ×wittrockiana*), petunia (*Petunia ×hybrida*), and gerbera (*Gerbera jamesonii*) plug production. Observed symptoms included distorted, curled, thickened and brittle leaves, abortion of apical meristems and proliferation of lateral shoots. The abnormal growth was present in varying severities and in no pattern within a plug flat or the greenhouse. In the past, the symptoms have been attributed to a deficiency of calcium (Ca) or boron (B), or a virus infection. Symptomatic plants tested negative for 16 common ornamental viruses. Calcium and B deficiency were induced; plants deficient in Ca became discolored and eventually necrotic, while symptoms on plants deficient in B were similar to those reported above. Persisting symptoms were also exhibited when plants were deprived of B for 1-wk intervals, after which B was reintroduced. These studies concluded that B deficiency is the cause of the observed abnormal growth in plug production and a temporary B disruption can cause persistent deficiency symptoms.

In the production of plugs, constant application of complete fertilizers containing B is commonly used. Therefore it is improbable that insufficient B is the cause of B deficiency unless the initiation of deficiency symptoms occurs as seeds germinate and before the first fertilizer application (7-10 d after sowing). Germination substrates are commonly amended with a nutrient charge to supply adequate nutrients including B before the first fertilizer application. Six germination substrates were sampled to test

how evenly the nutrient charge was distributed when 288-plug flats were filled. In all substrates, there was no significant difference in the distribution of any of the nutrients. High substrate pH causes B in the substrate to be unavailable to the plant. When germination substrate was incorporated with four increasing rates of dolomitic limestone, substrate pH increased and the shoot tissue concentration of B decreased. Plants take up B passively through the transpiration stream, therefore B uptake is closely linked to the rate of transpiration. Plants were exposed to three degrees of drought stress either by drying the substrate or the use of polyethylene glycol. Shoot concentrations of B were not affected by either drought stress treatment. Abscisic acid was applied as either a foliar spray or substrate drench, both at concentrations of 150 or 300 $mg \cdot L^{-1}$ in order to decrease transpiration. Abscisic acid resulted in a decrease of both transpiration and shoot B concentration.

High relative humidity (RH) can also decrease plant transpiration; plug production requires elevated RH to ensure germination and to maintain hydration of young seedlings. Plants were grown in ambient or high RH; plants grown in high RH had lower shoot tissue concentrations of B. To attempt to counteract the effect of high RH, air flow was increased on a constant basis. Providing constant airflow did not increase transpiration nor shoot tissue concentration of B.

Physiological and Environmental Factors Affecting Shoot Tissue Boron Concentration of Pansy (*Viola ×wittrockiana*), Petunia (*Petunia ×hybrida*), and Gerbera (*Gerbera jamesonii*) Plugs

by

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Dedication

This dissertation is dedicated to my loving wife

Tina

I would not have been able to accomplish this without her help and support.

Biography

Brian Alan Krug was born in Pella, Iowa to Jerry and JoEllen Krug on 18 July 1976. He lived there with his parents and older sister Pamela until 1982 when they moved to Mt. Auburn, Iowa, so his father could take over the family farm when Brian's grandparents retired. Brian graduated from Union High School in La Porte City, Iowa in May of 1995. He graduated with his undergraduate degree in Horticulture from Iowa State University at Ames, Iowa in December of 2000. After graduation Brian took the Head Grower position at DeJong Greenhouse's Oskaloosa, Iowa facility. In 2004, he completed the requirements of the degree of Master of Science in the floriculture program under the direction of Dr. Brian E. Whipker. He remained at North Carolina State University under the direction of Dr. Whipker to pursue a Ph.D. degree. In January 2008 Brian will begin an academic career as an Extension Specialist in the areas of floriculture and greenhouse production at the University of New Hampshire.

On March 19th, 2005 he made Tina Wilkinson his wife. Tina gave birth to their first child Henry Alan Krug on December 10, 2007.

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Chapter 1

Physiological Aspects of Calcium and Boron in Plants

During the past five years, greenhouse growers and seed company technical representatives have reported concerns about distorted terminal growth of pansy, petunia, and gerbera plants. The problem is thought to be caused by a deficiency of calcium (Ca) or boron (B). The occurrence and severity of the problem can vary: from year to year, within a particular period of the season, by stage of plant development, by geographical distribution (with problems reported in NC, IN, OH, TX, CA, VA, WV, Canada, The Netherlands, and Italy), and with respect to pansies by bloom color, more specifically, to particular cultivar (B. Whipker, personal communication). This research proposes to investigate what is causing the growth distortion and how to prevent it.

Roles Within Plants

Calcium

Calcium has been and continues to be studied extensively in regard to its role in plant nutrition and plant physiology. In general, the roles of Ca can be categorized in three main topics: structure, signaling, and protection from stresses. The following is not a complete review of every function of Ca, but a review of the major points which can be grouped in these categories.

Calcium is considered to be the key element in the primary walls of plant cells (Pilbeam and Morely, 2007). Polygalacturonase, which aids in the hydrolysis of cell walls, is inhibited by the presence of Ca; therefore one symptom of Ca deficiency is the breakdown of tissue (Marschner, 1995). In addition, Ca aids in cell wall biosynthesis (Roux and Slocum, 1982). By bridging phosphate and carboxylate groups in phospholipids and creating a linkage between polygalacturonic acids (pectins), Ca provides rigidity to cell walls (Marschner, 1995; Palta, 1996; Pilbeam and Morely, 2007; Taiz and Zeiger, 2002).

Calcium is important for the structure and function of membranes (Palta, 1996; Pilbeam and Morely, 2007; White and Broadley, 2003). Calcium establishes and maintains the negative electrochemical potential gradient across the plasma membrane by being a counter-cation for organic and inorganic anions in the vacuole (Roux and Slocum, 1982; White and Broadley, 2003). Also, concentrations of Ca needed for pollen germination range from 50 to 5000 ppm in 86 different species tested (Brewbaker and Kwack, 1963). Once pollen is germinated, Ca is required for pollen tube elongation, rigidity and straightness (Brewbaker and Kwack, 1963; White and Broadley, 2003).

Free Ca can be found in micromolar and submicromolar concentrations within a plant; cytosolic concentrations of Ca are 10^{-7} M while apoplastic concentrations range from 10^{-4} to 10^{-3} M (Bush, 1995; Roux and Slocum, 1982). These concentrations are strictly regulated by Ca ATPases and H^{+}/Ca antiporters (White and Broadley, 2003). Minute changes in cytosolic concentrations can signal a plentitude of reactions within plants. Probably the most researched aspect of Ca signaling within plants is the interaction between Ca and the protein calmodulin (CaM). Calcium involves signaling ranging from the plant's responses to touch, gravity, light, temperature, and hormones, as well as ion balance, gene expression, carbohydrate metabolism, mitosis, and cytokenisis (Bush, 1995; Helper and Wayne, 1985; Palta, 1996).

Calcium is involved in protecting plants from stress. This process can be by structural or physiological means or through signaling a response. Increased Ca concentrations are beneficial in decreasing the incidence of potato disorders such as internal brown spots, hollow heart, and increasing tuber quality and storability (Palta, 1996). These benefits are probably due to increased cell wall integrity caused by the presence of high Ca.

Calcium regulates turgor pressure in response to salt stress and is involved in stomatal opening and closing, which protects the plant from desiccation (Taiz and Zeiger, 2002; White and Broadley, 2003). Calcium also is involved in relieving plants from heat stress, cold shock, mechanical stress, ozone, blue light, ultraviolet radiation, toxic ions, and low pH (Clarkson and Hanson, 1980; Pilbeam and Morely, 2007; Taiz and Zeiger, 2002).

Boron

Although a micronutrient, B affects the physiology of plants in many ways. Boron has been a difficult element to research due to the small quantities needed for normal plant function and the lack of a radioactive isotope. Because no radioactive isotope is available, most of the information known about B's role has been gained through observations of plants deficient in B. Therefore, the reported roles of B have the potential of being indirectly caused by B, rather then directly.

Despite the challenges in research a large amount of work has been accomplished; however, many reports have been conflicting. For example, Clarkson and Hanson (1980) concluded that B had no structural function while Hu and Brown (1997) and Matoh (1997) state that the primary role of B is structural. Clarkson and Hanson (1980) and Marschner (1995) conclude that B has no enzymatic function while Lovatt and Dugger (1984), Shelp (1993), and Dave (1996) report B does. The contradictory reports could be due to technological advances over the years.

A review of the literature reveals it is probable that B is involved in structural roles, especially in cell walls. Boron has been reported to complex with the cell wall and many of its constituents (Marschner, 1995; Matoh, 1997; Römheld and Marschner, 1991; Taiz and Zeiger, 2002; Thellier et al., 1979). A deficiency in B also leads to a number of changes in

cell walls; these changes may not be caused directly by B deficiency but by a cascade of events catalyzed by the lack of B (Marschner, 1995). Plants deficient in B also have an increase in hemicellulose, pectin, callouse, and lignin (Lovatt and Dugger, 1984; Neales, 1960; Rajaratnam and Lowry, 1974; Römheld and Marschner, 1991; Van de Venter and Currier, 1977). Cells which have been deprived of B often have thickened walls and are unable to elongate or divide (Dell and Huang, 1997).

Boron deficiency also influences cellulose synthesis. When B is not present, uridine diphosphate glucose (UDPG)-pyrophosphorylase is inhibited, causing a decrease in UDPG for cellulose synthesis (Shelp, 1993). All of these effects can cause changes in growth of the cells and tissue including a shift in orientation of cell division, hindered cell elongation or division, inhibition of cell growth, and altered tissue growth (Dell and Huang, 1997; Hu and Brown, 1994; Lovatt and Dugger, 1984; Marschner, 1995; Shelp, 1993).

Membrane integrity and function of the plasma membrane is affected by B (Agarwala and Chatterjee, 1996; Marschner, 1995; Römheld and Marschner, 1991). Specifically, ATPase activity decreases as B concentration decreases, which in turn affects transport of ions, metabolites, and hormones, and alters the electrical potential gradients across membranes (Agarwala and Chatterjee, 1996; Blaser-Grill et al., 1989; Marschner, 1995; Römheld and Marschner, 1991; Schon et al., 1990; Shelp, 1993).

The role of B in enzymatic activity has also been in disagreement. Marschner (1995) and Clarkson and Hanson (1980) suggest that B has no enzymatic function in plants. Conversely, there are many reports that B increases or decreases the activity of various enzymes (Dave, 1996; Lovatt and Dugger, 1984; Shelp, 1993). According to Lovatt and

Dugger (1984), B can decrease or completely inhibit the activity of a number of oxidases, phosphatases, and dehydrogenases.

The reproductive system of plants is affected by B deficiency. The meristems are often aborted and the formation of flowers are inhibited (Agarwala and Chatterjee, 1996; Kamali and Childers, 1970). Even when flowers do form, B deficiency can still affect the reproduction cycle. The development of pollen and pollen germination is sensitive to a lack of B (Agarwala and Chatterjee, 1996; Dell and Huang, 1997; Kamali and Childers, 1970; Roux and Slocum, 1982; Schmucker, 1932; Vasil, 1964).

One of the first observed effects of B deprivation is a decrease in nucleic acid biosynthesis, especially in root cells (Lovatt and Dugger, 1984). This is not a surprise, as one of the major symptoms of B deficiency is short, club-like roots.

Boron has been reported to complex with various carbohydrates, resulting in altered metabolism and/or translocation (Agarwala and Chatterjee, 1996; Lovatt and Dugger, 1984; Taiz and Zeiger, 2002). The specific role B has in carbohydrate metabolism can be dependent on species; B may cause an accumulation of sugars and starches or a reduction of sucrose (Agarwala and Chatterjee, 1996). The effect of B on sugars and other carbohydrates could cause cascading effects altering cell walls (Marschner, 1995; Shelp, 1993).

Another subject surrounded with disagreement is the affect B has on indole-3-acetic acid (IAA). When present in stems IAA promotes growth, but when present in roots it inhibits growth (Taiz and Zeiger, 2002). One hypothesis is that B suppresses IAA oxidase activity, therefore when B is in adequate supply IAA is in ample supply (Agarwala and Chatterjee, 1996; Lovatt and Dugger, 1984; Marschner, 1995; Römheld and Marschner, 1991). Conversely, Li et al. (1997) report that when B is present IAA oxidase activity

increases. This discussion is addressed by Lovatt and Dugger (1984), who report that IAA content can vary in different plant parts and species. They suggest that a single hypothesis of the connection between B and IAA is doubtful. If IAA levels in the plant increase when B is deficient, IAA could be linked to B deficiency symptoms such as proliferating shoots and short club-like roots.

Plant Uptake

Calcium

Calcium is available to plants as the divalent cation, Ca^{2+}. Calcium moves through the soil solution to the surface of a plant root through mass flow (Pilbeam and Morely, 2007). Once Ca reaches the surface of the root it enters passively into the apoplasm of the cortex. The movement of Ca through the root cortex is facilitated by diffusion and by displacements and exchanges within the cation exchange sites in the free space (Bangerth, 1979). In mature root tissue, the Casparian strip (a suberized barrier around root cells) prevents water and solutes from entering the stele through the apoplasm (Bangerth, 1979). Calcium must enter into the stele through the apoplasm, therefore it moves to the root tip where the Casparian strip is not yet intact and Ca is free to enter in the stele (Bangerth, 1979; Roux and Slocum, 1982). There are also breaks in the Casparian strip where lateral roots are initiated and the suberized region has yet to reformed thus allowing Ca to enter (White and Broadley, 2003). After entering the stele, Ca is free to enter into the xylem to be transported throughout the shoot of the plant.

Boron

Boron is present in the soil solution as $B(OH)_3$, or boric acid, and is available in this form for uptake by plant roots (Jiao et al., 2005). The mechanism by which B moves into

plant roots is not well documented and is a subject which has been highly disputed in literature (Blevins and Lukaszewski, 1998). At the center of the debate is whether B is taken up actively or passively. The case for B being taken up passively is well supported. Boric acid is readily able to diffuse across the plasma membrane of cells (Kochian, 1991). Hu and Brown (1997) and Halbrooks et al. (1986) concluded that B is taken up passively through the transpiration stream. Marschner (1995) states that internal concentrations of B appear to be dependent on the external concentrations, giving strength to the idea that B moves down a concentration gradient. This idea is supported by the report that in walnut plants the tissue concentration of B increased linearly as the B concentration in the soil increased (Picchioni and Miyamoto, 1991).

Despite this evidence, it seems that an active mechanism might also play a part in the uptake of B because it has been reported that tissue concentrations can be higher than those in the soil (Bowen, 1972; Raven, 1980). Also, not all plants take up the same concentration of B while being treated with the same concentration in the growing substrate (Nable, 1991). The theory of active uptake has been rebutted with several explanations. Boron has been known to complex with *cis*-diols and constituents of cell walls. Complexing lowers the gradient within the cell, which would allow more B to enter (Jiao et al., 2005). Plants of different species, or even different genotypes of the same species, may have different membrane permeabilities which would account for differences in rates of uptake (Jiao et al., 2005; Marschner, 1995). The uptake of B has not been shown to stop when metabolic inhibitors are used, which would point towards the theory of passive uptake being correct (Bingham et al., 1970). However, a third theory has emerged where both passive and active uptake are present in plants (Dannel et al., 1997; Dannel et al., 2000). By pretreating plants

with sufficient or low concentrations of B before depriving the plants of B, studies indicated that under circumstances where B is in adequate supply it is taken up in a linear trend. Conversely, when plants are pretreated with low concentrations of B, the concentration in the plant tissue exceeds that of the external concentration. This suggests that at sufficient levels, B is taken up passively, but when marginal levels of B are present, it is taken up actively against the concentration gradient. Although evidence supports the idea of an active process taking up B, no specific mechanism has been identified (Pfeffer et al., 1997).

Long and Short Distance Transport

Calcium

Calcium is an immobile element which is primarily moved from the roots to the shoot via the xylem for long-distance transport (Bangerth, 1979; Hanger, 1979; Marschner, 1995; Roux and Slocum, 1982; White and Broadley, 2003). The movement of Ca through the xylem to the leaves is dependent on the transpiration stream during the day when leaves are actively transpiring; during the night when transpiration is low or non-existent, Ca moves into buds and meristems by root pressure (Clarkson, 1984; Marschner, 1995). Although Ca is dependent on the transpiration stream, it does not move via mass flow but instead by jumping from exchange site to exchange site in the xylem (Bangerth, 1979; Clarkson, 1984; Hanger, 1979). Factors which aid the movement through the transpiration stream are the velocity of the water, the concentration of Ca, and the presence of chelators like malic and citric acids, which keep Ca off exchange sites (Bangerth, 1979; Clarkson, 1984). Calcium is not mobile in the phloem, as reported by Hanger (1979) who applied Ca only as a foliar spray and observed no Ca movement out of the leaves into other parts of the plant. Bangerth (1979) supports Hanger's work by reporting low Ca concentrations in the phloem sap, no Ca

movement out of leaves when it is applied to the foliage, no Ca accumulated above a girdle, and no Ca moved out of leaves before senescence. Calcium can be moved in short-distance transportation via the symplasm. However, the low solubility of Ca in the cytoplasm excludes symplastic transport as a viable method to supply adequate Ca to the plant (Bangerth, 1979). Calcium moves across the plasma membrane via Ca ATPase, Ca/H^+ anti-porters, and channels that regulate cytosolic Ca levels used in signaling (Bush, 1995).

Boron

Boron is moved through the plant as boric acid, but little work has been conducted on the transportation of B (Kochian, 1991). To move into cells, B can simply diffuse across the plasma membrane (Kochian, 1991). It has been reported that different species and genotypes vary in membrane permeability and cause different concentrations and rates of B to be transported into cells (Jiao et al., 2005; Marschner, 1995). Most of the B movement from the roots to the shoots is done through the transpiration stream via the xylem (Jones, 1991; Kochian, 1991; Kohl and Oertli, 1961; Raven, 1980). Boron is an immobile element and is not transported through the phloem (Blevins and Lukaszewski, 1998; Kochian, 1991; Kohl and Oertli, 1961; Woodbridge et al., 1971). Because it is immobile, B tends to accumulate in the leaf tips and margins of many plants (Blevins and Lukaszewski, 1998). However, most plants do not accumulate enough B under normal circumstances to cause toxicity symptoms. This may be due to the loss of B through guttation protecting against an over-accumulation (Kohl and Oertli, 1961). Although B is generally thought to be immobile, there have been studies suggesting otherwise. Kochian (1991) reports that B can be complexed with manitol in carrot, celery, bean and cauliflower plants and then moved throughout the plant via the

phloem. Boron has also been reported to be phloem mobile when complexed with sorbitol in *Pyrus*, *Malus*, and *Prunus* (Brown and Hu, 1996).

Deficiency Symptoms

Calcium

Calcium is usually supplied to the plant in adequate amounts by soils, yet Ca deficiencies are common. Calcium deficiency is often caused by a local deficiency within particular plant cells or tissues brought about by difficulties in the transport of Ca within the plant because of environmental factors (Kirkby and Pilbeam, 1984). In general dicotyledons require higher concentrations of Ca than do monocotyledons (Marschner, 1995).

Visual symptoms include deformed, strap-like leaves; chlorosis; and curled leaves that develop yellow-to-tan margins, eventually becoming necrotic (Nelson, 2003). Symptoms will occur on the growing tips and the youngest leaves due to calcium's immobility (Mengel and Kirkby, 2001). A low supply of Ca will lead to enhanced plant senescence and the plant may also be more susceptible to fungal infections (Marschner, 1995; Sorokin and Sommer, 1940). A number of disorders in various crops have been reported to be caused by a deficiency of Ca, including marginal bract necrosis (poinsettias), bitter pit (apples), blossom-end rot (tomatoes and peppers), and blackheart (celery) (Bierman et al., 1989; Mengel and Kirkby, 2001; Woltz and Harbaugh, 1986). Shear (1975) reports more than 30 Ca-deficient conditions disorders on fruits and vegetables.

Responses to Ca deficiency by plants can be very rapid. When plants are deprived of Ca, root extension and cell extension cease within a few hours (Marschner, 1995). Under conditions of low Ca, apples have increased rates of respiration (Bangerth et al., 1972).

Hecht-Buchholz (1979) reports that under Ca deficient conditions membranes begin to breakdown and cellular leakage increases.

Boron

Boron deficiency is more prevalent than any other essential micronutrient deficiency (Gupta, 1993). Reported values at which plants are considered to be deficient in boron vary greatly (Jones, 1991; Mengel and Kirkby, 2001; Römheld and Marschner, 1991). This variation is indicative of the wide difference in B requirements for normal growth among species. To further complicate the issue, B deficiency symptoms have been reported to be inconsistently present from season to season as well as year to year in several crops (Eaton, 1944).

Symptoms of B deficiency typically manifest in the young leaves and, unlike other micronutrient deficiencies, in the roots (Gupta, 1993). Many of the symptoms can be seen by the naked eye, including aborted growing tips, fast-growing auxiliary shoots, strapped or crinkled leaves, stunted leaves, upward-cupping leaves, chlorosis of upper leaves, and decreased leaf expansion (Jiao et al., 2005; Laffe and Styer, 1989; Mengel and Kirkby, 2001; Stuart, 1991). Symptoms appearing on the roots include: brown discoloration of tissue and reduced growth (Blevins and Lukaszewski, 1998; MacInnes and Albert, 1969; Pilbeam and Morely, 2007; Pitchay, 2002; Sanzonowicz et al., 1998).

Besides the visual symptoms, B deficiency can be detected at the cellular and metabolic levels. Plants deficient in B have decreased RNA content in root tips, thickening of cell walls, less tightly packed golgi bodies, slower and/or ceased pollen tube growth, and decreased intercellular spaces (Loomis and Durst, 1992; MacInnes and Albert, 1969; Matoh, 1997).

Summary

Both Ca and B are primarily taken up passively by plant roots and are dependent on water availability. Drought-induced B deficiency has been reported in birdsfoot trefoil (*Lotus corniculatus*) (MacQuarrie et al., 1983) similarly, the incidence of blossom end rot, a symptom of Ca deficiency, can be decreased with increased irrigation (Franco et al., 1999). However, these two characteristics are the only similarities between B and Ca uptake.

As mentioned above, the majority of Ca can only enter the apoplast. Small amounts of Ca, needed for signaling, can be moved across the plasma membrane into the cytosol using specific Ca transporters (Pilbeam and Morely, 2007). The Casparian strip inhibits Ca from entering the xylem from any other location than the root tips and points of new lateral root initiations. Boron is readily diffused across the plasma membrane and can enter the symplasm without any expense of energy by the plant.

Calcium and B both are moved long distances through the xylem in the transpiration stream. However, B is moved via mass flow whereas Ca moves by binding to sequential exchange sites. With the exception of B in manitol- and sorbitol-rich plants, both B and Ca are phloem-immobile. Calcium and B are different in the way they are transported across short distances. Calcium requires specific transporters, some of which require the use of energy, were as B strictly moves passively, by diffusion.

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Chapter 2

Characterization of Calcium and Boron Deficiency and the Effects of Temporal Disruption of Calcium and Boron Supply on Pansy, Petunia, and Gerbera Plugs

(In the format appropriate for submission to HortScience)

Submitted to HortScience

Characterization of Calcium and Boron Deficiency and the Effects of Temporal Disruption of Calcium and Boron Supply on Pansy, Petunia, and Gerbera Plugs

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Summary

'Dynamite Yellow' pansies (*Viola ×wittrockiana*), 'White Storm' petunias (*Petunia ×hybrida*), and 'Festival Apricot' gerbera daisies (*Gerbera jamesonii*) plants were grown hydroponically to characterize the deficiency symptoms caused by the absence of calcium (Ca) or boron (B). Primary symptoms occurred on the youngest tissue for both elements but distinct differences between Ca and B deficiencies were observed. Plants responding from Ca deficiency exhibited discoloration and upward rolling of leaves and ultimately necrosis. Plants responding from B deficiency exhibited minor chlorosis, upward curling and thickening of leaves, distorted meristems, and straplike leaves. The major differentiating factor between Ca and B deficiencies was the presence of necrosis in the Ca deficient plants and the distorted and thicken leaves that lack necrosis in the B deficient plants.

A second experiment was conducted using the same species, cultivars, and growing practices to investigate how a temporary disruption of Ca or B affects the plant throughout the crop cycle. Either Ca or B was removed from the nutrient solution for a 7-d period from d 15 to d 21, d 22 to d 28, or d 29 to d 35 after sowing. After a 7-d disruption, the respective element was reintroduced to the plants. Regardless of when the plants were deprived of Ca or B, the symptoms of the respective deficiency were present at the end of the experiment.

Introduction

During the past five years, greenhouse growers and seed company technical representatives have reported concern about distorted terminal growth of pansy, petunia, and gerbera plants. The problem is thought to be caused by a deficiency of calcium (Ca) or boron (B). The occurrence and severity of the problem can vary: from year to year; within a particular period of the season; by stage of plant development; by geographical distribution and with respect to pansy cultivar.

Because Ca is an immobile element, deficiency symptoms appear primarily on the upper leaves. Visual symptoms include deformed, straplike leaves; chlorosis; and leaves that develop yellow-to-tan margins, eventually becoming necrotic (Nelson, 2003).

Symptoms of B deficiency typically manifest in the young leaves and, unlike other micronutrient deficiencies, in the roots (Gupta, 1993a). Many of the symptoms can be seen by the naked eye including: aborted growing tips, fast growing auxiliary shoots, and leaves that are strapped, crinkled, stunted, thickened, or upward cupping, chlorosis of upper leaves and restricted leaf expansion (Jiao et al., 2005; Laffe and Styer, 1989; Mengel and Kirkby, 1987; Stuart, 1991)

Plants with either Ca or B deficiencies can produce similar symptoms, leading to confusion. When comparing the symptoms of the two deficiencies one symptom stands out to easily identify which deficiency is affecting the plant. Calcium deficient plants will typically develop necrosis earlier in the deficiency syndrome. The initial objective of this study was to characterize Ca and B deficiencies in pansies, petunias, and gerbera to improve the process of differentiation of the two deficiencies. The second objective of this study was

to determine if a temporary disruption of Ca or B availability could cause a lasting deficiency in that element throughout the crop cycle.

Materials and Methods

Experiment 1

Excluding calcium or boron. Oasis foam (Smithers-Oasis, Kent, Ohio) was used as the growing medium. The foam was cut to fit into a 2-ml microcentrifuge tubes that had approximately the bottom 3 mm removed. 'Dynamite Yellow' pansy, 'White Storm' petunia, and 'Festival Apricot' gerbera were sown, one seed per tube. There were 36 tubes per replication. The experiment was a completely randomized design with 3 replications of each treatment. Once sown, seeds were placed in a germination chamber with a temperature set point of 20 ºC. Light was provided by fluorescent bulbs with a PPFD of 24 to 75 $\mu mol \cdot m^{-2} s^{-1}$ at plant canopy for 12 h per d. Oasis foam was kept moist using deionized water until seeds germinated. After germination plant tubes were transferred into 2.3-L plastic containers with holes drilled in the lids to hold the microcentrifuge tubes. Plants were then moved into a greenhouse and grown in a complete modified Hoagland's all nitrate solution, minus Ca or B, and 0.25x Ca or B: (macronutrients in mM) 15 NO_3-N, 1.0 PO_4-P, 6.0 K, 5.0 Ca, 2.0 Mg, and 2.0 SO_4-S (Hoagland and Arnon, 1950), plus μM concentrations of micronutrients, 72 Fe, 9.0 Mn, 1.5 Cu, 1.5 Zn, 45.0 B, and 0.1 Mo. The following reagent grade chemicals KNO_3, $Ca(NO_3)_2$, KH_2PO_4, $MgSO_4$, $NaNO_3$, FeDTPA, $MnCl_2$, $ZnSO_4$, CuCl, H_3BO_3, $Na_2 2MoO_4$ and deionized water was used to formulate treatment solutions. When Ca, supplied in the form of $CaNO_3$, was removed from the solution, the nitrate was replaced with $NaNO_3$. Because the plants were small and did not use large quantities of nutrient solutions, solutions were replaced and were adjusted to pH 5.8 using 0.1M NaOH every two weeks. Petunias

and gerberas were only treated with a complete, minus Ca, or minus B solution. Plants were grown in a greenhouse with day/night temperature set points of 23.9/17.8 ºC, under high pressure sodium lights to provide a minimum PPFD of 100 $\mu mol \cdot m^{-2}s^{-1}$ at plant canopy for 12 h per d. Plant tissue was harvested 35 d after sowing (DAS). Tissue samples were taken by removing the two most recently mature leaves from the control, 0.25x B and Ca treatments, and the entire plant, excluding the cotyledons, for the minus B and Ca treatments.

Experiment 2

Temporary disruption of calcium or boron availability. Seeds of 'Dynamite Yellow' pansy, 'White Storm' petunia, and 'Festival Apricot' gerbera were sown and grown similarly, except for fertilization, to those in Experiment 1. Each replication consisted of a tub holding 36 seedlings. The experiment was a completely randomized design with 4 replications of each treatment. All plants were provided with a complete modified Hoagland's solution except from 15 to 21 DAS (week 3), 22 to 28 DAS (week 4), or 29 to35 DAS (week 5). At those times the complete solution was replaced with minus Ca or minus B solutions. At the beginning and end of the fertilization disruption phase, the oasis was flushed three times with the replacement solution to leach the substrate. A control treatment that received a complete solution for the entire duration of the experiment was also included. After plants had been deprived of Ca or B for 7 d, a complete solution was reintroduced. Plants that were deprived of Ca during week 3 were harvested 28 DAS due to the severe effects the treatment had and the decline of the plants. For the other two treatments tissue was harvested 42 DAS after germination by removing the entire plant excluding the cotyledons.

Tissue Analysis

The tissue samples for both experiments were first rinsed in deionized water, then washed in 0.2 N HCl for 30 s, re-rinsed with deionized water, and dried at 70 ºC for 72 h. Dried tissue was ground in a stainless steel Wiley mill through a 1-mm screen (20-mesh) and 0.1 g was digested in a microwave digester (MARS; CEM Corp, Matthews, N.C.) using a modified EPA method (EPA method 3051 with additional peroxide step). Nutrient concentration, except N, was determined with inductively coupled plasma optical emission spectroscopy (ICP-OES; Model IRIS Intrepid II, Thermo Corp., Waltham, Mass.). A quality control was run every ten samples and if any element was determined to be more than 10% higher or lower than the standard value, the instrument was recalibrated. Tomato standards (NIST reference material 1573) were compared every 20 samples and tomato and spinach standards (NIST reference material 1570a) were compared every 40 samples. Tissue was analyzed for P, K, Ca, S, Mg, B, Cu, Fe, Mn, and Zn.

Data analysis

Data were tested by analysis of variance (ANOVA) using general linear model (SAS Institute, Cary, N.C.) and means were separated by least significant differences (LSD) at $P \leq 0.05$. When tissue concentrations of an element were not detected a value half that of the lowest detectable level for the respective element was used for statistical analysis.

Results and Discussion

Experiment 1

Pansies Minus Calcium

Plants with slight Ca deficiency turned purple-brown on the petioles and leaf tips, and had a slight upward leaf curl (Fig. 1A). Moderate symptoms included greater discoloration

and more prominent upward leaf curling as well as necrosis of the leaf tips. At advanced stages of the deficiency, whole leaves and the apical meristem became necrotic (Fig. 1B). Plants receiving treatments of 0.25x Ca and minus Ca had tissue concentrations of 0.27 and 0.03% Ca, respectively, which were significantly ($P \leq 0.001$) lower than the control (Table 1). These values for plugs were below previous reported tissue concentrations for early and advanced Ca deficiency symptoms for fully expanded mature leaves from transplanted pansies (46 DAS), which were 0.45% and 0.61%, respectively (Pitchay, 2002). The tissue concentration of S, Mg, B, and Zn were inversely related to the amount of Ca in the solution (Table 1). The lower concentration of Mg, B, and Zn may be attributed to the known antagonistic role Ca has on those elements (Gupta, 1993b; Jones and Scarseth, 1944; Marschner, 1995; Robson and Pitman, 1983).

Pansies Minus Boron

Plants exhibiting a slight B deficiency had leaves that were beginning to curl upward (Fig. 1D). Plants with moderate B deficiency symptoms were thicker and had leaves more prominently curled upward. Severe deficiency symptoms included thickened, smaller leaves as compared to the control, and distorted meristems and young leaves (Fig. 1E). Plants receiving treatments of 0.25x B and minus B had tissue concentrations of 12.64 and $\leq$ 1.70 $mg \cdot L^{-1}$ B, respectively, which were significantly ($P \leq 0.001$) lower than the control. These concentrations were to be expected as Pitchay (2002) reported symptoms appearing when tissue concentrations of B were between 9.7-10.9 $mg \cdot L^{-1}$, for fully expanded mature leaves from transplanted pansies (72 DAS). As the concentration of B supplied diminished, the tissue concentration of P, K, Ca, S, and Mg increased (Table 1). Mishra et al. (unpublished data) report similar results were geranium tissue with lower concentrations of B also had

higher concentrations of P and S. However in their study K, Ca, and Mg decreased with decreased B concentrations.

Petunias Minus Calcium

Plants not receiving Ca were smaller than those receiving Ca. Plants exhibiting a slight deficiency were chlorotic and beginning to become necrotic (Fig. 2A). Plants with moderate Ca deficiency symptoms had distinct necrotic regions on the leaves, primarily on the tips. Plants that exhibited severe Ca deficiency symptoms were almost completely necrotic (Fig. 2B). Tissue concentrations of Ca in plants treated with a nutrient solution minus Ca (0.05%) were significantly ($P \leq 0.01$) lower than those treated with a complete nutrient solution (1.54%) (Table 2). These values were below previous reported tissue concentrations for early and advanced Ca deficiency symptoms, which were 0.32% and 0.38%, respectively, for fully expanded mature leaves from transplanted petunias (88 DAS) (Pitchay, 2002).

Tissue concentrations of Zn were significantly ($P \leq 0.01$) greater in plants treated with a nutrient solution minus Ca, which could be due to the lack of Ca antagonism previously mentioned (Table 2). Potassium values in plants treated with the minus Ca solution were significantly ($P \leq 0.05$) lower than those treated with the control (Table 2).

Petunia Minus Boron

Plants not receiving B were smaller than those receiving B. Plants exhibiting slight signs of B deficiency had thickened brittle leaves (Fig. 2D). Moderate symptoms included, in addition to thickened brittle leaves, distortion, and straplike young leaves. Plants exhibiting severe symptoms were the smallest in size; all leaves and the apical meristem exhibited signs of distortion (Fig. 2E). Tissue concentrations of B were too low to be

detected (lowest detectable level of B = 1.7 $mg \cdot L^{-1}$) in plants treated with the minus B solution and control plants had B tissue concentrations of 29.70 $mg \cdot L^{-1}$ which is within the acceptable range reported by Pitchay (2002) for fully expanded mature leaves from transplanted petunias (102 DAS) (Table 2). Treatments had no effect on any other analyzed element.

Gerbera Minus Calcium

Plants exhibiting a slight deficiency had leaves that had some bronzing on the margins of older leaves and younger leaves were curled and necrotic on the edges (Fig. 3A). The bronzing color was more pronounced on plants with moderate symptoms and necrosis was also more pronounced on younger leaves. Plants with severe symptoms had fewer leaves and severe bronzing on older leaves and younger leaves were completely necrotic (Fig. 3B). Tissue concentrations of plants not receiving Ca (0.10%) were lower than the sufficient range reported by Jones et al. (1991) and was significantly ($P \leq 0.001$) lower than those in the control (1.08%) (Table 3). Plants treated with nutrient solution minus Ca also had significantly ($P \leq 0.05$) higher concentrations of Mg and B and significantly ($P \leq 0.01$) lower concentrations of S than the control plants (Table 3). As noted above, Ca can have an antagonistic effect on Mg and B, therefore when Ca is not present tissue concentrations of Mg and B would be expected to be higher (Gupta, 1993b; Jones and Scarseth, 1944; Marschner, 1995; Robson and Pitman, 1983).

Gerbera Minus Boron

Plants not receiving B that exhibited slight deficiency symptoms were chlorotic and had thicker, more brittle leaves than those receiving a complete solution (Fig. 3D). Plants with more severe symptoms had upward cupped leaves that were wavy and uneven (Fig. 3E).

Tissue concentrations of B were too low to be detected in plants treated with the minus B solution and control plants had B tissue concentrations of 37.39 $mg \cdot L^{-1}$ (Table 3). The B tissue concentration of B in the plants treated with the minus B solution were lower than previously published sufficiency range (Jones et al., 1991). Treatments had no effect on any other analyzed element.

Experiment 2

Pansies Minus Calcium

Plants that were deprived of Ca during the third week of greenhouse production had to be harvested after the fourth week (32 DAS) due to the advanced stages of deficiency present (almost completely necrotic). A set of controls were harvested at the same time. The tissue concentration of the deficient plants (0.56%) was significantly ($P \leq 0.05$) lower than the control (0.80%) (Table 4). The tissue concentration of K, S, Mg, Cu, Fe, Mn, and Zn were inversely related to the amount of Ca in the solution (Table 4). Lower concentrations of Mg, Fe, Mn, and Zn may be attributed to the known antagonistic role Ca has on these elements (Gupta, 1993b; Jones and Scarseth, 1944; Marschner, 1995; Robson and Pitman, 1983). The quick onset of Ca deficiency symptoms were similar to those reported by Pitchay (2002). In that study in which 4 week old pansies began discoloring 8 d after calcium deficient conditions were introduced and necrosis appeared 9 d after treatment initiation. Concentrations of Ca in plant tissue of plants deprived of Ca during week 3 or 4 were 0.83 and 0.81%, respectively, and were significantly ($P \leq 0.05$) lower than the concentration of the controls (1.18%) (Table 4).

Pansies Minus Boron

There was no difference in B concentration between the control plants and plants deprived of B during week 3. Plants deprived of B during week 4 or 5 had B tissue concentrations of 64.80 and 66.08 $mg \cdot L^{-1}$, respectively, which were significantly ($P \leq 0.05$) lower than the control (78.88 $mg \cdot L^{-1}$) (Table 5). Concentrations of P for plants deprived of B in week 4 were significantly ($P \leq 0.05$) higher than the controls (Table 5), however all values were within an acceptable range for fully expanded mature leaves from transplanted pansies (72 DAS) (Pitchay, 2002).

Petunias Minus Calcium

There was no difference in Ca concentration between the control and plants deprived of Ca during week 3. Plants that did not receive Ca during week 4 or 5 had Ca tissue concentrations of 1.50 and 1.44%, respectively, which were significantly ($P \leq 0.001$) lower than the concentration of the controls (2.09%) (Table 6). Although lower than the controls, Ca values for plant not receiving Ca during week 4 or 5 were within the recommended range (Pitchay, 2002). The tissue concentration of K, S, and Mg, were inversely related to the amount of Ca in the solution (Table 6). As mentioned above, lower concentrations of Mg may be attributed to the antagonistic effect from Ca (Gupta, 1993b; Jones and Scarseth, 1944; Marschner, 1995; Robson and Pitman, 1983).

Petunias Minus Boron

There were no measurable differences in B tissue concentration among any of the treatments. In fact, the only treatment where B was detected was in the control, but even that was well below the level where deficiency symptoms would be expected. Analyzing petunias for B has created problems in the past and a suitable detection method using the

ICP-OES for B in petunias has not been developed (J. Frantz, unpublished data). No matter when B was deprived, both P and S concentrations were significantly ($P \leq 0.05$) lower than the concentration of the controls (Table 6).

Gerbera Minus Calcium

Plants deprived of Ca during any interval had tissue concentrations within the sufficiency range reported by Jones et. al (1991) and did not have significantly ($P \leq 0.05$) different tissue concentrations of Ca than the control (Table 7). Plants deprived of Ca during weeks 4 or 5 had significantly lower concentrations of Mg than the control (Table 7). The tissue concentration of B, were inversely related to the amount of Ca in the solution (Table 7).

Gerbera Minus Boron

Plants deprived of B at any interval did not have significantly ($P \leq 0.05$) different tissue concentrations of B than the control (Table 7), however deficiency symptoms appeared. However, this is consistent with tissue test done on plants in greenhouse production, by the time tissue samples were taken in this experiment and by growers, B could have been taken up by the plant, masking the deficiency (unpublished data). The concentrations of B in all treatments were within the published sufficiency range (Jones et al., 1991). There were significant ($P \leq 0.05$) differences in the concentrations of S among treatments; these concentrations were inversely related to the amount of B in the solution (Table 7).

Regardless of when the pansy, petunia, or gerbera plants were deprived of Ca or B the symptoms of the respective deficiency were present. Symptoms for Ca deficiency included: chlorosis along leaf margins, upward curling of leaves, and necrosis. While symptoms for B

deficiency included: leaf curling, distorted apical meristems, and proliferation of auxiliary shoots. As in experiment 1, plants deprived of Ca exhibited necrosis and plants deprived of B had no necrosis. The plants did not recover from the symptoms even when Ca or B was reintroduced in the nutrient solutions.

Conclusion

The results from experiment 1 demonstrated the clear differences between symptoms caused by Ca and B deficiencies. Pansy, petunia, and gerbera plants experiencing Ca deficiency were generally smaller than control plants, exhibited discoloration and ultimately necrosis. Plants that experienced B deficiency typically had distorted growth of the newest tissue and rarely had any necrosis present.

The tissue concentrations of Ca and B in the three species in experiment 2 were often within the recommended sufficiency range for Ca or B even when the respective element had been temporarily excluded. However, the plants still exhibited symptoms of being deficient in either Ca or B. Since tissue was analyzed after the period when the nutrients were re-supplied to the plants, it is likely that subsequent Ca or B uptake masked lower nutrient concentrations present during the periods of deprivation. Mishra et al. (unpublished data) report that geranium root swelling was observed within 1 d of withdrawing boron from the nutrient solution. The observation of deficiency symptoms 1 d after B deprivation supports our findings of symptoms persisting after a complete nutrient solution was reintroduced.

In these two studies we have shown that the symptoms that have been observed in plug production greenhouses were most similar to those symptoms caused by B deficiency. Also, a temporary disruption of either Ca or B can cause lasting symptoms throughout the plug production cycle.

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Table 1. 'Dynamite Yellow' pansy tissue concentrations at 35 d after sowing (DAS) treated with a complete, 0.25x calcium (Ca), minus Ca, 0.25x boron (B), or minus B modified Hoagland's solution.

	Percent dry wt.					$mg \cdot L^{-1}$ dry wt.				
Treatment	**P**	**K**	**Ca**	**Mg**	**S**	**B**	**Cu**	**Fe**	**Mn**	**Zn**
					Calcium Treatments					
Complete	0.63b	3.24a	0.97a	0.32b	0.30b	31.76b	2.98	190.51	62.16	12.46b
0.25x Ca	0.78a	2.91b	0.27b	0.33b	0.31b	33.76b	3.96	198.16	58.09	15.72b
Minus Ca	0.57c	3.41a	0.03c	0.48a	0.46a	46.15a	5.07	164.70	45.95	24.42a
P-value [z]	***	**	***	***	***	**	NS	NS	NS	**
					Boron treatments					
Complete	0.63b	3.24b	0.97b	0.32b	0.30b	31.76a	2.98	190.51	62.16	12.46
0.25x B	0.68b	3.17b	0.89b	0.30b	0.30b	12.64b	≤0.55	152.51	59.37	15.15
Minus B	0.81a	3.55a	1.30a	0.35a	0.40a	≤1.70c	3.54	169.79	53.43	22.50
P-value [z]	***	*	**	**	***	***	NS	NS	NS	NS

[z] NS, *, **, ***, Not significant or significant at $P \leq 0.05$, ≤ 0.01, or ≤ 0.001
Mean separations are shown by Ca or B treatment and are in columns under each element

Table 2. 'White Storm' petunia tissue concentrations at 35 d after sowing (DAS) treated with a complete, minus calcium, or minus boron modified Hoagland's solution.

	Percent dry wt.					mg·L^{-1} dry wt.				
Treatment	**P**	**K**	**Ca**	**Mg**	**S**	**B**	**Cu**	**Fe**	**Mn**	**Zn**
					Calcium Treatment					
Complete	0.86	7.94a	1.54a	0.41	0.31	29.70	7.44	≤46.00	≤16.00	12.76b
Minus Ca	0.53	3.58b	0.05b	0.50	0.35	36.37	7.57	52.40	≤16.00	20.51a
P-value [z]	NS	*	**	NS	NS	NS	NS	NS	NS	**
					Boron Treatment					
Complete	0.86	7.94	1.54	0.41	0.31	29.70a	7.44	≤46.00	≤16.00	12.76
Minus B	0.58	5.77	1.33	0.34	0.26	≤1.70b	4.55	≤46.00	≤16.00	23.44
P-value [z]	NS	NS	NS	NS	NS	***	NS	NS	NS	NS

[z] NS, *, **,**, Not significant or significant at $P \leq 0.05$, ≤ 0.01, or ≤ 0.001

Mean separations are shown by Ca or B treatment and are in columns under each element

Table 3. 'Festival Apricot' gerbera tissue concentrations at 35 d after sowing (DAS) treated with a complete minus calcium, or minus boron modified Hoagland's solution.

	Percent dry wt.					mg·L^{-1} dry wt.				
Treatment	**P**	**K**	**Ca**	**Mg**	**S**	**B**	**Cu**	**Fe**	**Mn**	**Zn**
					Calcium Treatments					
Complete	0.42	2.88	1.08a	0.33b	0.31a	37.39b	4.26	49.34	≤16.00	13.34
Minus Ca	0.41	3.13	0.10b	0.40a	0.25b	46.92a	4.85	≤46.00	25.45	18.76
P-value [z]	NS	NS	***	*	**	*	NS	NS	NS	NS
					Boron Treatments					
Complete	0.42	2.88	1.08	0.33	0.31	37.39a	4.26	49.34	≤16.00	13.34
Minus B	0.40	2.80	1.03	0.30	0.29	≤1.70b	4.67	≤46.00	≤16.00	18.92
P-value [z]	NS	NS	NS	NS	NS	***	NS	NS	NS	NS

[z] NS, *, **, ***, Not significant or significant at $P \leq 0.05$, ≤ 0.01, or ≤ 0.001

Mean separations are shown by Ca or B treatment and are in columns under each element

Table 4. 'Dynamite Yellow' pansy tissue concentrations at 28 d after sowing (DAS) treated with a complete modified Hoagland's solution and calcium supply disrupted from d 15 to d 21 (week 3) after sowing; and tissue concentrations for pansy plants at 42 d of age treated with a complete modified Hoagland's solution and Ca supply disrupted from 15 to 21 DAS (week 3), 22 to 28 DAS (week 4), or 29 to 35 DAS (week 5).

	Percent dry wt.					$mg \cdot L^{-1}$ dry wt.				
Treatment	**P**	**K**	**Ca**	**Mg**	**S**	**B**	**Cu**	**Fe**	**Mn**	**Zn**
Complete	0.33	2.53b	0.80a	0.28b	0.22b	42.58	3.03b	90.20b	25.28b	5.04b
Week 3	0.40	3.73a	0.56b	0.44a	0.51a	47.98	8.50a	179.13a	41.15a	25.50a
P-value [z]	NS	***	*	***	***	NS	***	*	*	*
Complete	0.50	3.92	1.18a	0.46	0.47	78.88	7.75	157.90	≤16.00	11.20
Week 4	0.48	3.46	0.83b	0.39	0.42	70.13	6.45	144.28	≤16.00	7.40
Week 5	0.57	3.77	0.81b	0.39	0.46	70.78	6.65	157.53	≤16.00	5.75
P-value [z]	NS	NS	*	NS	NS	NS	NS	NS	NS	NS

[z] NS, *, ***, Not significant or significant at $P \leq 0.05$, or ≤ 0.001

Mean separations are shown by treatment and are in columns under each element

Table 5. 'Dynamite Yellow' pansy tissue concentrations at 42 d after sowing (DAS) treated with a complete modified Hoagland's solution and boron supply disrupted from 15 to 21 DAS (week 3), 22 to 28 DAS (week 4), or 29 to 35 DAS (week 5).

	Percent dry wt.					$mg \cdot L^{-1}$ dry wt.				
Treatment	**P**	**K**	**Ca**	**Mg**	**S**	**B**	**Cu**	**Fe**	**Mn**	**Zn**
Complete	0.50b	3.92	1.18	0.46	0.47	78.88a	7.75	157.90	≤16.00	11.20
Week 3	0.57ab	4.11	1.02	0.42	0.40	77.50a	5.58	98.98	≤16.00	27.50
Week 4	0.63a	4.03	1.16	0.45	0.43	67.80b	4.95	118.18	≤16.00	8.00
Week 5	0.58ab	4.10	1.22	0.46	0.46	66.08b	6.55	160.63	≤16.00	28.10
P-value [z]	*	NS	NS	NS	NS	*	NS	NS	NS	NS

[z] NS, *, Not significant or significant at $P \leq 0.05$

Mean separations are shown by treatment and are in columns under each element

Table 6. 'White Storm' petunia tissue concentrations at 42 d after sowing (DAS) treated with a complete modified Hoagland's solution and calcium or boron supply disrupted from 15 to 21 DAS (week 3), 22 to 28 DAS (week 4), or 29 to 35 DAS (week 5).

	Percent dry wt.					mg·L^{-1} dry wt.				
Treatment	**P**	**K**	**Ca**	**Mg**	**S**	**B**	**Cu**	**Fe**	**Mn**	**Zn**
					Calcium Treatments					
Complete	0.98	6.28ab	2.09a	0.54b	0.33ab	≤1.70	4.22	69.77	22.40	75.14
Week 3	1.13	6.82a	2.14a	0.62a	0.37a	≤1.70	4.77	85.75	23.63	25.63
Week 4	0.85	5.27c	1.50b	0.64a	0.29b	≤1.70	4.22	96.13	16.69	20.48
Week 5	0.80	5.56bc	1.44b	0.63a	0.29b	≤1.70	4.32	57.16	25.60	14.83
P-value [z]	NS	**	***	*	**	NS	NS	NS	NS	NS
					Boron Treatments					
Complete	0.90a	6.45	2.01	0.55	0.37a	≤1.70	5.19	302.20	20.61	6.28
Week 3	0.77b	6.39	2.07	0.54	0.32b	≤1.70	3.96	48.67	21.01	8.31
Week 4	0.78b	6.24	2.08	0.54	0.33b	≤1.70	4.04	57.08	20.98	8.37
Week 5	0.74b	6.11	2.13	0.56	0.33b	≤1.70	4.39	49.79	25.03	7.91
P-value [z]	*	NS	NS	NS	*	NS	NS	NS	NS	NS

[z] NS, *, **, ***, Not significant or significant at $P \leq 0.05$, ≤ 0.01,or ≤ 0.001

Mean separations are shown by Ca or B treatment and are in columns under each element

Table 7. 'Festival Apricot' gerbera tissue concentrations at 42 d after sowing (DAS) treated with a complete modified Hoagland's solution and calcium or boron supply disrupted from 15 to 21 DAS (week 3), 22 to 28 DAS (week 4), or 29 to 35 DAS (week 5).

	Percent dry wt.					mg·L^{-1} dry wt.				
Treatment	**P**	**K**	**Ca**	**Mg**	**S**	**B**	**Cu**	**Fe**	**Mn**	**Zn**
					Calcium Treatments					
Complete	0.51	3.24	1.21	0.38c	0.34	21.39b	4.25	97.55	29.93	1.57
Week 3	0.60	3.42	1.11	0.44bc	0.33	46.91a	3.68	96.64	30.47	2.47
Week 4	0.52	3.18	1.16	0.50ab	0.40	39.69a	4.57	130.96	31.66	2.50
Week 5	0.62	3.64	1.04	0.57a	0.44	43.24a	4.36	120.26	35.35	1.93
P-value [z]	NS	NS	NS	*	NS	***	NS	NS	NS	NS
					Boron Treatments					
Complete	0.60	3.67	1.47	0.44	0.31b	39.84	3.04	55.66	22.57	8.77
Week 3	0.63	3.81	1.65	0.49	0.38a	48.24	4.33	54.18	30.22	24.76
Week 4	0.55	3.53	1.56	0.46	0.40a	39.77	4.50	82.40	30.71	85.08
Week 5	0.56	3.47	1.61	0.43	0.40a	34.57	3.48	71.23	22.93	51.03
P-value [z]	NS	NS	NS	NS	*	NS	NS	NS	NS	NS

[z] NS, *, ***, Not significant or significant at $P \leq 0.05$, or ≤ 0.001

Mean separations are shown by Ca or B treatment and are in columns under each element

Fig. 1. 'Dynamite Yellow' pansy plants 39 d after sowing with initial (A) or advanced (B) calcium deficiency symptoms, control (C), initial (D), or advanced (E) boron deficiency symptoms.

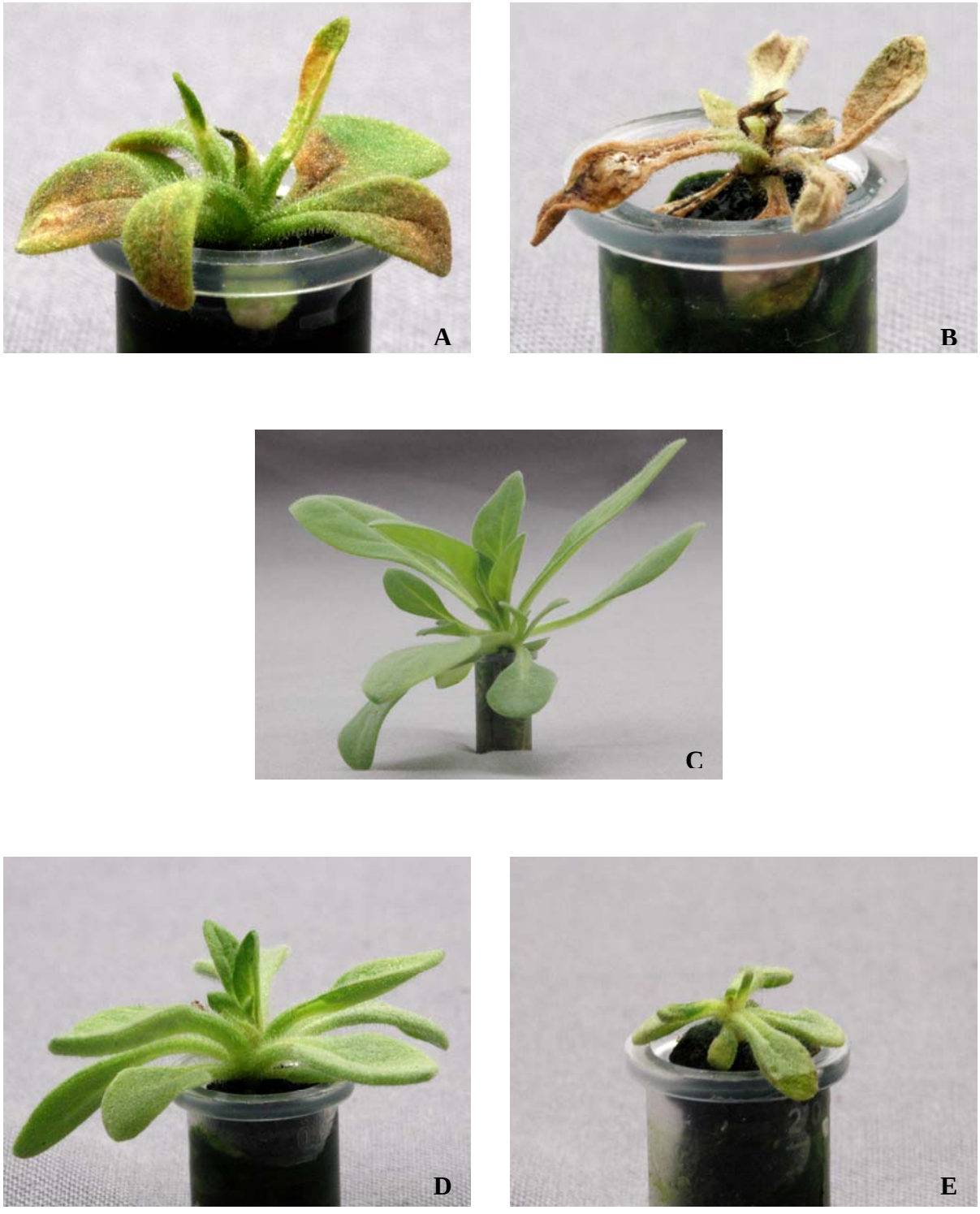

Fig. 2. 'White Storm' petunia plants 39 d after sowing with initial (A) or advanced (B) calcium deficiency symptoms, control (C), initial (D), or advanced (E) boron deficiency symptoms.

Fig. 3. 'Festival Apricot' gerbera plants 39 d after sowing with initial (A) or advanced (B) calcium deficiency symptoms, control (C), initial (D), or advanced (E) boron deficiency symptoms.

Chapter 3

Incidence of Boron Deficiency in Bedding Plants Caused by Drought Stress

(In the format appropriate for submission to HortScience)

Submitted to HortScience

Incidence of Boron Deficiency in Bedding Plants Caused by Drought Stress

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Incidence of Boron Deficiency in Bedding Plants Caused by Drought Stress

Additional Index Words. Hydroponics, Hoagland's, Polyethylene Glycol, PEG, *Viola ×wittrockiana*

Summary

Reports of boron (B) deficiency have become more prevalent over that past five years in pansy (*Viola ×wittrockiana)* plug production, specifically in plugs grown in the high heat and humidity conditions of summer. Past studies have reported that soil moisture has an impact on boron availability. To simulate drought conditions, 'Dynamite Yellow' pansy plants were grown in a peat based substrate which was allowed to dry down to 40, 30, or 20% container capacity (CC), 10 or 20 days after sowing (DAS) or on a continual basis. Plants were also grown hydroponically and exposed to polyethylene glycol (PEG) 1,000 for 1 h to exert an osmotic potential similar to those when the substrate is dried to 40, 30, or 20% CC (-2, -5, or -20 kPa, respectively). Boron tissue concentration was not affected by any induced drought stress. To simulate a physiological response to drought stress, exogenous abscisic acid (ABA) was applied as a substrate drench or foliar spray at concentrations of 150 or 300 $mg \cdot L^{-1}$ to 'Dynamite Yellow' pansy plants grown in a peat based substrate 10 or 20 DAS. All treatments resulted in lower tissue concentrations of B compared to an untreated control. Plants treated 10 DAS with a substrate drench (300$mg \cdot L^{-1}$) or a foliar spray (150 $mg \cdot L^{-1}$) and plants treated 20 DAS with a foliar spray (150 or 300 $mg \cdot L^{-1}$) had a reduction of transpiration. Plants had lower ratios of transpiration/leaf area when ABA was applied as a 300 $mg \cdot L^{-1}$ substrate drench 10 DAS and as foliar spray at both concentrations at either application time. The lower B tissue concentrations coupled with lower transpiration rates

were similar to circumstances in greenhouse production of fall pansy crops. Boron deficiency is most common in August when high temperatures and relative humidity cause the plants to transpire less.

Introduction

Moisture levels affect B availability more than any other micronutrient. It is generally accepted that B availability decreases under dry soil conditions (Fleming, 1980; Gupta, 1993). Under drought conditions mass flow is reduced; subsequently reducing B in contact with the roots to be taken up by diffusion (Kluge, 1971). Gupta et al. (1993) found that even when B is adequately available in the soil, concentrations in barley plants were lower when soil moisture was low. Mortvedt and Osborn (1965) reported that increasing soil moisture from 12% to 20% increased B dispersal and movement.

Drought stress can commonly occur in field production; however, in greenhouse production, the grower is in better control of moisture levels and water stress is rarely allowed. It is unlikely that B deficiency is being induced by dry conditions during seed germination of pansy plugs. Nevertheless, Hobbs and Bertramson (1949) reported that tomato plants grown in greenhouse culture became deficient in B with inadequate moisture in the topsoil. With the small volume of soil contained in a plug container, it is possible that the soil surface dries enough to restrict B movement into the plant.

The objective of these studies were to investigate how single or repeated water stresses affect B concentrations in pansy seedling tissue.

Materials and Methods

Experiment 1

Drought stress – 'Dynamite Yellow' pansy seeds were sown in 288-plug trays cut into 6x6 cell flats (each cell: 2 cm × 2 cm × 3 cm deep), with one 6x6 flat being considered a replication. The germination substrate was Berger BM 2 (Berger Peat Moss, St. Modestede, Quebec, Canada). Once sown, seeds were placed in a germination chamber with a temperature set point of 20 ºC. Light was provided by fluorescent bulbs with a PPFD of 24 to 75 $\mu mol \cdot m^{-2} s^{-1}$ for 12 h per d. The substrate was kept moist using tap water until seeds germinated. After seeds had germinated plants were moved into a greenhouse with day/night temperature set points of 23.9/17.8 ºC. Plants were fertilized at each watering after germination with 50 $mg \cdot L^{-1}$ N from Champion 13-2-13 Plug Special (Scotts, Marysville, Ohio) (13N-0.86P-10.79K).

Using methods described by Milks et. al (1989) and Van Genuchten (1980) water content was determined for the rooting substrate (Berger BM 2) at a range of pressures. The substrate was allowed to dry to 40, 30, or 20% of container capacity (CC) (-2, -5 or -20 kPa osmotic pressure, respectively) to obtain the desired drought stress. Plants were exposed to this stress at 10 or 20 days after sowing (DAS) or after every irrigation. An untreated control was also included. The experiment was a completely randomized design with 4 replications (6x6-cell flats) of 10 treatments. Plants were harvested 33 DAS. Tissue samples were taken by removing the entire shoot.

Experiment 2

'Dynamite Yellow' pansy seeds were grown as in Experiment 1. Abscisic acid was applied 10 or 20 DAS as either a drench or a foliar spray at a concentration of 150 or 300

$mg \cdot L^{-1}$. Drenches were pipetted onto each individual plug cell at a volume of 0.35 mL/cell (5% of the substrate volume). Foliar sprays were applied at a volume of 306 $ml \cdot m^{-2}$. Untreated controls were also included. The experiment was a completely randomized design with 4 replications (6x6-cell flats) of 9 treatments. Plants were harvested 33 DAS. Tissue samples were taken by removing the entire shoot.

Experiment 3

Polyethylene Glycol - Oasis foam (Smithers-Oasis, Kent, Ohio) was used as the growing medium for 'Dynamite Yellow' pansy seeds. The foam was contained by 2-ml microcentrifuge tubes which had approximately the bottom 3-mm removed. Once sown seeds were placed in a germination chamber with a temperature set point of 20 ºC. Light was provided by fluorescent bulbs with a PPFD of 24 to 75 $\mu mol \cdot m^{-2} s^{-1}$ at plant canopy for 12 h per d. Oasis foam was kept moist using deionized water until seeds germinated. After germination, plants were transferred into 2.3-L plastic containers with holes drilled in the lids to hold the microcentrifuge tubes. Plants were then moved into a greenhouse and grown in a complete modified Hoagland's all nitrate solution: (macronutrients in mM) 15 NO_3-N, 1.0 PO_4-P, 6.0 K, 5.0 Ca, 2.0 Mg, and 2.0 SO_4-S (Hoagland and Arnon, 1950), plus µM concentrations of micronutrients, 72 Fe, 9.0 Mn, 1.5 Cu, 1.5 Zn, 45.0 B, and 0.1 Mo. The following reagent grade chemicals KNO_3, $Ca(NO_3)_2$, KH_2PO_4, $MgSO_4$, $NaNO_3$, FeDTPA, $MnCl_2$, $ZnSO_4$, CuCl, H_3BO_3, $Na_2 2MoO_4$ and deionized water was used to formulate treatment solutions. Plants were grown in a greenhouse with day/night temperature set points of 23.9/17.8 ºC, under high pressure sodium lights to provide a minimum PPFD of 100-200 $\mu mol \cdot m^{-2} s^{-1}$ at plan canopy for 12 h per d. Because the plants were small and did not use

large quantities of nutrient solutions, solutions were replaced and were adjusted to pH 5.8 using 0.1M NaOH every two weeks.

A drought stress was applied to the seedling 10 or 20 DAS using polyethylene glycol (PEG) 1,000 (Fluka BioUltra, Steinheim, Germany). Solutions of PEG were prepared to obtain an osmotic potential of -2, -5 or -20 kPa as described by Money (1989). The osmotic potentials in this experiment and Experiment 1 were similar. Untreated controls for each application day were also included. The experiment was a completely randomized design with 4 replications of 8 treatments. Each replication consisted of a container holding 36 seedlings. Before treating the plants, the tubes were removed from the nutrient solution and any remaining free water was removed by moving the tubes in a sharp repeating downward motion. The PEG solutions were pipetted (2 mL per plant) into the rooting substrate of each individual plant. The PEG solutions were left in the substrate for 60 min, any excess solution was allowed to drain from the bottom of the substrate. The solutions were then removed as described above, and deionized water was pipetted (3 mL) onto the rooting substrate to flush any remaining PEG solution from the substrate. The plants were then returned to the nutrient solution until plants were harvested. Plant tissue was harvested 40 DAS. Tissue samples were taken by removing the entire shoot.

Tissue Analysis

The tissue samples for all experiments were rinsed in deionized water, then washed in 0.2 N HCl for 30 s, re-rinsed with deionized water, and dried at 70 ºC for 72 h. Dried tissue was ground in a stainless steel Wiley mill through a 1-mm screen (20-mesh) and 0.1 g was digested in a microwave digester (MARS; CEM Corp, Matthews, N.C.) using a modified EPA method (EPA method 3051 with additional peroxide step). Nutrient concentration,

except N, was determined with inductively coupled plasma optical emission spectroscopy (ICP-OES; Model IRIS Intrepid II, Thermo Corp., Waltham, Mass.). A quality control was run every ten samples and if any element was determined to be more than 10% higher or lower than the standard value, the instrument was recalibrated. Tomato standards (NIST reference material 1573) were compared every 20 samples and tomato and spinach standards (NIST reference material 1570a) were compared every 40 samples. Tissue was analyzed for P, K, Ca, S, Mg, B, Cu, Fe, Mn, and Zn.

Transpiration

Transpiration was quantified gravimetrically for experiments 1 and 2, 3 times/wk for the duration of the experiments. Values were averaged over 10 or 11 d, for experiments 1 and 2, respectively. Flats containing plants and those containing only substrate were weighed at dawn and at dusk (07:00 to 8:30 and 17:30 to 19:00, depending on day length). The difference between the two weights was the amount of water loss to evapotranspiration. The average difference of the flats with only substrate was subtracted from the individual differences of the flats with plants, leaving the amount of water lost due to transpiration alone.

The area of the plant canopy was determined using digital photography and PixelCounter 1.0 (North Carolina State University, Raleigh, N.C.) as described by Steward et al. (2007). The plant canopy area values were used along with the amount of water loss due to transpiration to calculate the amount of water loss/cm^2 of plant canopy.

Data analysis

Data were tested by analysis of variance (ANOVA) using general linear model (SAS Institute, Cary, N.C.) and means were separated by least significant differences (LSD) at $P \leq 0.05$.

Results and Discussion

Experiment 1

When a drought stress was applied 10 DAS, flats allowed to dry to 20 or 30 % CC loss significantly ($P \leq 0.001$) less water due to transpiration (0.64 and 1.32 ml, respectively) compared to the untreated control (4.50 ml) (Table 8). Flats dried to 20% CC 20 DAS loss significantly ($P \leq 0.001$) less water due to transpiration (1.88 ml) compared to the untreated control (5.94 ml) (Table 8). When the drought stress was applied on a continuous basis, flats dried to 20 or 30% CC loss significantly ($P \leq 0.001$) less water due to transpiration (1.00 and 0.25 ml, respectively) compared to the untreated control (4.55 ml) (Table 8). Although there were differences in the total amount of water loss due to transpiration among treatments, the amount of water loss due to transpiration/canopy area was not different from the controls for any of the treatments with the exceptions of plants dried to 20 or 30% CC 10 DAS (Table 8). As plants experienced harsher drought conditions some fatalities occurred as well as smaller leaf areas on surviving plants. The smaller leaf canopies explain why there was significantly less transpiration, but no differences in transpiration/area ratios for many of the treatments.

The only treatment which had significantly ($P \leq 0.05$) different concentrations of B was that which was allowed to dry to 40% CC on 20 DAS. The B concentration (45.09 $mg \cdot L^{-1}$) was greater than the untreated control (24.52 $mg \cdot L^{-1}$) (Table 8). When plants were allowed to dry to 20% CC on 10 DAS K, S, and Cu concentrations were greater than any

other treatment and the concentration of P was greater than plants dried to 30% and 40% (Table 8). Plants dried to 20% CC on 20 DAS had greater concentrations of P, S, and Cu than any other treatment; the Mn concentration was significantly lower than the untreated controls and plants dried to 40% CC (Table 8). Plants dried to 40% CC on 20 DAS had shoot tissue K concentrations significantly greater than the untreated controls and plants dried to 30% CC. The 20% and 40% CC treatments on 20 DAS had greater Ca concentrations than the untreated controls and the 30% CC treatment.

Plant continually allowed to dry to 20% and 30% CC had greater concentrations of P and S than the untreated controls and 40% CC treatment. When plants were dried continually to 30% CC the concentrations of Cu and Fe were greater than the untreated control and 40% CC treatment. Plants dried to 40% CC continually, had a greater concentration of K than any other treatment (Table 8).

The concentrations of B, and all other elements, were above those determined to cause deficiency symptoms on fully expanded mature leaves from transplanted pansies (72 DAS) by Pitchay (2002). Adequate tissue concentration values were expected as there were no symptoms of nutrient deficiency.

Experiment 2

When ABA was applied 10 DAS as a drench of 300 $mg \cdot L^{-1}$ or a foliar spray of 150 or 300 $mg \cdot L^{-1}$ there was significantly ($P \leq 0.001$) less water loss due to transpiration (-2.42, -0.90 and -0.08 ml, respectively) compared to the untreated control (1.91 ml) (Table 9). When applied 20 DAS only the foliar sprays (150 and 300 $mg \cdot L^{-1}$) lost significantly ($P \leq 0.05$) less water due to transpiration (-0.25 and -1.20 ml, respectively) than the untreated control (1.83 ml) (Table 9). The negative value for transpiration indicates that transpiration

was effectively stopped; with no canopy cover, the fallow flats lost more water due to evaporation than the flats with plants lost to evaporation and transpiration (Table 10).

Transpiration/area ratios were significantly lower ($P \leq 0.01$) for plants treated on 10 DAS with an ABA substrate drench of 300 $mg \cdot L^{-1}$ and both foliar spray concentrations (150 and 300 $mg \cdot L^{-1}$) all other treatments. Only plants treated with ABA foliar sprays (150 or 300 $mg \cdot L^{-1}$) on 20 DAS had significantly ($P \leq 0.05$) lower ratios of transpiration/area than the untreated control (Table 9).

None of the plants displayed symptoms of B deficiency, however B tissue concentrations from all treatments were significantly ($P \leq 0.05$) lower than that of the untreated control (Table 9). With the exception of P in the plants treated 20 DAS, none of the elements were significantly different in the ABA treated plants when compared to the untreated controls. Tissue concentrations of P were significantly ($P \leq 0.05$) higher than the untreated control when plants were treated 20 DAS with an ABA substrate drench of 300 $mg \cdot L^{-1}$ and ABA foliar sprays (150 and 300 $mg \cdot L^{-1}$).

Experiment 3

The interaction between the day of treatment and osmotic potential the plants were exposed to was not significant ($P \leq 0.05$); therefore tissue value means for treatments applied 10 and 20 DAS were pooled in the statistical analysis (data not shown). As the osmotic potential increased there was no significant ($P \leq 0.05$) affect on the tissue concentration for Ca, Mg, S, B, Cu, Fe, Mn, or Zn. As the osmotic potential increased (0, -2, -5, -20), the tissue concentrations of P decreased (0.55, 0.50, 0.48, 0.43%, respectively) as did the tissue concentrations of K (3.11, 3.09, 2.92, 2.78%, respectively). It is reported that soil moisture has an effect on plant uptake of P; however, soil moisture has more to do with governing the

release of P from the soil (Jones, 1998; Sanchez, 2007). In this experiment P was in a water soluble form and was readily available to the plant.

Conclusion

Drought stress alone appears to have less effect on the tissue concentration of B than does the rate of transpiration. Increasing osmotic potentials to simulate water stress had no affect on the concentration of B in tissue. Unlike previous research by Hobbs and Bertramson (1949) and Gupta et al. (1993) on tomatoes and barley, respectively, there was no evidence that dry substrate conditions had any effect on boron tissue concentrations in pansy seedlings. Drought conditions did affect the amount of water loss due to transpiration by the plants but did not effect transpiration per leaf area. The application of ABA on pansy seedlings produced more conclusive results, with a reduction of transpiration in plants. Transpiration and transpiration/leaf area ratios were both reduced when ABA was applied, which also resulted in lower concentrations of B in leaves.

These studies indicate, that rather than water stress, lower transpiration and more specifically lower ratios of transpiration/leaf area might cause B deficiency occurring in pansy production.

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Table 8. Nutrient concentration of 'Dynamite Yellow' pansy shoots, water loss (ml) due to transpiration (average of 10 d), and water loss (ml) due to transpiration/area of substrate covered by leaves (cm^2) (average of 10 d) from plants 36 DAS allowed to dry to 40, 30, or 20% container capacity (CC); 10 or 20 days DAS or on a continual basis.

	Percent dry wt.					mg·L^{-1} dry wt.						
Treatment	**P**	**K**	**Ca**	**Mg**	**S**	**B**	**Cu**	**Fe**	**Mn**	**Zn**	**Trans**	**Trans/ Area[z]**
						Treated 10 DAS						
Control	0.27ab	3.76b	0.50	0.60	0.31b	24.52	10.90b	67.31	161.44	50.08	4.42a	0.066a
40% CC	0.25b	4.04b	0.54	0.62	0.31b	23.42	10.77b	102.44	183.76	60.23	3.77a	0.045ab
30% CC	0.24b	3.63b	0.56	0.61	0.32b	22.20	10.76b	72.83	213.04	71.66	1.18b	0.009bc
20% CC	0.30a	4.80a	0.57	0.68	0.42a	23.29	16.51a	154.47	197.25	66.42	0.48b	-0.018c
***P-value*[y]**	*	**	NS	NS	**	NS	*	NS	NS	NS	***	*
						Treated 20 DAS						
Control	0.27b	3.76b	0.50b	0.60	0.31b	24.52b	10.90b	67.31	161.44a	50.08	5.94a	0.059
40% CC	0.31b	4.52a	0.65a	0.67	0.32b	45.09a	12.15b	87.03	166.02a	52.62	6.83a	0.039
30% CC	0.29b	3.94b	0.53b	0.59	0.31b	22.46b	10.58b	82.73	132.38ab	47.33	5.32a	0.050
20% CC	0.40a	4.22ab	0.68a	0.68	0.42a	24.40b	17.69a	75.58	107.32b	52.16	1.88b	0.056
***P-value*[y]**	**	*	**	NS	*	**	*	NS	*	NS	***	NS
						Treated Continuously						
Control	0.27c	3.76b	0.50	0.60	0.31c	24.52	10.90b	67.31b	161.44	50.08	4.42a	0.067
40% CC	0.29c	4.32a	0.58	0.64	0.31c	23.37	10.68b	71.73b	177.05	39.13	3.61a	0.076
30% CC	0.48a	3.41b	0.61	0.73	0.67a	20.87	25.08a	254.47a	90.32	54.57	0.28b	-0.053
20% CC	0.40b	3.22b	0.59	0.69	0.52b	21.39	17.91ab	167.57ab	115.21	49.85	0.95b	0.099
***P-value*[y]**	***	**	NS	NS	***	NS	**	*	NS	NS	***	NS

[z] values with negative numbers indicate a net gain of water

[y] NS, *, ***, Not significant, significant at $P \leq 0.05$, or $P \leq 0.001$

Mean separations are shown by day of treatment in columns under each element

Table 9. Nutrient concentration of 'Dynamite Yellow' pansy shoots, water loss (ml) due to transpiration (average of 11 d), and water loss (ml) due to transpiration/area of substrate covered by leaves (cm^2) (average of 11 d) from plants 36 DAS treated with abscisic acid as a drench (150 or 300$mg \cdot L^{-1}$) or a foliar spray (150 or 300 $mg \cdot L^{-1}$) 10 or 20 DAS.

Treatment	Percent dry wt.					$mg \cdot L^{-1}$ dry wt.					Trans[z]	Trans/ Area[z]
	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn		
Treated 10 DAS												
Control	0.30	4.50	0.56	0.67	0.32	32.75a	11.08	59.88	203.85	58.77	1.91a	0.038a
Drench ($mg \cdot L^{-1}$)												
150	0.34	5.18	0.58	0.74	0.35	22.71b	11.76	64.36	211.82	55.79	0.76ab	0.018a
300	0.35	5.14	0.58	0.74	0.38	23.58b	12.71	85.77	196.20	60.12	-2.42d	-0.048c
Foliar Spray ($mg \cdot L^{-1}$)												
150	0.32	4.81	0.58	0.72	0.36	24.65b	12.29	79.57	204.07	67.52	-0.90c	-0.028bc
300	0.34	5.07	0.60	0.73	0.35	24.95b	12.43	79.96	185.15	68.28	-0.08bc	-0.015b
P-value[y]	**NS**	**NS**	**NS**	**NS**	**NS**	*	**NS**	**NS**	**NS**	**NS**	***	***
Treated 20 DAS												
Control	0.30b	4.50	0.56	0.67	0.32	32.75a	11.08	59.88	203.85	58.77	1.83a	0.018a
Drench ($mg \cdot L^{-1}$)												
150	0.34ab	5.11	0.57	0.71	0.34	24.68b	12.09	57.34	155.21	64.21	0.72ab	0.001ab
300	0.37a	5.19	0.63	0.75	0.34	24.78b	13.26	84.90	201.81	69.73	0.32ab	-0.001ab
Foliar Spray ($mg \cdot L^{-1}$)												
150	0.37a	5.19	0.61	0.73	0.34	24.77b	13.15	106.95	182.35	68.57	-0.25b	-0.012b
300	0.38a	5.09	0.59	0.71	0.34	23.39b	12.23	137.31	192.51	63.07	-1.20b	-0.020b
P-value[y]	*	**NS**	**NS**	**NS**	**NS**	*	**NS**	**NS**	**NS**	**NS**	*	*

[z] values with negative numbers indicate a net gain of water

[y] NS, *, ** Not significant, significant at $P \leq 0.05$, or $P \leq 0.01$

Mean separations are shown by day of treatment in columns under each element

Table 10. Differences in weight (g) from sunrise to sunset for flats with plants or flats without plants when flats were treated with abscisic acid as a substrate drench (150 or 300 $mg \cdot L^{-1}$) or a foliar spray (150 or 300 $mg \cdot L^{-1}$) 10 or 20 DAS.

	19-Feb	**21-Feb**	**26-Feb**	**28-Feb**	**2-Mar**	**5-Mar**	**7-Mar**	**9-Mar**	**12-Mar**	**14-Mar**
					Control					
Plants	34.2	27.4	26.3	33.6	42.3	40.1	39.9	39.9	39.5	46.2
Blanks	31.9	15.1	24.4	32.9	41.4	38.1	38.4	40.6	37.6	39.6
					Treated 10 DAS					
Drench 150 ($mg \cdot L^{-1}$)										
Plants	33.6	27.6	26.1	32.5	39.7	37.7	37.8	39.7	39.4	44.5
Drench 300 ($mg \cdot L^{-1}$)										
Plants	30.8	26.5	24.5	30.1	37.1	34.9	33.6	35.5	35.4	40.1
Foliar Spray ($mg \cdot L^{-1}$)										
Plants	29.4	25.2	24.8	31.6	38.7	36.8	37.6	37.3	36.3	46.2
Foliar Spray 300 ($mg \cdot L^{-1}$)										
Plants	30.5	26.8	25.2	31.6	39.2	37.6	38.1	39.2	39.3	45.2
					Treated 20 DAS					
Drench 150 ($mg \cdot L^{-1}$)										
Plants	No data taken before treatment			31.1	37.3	38.2	39.9	40.9	39.5	46.8
Drench 300 ($mg \cdot L^{-1}$)										
Plants	No data taken before treatment			31.9	37.9	36.9	38.3	40.9	39.2	45.9
Foliar Spray 150 ($mg \cdot L^{-1}$)										
Plants	No data taken before treatment			30.1	36.4	36.6	39.3	39.2	39.7	45.5
Foliar Spray 300 ($mg \cdot L^{-1}$)										
Plants	No data taken before treatment			30.2	36.7	36.0	35.0	39.7	38.4	44.3

Chapter 4

The Effect of Relative Humidity on the Incidence of Boron Deficiency in Bedding Plants

(In the format appropriate for submission to HortScience)

Submitted to HortScience

The Effect of Relative Humidity on the Incidence of Boron Deficiency in Bedding Plants

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The Effect of Relative Humidity on the Incidence of Boron Deficiency in Bedding Plants

Additional Index Words. *Gerbera jamesonii*, horizontal air flow fans, HAF fans, *Petunia ×hybrida*, *Viola ×wittrockiana*

Summary

Reports of boron (B) deficiency have become more prevalent over the past five years in pansy (*Viola ×wittrockiana*), petunia (*Petunia ×hybrida*), and gerbera (*Gerbera jamesonii*) plug production, specifically to pansy plugs grown in high heat and humidity conditions of summer. Past studies have reported high relative humidity (RH) can cause lower concentrations of B in leaf tissue. It is common practice to control excess temperatures by applying mist irrigation which artificially increases the RH; this may be a cause of B deficiency in these crops. 'Dynamite Yellow' pansy, 'White Storm' petunia, and 'Festival Apricot' gerbera plugs were grown in high or ambient RH conditions. Plants grown in high RH conditions had lower shoot tissue concentrations of B than those grown in the ambient RH conditions.

Plants take up B passively through the transpiration stream in a similar fashion to calcium (Ca). Past studies have also reported that increased transpiration can increase the uptake of Ca. To determine if increased transpiration would increase the uptake of B, 'Dynamite Yellow' pansy plants were grown in high or ambient RH conditions with or without increased air velocity. The constantly increased air velocity did not result in a transpiration increase or an increase in B shoot tissue concentration due to stomatal closure. Intermittent air flow may be more appropriate.

Introduction

Boron (B) moves into plants from the roots to the shoots through the transpiration stream via the xylem (Jones, 1991; Kochian, 1991; Kohl and Oertli, 1961; Raven, 1980). Relative humidity (RH) can be a major factor controlling the rate a plant can transpire and therefore the amount of B in a plant. The uptake of B has been reported to be affected by RH in a number of studies. Bowen (1972) reports that in high RH conditions B uptake is reduced; this is also supported by studies by Halbrooks et al. (1986). Oertli (1963) also reports lower concentrations of B in leaves when plants were grown in high RH conditions than those grown in lower RH, but also conclude that the lower concentration may be due to B being lost through guttation in high RH conditions. Plant uptake of calcium (Ca) has been extensively studied and it is known that Ca is taken up passively with water and is closely linked to transpiration (Clarkson, 1984; Marschner, 1995). Chang and Miller (2004) reported that calcium uptake increases with higher transpiration rates.

During germination, seeds of bedding plants are maintained in growing conditions near 100% RH. Once the plants have germinated they are moved into a greenhouse and it is common practice to mist the plants to keep the RH high, particularly with pansy seedlings grown during hot and humid conditions. This high RH environment could be a cause of the B deficient symptoms occurring in commercial production.

Pansy plants exhibiting symptoms of B deficiency have been observed in a large scale plug production facility; the symptoms were more pronounced and common in an area of the greenhouse range where airflow was impeded. However within each area of the greenhouse where symptoms were present the symptoms appeared in a random pattern and varied in severity.

The first objective was to determine if elevated RH levels could cause lower tissue concentrations of B and lead to the development of visual symptoms of B deficiency. The second objective was to determine if growers could increase air flow to avoid B deficiency during seedling production.

Materials and Methods

Experiment 1

'Dynamite Yellow' pansy, 'White Storm' petunia, and 'Festival Apricot' gerbera seeds were sown in 288-plug trays cut into 2x2-cell flats (each cell: 2 cm × 2 cm × 3 cm deep) on 5 Jan. 2007. The germination substrate was Berger BM 2 (Berger Peat Moss, St. Modestede, Quebec, Canada). Once sown, seeds were placed in a germination chamber with a temperature set point of 20 ºC. Light was provided by fluorescent bulbs with a PPFD of 24 to 75 $\mu mol \cdot m^{-2} s^{-1}$ at plant canopy for 12 h per d. The substrate was kept moist using tap water until seeds germinated. When the first true leaves began to emerge the plants were moved to one of two environments in the greenhouse, ambient humidity (AH) or high humidity (HH) with day/night temperature set points of 23.9/17.8 ºC. The experiment was a completely randomized design with 36 flats (2x2) of each treatment. A 122-cm plastic high curtain was used to create the two environment chambers, each 152 cm × 267 cm, plastic lined the bench to prevent airflow from below but the top of the chamber was open. On average the RH of the AH chamber was 65% and the RH was raised to 102% using humidifiers (Model 707; Fedders Corp, Liberty Corner, N.J.) in the HH chamber. Relative humidity and temperature were monitored using Hobo H8 data loggers (Onset Computer Corp, Bourne, Mass.). Plants were fertilized at each watering after germination with 50 $mg \cdot L^{-1}$ N from Champion 13-2-13 Plug Special (Scotts, Marysville, Ohio) (13N-0.86P-

10.79K). Plants were harvested 35 d after sowing (DAS). Tissue samples were taken by removing the entire plant. To ensure sufficient tissue was available for tissue analysis 6 2x2-cell flats were combined and used as 1 replication for a total of 6 replications per treatment. The experiment was repeated with an initiation date of 10 May, 2007 and harvest occurring 41 DAS. The RH was 81% and 101%, on average, for the AH and HH treatments, respectively. The experiment was a completely randomized design with 27 flats (2x2) of each treatment. To ensure sufficient tissue was available for tissue analysis 9 2x2-cell flats were combined and used as 1 replication for a total of 3 replications per treatment.

Experiment 2

'Dynamite Yellow' pansy seeds were sown in the same flats with the same germination substrate as used in Experiment 1; the germination environment and fertilizing procedure were also the same. When the first true leaves began to emerge the plants were moved to one of four environments in the greenhouse. Horizontal airflow fans (HAF) were used to increase the air velocity in two of the environments. Air speed was measured as 1-2 mph using a Dwyer Wind Meter (Dwyer Instruments, Inc. Michigan City, Ind.) The treatments included: AH with HAF (AH-F), AH without HAF fans (AH-NF), HH with HAF fans (HH-F), or HH without HAF fans (HH-NF) with day/night temperature set points of 23.9/17.8 ºC. The experiment was a completely randomized design with 27 flats (2x2) of each treatment. The chambers were identical to those used in Experiment 1. The RH humidity for the AH-F, AH-NF, HH-F, and HH-NF were 85%, 83%, 101% and 98%, respectively. Plants were harvested 35 DAS. Tissue samples were taken by removing the entire plant. To ensure sufficient tissue was available for tissue analysis 9 2x2-cell flats were combined and used as 1 replication for a total of 3 replications per treatment.

Transpiration

Transpiration was quantified gravimetrically for both experiments 3 times/wk for the duration of the experiments. Values were averaged over 10 or 8 d for the first and second run of experiment 1, respectively, and 8 d for experiment 2. Flats containing plants and those containing only substrate were weighed at dawn and at dusk (07:00 to 8:30 and 17:30 to 19:00, depending on day length). The difference between the two values was calculated to be the amount of water loss to evapotranspiration. The average difference of the flats with only substrate was subtracted from the individual differences of the flats with plants, leaving the amount of water loss due to transpiration alone. Some reported values appear as negative numbers; these indicate when plants gained water over the course of the day.

The area of the plant canopy was determined using digital photography and PixelCounter 1.0 (North Carolina State University, Raleigh, N.C.) as described by Steward et al. (2007). These areas were used in with the amount of water loss due to transpiration to obtain the amount of water loss/cm^2 of plant canopy.

Tissue Analysis

The tissue samples for both experiments were first rinsed in deionized water, then washed in 0.2 N HCl for 30 s, re-rinsed with deionized water, and dried at 70 ºC for 72 h. Dried tissue was ground in a stainless steel Wiley mill through a 1 mm screen (20-mesh) and 0.1 g was digested in a microwave digester (MARS; CEM Corp, Matthews, N.C.) using a modified EPA method (EPA method 3051 with additional peroxide step). Nutrient concentration, except N, was determined with inductively coupled plasma optical emission spectroscopy (ICP-OES; Model IRIS Intrepid II, Thermo Corp., Waltham, Mass.). A quality control was run every ten samples and if any element was determined to be more than 10%

higher or lower than the standard value, the instrument was recalibrated. Tomato standards (NIST reference material 1573) were compared every 20 samples and tomato and spinach standards (NIST reference material 1570a) were compared every 40 samples. Tissue was analyzed for P, K, Ca, S, Mg, B, Cu, Fe, Mn, and Zn.

Data analysis

Data were tested by analysis of variance (ANOVA) using general linear model (SAS Institute, Cary, N.C.) and means were separated by least significant differences (LSD) at $P \leq 0.05$. When tissue concentrations of an element were not detected a value half that of the lowest detectable level for the respective element was used for statistical analysis.

Results and Discussion

Experiment 1

The amount of water loss due to transpiration as well as the amount of the amount of water loss due to transpiration/canopy area (trans/area) were significantly less in the HH treatments as compared to the AH for both runs of the experiment and for all three species. Note that the value for transpiration and trans/area ratio for pansies in the HH treatment in the first run are negative numbers (Table 11). As stated above, a negative number indicates a gain in water. No watering occurred between sunrise and sunset on days where transpiration data was taken; therefore, the gain was not due to irrigation. However, it is possible that the plants and/or the substrate absorbed moisture from the surrounding air. The substrate of the HH treatments never dried and condensation was observed on a regular basis on the leaves of the plants, with a RH of 102% it is reasonable that water also condensed on the soil surface.

Pansy

No symptoms of B deficiency were observed in the first run of the experiment, but B symptoms were observed in the HH treatment during the second run of the experiment. Symptoms included: distorted leaves and meristems, and the proliferation of auxiliary shoots. However, the shoot tissue concentrations of B in the first and second run of the experiment were significantly ($P < 0.001$ and $P < 0.01$, respectively) less in the HH treatments as compared to the AH treatments (Table 11 and Table 12). The B concentration in HH treatment in the first run were lower than those reported to result in B deficiency on fully expanded mature leaves from transplanted pansies (72 DAS) (Pitchay, 2002). Plants from both runs in the HH treatment had significantly lower concentrations of K and Mg when compared to the AH treatment (Table 11 and Table 12). In the first run of the experiment tissue concentrations of P and Ca were also significantly lower in the HH treatment as compared to the AH treatment, but the concentrations of Cu, Fe, and Mn were significantly higher in the HH treatment as compared to the AH treatment (Table 11). With the exception of Ca in the HH treatment in the first run of the experiment these nutrients were above the levels in which deficiencies were observed on fully expanded mature leaves from pansy transplants (46 DAS) (Pitchay, 2002).

Petunia

Symptoms of B deficiency, including thickened, distorted, and upward curled leaves, were observed in the HH treatment in both runs of the experiment. Shoot tissue concentrations of B in the HH treatments for the first and second runs of the experiment were significantly lower than the AH treatments; the values (10.56 and 14.82 $mg \cdot L^{-1}$, respectively) were approaching levels where B deficiency (10.3 $mg \cdot L^{-1}$) was observed in fully expanded

mature leaves from petunia transplants (102 DAS) (Pitchay, 2002). Shoot tissue concentrations of P, Mg, S, Cu, Fe, Mn, and Zn in the first run of the experiment and P, Ca, Mg, S, and Zn in the second run were significantly different when the AH treatments and HH treatments were compared (Table 11 and Table 12). However, none of these values were below those reported by Pitchay (2002) to cause the respective deficiency in fully expanded mature leaves from petunia transplants.

Gerbera

Symptoms of B deficiency were observed in the HH treatment for both runs of the experiment. Symptoms included: thickened, distorted, and upward curled leaves. Shoot tissue concentrations of B in the HH treatments for the first and second runs of the experiment were significantly lower ($P \leq 0.01$) than those in the AH treatments (Table 11 and Table 12). In the first run of the experiment, shoot tissue concentrations of P, K, Ca, Mg, and Mn were significantly lower in the HH treatment than in the AH treatment, but Cu and Fe were significantly higher in the HH treatment than in the AH treatment (Table 11). Calcium concentrations for both treatments in both runs of the experiment were lower than optimal levels for gerberas 2 weeks after transplant when fertilized with 50-75 $mg \cdot L^{-1}$ N (Whipker et al., 2007). Concentrations of B for the HH treatment in run 1 and both treatments in run 2 were below those reported by Whipker et. al. (2007). However, the value for the AH treatment in run 2 (24.37 $mg \cdot L^{-1}$) was only slightly lower than the published value (26.60 $mg \cdot L^{-1}$).

Experiment 2

It was anticipated that when airflow was increased around the plants the amount of water loss due to transpiration would increase. The increase in transpiration would in turn

result in higher concentrations of B in the shoot tissue due to B uptake being linked with transpiration (Halbrooks et al., 1986; Hu and Brown, 1997).

There was a significant difference in the amount of water loss due to transpiration ($P \leq 0.001$), but the expected trend was not observed in this experiment. The amount of water loss due to transpiration in the AH-NF treatment was the highest and the lowest amount of water loss due to transpiration was in the HH-F treatment (Table 13). Also, both the AH-F and HH-F treatments had significantly ($P \leq 0.01$) lower transpiration/area ratios. Because of the high substrate surface area to substrate volume ratio in a plug tray, the plug trays without plants (no canopy cover) in the AH-F treatment lost significantly ($P \leq 0.001$) more water due to surface evaporation than the plug trays with no plants in the AH-NF treatment (Table 13). This causes the calculation for determining transpiration as described in the materials and methods to result in lower transpiration rates. Although increased air velocity increases transpiration, it is most effective at low velocities; if the plants are exposed to higher air velocities stomata close and transpiration decreases (Kramer, 1983). With the transpiration values lower in treatments with constant air flow modified by HAF fans, it appears that the velocities of air movement in this experiment (1-2 mph) were too high and resulted in stomatal closure, thus negatively influencing B uptake.

Experience from previous experiments was cause to expect higher concentrations of B in both AH treatments as compared to the HH treatments. The B concentration for the AH-NF treatment was significantly higher ($P \leq 0.01$) than all other treatments (Table 13). However the AH-F, HH-NF, and HH-F treatments had similar B concentrations. Although there were lower concentrations of B in these treatments, no B deficiency symptoms were observed. The tissue concentrations of B were above those determined to cause deficiency

symptoms on fully expanded mature leaves from pansy transplants (72 DAS) by Pitchay (2002). The lack of observed B deficiency symptoms could be due to the relatively small number of plants in the study. As mentioned above, in large scale commercial production, symptoms are not typically found consistently throughout a crop. This experiment consisted of less than 500 plants which may have not an adequate number of individuals to exhibit noticeable symptoms as compared to hundreds of thousands of plants in a commercial setting. Differences in tissue concentrations among treatments were found for P, K, Ca, Mg, and S (Table 13). There were no differences in Cu, Fe, Mn, or Zn shoot tissue concentrations (data not shown).

Conclusions

Results from Experiment 1 indicate that an increase in RH decreases the amount of water the plant loses due to transpiration resulting in lower concentrations of B in shoot tissue and ultimately increasing the occurrence of B deficiency symptoms. To aid in the uptake of B by pansy, petunia, and gerbera plugs growers should maintain a lower RH when propagating, but ensuring that RH sufficiently high enough to avoid compromising germination rate or plant quality.

An increase in air velocity did not increase the B concentration in shoot tissue. However, in this study when the constant air velocity was increased it may have been too high and actually caused the plants to transpire less than those when the air velocity was not increased. Future studies using lower air velocities or intermitted increases of air velocities should be conducted.

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Table 11. Experiment 1, run 1 nutrient concentration of tissue, water loss (ml) due to transpiration (averaged of 10 d), and water loss (ml) due to transpiration /area of substrate covered by leaves (cm^2) (average of 10 d) from 'Dynamite Yellow' pansy, 'White Storm' petunia, and 'Festival Apricot' gerbera plants 35 d after sowing grown in ambient (65%) relative humidity (RH) or high (102%) RH.

	Percent dry wt.					$mg \cdot L^{-1}$ dry wt.						
Treatment	**P**	**K**	**Ca**	**Mg**	**S**	**B**	**Cu**	**Fe**	**Mn**	**Zn**	**Trans[z]**	**Trans/area[z]**
						Pansy						
Ambient	0.29a	2.55a	0.49a	0.46a	0.28	19.94a	9.76b	61.66b	121.54b	45.15	1.63a	0.121a
High	0.24b	1.82b	0.35b	0.32b	0.27	9.43b	12.34a	120.26a	137.24a	32.69	-0.008b	-0.152b
P-value[y]	**	***	***	***	NS	***	***	***	*	NS	***	***
						Petunia						
Ambient	0.23b	3.12	0.63	0.45a	0.33b	25.49a	9.23b	67.47b	48.01b	28.82b	1.43a	0.081a
High	0.26a	3.35	0.61	0.35b	0.43a	10.56b	18.12a	96.98a	59.92a	68.68a	0.05b	0.013b
P-value[y]	*	NS	NS	***	***	***	***	*	**	***	***	*
						Gerbera						
Ambient	0.39a	3.86a	0.72a	0.50a	0.35	42.71a	13.41b	123.00b	76.97a	50.32	0.46a	0.040a
High	0.28b	2.77b	0.61b	0.35b	0.35	17.81b	18.31a	161.09a	64.53b	53.29	0.03b	-0.002b
P-value[y]	***	***	***	***	NS	**	***	**	**	NS	***	***

[z] Values with negative numbers indicate a net gain of water

[y] NS, *, **, ***, Not significant, significant at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$

Mean separations are shown by species in columns under each element

Table 12. Experiment 1, run 2 nutrient concentration of tissue, water loss (ml) due to transpiration (average of 8 d), and water loss (ml) due to transpiration /area of substrate covered by leaves (cm^2) (average of 8 d) from 'Dynamite Yellow' pansy, 'White Storm' petunia, and 'Festival Apricot' gerbera plants 41 d after sowing grown in ambient (81%) relative humidity (RH) or high (101%) RH.

	Percent dry wt.					$mg \cdot L^{-1}$ dry wt.						
Treatment	**P**	**K**	**Ca**	**Mg**	**S**	**B**	**Cu**	**Fe**	**Mn**	**Zn**	**Trans**	**Trans/area**
						Pansy						
Ambient	0.21	3.99	0.49	0.45	0.26	19.66a	3.94	34.78	36.66	47.03	1.29a	0.108a
High	0.23	3.05	0.46	0.41	0.27	12.66b	3.97	95.59	36.52	56.34	0.384b	0.055b
P-value[z]	NS	***	NS	*	NS	**	NS	NS	NS	NS	***	***
						Petunia						
Ambient	0.11b	3.75	0.49b	0.26b	0.25b	20.89a	5.68	45.42	22.96	34.88b	2.05a	0.167a
High	0.17a	3.98	0.70a	0.32a	0.32a	14.82b	6.99	82.19	25.90	53.44a	0.52b	0.083b
P-value[z]	***	NS	***	*	***	**	NS	NS	NS	***	***	***
						Gerbera						
Ambient	0.16	3.16	0.60	0.35	0.21	24.37a	3.34	88.76	31.95	31.83	0.79a	0.049a
High	0.14	3.52	0.84	0.34	0.21	13.33b	3.63	135.25	35.05	38.16	0.165b	0.010b
P-value[z]	NS	NS	NS	NS	NS	**	NS	NS	NS	NS	***	***

[z] NS, ***, Not significant, significant at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$
Mean separations are shown by species in columns under each element

Table 13. Nutrient concentration of tissue, water loss (ml) due to transpiration (average of 8 d), and water loss (ml) due to transpiration /area of substrate covered by leaves (cm^2) (average of 8 d), and water loss from flats without plants (FWOP) (average of 8 d) for 'Dynamite Yellow' pansy plants 41 d after sowing; grown in ambient relative humidity (RH) with or without air horizontal air flow (HAF) fans (85%, and 83% RH, respectively) or high RH with or without HAF fans (101% and 98% RH, respectively).

	Percent dry wt.					$mg \cdot L^{-1}$ dry wt.				
Treatment	**P**	**K**	**Ca**	**Mg**	**S**	**B**	**Trans**	**Leaf area (cm)**	**Trans/area**	**FWOP**
Ambient	0.23ab	4.84a	0.61a	0.54ab	0.29c	17.63a	1.11a	14.45a	0.073a	2.80b
Ambient Fan	0.26a	4.93a	0.66a	0.61a	0.32b	16.05ab	0.66b	14.26a	0.033b	3.00a
High	0.22b	3.87b	0.48b	0.47c	0.29bc	13.95b	0.69b	11.70b	0.073a	1.64c
High Fan	0.21b	3.87b	0.52b	0.50bc	0.39a	13.87b	0.31c	11.71b	0.022b	1.62c
P-value[z]	*	***	***	**	***	**	***	***	**	***

[z] NS, *, **, ***, Not significant, $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$
Mean separations are shown by species in columns under each element

Chapter 5

Boron Distribution and the Effect of pH on Boron Uptake by Pansy, Petunia and Gerbera Plants

(In the format appropriate for submission to HortScience)

Submitted to HortScience

Boron Distribution and the Effect of pH on Boron Uptake by Pansy, Petunia and Gerbera Plants

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Boron Distribution and the Effect of pH on Boron Uptake by Pansy, Petunia and Gerbera Plants

Additional Index Words. Dolomitic limestone, *Gerbera jamesonii*, germination substrate, *Petunia ×hybrida*, *Viola ×wittrockiana*

Summary

Reports of boron (B) deficiency have become more prevalent over the past five years in pansy (*Viola ×wittrockiana)*, petunia (*Petunia ×hybrida*), and gerbera (*Gerbera jamesonii*) plug production. When symptoms are observed in production the presence and severity of symptoms have no pattern, symptomatic plants can be located adjacent to asymptomatic plants in the same plug flat.

The availability of B in the soil decreases as soil pH increases. 'Yellow Dynamite' pansy, 'White Storm' petunia, and 'Festival Apricot' gerbera seedlings were grown in a peat:perlite substrate which was amended with pulverized dolomitic limestone at a rate of 6, 8, 10, or 20 lbs/yd^3. As the rate of lime incorporated into the substrate increased so did soil pH and shoot tissue concentrations of Ca while shoot tissue concentrations of B decreased.

Many commercially available germination substrates are amended with macro and micro nutrient fertilizers. Relatively small amounts of each element are incorporated into the substrate and uneven mixing could result in varying amounts of nutrients in individual cells of a plug flat. This variability could lead to nutrient deficiencies early in the crop cycle. Six commercially available germination substrates were used to fill plug flats and individual cells were sampled and analyzed for nutrient concentrations. Of the substrates tested, there was no variation in any nutrient concentration from cell to cell.

Introduction

Boron is available to plants as boric acid [$B(OH)_3$] (Marschner, 1995). As with most other elements, boron availability is pH dependent and is more readily available with pH values <6.0 (Gupta, 1993). The pKa of B is at pH 9.25, therefore B is found as boric acid at lower pH values and as unavailable borate at higher pH values. The application of lime has been observed to cause temporary B deficiency in plants (Goldberg, 1993).

The pH of a substrate may increase over time due to the basic effects of alkalinity in irrigation water or the practice of liming substrate. Irrigation water alkalinity would only account for the occurrence of boron deficiency late in the production cycle due to the lag time required for the alkalinity to increase the substrate pH. In addition, if one would expect this to be the primary cause of boron deficiency, its prevalence should be much greater in the Midwest and Plains states where high alkalinity is a problem – and practically nonexistent in the Southeast where the water is almost pure (little to no alkalinity). Observations of pansy, petunia, and gerbera crops over the past several years do not support this; severe incidents have been seen at a large North Carolina greenhouse with excellent water quality (personal communications, Sim McMurray, Metrolina Greenhouses, Huntersville, N.C.). Whereas liming of the substrate, if done well in advance of sowing, could cause boron to become unavailable earlier in the production cycle. The use of sphagnum peat for a root substrate makes liming a common practice in greenhouse operations, and the use of finer grades of lime can reduce the time needed to raise the substrate pH. Plug producers have developed production guidelines which control excessive alkalinity in the irrigation water (target level of 0.8 to 1.3 meq for most crops) and monitor the substrate pH to ensure it is within the acceptable pH range of 5.4 to 6.0 (Dole and Wilkins, 2005).

In addition to being amended with lime, substrates are also amended with macro and micro nutrients. Typically the amount of B added to a substrate before use is 9 g per cubic yard (yd^3). This requires that 9 g of B be evenly distributed so that when 288-plug flats are filled there is a consistent amount of B and all other nutrients in each cell. In previous studies we were able to determine that when the plant is temporarily deprived of B the plant can exhibit B deficiency symptoms which persist after B was reintroduced. Even though growers are applying B to plugs as a liquid feed, this does not usually occur until several days after germination. If the B distribution is not consistent when plug flats are filled this could be a cause of B deficiency in plug crops beginning at germination and explain the erratic pattern of symptoms within a plug flat.

High calcium (Ca) levels in the substrate can also negatively affect the levels and availability of B. Soils high in Ca generally will have a high pH, making B less available to the plant (Gupta, 1993). In tomato plants it has been reported that as Ca concentrations increased, so did the severity of B deficiency (Reeve and Shive, 1944). Like B, Ca is also commonly added to the substrate before use and distribution could be inconsistent.

The first objective of these studies was to determine how different liming rates, and in turn the substrate pH, can affect the plant availably of B. The second objective was to determine if Ca and B distribution was consistent throughout a plug flat in several different commercially available germination substrates.

Materials and Methods

Experiment 1

Berger peat moss (Berger Peat Moss, St. Modestede, Quebec, Canada) was combined with perlite to create a 80:20 peat:perlite mix (by volume). The substrate was amended with

pulverized dolomitic limestone at a rate of 6, 8, 10, or 20 lbs/yd^3. On 09 May, 2007 'Dynamite Yellow' pansy, 'White Storm' petunia, and 'Festival Apricot' gerbera seeds were sown in 288-plug trays cut into 6x6 cell flats (each cell: 2 cm × 2 cm × 3 cm deep), one seed per cell. The experiment was a completely randomized design with 8 flats (36-cell) of each treatment. Once sown, the seeds were placed in a growth chamber with temperature set point of 20 °C. Light was provided by fluorescent bulbs with a PPFD of 24 to 75 $\mu mol \cdot m^{-2} s^{-1}$ at plant canopy for 12 h per day. On 17 May the pansy, petunia and gerbera seedlings were moved to a greenhouse with the day/night temperature set points of 23.9/17.8 ºC. Plants were fertilized at each watering after germination with 50 $mg \cdot L^{-1}$ N from Champion 13-2-13 Plug Special (Scotts, Marysville, Ohio) (13N-0.86P-10.79K). A fifth treatment with 8 replications was included where the substrate was amended with 20 lbs/yd^3 lime and weekly foliar sprays of B at 0.25 $mg \cdot L^{-1}$ were applied.

Electrical conductivity (EC) and pH were determined weekly using a Cardy Twin EC (Spectrum Technologies, Inc. Plainfield, Ill.) and a Cardy Twin pH Meter (Spectrum Technologies, Inc. Plainfield, Ill.), respectively, according to the Press Extraction Method (PEM) as described by Scoggins et al. (2000).

Pansy, petunia and gerbera plants were harvested on 19 June, 2007. Plants were severed at the base of the stem and plants from two individual flats, excluding the outside edges (32 plants), were combined into one replication.

Experiment 2

Five 288-plug flats were filled from each of 6 different commercially available germination substrates. The substrates included Berger BM2, Fafard Superfine GM (Fafard Anderson S.C.), Sun-Gro Redi-earth, LG-3 and LP-5 (Sun-Gro Horticulture, Bellevue,

Wash.), Premier Pro-Mix PGX (Premier Horticulture, Dorval, Quebec, Canada). Substrate was collected from 9 cells from each flat. Using the procedure described in Experiment 1, 9 cells of each flat were tested for EC and pH. For statistical comparison of nutrient concentration, pH and EC a total of 3 blocks were taken from each soil mix, with a block being a combination of 3 subsamples from each of 5 separate flats.

Tissue Analysis

To determine tissue nutrient concentration, tissue samples were rinsed in deionized water, then washed in 0.2 N HCl, and again rinsed in deionized water. The samples were then oven derived at 70 °C for 72 h. Dried tissue was ground in a stainless steel Wiley mill through a 1 mm screen (20-mesh) and 0.1 g was digested in a microwave digester (MARS; CEM Corp, Matthews, N.C.) using a modified EPA method (EPA method 3051 with additional peroxide step). Nutrient content, except N, was determined with inductively coupled plasma optical emission spectroscopy (ICP-OES; Model IRIS Intrepid II, Thermo Corp., Waltham, Mass.). A quality control was run every ten samples and if any element was determined to be more than 10% higher or lower than the standard value, the instrument was recalibrated. Tomato standards (NIST reference material 1573) were compared every 20 samples and tomato and spinach standards (NIST reference material 1570a) were compared every 40 samples. Tissue was analyzed for P, K, Ca, S, Mg, B, Cu, Fe, Mn, and Zn.

Soil Analysis

To determine the plant available nutrient concentrations of the substrates a saturated media extract (SME) as described by Warncke (1986) was used. Using the collected solution from the SME nutrient concentration, except N, was determined with inductively coupled plasma optical emission spectroscopy (ICP-OES; Model IRIS Intrepid II, Thermo Corp.,

Waltham, Mass.). A quality control was run every ten samples and if any element was determined to be more than 10% higher or lower than the standard value, the instrument was recalibrated. Tomato standards (NIST reference material 1573) were compared every 20 samples and tomato and spinach standards (NIST reference material 1570a) were compared every 40 samples. Tissue was analyzed for P, K, Ca, S, Mg, B, Cu, Fe, Mn, and Zn.

Data analysis

Data were tested by analysis of variance (ANOVA) using general linear model (SAS Institute, Cary, N.C.) and means were separated by least significant differences (LSD) at $P \leq 0.05$.

Results and Discussion

Experiment 1

Plant species had no significant effect on the substrate pH; therefore, the means of the species, over time, were pooled. The substrate pH increased as lime rate increased (Fig. 4). The lowest lime rate (6 lbs/yd^3) resulted in a pH of 5.82 while the highest treatment rate (20 lbs/yd^3) resulted in a pH of 7.19. There was no effect on EC either by species or lime rate (data not shown).

Symptoms of B deficiency were not observed in lime rate or species. The 20 lbs/yd^3 treatments had a B concentration lower than any other treatment in the study for all three species (Fig. 5). Shoot tissue B concentrations for the 20 lbs/yd^3 treatments in pansies and petunias were only slightly higher than those reported to cause B deficiency symptoms in fully expanded mature leaves from transplanted pansy or petunia plants, 72 or 102 DAS, respectively (Pitchay, 2002). Shoot tissue B concentrations for the 10 and 20 lbs/yd^3 treatments in gerberas were lower than optimal levels for gerberas 2 weeks after transplant

when fertilized with 50-75 $mg \cdot L^{-1}$ N (Whipker et al., 2007). The negative affect on B concentration by the 20 lbs/yd^3 lime rate was overcome by the weekly foliar applications of B in all three species [22.65, 16.13, and 24.09 $mg \cdot L^{-1}$, respectively for pansy, petunia and gerbera ($P \leq 0.05$)]. The increase in substrate pH due to increased lime rates could be one cause of lower concentration of B, as B is less available at higher pH values (Peterson, 1982). Dolomitic limestone is a source of Ca; consequently as the rate of lime incorporated into the substrate increased so did the Ca concentrations in all three species (Fig. 5). Increased Ca uptake can have an antagonistic effect on B causing a decrease in the uptake of B by the plant (Reeve and Shive, 1944). Individual differences in other nutrient concentrations are reported for each species below.

Pansies

Tissue concentrations of P, Cu and Zn (Table 14) were inversely related to the rate of lime incorporated into the substrate as well at substrate pH; these elements are less available to the plant as pH increases (Peterson, 1982). The tissue concentration of K also decreased as lime rate and pH increased, but pH has little effect on K availability (Peterson, 1982). Although Mg is provided by dolomitic limestone, shoot concentrations of Mg decreased as lime rate increased. The decreasing Mg concentrations can be explained by the effect of increased Ca uptake causing Mg uptake to decrease (Marschner, 1995). Sulfur concentrations also decreased as lime rate and pH increased; however, S uptake is not sensitive to pH and no plant nutrient is known to affect S uptake (Mengel and Kirkby, 1982).

Petunias

Concentrations of P and Mn increased as the rate of lime increased. Both elements become less available to plants as the pH increases, it is unclear why these two

concentrations increased with lime rate. With the exception of the lime rate of 20 lbs/yd^3, the concentration of Cu decreased as lime rate increased (Table 14). Copper is less available at higher pH values (Peterson, 1982). The highest two lime rates (10 and 20 lbs/yd^3) resulted in Cu tissue values of lower than those reported to cause deficiency symptoms in fully expanded mature leaves from transplanted petunia plants (102 DAS) (Pitchay, 2002); however, no deficiency symptoms were observed.

Gerbera

The trends for P, K, Mg, and Fe tissue concentrations were similar to those in pansies (Table 14). Phosphorus and Fe are less available in the substrate for plant uptake as pH increases (Peterson, 1982), and increased uptake of Ca is antagonistic to the uptake of Mg (Marschner, 1995). As with the pansies the cause for a decrease in K is unclear as pH has little effect on K availability (Peterson, 1982).

Experiment 2

There were no significant ($P \leq 0.05$) differences in plant available nutrient concentrations, substrate pH or substrate EC for any of the six substrates in this experiment (Table 15 and Table 16). With the exception of Premier Pro-Mix PGX, the Ca concentrations of the tested substrates were below the optimal range for greenhouse substrates (Warncke and Krauskopf, 1983). Fafard Superfine GM was the only substrate with values lower than the range for B (0.05-0.5 mg·L^{-1}) as recommended by the Scotts Co. (Keith Santner, personal communication) (Table 15 and Table 16).

Conclusion

Increasing the amount of lime incorporated into the germination substrate increased substrate pH and therefore decreased the tissue concentration of B in shoot tissue. The

decrease in B shoot tissue was due to the antagonistic effect of Ca as well as the higher substrate pH, both caused by increased lime applications. Plug growers should only incorporate sufficient lime to raise the substrate pH to 5.5 to 6.0. In addition, growers should monitor substrate pH and take any corrective measures to maintain a proper substrate pH. Plug growers can apply foliar sprays to increase B concentration in shoot tissue and may consider applying a supplemental drench of B at the time of sowing to ensure an adequate amount of B is available to the plant at germination.

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Table 14. 'Dynamite Yellow' pansy, 'White Storm' petunia, and 'Festival Apricot' gerbera tissue concentrations at 38 d after sowing grown a peat-based substrate amended with pulverized dolomitic limestone at a rate of 6, 8, 10, or 20 lbs/yd^3.

lbs/yd^3	Percent dry wt.				mg·L^{-1} dry wt.			
Treatment	**P**	**K**	**Mg**	**S**	**Cu**	**Fe**	**Mn**	**Zn**
				Pansy				
6	0.163a	3.61a	0.47a	0.26a	4.67a	≤46.00	120.93	55.09a
8	0.130c	3.27ab	0.41b	0.22b	3.78b	≤46.00	110.95	51.42a
10	0.138bc	3.21b	0.41b	0.22b	3.39b	≤46.00	119.86	45.33ab
20	0.140b	3.03b	0.37c	0.21c	3.00b	130.89	114.85	31.19b
P-value[z]	***	*	***	***	**	NS	NS	*
				Petunia				
6	0.09b	3.40	0.33	0.21	3.94ab	50.11	28.92c	21.68
8	0.10a	3.23	0.32	0.21	4.42a	69.65	36.77b	21.97
10	0.10ab	3.11	0.32	0.22	2.77c	46.42	41.40b	18.95
20	0.11a	3.44	0.33	0.22	3.13bc	73.18	47.68a	24.82
P-value[z]	*	NS	NS	NS	*	NS	***	NS
				Gerbera				
6	0.19a	3.39a	0.48a	0.25	5.37	97.02a	64.36	39.09
8	0.16b	3.21ab	0.41b	0.22	3.84	84.14ab	59.29	32.83
10	0.17ab	3.12b	0.44ab	0.23	4.27	52.80bc	64.23	32.22
20	0.16b	3.10b	0.40b	0.23	2.91	≤46.00c	59.19	32.08
P-value[z]	*	*	**	NS	NS	*	NS	NS

[z] NS, *, Not significant or significant at $P \leq 0.05$, $P \leq 0.01$, or $P \leq 0.001$
Mean separations are shown by species in columns under each element

Table 15. Substrate plant available nutrient concentrations (saturated media extract), pH and electrical conductivity (EC) for Berger BM2, Sun-Gro LG3, and Sun-Gro LP5 soilless germination substrates.

	mg·L^{-1}											
Block[z]	**P**	**K**	**Ca**	**Mg**	**S**	**B**	**Cu**	**Fe**	**Mn**	**Zn**	**pH**	**EC**
					Berger BM2							
1	3.99	31.21	71.49	65.12	91.17	0.13	0.04	0.45	0.35	0.46	6.05	0.91
2	3.88	31.27	72.76	65.45	92.42	0.12	0.04	0.44	0.33	0.49	6.01	0.91
3	3.89	30.78	69.94	62.86	88.52	0.13	0.04	0.45	0.33	0.44	5.99	0.91
P-value[y]	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
					Sun-Gro LG3							
1	7.33	69.86	48.89	94.49	123.64	0.08	0.02	0.55	0.25	0.17	5.32	1.06
2	8.48	74.5	50.71	99.33	128.26	0.07	0.01	0.43	0.24	0.14	5.10	1.06
3	7.76	77.13	53.83	103.83	129.71	0.08	0.02	0.4	0.28	0.14	5.18	1.05
P-value[y]	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
					Sun-Gro LP5							
1	8.71	46.26	44.33	70.37	99.68	0.07	0.02	0.13	0.14	0.13	5.55	0.98
2	9.08	44.93	41.40	67.93	94.81	0.07	0.01	0.17	0.13	0.14	5.47	0.93
3	9.11	45.76	43.70	69.15	99.99	0.07	0.01	0.09	0.14	0.14	5.43	0.96
P-value[y]	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

[z] Blocks were taken from each soil mix, with a block being a combination of 3 subsamples from each of 5 separate flats
[y] NS – Not significant
Means are compared by substrate in columns under each element

Table 16. Substrate plant available nutrient concentrations (saturated media extract), pH and electrical conductivity (EC) for Premier Pro-Mix PGX, Sun-Gro Redi-earth, and Fafard Superfine GM germination substrates.

	mg·L^{-1}											
Block[z]	**P**	**K**	**Ca**	**Mg**	**S**	**B**	**Cu**	**Fe**	**Mn**	**Zn**	**pH**	**EC**
					Premier Pro-Mix PGX							
1	9.47	176.64	133.59	84.8	82.36	0.13	0.03	0.04	0.32	0.66	6.56	1.65
2	8.45	160.04	117.87	74.14	73.65	0.12	0.04	0.08	0.48	0.81	6.52	1.86
3	8.33	151.64	117.87	73.65	71.64	0.12	0.04	0.14	0.47	0.73	6.47	1.74
P-value[y]	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
					Sun-Gro Redi-earth							
1	3.66	109.13	66.83	120.21	137.58	0.08	0.03	0.75	0.54	0.32	5.58	1.85
2	3.63	129.13	80.9	146.2	165.3	0.09	0.03	0.59	0.65	0.12	5.46	1.88
3	3.97	136.07	85.98	148.4	167.73	0.08	0.03	0.57	0.68	0.08	5.46	1.93
P-value[y]	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**
					Fafard Superfine GM							
1	10.3	69.82	67.67	89.63	160.36	0.04	0.01	1.15	0.24	0.22	5.70	1.11
2	10.79	67.5	66.43	85.64	158.54	0.04	0.01	0.83	0.26	0.23	5.56	1.13
3	14.51	76.72	69.03	87.25	164.93	0.05	0.02	0.88	0.23	0.23	5.57	1.13
P-value[y]	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**

[z] Blocks were taken from each soil mix, with a block being a combination of 3 subsamples from each of 5 separate flats
[y] NS – Not significant
Means are compared by substrate in columns under each element

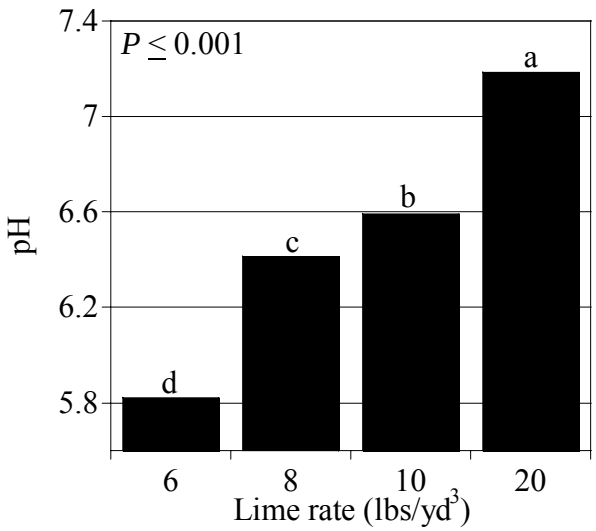

Fig. 4. Substrate pH when 'Dynamite Yellow' pansy, 'White Storm' petunia, and 'Festival Apricot' gerbera were grown in substrate amended with pulverized dolomitic limestone at a rate of 6, 8, 10, or 20 lbs/yd^3. Values are pooled means across species (pansy, petunia, and gerbera), n=96.

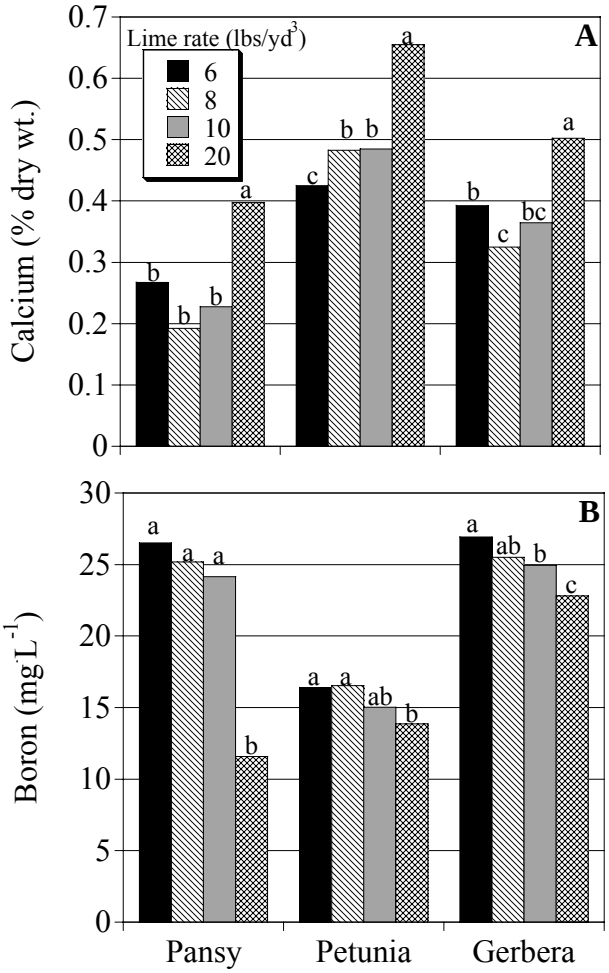

Fig. 5. Calcium (A) and boron (B) tissue concentrations for 'Dynamite Yellow' pansy, 'White Storm' petunia, and 'Festival Apricot' gerbera plants at 38 d after sowing grown in a peat-based substrate amended with pulverized dolomitic limestone at a rate of 6, 8, 10, or 20 lbs/yd^3. Means separation conducted by LSD ($P \leq 0.05$) within plant species.

Chapter 6

Investigation of Causal Agents of Leaf Distortion Through Histological Examination of Leaf Pectin and Lignin Content and Screening of Plant Viruses

(In the format appropriate for submission to HortScience)

Submitted to HortScience

Investigation of Causal Agents of Leaf Distortion Through Histological Examination of Leaf Pectin and Lignin Content and Screening of Plant Viruses

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Investigation of Causal Agents of Leaf Distortion Through Histological Examination of Leaf Pectin and Lignin Content and Screening of Plant Viruses

Additional Index Words. *Gerbera jamesonii*, Hoagland's, *Petunia ×hybrida*, Toluidine Blue O, *Viola ×wittrockiana*

Summary

Reports of pansy (*Viola ×wittrockiana*), petunia (*Petunia ×hybrida*), and gerbera (*Gerbera jamesonii*) seedlings in plug production with curled, thickened, and distorted leaves have become more prevalent over the past five years. Although there is strong evidence that these symptoms are due to boron (B) deficiency, the possibility of a viral cause has been suggested. 'Dynamite Yellow' pansy, 'White Storm' petunia, and 'Festival Apricot' gerbera seedlings were grown hydroponically with nutrient solutions with or without B to induce the previously mentioned symptoms. Plants were tested for 16 common ornamental viruses and no positive detections were found in symptomatic or asymptomatic plants.

To investigate the cause of the morphological symptoms, tissue exhibiting symptoms and tissue with normal growth were sectioned and stained with Toluidine Blue O. A region of darker stained cells below the epidermis of symptomatic tissue was observed indicating a higher concentration of pectin. It is likely that this cellular region is causing symptoms such as curled, thickened and distorted leaves.

Introduction

During the past five years, greenhouse growers and seed company technical representatives have reported concern about distorted terminal growth of pansy, petunia, and gerbera plants. The distorted growth has been determined to be caused by boron (B)

deficiency (Krug et al., 2007). Many of the symptoms of B deficiency can be seen with the naked eye including: aborted growing tips, fast growing auxiliary shoots, and leaves that are strapped, crinkled, stunted, thickened, or upward cupped, chlorosis of upper leaves and restricted leaf expansion (Jiao et al., 2005; Laffe and Styer, 1989; Mengel and Kirkby, 1987; Stuart, 1991).

Boron has been reported to lead to a number of changes in cell walls and may be responsible for many of the symptoms observed in B deficiency tissue. Plants deficient in B also have an increase in hemicellulose, pectin, callouse, and lignin (Lovatt and Dugger, 1984; Neales, 1960; Rajaratnam and Lowry, 1974; Römheld and Marschner, 1991; Van de Venter and Currier, 1977). Cells which have been deprived of B often have thickened walls and are unable to elongate or divide (Dell and Huang, 1997). Although the changes may not be caused directly by a lack of B they may be caused by a cascade of events catalyzed by B deficiency (Marschner, 1995).

There has also been speculation by growers and seed producers that the symptoms may be caused by a virus. Therefore, the first objective was to determine if a virus is present in plants with B deficiency symptoms. The second objective was to determine if B deficiency leads to a change in pectin and lignin content in pansy leaves; therefore, leading to the malformation associated with B deficiency.

Materials and Methods

Experiment 1

Oasis foam (Smithers-Oasis, Kent, Ohio) was used as the growing medium. The foam was cut to fit into a 2-ml microcentrifuge tubes that had approximately the bottom 3 mm removed. 'Dynamite Yellow' pansy, 'White Storm' petunia, and 'Festival Apricot'

gerbera were sown, one seed per tube, and 36 tubes per replication. The experiment was a completely randomized design with 2 replications of each treatment. Once sown, seeds were placed in a germination chamber with a temperature set point of 20 ºC. Light was provided by fluorescent bulbs with a PPDF of 24 to 75 $\mu mol \cdot m^{-2}s^{-1}$ for 12 h per d. Oasis foam was kept moist using deionized water until seeds germinated. After germination, plants were transferred into 2.3-L plastic containers with holes drilled in the lids to hold the microcentrifuge tubes. Plants were then moved into a greenhouse and grown in a complete modified Hoagland's all nitrate solution, or a formulation minus B: (macronutrients in mM) 15 NO_3-N, 1.0 PO_4-P, 6.0 K, 5.0 Ca, 2.0 Mg, and 2.0 SO_4-S (Hoagland and Arnon, 1950), plus μM concentrations of micronutrients, 72 Fe, 9.0 Mn, 1.5 Cu, 1.5 Zn, 45.0 B, and 0.1 Mo. The following reagent grade chemicals KNO_3, $Ca(NO_3)_2$, KH_2PO_4, $MgSO_4$, $NaNO_3$, FeDTPA, $MnCl_2$, $ZnSO_4$, CuCl, H_3BO_3, Na_22MoO_4 and deionized water was used to formulate treatment solutions. Because the plants were small and did not use large quantities of nutrient solutions, solutions were replaced and were adjusted to pH 5.8 using 0.1M NaOH every two weeks. Plants were grown in a greenhouse with day/night temperature set points of 23.9/17.8 ºC, under high pressure sodium lights to provide a minimum PPFD of 100 $\mu mol \cdot m^{-2}s^{-1}$ at plant canopy for 12 h per d. Plant tissue was harvested 41 days after sowing (DAS). Tissue samples for the virus screens were taken by removing tissue from each plant exhibiting symptoms of B deficiency. The tissue from the two replications were combined and sent to Agdia Inc. (Elkhart, Ind.) for testing. The remaining tissue was harvested by removal at the base of the plant to be analyzed for nutrient concentrations to confirm lower concentrations of boron in the tissue.

Experiment 2

'Dynamite Yellow' pansies were grown in an identical fashion as in Experiment 1 to obtain plants exhibiting normal growth or B deficiency symptoms. The experiment was a completely randomized design with 3 replications per treatment. Additional plants ('Matrix Orange' and 'Supreme Orange') were collected from a commercial pansy plug producer. Plants which exhibited normal growth and those exhibiting symptoms of B deficiency were collected. Young leaf samples from 'Dynamite Yellow', 'Matrix Orange', and 'Supreme Orange' (48, 32, and 32 DAS, respectively) from B deficient and normal plants were placed in fixative containing 18:1:1 parts of 70% ethyl alcohol:glacial acetic acid:formaldehyde. The samples were kept at room temperature for 48 h and transferred to 70% alcohol and kept at 4 °C until they were processed for dehydration and embedding. After selected tissue was collected the remaining tissue from 'Dynamite Yellow' was harvested for tissue nutrient concentration analysis.

Leaf samples were dehydrated according to the procedures detailed by Johansen (1940) using a series of ethyl alcohol and tertiary butyl alcohol with a final infiltration step in paraffin and embedded in Paraplast Plus. Embedded sample blocks were sectioned in a Leica RM 2255 rotary microtome (Leica Microsystems, Wetzlar, Germany) at a thickness of 15 µm. The resulting parafifin ribbon containing serial sections was placed on a glass slide coated with Haupt's adhesive, flooded with 3% formalin solution and transferred to a slide warmer at 41 °C (Johansen, 1940). Dried slides were stored at room temperature until stained. The slides were left overnight in Xylene to remove paraffin before sections were stained with Toluidine Blue O (stains lignified tissues bluish green and pectin and pectic substances pink to purple). Once stained, sections were viewed on a Zeiss Photomicroscope

III (Carl Zeiss, Jena, Germany) and photographs were taken with a Sony DSC-707 digital camera (Sony Corp. Tokyo, Japan) attached to the microscope.

Tissue Analysis

The tissue samples for both experiments were first rinsed in deionized water, then washed in 0.2 N HCl for 30 s, re-rinsed with deionized water, and dried at 70 ºC for 72 h. Dried tissue was ground in a stainless steel Wiley mill through a 1 mm screen (20-mesh) and 0.1 g was digested in a microwave digester (MARS; CEM Corp, Matthews, N.C.) using a modified EPA method (EPA method 3051 with additional peroxide step). Nutrient concentration, except N, was determined with inductively coupled plasma optical emission spectroscopy (ICP-OES; Model IRIS Intrepid II, Thermo Corp., Waltham, Mass.). A quality control was run every ten samples and if any element was determined to be more than 10% higher or lower than the standard value, the instrument was recalibrated. Tomato standards (NIST reference material 1573) were compared every 20 samples and tomato and spinach standards (NIST reference material 1570a) were compared every 40 samples. Tissue was analyzed for P, K, Ca, S, Mg, B, Cu, Fe, Mn, and Zn.

Data Analysis

Tissue data for Experiment 2 were tested by analysis of variance (ANOVA) using general linear model (SAS Institute, Cary, N.C.) and means were separated by least significant differences (LSD) at $P \leq 0.05$. When tissue concentrations of an element were not detected a value half that of the lowest detectable level for the respective element was used for statistical analysis.

Results and Discussion

Experiment 1

Boron concentrations in pansies (< 1.70 $mg \cdot L^{-1}$), petunias (5.13 $mg \cdot L^{-1}$) and gerberas (< 1.70 $mg \cdot L^{-1}$) treated with a minus B solution were lower than the controls (42.60, 41.05, and 39.29 $mg \cdot L^{-1}$, respectively). Tissue levels of B for pansies and gerberas in the minus B treatments were below the detectable levels (1.70 $mg \cdot L^{-1}$) using the ICP-OES. The levels for pansies, petunias, and gerberas were lower than published values to cause B deficiency in fully expanded mature leaves from transplants (72 DAS) (Jones et al., 1991; Pitchay, 2002). None of the tested tissue was found to be infected with a virus (Table 17). Although this virus screen rules out infection from any of the 16 common ornamental viruses, it does not rule out any viruses not listed in Table 17.

Experiment 2

Shoot tissue concentrations of B were significantly ($P \leq 0.001$) lower in 'Dynamite Yellow' pansies in the minus B treatment than the control (Table 18). When the sectioned tissue was stained with Toluidine Blue O, very little ligin was stained. As the leaf tissue of a pansy seedling is primarily soft vegetative growth it would be unusual to observe large quantities of lignified tissue. Some lignified tissue was observed in the vascular tissue but there was no obvious difference between the two treatments. However, regions of darker stained cells (approximately 200 μm wide and highlighted by arrows) were visible just under the epidermis in tissue exhibiting B deficiency symptoms (Fig. 6). The darker stained region indicates a higher concentration of pectin. This increase in pectin could be a result of alterations of the Golgi apparatus in the pansy leaves. The Golgi apparatus is where the assembly of pectin occurs (Northcote, 1986), and Kouchi and Kumazawa (1976) report that

root tips of tomatoes grown without B had an increased number and size of secretory vesicles of the Golgi apparatus. This change in the Golgi apparatus could increase the amount of pectins produced in B deficient tissue (Römheld and Marschner, 1991).

Conclusion

Although the symptoms of B deficiency can mimic some viral symptoms none of the tissue tested were positive for the 16 common ornamental viruses tested in this study. It is possible that an unknown virus which was not tested was the cause of the symptoms observed. However, this is unlikely because if a virus was the cause, symptoms would have also been observed in the untreated control plants.

Some of the visual symptoms of B deficiency, curled, thickened, distorted leaves, could be caused by a region of cells under the leaf epidermis which has a higher concentration of pectin. If a layer of cells with higher concentration of pectin occurred across the entire leaf or large sections of a leaf it would cause morphological changes.

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Table 17. Results from virus screen from 'Dynamite Yellow' pansy, 'White Storm' petunia, and 'Festival Apricot' gerbera plants grown a complete or minus boron modified Hoagland's solution.

	Pansy		Petunia		Gerbera	
Virus Tested	**Complete**	**Minus B**	**Complete**	**Minus B**	**Complete**	**Minus B**
Arabis Mosaic	Negative	Negative	Negative	Negative	Negative	Negative
Alfalfa Mosaic	Negative	Negative	Negative	Negative	Negative	Negative
Broad Bean Wilt	Negative	Negative	Negative	Negative	Negative	Negative
Calibrachoa Mottle	Negative	Negative	Negative	Negative	Negative	Negative
Chrysanthemum Virus B	Negative	Negative	Negative	Negative	Negative	Negative
Cucumber Mosaic	Negative	Negative	Negative	Negative	Negative	Negative
Impatiens Necrotic Spot	Negative	Negative	Negative	Negative	Negative	Negative
Prunus Necrotic Ringspot	Negative	Negative	Negative	Negative	Negative	Negative
Tobacco Mosaic	Negative	Negative	Negative	Negative	Negative	Negative
Tobacco Ringspot	Negative	Negative	Negative	Negative	Negative	Negative
Tobacco Streak	Negative	Negative	Negative	Negative	Negative	Negative
Tomato Aspermy	Negative	Negative	Negative	Negative	Negative	Negative
Tomato Mosaic	Negative	Negative	Negative	Negative	Negative	Negative
Tomato Ringspot	Negative	Negative	Negative	Negative	Negative	Negative
Tomato Spotted Wilt	Negative	Negative	Negative	Negative	Negative	Negative
Potyvirus Group	Negative	Negative	Negative	Negative	Negative	Negative

Table 18. 'Dynamite Yellow' pansy tissue concentrations at 47 d of age treated with a complete or minus boron modified Hoagland's solution.

	Percent dry wt.					$mg \cdot L^{-1}$ dry wt.				
Treatment	**P**	**K**	**Ca**	**Mg**	**S**	**B**	**Cu**	**Fe**	**Mn**	**Zn**
Complete	0.45	3.35	1.35b	0.43b	0.31	50.69a	4.48	115.48	<16.00	6.83
Minus B	0.50	3.63	1.62a	0.49a	0.34	<1.70b	4.55	94.37	<16.00	9.18
P-value [z]	NS	NS	**	*	NS	***	NS	NS	NS	NS

[z] NS, *, **, ***, Not significant or significant at $P \leq 0.05$, ≤ 0.01,or ≤ 0.001
Mean separations are shown in columns under each element

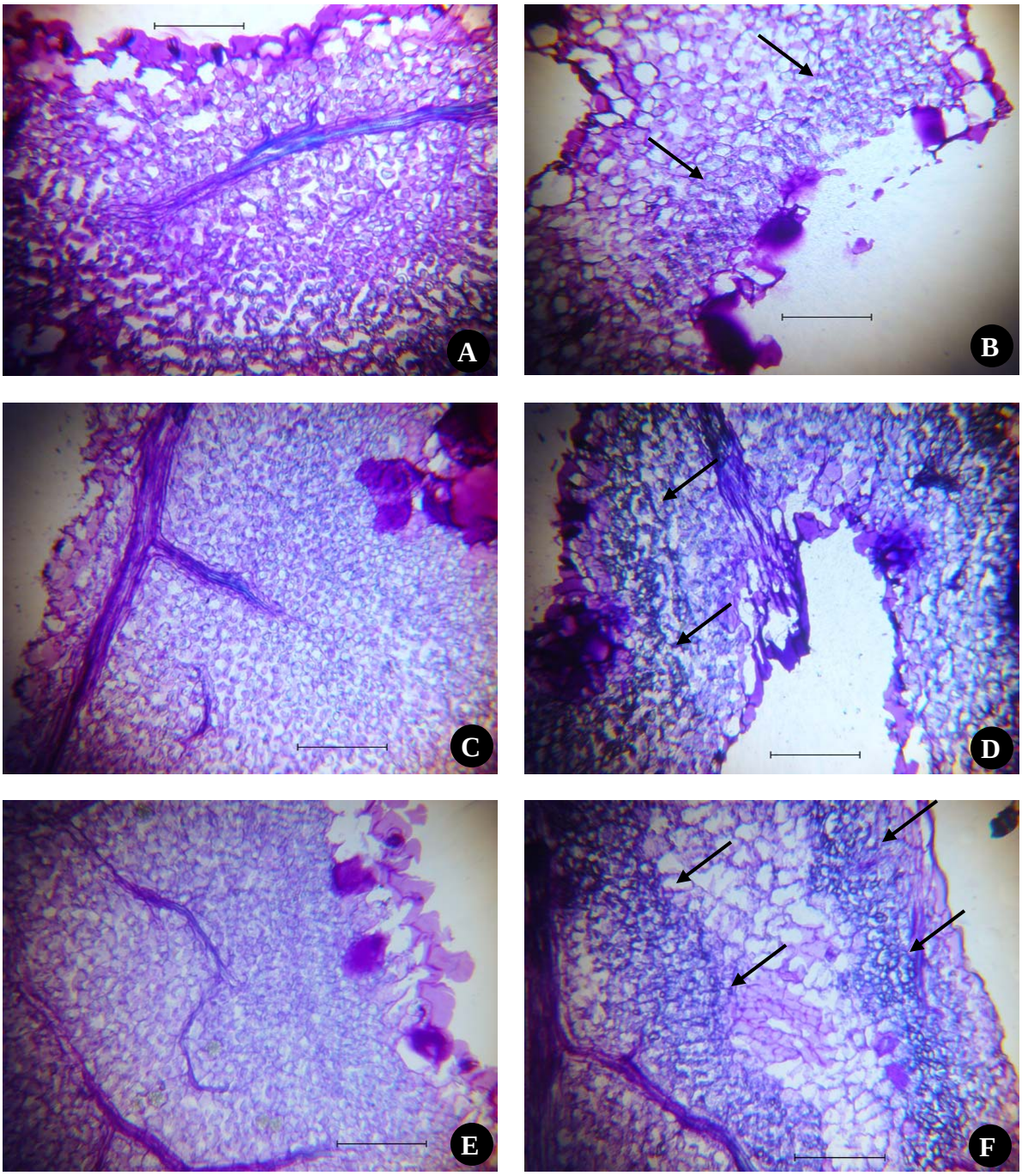

Fig. 6. Longitudinal sections of 'Dynamite Yellow' (A and B), 'Matrix Orange' (C and D), and 'Supreme Orange' (E and F) pansy leaf tissue stained with Toluidine Blue O. Plants which were asymptomatic of boron deficiency (A, C, and E) and plants exhibiting symptoms of boron deficiency (B, D, and F). Arrows indicate areas which stained darker and have higher concentrations of pectin. Scale bar = 200 µm.

Chapter 7

Grower Recommendations

Through this series of studies, boron (B) deficiency has been identified as the likely cause of distorted growth in pansy, petunia, and gerbera plug production. The cause of B deficiency in these crops was also investigated to determine how plug producers can best avoid the problem. Boron is needed at very low concentrations in the substrate for adequate plant growth (Gupta, 1993); it is common practice to apply a complete fertilizer at every irrigation. This led us to hypothesize that B deficiency is not due to the lack of B in the substrate but instead to the inability of the plants to take up B from the substrate.

Substrate pH has an affect on availability of B. At higher pH values B becomes less available to plants (Peterson, 1982). When pansy, petunia, and gerbera were grown in a substrate amended with increasing rates of dolomitic limestone, substrate pH increased and the shoot tissue concentration of B decreased. The negative effect on B concentration with the highest rate of lime was overcome by weekly foliar applications of B.

Boron is taken up passively via the transpiration stream and is only mobile through xylem of the plant (Jones, 1991; Kochian, 1991; Kohl and Oertli, 1961; Raven, 1980). Low substrate moisture level can negatively affect B availability to the plant (Fleming, 1980; Gupta, 1993). However, when pansy plants grown in plug flats were allowed to dry to 40, 30 or 20% container capacity (-2, -5, or -20 kPa osmotic pressure, respectively) at 10 or 20 days after sowing (DAS), or on a continual basis, B concentration in shoot tissue was not affected. Likewise, when polyethylene glycol was used to exert a drought stress on pansy plants (-2, -5, or -20 kPa osmotic pressure) B concentration in shoot tissue was not affected. However, when abscisic acid was applied as either a drench or foliar spray to induce stomatal closure, transpiration decreased as did B concentration in shoot tissue. Therefore, in greenhouse production of pansy plugs, drought stress appears not to be the primary cause of lower

concentrations of B in shoot tissue, whereas a decreased transpiration rate leads to a decreased concentration of B in shoot tissue.

In commercial production of pansy, petunia, and gerbera plugs cyclic applications of water are applied as a fine mist to increase relative humidity, providing adequate moisture for germination and reduced air temperatures. Relative humidity can be a major factor controlling transpiration rate. The uptake of B has been reported to be negatively affected by high relative humidity (Bowen, 1972; Halbrooks et al., 1986). In our studies we grew pansy, petunias, and gerberas in ambient humidity (AH) or high humidity (HH). Plants grown in HH had lower concentrations of B in shoot tissue and had lower rates of transpiration. It has been reported that increasing air movement around plants can increase the amount of transpiration (Kramer, 1983). Therefore we grew pansy plants in the same humidity conditions, AH or HH, with or without increased air circulation using horizontal airflow fans. However, when airflow was continually increased using fans, the transpiration rate decreased and no effect on B concentration in the shoot tissue was observed. This decrease was thought to be due to the excessive air velocity which resulted in stomatal closure. Lower air velocities or the use of periodic airflow still needs to be studied.

Based on these experiments, plug growers have options to avoid B deficiency in pansy, petunia, and gerbera crops. Growers are already applying pre-plant drenches and post-germination foliar sprays of B; this practice should be continued but with caution to avoid over-application of B, which could lead to toxicity. The amount of B applied should not be greater than 0.5 $mg \cdot L^{-1}$ (including irrigation water levels). Managing the amount of lime incorporated into the substrate and monitoring the substrate pH over time is an

important step to ensure that B in the substrate is available to the plant; maintaining the substrate pH between 5.5 and 6.0 will guarantee B is in an available form.

The most important measure growers can take to avoid B deficiency in pansy, petunia, and gerbera plugs is to properly manage the relative humidity in the greenhouse. Using mist to raise the relative humidity is important to obtain consistent germination of seeds and to lower the air temperature in hot conditions. In doing this, growers must only use the minimum amount of mist required so that the elevated relative humidity does not decrease transpiration and therefore decrease B concentration in shoot tissue. Growers should also manage the irrigation applications so that the substrate is not waterlogged and the plug is not saturated during the evening hours.

Although our studies concluded that continually increasing the airflow did not increase transpiration or B concentration in shoot tissue, we recommend that growers periodically increase the airflow around the crop to encourage higher transpiration rates and therefore allow the plants to take up more B. It has been reported that too high of air velocities will decrease the transpiration rate (Kramer, 1983) and the constant velocity of 1 to 2 mph used in our study may have been too great. Therefore, lower velocities or intermittent increases of airflow should be used. Further study of the effects of intermittent increases of airflow and lower velocities on transpiration should be conducted.

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