ENVIRONMENTAL FACTORS INFLUENCING THE PHYSIOLOGICAL DISORDERS OF EDEMA ON IVY GERANIUM (*PELARGONIUM PELTATUM*) AND INTUMESCENCES ON TOMATO (*SOLANUM LYCOPERSICUM*)

by

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Abstract

Ivy geranium (*Pelargonium peltatum* L'Herr ex. Ait.) and tomato (*Solanum lycopersicum* L.) are two economically important greenhouse crops known to be affected by non-pathogenic lesions on leaf tissues. These physiological disorders are often termed edema (oedema) or intumescences, but several other names have been used including enations, non-pathogenic galls or tumors, and neoplasms. These lesions, characterized by small protrusions on leaf tissues that become necrotic over time, are considered to be the result of environmental factors. Our research focused on determining what environmental factors affect these disorders on ivy geranium and tomato.

The physiological disorder of ivy geranium is thought to be the result of water uptake exceeding transpiration, resulting in a build-up of water and solutes in leaf tissue that results in the blister-like protrusions in the epidermal layer. Current convention suggests that susceptible plants be grown in an environment that promotes transpiration with low humidity and infrequent watering. Over four experiments, we evaluated the effects of four root medium water contents, five rates of supplemental calcium application and two vapor pressure deficit (VPD) environments on three cultivars of ivy geranium. Our results indicate that high root medium water contents do not increase the incidence of edema on ivy geranium, but increase overall plant growth. Supplemental calcium had no affect on edema or growth, while our VPD results were inconclusive. These results suggest that current convention regarding cultural practices that abate the disorder be revisited.

In tomato var. *hirsutum* 'Maxifort', the physiological disorder is characterized by individual epidermal cells swelling, which is unlike the disorder in ivy geranium where solutes build up across a group of epidermal cells. The environmental factors we focused on were two root medium water contents and supplemental UVB light. Our results suggest that root medium water content may play a role in development of tomato intumescences based on visual observation, and UVB light supplementation helps prevent the lesions from forming.

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Dedication

There are lots of people whom I can dedicate this thesis to, but two were the most instrumental in my life. I dedicate this thesis to my mom, Kathy Owens, who supported me all throughout my life and to my husband, Ken Nelson, who has supported me without fail for all our years together.

CHAPTER 1 - Literature Review

Edema and intumescence development are disorders of greenhouse grown crops, characterized by non-pathogenic bumps, galls, or blisters on plant tissues. Often, the terms edema (oedema) and intumescences are used interchangeably with several other terms, which include enations, genetic tumors, neoplasms, and non-pathogenic galls. While these terms are used interchangeably, the term edema is most used in ivy geranium (*Pelargonium peltatum* L'Herr ex. Ait.), while in tomato (*Solanum lycopersicum* L.) the most common term used is intumescences.

Research by previous investigators has suggested several environmental that contribute to these disorders. Pinkard et al. (2006) provide an extensive review of economically important plant species known to develop intumescences as well as their probable triggers, which include mechanical or chemical injury, nutrient status, hormones, genetics, insect and fungal injury, air quality, light quality and availability temperature, excess water and unknown factors. This literature review will discuss the environmental conditions that have been associated with edema and intumescence development in relation to the ivy geranium and tomato experiments presented in this thesis.

Ivy Geranium

Edema is most commonly associated in the horticulture industry with geranium (*Pelargonium*) species. Ivy geranium, a susceptible ornamental crop, is native to costal South Africa, where temperatures tend to be mild and salt levels in the growing environment may be on the high side. These environmental conditions have likely led to the morphology of these plants having thick, waxy cuticles, which aid in water retention. In ivy geranium, epidermal cells are stretched over an area in which solutes have built up, much like a blister. While mild symptoms are considered acceptable, moderate to severe symptoms compromise the aesthetics of the plant. This results in decreased sales of affected plants and economic losses (Balge et al. 1969).

The first known mention of edema was in 1905, when Dale (1905) suggested environmental conditions as a trigger of edema on *Hibiscus vitifolius* Linn., as opposed to pathogens which at the time were thought to be the cause. In her study, moist air was shown to be a cause of this disorder, while dry air and submergence into water were not.

Later studies focused on other environmental causes of edema. The primary focus was on root medium water content and relative humidity. Balge et al. (1969) found that zonal geraniums (*Pelargonium hortorum* Ait.) grown in warm, moist soil were more prone to the occurrence of edema, while geraniums grown in a soil with a water content substantially below container capacity had fewer edemata.

Metwally et al. (1970a) examined the role of root medium water content further on zonal geranium. Watering frequencies, supplemental fertilization, media characteristics, and light intensities were studied. They found that the environmental factors restricting transpiration led to an increased susceptibility in edema development. Plants grown in an environment with high air moisture, high soil water contents, low light intensities, or combinations of these factors had increased edema injury.

A second study by Metwally et al. (1970b) looked at anatomical differences between zonal geraniums grown with different root medium water contents. Geraniums grown in a high moisture regime were found to have larger xylem elements and smaller phloem elements, as well as a larger overall cell size, compared to plants grown under drought stress. They suggest that these anatomical changes caused increased water movement and decreased movement of photosynthates within the plant, resulting in abundant water levels in the plant forming edemata.

To elucidate a possible mechanism behind edema formation, Metwally et al. (1971) researched the formation of stomata on zonal geraniums grown under different soil moisture regimes. Cultivar and soil moisture content played a role in number of stomata, and the number of stomata played a role in edemata formation. Higher soil moisture contents led to more stomata per area in young tissues than those grown in a drier environment. However, higher soil moisture contents also lead to thinner epidermal cell walls causing a possible trade-off between growing geraniums wet versus dry in terms of edemata formation. They also found a relationship between cultivars and susceptibility, suggesting a genetic component to this disorder.

Jonas (2000) contradicts the findings by Metwally et al. (1970a, 1970b, 1971) that suggest high root medium water contents are the culprit in edema formation. In her thesis, Jonas found higher root medium water contents did not increase the incidence of edema in ivy geranium. Further, she found that plants receiving the least frequent irrigation resulted in the highest levels of edema development, concluding that large fluctuations in root medium water may play a stronger role than having a high root medium water content overall.

Other than the Jonas thesis, only one paper has been published on edema development on ivy geranium (*Pelargonium peltatum* L'Hér. ex Ait). Rangarajan and Tibbitts (1994) studied the effect of far-red radiation (700-800 nm) and that plants were exposed to this range of light wavelengths, plants did not develop edema. Morrow and Tibbitts (1988) found a possible link to intumescence development in tomato (*Solanum lycopersicum* var. *hirsutum* L. 'Oxheart'.), in which far-red radiation prevented its occurrence and suggested a possible relation to phytochrome. It was thought at the time that intumescence development and edema were the same disorder, so this study was repeated on ivy geraniums. However, far-red radiation had no effect on prevention of edema in ivy geranium in their study.

The findings, predominately by Metwally et al. (1970a, 1970b, 1971) led to the recommendation from several sources, ranging from textbooks to extension publications, for commercial growers to grow susceptible species in as dry an environment as possible without stressing the plant and keeping pest feeding at a minimum. Feeding from insects such as thrips (*Frankliniella occidentalis* (Pergande) Thysanoptera: Thripidae) and spider mite (*Tetranychus urticae* (Koch) Arachnida: Acari: Tetranychidae) can cause edema-like symptoms (Burns, 2002). Suggestions included maintaining low humidity and a low root medium moisture content; watering during morning hours to provide sufficient time for crops to transpire excess water (Anonymous 10/1999a; Anonymous 10/1999b; Anonymous 2001; Dole and Wilkins, 1999; Dudek, 2004; Lemon, 1996; Micheal, 1996; Moorman, 1993; Ogelvee, 2003; Pataky, 1998; Wiles, 1997); and providing supplemental calcium to strengthen cell walls (Micheal, 1996). Despite these studies, the factors that contribute edema are still not well understood. Further research should be undertaken to determine what environmental and genetic factors cause this disorder so economic losses in the future can be prevented.

Tomato

Intumescences, like edema, are a result of a physiological disorder. In the case of intumescences, several individual cells swell in a given area, opposed to edema where several epidermal cells are stretched over what appears to be a pocket of water or solutes forming a single, blister-like edemata. Intumescences have been found to primarily affect solanaceous crops grown in controlled environments. However, a few other genera (*Populus*, *Hibiscus*, *Eucalyptus*, *Ipomoea*, *Cuphea*) include certain species known to exhibit intumescence-like

development. Further, intumescence development is highly dependent on cultivar, as Jarwoski et al. (1988) found on *Cuphea* species, suggesting a genetic component.

As with edema, while mild symptoms of intumescence development may be tolerated, moderate to severe symptoms, including leaf chlorosis, leaf necrosis, and leaf drop, are not acceptable. Chlorosis and necrosis, along with the finding that intumescence development lowers the number of chloroplasts in palisade cells (Eliza and Dobrenz, 1971), may cause plants to grow slowly and to have lower yields than unaffected plants. In addition, plants are severely affected aesthetically, resulting in economic loss in plant sales to consumers.

The exact environmental triggers of intumescence development are unknown. Previous authors have suggested many possible factors that can lead to the development of intumescences. La Rue (1933) noted that intumescences on *Populus* species were usually the result of excess moisture in the air or on a leaf surface. In his study, intumescences were readily produced in artificial environments where humidity and moisture were high and they were only found in the field on leaves that had not yet unfurled completely and were not exposed to the drier air conditions. Eliza and Dobrenz (1971) observed that "oedema" on eggplant (*Solanum melongena* L.) was induced in hot, humid environments within a plastic greenhouse. Their anatomical study indicated lesions were caused by hypertrophy (cellular swelling) of palisade cells, which then ruptured the epidermal layer. Given their description of the disorder and the anatomical analysis, we believe they were actually studying intumescence development. Pinkard et al. (2006) found that in eucalyptus (*Eucalyptus nitens* Libill. and *Eucalyptus globulus* Deane and Maiden) intumescence growth was actually the formation of lenticel-like structures on plant leaves as a result of high relative humidity, which is a way to facilitate gas exchange.

Air pollution containing ethylene was a probable cause in the development of enations on potatoes (*Solanum tuberosum* L. 'Russet Burbank') grown in a growth chamber according to Kirkham and Keeney (1974). Petitte and Ormrod (1986) also found that air contaminants SO_2 and NO_2 had an effect on intumescence development on potato, as well as propagation method. In this case, potatoes ('Russet Burbank,' 'Norchip,' and 'Kennebec') propagated from a high carbohydrate source (tuber) generated more intumescences than potatoes propagated via stem cutting. However, Lang and Tibbitts (1983) found air pollution to be an inconclusive cause on tomato plants (*Solanum lycopersicum* var. *esculentum* L. 'Oxheart').

Also in the Lang and Tibbitts (1983) paper, relative humidity and radiance levels did not have an effect on intumescence development, but temperature and ultraviolet wavelengths did. Their finding that high temperatures (30°C) resulted in lessened occurrence of intumescences is contrary to Eliza and Dobrenz (1971) who suggested high temperatures resulted in worse symptoms. Lang et al. (1983) performed an anatomical study and found results for tomato to be similar to those of Eliza and Dobrenz (1971) for eggplant. That is, the palisade cells were hypertrophic, swelling to almost double in size, and they ruptured through the leaf epidermis. In sweet potato (*Ipomoea batatas* L. Lam), a different anatomy of the disorder was described by Wetzstein and Frett (1984), where both hypertrophy (cell swelling) and hyperplasia (cell proliferation) were evident within mesophyll cells. Hyperplasia has not been reported in any other paper discussing anatomy of intumescences or edema.

Certain wavelengths of light have been shown to cause or prevent intumescences. The removal of blue-green light with a yellow filter was found to reduce the formation of intumescences of potato ('AC Brador' and 'Shepody') in a study performed by Seabrook and Douglass (1998). This is contrary to a finding by Morrow and Tibbitts (1988), where the addition of red light promoted intumescence development on potato, while blue and green lights had no effect. As noted above, Lang and Tibbitts (1983) found that UV light affected intumescence formation. When they exposed tomato plants to cool while fluorescent lights in a growth chamber, intumescence development was prevented. A further link to light was found on work by Frantz (2009) and Frantz et al. (2007) when working with *Capsicum annuum* L. 'Triton.' Extreme amounts of intumescences developed on the plants when grown under high pressure sodium lights in a growth chamber, but did not develop in a greenhouse.

Several studies, cited in this literature review, mentioned that intumescence development occurred only in controlled environments, such as greenhouses and growth chambers (Jaworski et al., 1987; Kirkham and Keeney, 1974; Lang and Tibbitts, 1983; Lang et al., 1983; Petitte and Ormrod, 1986). With the majority of the papers, the exception being LaRue (1933), intumescence symptoms only seem to occur in controlled environments, which suggest that this disorder is the result of a difference between field and controlled environment. Further research should be undertaken to determine what differences between the environments contribute to intumescence development so it can be prevented in the future.

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CHAPTER 2 - Root Medium Water Content and Supplemental Calcium do not Influence the Occurrence of Edema in Ivy Geranium

Introduction

Edema is a physiological disorder characterized by blister-like formations on surfaces of plant tissue that may become necrotic and form corky lesions. When severe, this damage reduces the economic value of the crop. Researchers have evaluated this disorder in zonal geranium, *Pelargonium hortorum* Ait., (Balge et al., 1969; Metwally et al., 1970; Metwally et al., 1970), but only one published study focuses on edema in ivy geranium, *Pelargonium peltatum* L'Hérr ex. Ait. (Rangarajan and Tibbitts, 1994), which is a highly susceptible species that contributes significantly to the economic value of geraniums because of their widespread use in hanging baskets (Holcomb and O'Donovan, 1993; NASS, 2008).

Current convention suggests that edema develops as a result of water uptake occurring faster than transpiration rate, resulting in accumulation of water and solutes in leaf epidermal tissue that gives rise to 'blisters' that eventually burst (Anonymous 1999a; Anonymous 1999b; Anonymous 2001; Balge et al., 1969; Dudek, 2004; Lemon, 1996; Lerner, 2006; Metwally et al., 1970; Micheal, 1996; Moorman, 1993; Ogelvee, 2003; Pataky, 1998; Pundt, 2005). It has also been suggested that stomata may become blocked, further reducing plant transpiration (Balge et al., 1969; Metwally et al., 1970). As a result, growers are encouraged to produce ivy geranium under conditions of low humidity, maintain low root medium moisture content so that excess water is not available for uptake, and irrigate in morning hours to provide sufficient time for plants to transpire excess water (Balge et al., 1969; Dole and Wilkins, 2004; Metwally et al., 1970; Metwally et al., 1970; Micheal, 1996; Moorman, 1993; Pataky, 1998). Applying supplemental calcium (Ca) and providing an appropriate physiochemical environment in the root medium for Ca uptake has also been suggested to abate the occurrence of edema (Micheal, 1996) through a mechanism of strengthened cell walls; however, no research has documented the effectiveness of this strategy. Therefore, the objectives of our research were to: 1) describe edemata by using scanning electron microscopy (SEM); 2) determine whether root medium water status plays a role in edema occurrence and severity; 3) evaluate the effectiveness of providing supplemental calcium in abating the occurrence of edema; and 4) determine how different plant transpiration rates affect the occurrence of edema.

Materials and Methods

Four experiments were conducted with ivy geranium (*Pelargonium peltatum* (L.) L'Hérr ex Ait.), each with varying cultivars, root medium water status and supplemental Ca treatments

to evaluate effect on the incidence and severity of edema. Day 1 for all experiments was the transplant date. The first experiment occurred from 21 Apr. (day 1) to 30 May 2008 (day 70, Spring 2008), the second from 4 Jun. (day 1) to 16 Aug. 2008 (day 74, Summer 2008), the third from 3 Oct.. (day 1) to 18 Dec. 2008 (day 77, Fall 2008), and the fourth from 18 Feb. (day 1) to 18 May 2009 (day 1, Spring 2009).

The Spring 2008 experiment was a completely random design (CRD) with three water status treatments (saturated, wet, and dry) x three cultivars (`Amethyst 96', `Lambada` and `Sybil Holmes') and four replications. The Summer 2008 experiment was a randomized complete block design (RCBD) with four blocks and the same treatments represented in the Spring 2008 experiment with the addition of two supplemental calcium (Ca) treatments of 0 and 200 $mg \cdot L^{-1}$ Ca. The Fall 2008 experiment added another water status treatment—optimal—and had a split plot design that consisted of two vapor pressure deficit (VPD) environments x four water status treatments x two Ca levels (0 or 300 $mg \cdot L^{-1}$) x four blocks on 'Amethyst 96' only. Finally, the Spring 2009 experiment was a RCBD with four blocks with three water status treatments (optimal, wet and saturated) x three Ca levels (0, 500, or 1000 $mg \cdot L^{-1}$) x two cultivars ('Amethyst 96' and 'Sybil Holmes').

Plants and growing environment

The three ivy geranium cultivars that were used, 'Amethyst 96', 'Lambada' and 'Sybil Holmes', all originated from Syngenta Flowers (formerly Fischer USA, Boulder, CO). Cuttings from stock plants were taken on 17 Jan. 2008 (Spring 2008), 5 May 2008 (Summer 2008) and 'Amethyst 96' only on 29 Aug. 2008 (Fall 2008). Cuttings were stuck into Oasis rooting cubes (Smithers-Oasis Company, Cuyahoga Falls, OH) and rooted under mist for four weeks. For the Spring 2009 experiment, rooted cuttings in Ellepots (Blackmore Company, Inc., Belleville, MI) were obtained from C. Raker & Sons (Litchfield, MI). Cuttings in all experiments were fertigated with 200 $mg \cdot L^{-1}$ N from 20N-4.4P-16.6K fertilizer (Peter's 20-10-20 Peat-Lite Special, Scotts Co., Marysville, OH) after transplant until experimental treatments were imposed.

Once rooted, cuttings were potted into round, green 16.5cm diameter x 11.5cm deep pots with a volume of 730 ml. Pots were filled with Fafard 2 root medium (Conrad Fafard, Inc., Agawam, MA), a peat-based mix with perlite, on 15 Feb. 2008 (Spring 2008), 4 Jun. 2008 (Summer 2008), 3 Oct. 2008 (Fall 2008) and 18 Feb. 2009 (Spring 2009). The plants were then

grown in a 7.6m x 7.6m glass greenhouse in the Throckmorton Plant Sciences range at Kansas State University, Manhattan, KS, for the duration of the experiments.

Controlled release fertilizer, 19N-2.6P-9.9K (Osmocote 19-6-12, Scotts Co., Marysville, OH) and a surfactant (Suffusion Granular, OHP Inc., Mainland, PA) were applied to each pot as a top-dress at rates of 7g and 3.7g per pot, respectively, on 31 Mar. 2008 (day 11, Spring 2008) and 27 Jun. 2008 (day 24, Summer 2008). Nine grams of fertilizer was applied per pot in the Fall 2008 and Spring 2009 experiments on 23 Oct. 2008 (day 21) and 23 Feb. 2009 (day 6), respectively, along with 3.7 grams of surfactant. The fertilizer rate was increased due to the low electrical conductivity (EC) readings of the previous two experiments. In addition to the nine grams of controlled release fertilizer in Spring 2009, supplemental N was added as KNO_3 at a rate of 200 $mg \cdot L^{-1}$ N as described below.

Natural day lengths were used in all experiments. However, shade cloth was installed and whitewash was applied to the outside surface of the greenhouse in an effort to control high temperatures during the Summer 2008 experiment, and supplemental lighting via high intensity discharge (HID) lights (Sun System III Model HPS 400 by Sunlight Supply Inc., Vancouver, WA) were used from 0730 HR to 1730 HR during the Fall 2008 experiment.

Temperature and humidity were monitored using HOBO Environmental Monitors (Onset Computer Corp., Bourne, MA). Average day : night temperatures, relative humidity (RH), and vapor pressure deficits (VPD) are stated in Table 1. Vapor pressure deficits were calculated by using Murray's Equation (Murray, 1967);

$$e_a = \frac{RH}{100}\left(0.61078 * e^{\frac{17.269 * TempAir}{(237.3 + TempAir}}\right).$$

Thrips (*Frankliniella occidentalis* (Pergande) Thysanoptera: Thripidae) is a phytophagous insect pest that causes feeding damage on ivy geranium very similar in appearance to the physiological disorder edema. Therefore, this pest was tightly controlled with inundative releases of predatory mites used as biological control agents. *Amblyseius cucumeris* ((Oudemans) Acarina: Phytoseiidae; Thripex, Koppert B.V., The Netherlands) was released on all pots on 28 Mar. 2008 (day 8, Spring 2008) and 9 Jul. 2008 (day 36, Summer 2008). Similarly, *Amblyseius swirskii* ((Athias-Henriot) Acarina: Phytoseiidae; Swirski-Mite, Koppert B.V., The Netherlands)

was released on 24 Oct. 2008 (day 22, Fall 2008) and 9 Apr. 2009 (day51, Spring 2009) to control thrips.

Leaves showing any symptoms of edema were marked prior to the start of the Summer 2008 experiment and removed at the start of the Fall 2008 and Spring 2009 experiments to ensure that all the edema at the termination of the experiment was a result of the experimental conditions.

Establishing root medium water content treatments

The watering regimes were established based on water loss by from pot weight, ±10%. Pots were weighed at the beginning of the experiment at container capacity; this starting weight was then used to determine the percent dry down between irrigation events that determined treatments. The dry, optimal, and wet treatments were irrigated after 65%, 30%, and 15% water loss, respectively, from container capacity. The saturated treatment was maintained by keeping the root medium uniformly and constantly moist by setting pots in a clear saucer (25.4 cm diameter) that was always filled with water.

Water loss was measured by weighing representative pots in each treatment on a regular basis, in conjunction with ECH$_2$O EC-5 probes (Decagon Devices Inc., Pullman WA). During the first two dry down cycles in each treatment, pots were weighed and probe readings were taken to correlate the % dry down by weight to the probe readouts; the probes were then used to provide a rough estimate of the % dry down. Once the probes began to give readings similar to those correlated with the pot weights, pots were weighed to determine the exact dry down percentages in grams, fine tuning the system to give the most exact watering dates without the need for daily weighing. This resulted in the wet treatments being watered about every to every-other day, the optimal treatments were watered about weekly, and dry treatments were watered every about every 11 to 14 days.

Except during Spring 2009, the fertilization regimen consisted solely of controlled release fertilizer applied at transplant, as described above. Plants were irrigated with municipal water (alkalinity as $CaCO_3$ ~ 30 mg·L^{-1} and Ca < 40 mg·L^{-1}) and a 15% (±5%) leaching fraction was maintained for all experiments except during Spring 2009 when a 0% leaching fraction was initially used. This lack of leaching, combined with supplemental fertilization with KNO_3 at a rate of 200 mg· L^{-1} N at each irrigation as described above, lead to excessive salt levels in the

root medium. Therefore, on day 52 of the Spring 2009 experiment, the optimal and wet treatments were thoroughly leached by successively applying 600 ml of tap water four times. After 20 days (day 72), a 20% leaching fraction (LF) was established and fertilizer was again applied as 20N-8.7P-16.6K at a rate of 200 mg$\cdot$ L^{-1} N at every other irrigation event.

Establishing Ca treatments

Calcium was a treatment factor in the Summer 2008, Fall 2008 and Spring 2009 experiments. Calcium was applied at each watering interval from $CaCl_2$ at a rate of 200 mg$\cdot$ L^{-1} Ca (Summer 2008) or 300 mg$\cdot$ L^{-1} Ca (Fall 2008) following the watering regimen described above; other plants were irrigated with municipal water. Since the saturated treatment did not have a dry-down interval like the other treatments, 150 ml of the Ca solution was applied to the treatment once weekly.

In the Spring 2009 experiment, Ca treatments included 0, 500 and 1000 mg $\cdot$ L^{-1} Ca and were applied as a part of the irrigation regimen. From 5 March 08 to 9 April 09 (days 16 to 51), each Ca treatment was applied with each irrigation. This was reduced to every other watering after a major leaching event was undertaken (10 April, day 52) to return EC levels to acceptable levels (described above) and was maintained until the termination of the experiment. Plants in the saturated water treatment received Ca applications on the same days as the optimal treatments.

Establishing vapor pressure deficit treatments

Two vapor pressure deficit (VPD) environments were created for the Fall 2008 experiment. Tent-like structures were built out of 1-inch PVC pipe (Charlotte Pipe and Foundry Co., Charlotte, NC) and plastic film (DuraGreen EM 3 Years OF D7/11; DuraGreen Marketing USA, Mount Dora, FL) with dimensions of 101.6 cm x 115.6 cm x 91.6 cm tall. While each treatment had the PVC cage around the plants, the high VPD environment had plastic film only on the top, in order to keep light quality as similar as possible between the two treatments; the low VPD experiment had plastic film on all but the bottom of the tent with access flaps on two opposite sides of the tent secured with Velcro. A mist system ran underneath the bench to provide supplemental humidity to the low VPD treatments and ran for 15 min hourly (0000,

0300, 0500, 0700, 0800, 0900, 1100, 1200, 1300, 1400, 1500, 1600, 1800, 2100 HR) on the 24 hr clock, while fans were set up to constantly blow air over the plants in the high VPD treatments.

Water stress was monitored in this experiment using Decagon's Leaf Porometer SC-1 (Decagon Devices, Inc; Pullman, WA). All plants were measured daily for 17 days (17 Nov. to 3 Dec. 2009, days 46 to 62) to determine if the root medium water status and VPD environments were affecting the stomatal resistance, and thus transpiration, of the plants.

Growth data at harvest

Plant height was taken by measuring from the root medium surface to the top of the foliage canopy (Fall 2008 and Spring 2009). Plant width was determined in all experiments by averaging two measurements taken at 90 degrees to each other. Fresh and dry weights were measured on whole, above-ground plant shoots at the termination of all experiments. Dry weights were obtained by drying the whole, above-ground plant shoots at 70^{o}C in a forced-air oven.

Tissue analysis

Whole shoots (Summer 2008) or youngest, fully-expanded leaves (Fall 2008 and Spring 2009) were collected for tissue analyses, washed in 0.1 N HCl and rinsed in distilled water twice, followed by briefly soaking in reverse osmosis water. Dried tissue was ground to pass a 20-mesh screen in preparation for tissue analysis for Ca and magnesium (Mg; Summer 2008, Fall 2008 and Spring 2009) using a nitric-perchloric acid digestion. ICP Spectrometry (Model 720-ES ICP Optical Emission Spectrometer, Varian Australia Pty Ltd, Mulgrave, Vic Australia) using Nitric-Perchloric digested plant material was used to analyze samples for Ca and Mg.

Root medium testing

Root media at the end of all experiments were extracted using the saturated media extract (SME) method (Warncke, 1986) and analyzed for pH and EC using an Extech Model 6595 pH/Conductivity/TDS/Temperature meter (Extech Instruments Corp.; Waltham, MA); Spring 2008 and Summer 2008) or an Accumet Excel XL20 pH/Conductivity meter (Thermo Fisher Scientific Inc.; Waltham, MA; Fall 2008 and Spring 2009). During Spring 2009, leachate was collected using the pour-through method during the experiment (Yeager, 1983) and at the termination of the experiment using the SME method and analyzed for soluble Ca content using

an inductively coupled plasma spectrometer (Model 720-ES ICP Optical Emission Spectrometer, Varian Australia Pty Ltd, Mulgrave, Vic Australia).

Edema characterization

Whole plant ratings were taken at harvest in the Spring 2008, Summer 2008 and Fall 2008 experiments. Given the lack of correlation between edema ratings and actual edema damage measured by the scanning procedure (described below) during Summer 2008 or Fall 2009, ratings were dropped in the Spring 2009 experiment in favor of the scanning procedure.

The whole-plant edema rating system, based on the incidence and severity of edema, is as follows: 0 = no edema; 1 = a few edemata beginning to form on 3 or fewer leaves; 2 = a few edemata forming on 4 or more leaves or numerous edemata on 3 or fewer leaves; 3 = numerous edemata on 4 or more leaves or callusing of edemata on 3 or fewer leaves; 4 = edema on all fully expanded leaves with some callusing on multiple leaves; 5 = edema on all fully expanded leaves and callusing on almost all leaves with some lesions forming on adaxial surface. The edema primarily forms on the abaxial surfaces of leaves in these cultivars of ivy geranium, until the lesions are so severe they form on the adaxial surface as well; therefore, the ratings primarily focused on abaxial surfaces until the damage became severe enough to affect the adaxial surface.

To truly quantify the edema damage, all leaves from Summer 2008, Fall 2008 and Spring 2009 experiments were scanned with a flatbed scanner (Epson Perfection 4990 Photo; Seiko Epson Corp., Suwa, Nagano, Japan) and evaluated for total leaf area and leaf area affected by edema using Adobe Photoshop 7.0 (Adobe Systems Inc., San Jose, CA) and Scion v. 4.0.3.2 (Scion Corp., Frederick, MD) following the procedure outlined in Chen and Williams (2006) and Appendix B. The procedure used to select damaged tissue for Spring 2009 was the 'lasso method', which was different compared to the 'color-range method' used for Summer 2008 and Fall 2008 (Appendix B).

Scanning electron microscopy (SEM) and dissection microscopy (DM)

Scanning electron micrographs were taken of tissue samples from the abaxial surface of selected ivy geranium leaves in the Spring 2009 experiment. Small pieces of leaf, approximately 75 mm^2, were glued onto a SEM slide using a graphite emulsion. The slides were placed into the scanning electron microscope (S-3500N Hitachi Science Systems Ltd., Hitachinaka, Japan) and

were rapid cooled using liquid nitrogen to fix the samples. Micrographs were taken under high vacuum using a backscatter detector (Robinson Detector ETP-USA/Electron Detectors Inc., Rocklin, CA).

A dissection microscope (Nikon SMZ1500; Nikon Corp., Kawasaki, Japan) with digital camera attachment (Nikon Digital Sight DS-5M; Nikon Instruments Inc., Melville, NY) was used to photograph edemata on the abaxial surface of ivy geranium leaves at magnifications of 3X and 8X.

Statistical procedures

Analysis of variance was conducted on results from each experiment using PROC GLM and PROC MIXED in SAS ver. 9.1.3 (SAS Institute Inc., Cary, NC). For Fall 2008, the data set was analyzed two ways: 1) the low VPD environment treatment was removed to better determine the role root medium water content had on oedema development; 2) the dry root medium water content treatments were removed as the treatment within the low VPD environments was compromised by the mist system.

Results and Discussion

Scanning electron microscopy (SEM) and dissection microscopy (DM)

As seen in Figs. 2.1 and 2.2, a single edemata is comprised of multiple cells. It appears that edemata are formed when water and solutes build up underneath or possibly within cells of the abaxial epidermis, causing epidermal cells to stretch. When viewed under a dissection microscope, as in Fig. 2.3 and 2.4, the edemata appear to collapse, not rupture. This lack of rupture is confirmed by SEM (Fig.2.5). This finding is different from current conventional wisdom that suggests that epidermal cells rupture (Balge et al., 1969) and result in the necrotic, scab-like lesions. Instead, the necrosis may be due to intracellular connections separating, irreparably damaging pathways of water and nutrient movement to affected cells. Stomatal blockage has also been implicated in this disorder (Balge et al., 1969; Metwally et al., 1970). However, our SEMs did not suggest that stomata were affected or blocked in the pre-necrotic stages of edemata formation.

Effect of root medium water content

Increased root medium water contents tended to result in larger plant size compared to 'dry' treatments (Tables 2.2 to 2.5). Surprisingly, plants maintained as the saturated treatment showed no signs of root rot in any of our experiments despite constant saturation of the root zone with water.

The wet and saturated root medium treatments resulted in similar growth and the dry treatment was smaller in the Spring 2008 experiment (Table 2.2). In Summer 2008 (Table 2.3), the saturated and wet root medium water content treatments again performed similarly based on all growth parameters, which included width, fresh weight, dry weight and leaf area, while the dry treatment was smaller. Root medium EC at the end of the experiment was slightly lower for the saturated treatment (1.2dS/m) compared to the wet and dry treatments (1.3 to 1.4 dS/m, Table 2.9).

In the Fall 2008 experiment, no growth differences occurred between root medium water content treatments when results from only the high VPD environment were analyzed (Table 2.4a), though the EC was lower in the wet treatment (0.5 dS/m) compared to the saturated, optimal, and dry treatments (0.7 to 0.8 dS/m, Table 2.10a). This low overall EC may have contributed to the lack of growth differences between water status treatments.

When VPD environment was included in the analysis, the interaction between VPD environment x root medium water content occurred across all growth parameters (Table 2.4b). Results were similar based on width, fresh weight, and leaf area: growth was similar across VPD environments and root medium water content treatments except that the wet treatment in the high VPD environment was smaller. Dry weight differences were not as clear cut: smallest plants were again the wet treatment in the high VPD environment, but these were similar in dry weight to the high VPD-optimal and low VPD-saturated plants (Table 2.4b). These growth results are easily explained by the excessive mechanical damage from moving tissue for daily watering that resulted in branches breaking off of plants in the high VPD-wet treatment, leading to less growth. Despite efforts to accomplish two distinctly different VPD environments in the Fall 2008 experiment, environmental conditions were not dramatically different between environments (Table 2.1). Stomatal conductance of optimal, wet, and saturated treatments was similar between the two environments (Fig. 2.6) which explains why there is not marked difference in plant response between the environments.

In Spring 2009, the saturated treatment resulted in larger plants compared to both the wet and optimal treatments, which were similar in size based on every parameter except width (Table 2.5). A water x cultivar interaction occurred for plant width (Table 2.5) because 'Amethyst 96' and 'Sybil Holmes' had similar plant widths in the saturated and optimal treatments, but 'Sybil Holmes' resulted in much greater width than 'Amethyst 96' when grown under the wet treatment regimen. The EC at the end of the experiment was different between root medium water content treatments: saturated was higher (3.4 dS/m) compared to wet and optimal (2.4 to 3.2 dS/m; Table 2.11).

Growing ivy geranium at reduced root medium water content did not result in preventing or reducing edema (Tables 2.2, 2.6 to 2.8). In Spring 2008, subjective, whole-plant edema ratings taken at the end of the experiment were not different between root medium water content treatments (Table 2.2). In Summer 2008, the cultivar x water interaction was significant: the dry treatment of only one cultivar, 'Amethyst 96', had more edema damage than other cultivars and root medium water content treatments (Table 2.6). One hypothesis is that the extreme dry down included in the Summer 2008 experiment may have aggravated the disorder on this one cultivar, 'Amethyst 96' (Table 2.6), possibly due to rapid uptake of water after the completion of the dry down cycle that exceeded plants' ability to transpire excess water. But if this theory was valid, the same result should have occurred on the dry root medium treatment of the other two cultivars, and it did not. In Fall 2008 and Spring 2009, water had no effect on percent edema damage (Tables 2.7a and b, 2.8). In Spring 2009, leaf area affected by edema was influenced by root medium water content (Table 2.8); however, differences in plant size between the water treatments (Table 2.5) explains this result as the total affected area and total leaf area were used to calculate percent damage.

Contrary to conventional wisdom derived from previous research (Balge et al., 1969; Metwally et al., 1970; Metwally et al., 1970; Metwally et al., 1971), the saturated treatment resulted in similar edema damage compared to the optimal and wet root medium water treatments. Even different transpiration rates measured in the Fall 2008 experiment between the dry versus optimal, wet and saturated treatments (Fig. 2.6) did not result in a difference in edema damage (Table 2.7a). Other researchers have suggested that water uptake that exceeds plant transpiration may contribute to causing this disorder (Balge et al., 1969; Khan and Conde, 2008; Metwally et al., 1970); our results do not support this theory but are inconclusive because

plant growth during the Fall 2008 experiment was not maximized by fertilization (as discussed earlier relating to EC levels in Tables 2.10a and 2.10b) and warmer production environment (Table 2.1).

Root medium water content treatments tended to affect medium pH, measured at the end of the experiment, differently between experiments. The dry root medium water content treatment in the Summer 2008 experiment resulted in a pH of 5.4, which was higher than for other treatments that ranged from 4.8 to 5.0 (Table 2.9). Also in Summer 2008, 'Sybil Holmes' resulted in a higher pH of 5.3 compared to 'Amethyst 96' and 'Lambada', which ranged from 4.9 to 5.0 (Table 2.9). There were no differences in pH in the Fall 2008 experiment (Tables 2.10a and 2.10b). In the Spring 2009 experiment, pH was highest in the saturated water status treatment at 5.0 compared to 4.3 to 4.5 for the wet and optimal treatments, respectively (Table 2.11). In Spring 2009 as in Summer 2008, 'Sybil Holmes' resulted in a higher pH (4.6) compared to 'Amethyst 96' (4.5, Table 2.11). Ultimately, these pH values are moderately low and may have contributed to reducing availability of Ca and Mg for uptake by the plant, as discussed later.

Effect of supplemental calcium

Application of supplemental Ca did not increase growth in any experiment (Tables 2.3 to 2.5). Supplemental Ca at a rate of 200 $mg \cdot L^{-1}$ in the Summer 2008 experiment resulted in a smaller leaf area at 552 cm^2 compared to 682 cm^2 in the 0 $mg \cdot L^{-1}$ Ca treatment (Table 2.3), though this response was implicated in a three-way interaction. No other growth characteristics were affected by supplemental Ca in that experiment and no growth characteristics were affected by supplemental Ca in the Fall 2008 experiment (Tables 2.4a and 2.4b).

In the Spring 2009 experiment, the highest level of 1000 $mg \cdot L^{-1}$ Ca resulted in smaller plants (Table 2.5) with an average fresh weight of 197 g compared to 235 g and 238 g in the 0 and 500 $mg \cdot L^{-1}$ Ca treatments, respectively. Dry weights in this experiment were not different while leaf areas followed a similar pattern to fresh weights (Table 2.5). This growth response was due to high salt levels in the root medium (Fig. 2.7, Table 2.11). The fertilization regimen was increased compared to previous experiments to provide luxurious nutrition. This resulted in EC levels being slightly high for ivy geraniums receiving the 0 and 500 $mg \cdot L^{-1}$ Ca treatments, but very high in the 1000 $mg \cdot L^{-1}$ Ca treatment, which explains the lack of vigor in that treatment compared to those receiving lower rates of supplemental Ca. The balancing anion in the

supplemental Ca solution was chloride, so the salt build up over time in relation to the supplemental Ca rate was not surprising.

Levels of Ca varied across experiments: tissue Ca averaged in the low 5% range in Summer 2008 (whole shoots, Table 2.9), low 1 to 1.5% in Fall 2008 (youngest, fully-expanded leaves, Tables 2.10a and 2.10b), and 1.0 to 1.8% in Spring 2009 (youngest, fully-expanded leaves, Table 2.11). In all experiments, tissue analyses suggested that adequate Ca was absorbed by plants that received no supplemental doses (0 $mg \cdot L^{-1}$ Ca treatments). Adequate Ca was provided by pre-plant dolomitic lime amendments to the root media and the irrigation water, which provided ~30 to 35 ppm Ca at each irrigation (municipal source, Manhattan, KS). Despite the fact that pH tended to be moderately low for optimal Ca uptake (Tables 2.9 to 2.11), tissue Ca concentrations fell within the sufficiency range of 0.9 to 1.4% for ivy geranium (Dole and Wilkins, 1999) in all treatments.

Supplemental Ca did not affect edema occurrence as no experiment resulted in significant percent damage based on Ca treatment (Tables 2.6 to 2.8). Therefore, either supplemental Ca did not strengthen epidermal cell walls in ivy geranium in these experiments, or strengthening cell walls in general did not affect edema occurrence. In the Fall 2008 and Spring 2009 experiments, supplemental Ca applications resulted in decreases in Mg concentrations within plant leaves, a result of antagonism of Ca uptake by Mg (Tables 2.10a, 2.10b and 2.11). The antagonism was not significant in the Summer 2008 experiment when only 200 $mg \cdot L^{-1}$ supplemental Ca was provided, but 300 $mg \cdot L^{-1}$ supplemental Ca and greater in the subsequent experiments resulted in this distinct effect.

Interactions between water x supplemental Ca were observed in the Summer 2008, Fall 2008 and Spring 2009 experiments (Tables 2.9, 2.10a and 2.11) for Ca and Mg tissue concentrations. In Summer 2008, the wet treatment resulted in higher tissue Ca (~7.4%) compared to the saturated and dry treatments (3.3 to 4.6%); the dry treatment resulted in most Mg (Table 2.9). In Fall 2009, Mg tissue concentrations were similar between the 0 $mg \cdot L^{-1}$ and 300 $mg \cdot L^{-1}$ supplemental Ca treatments in the saturated and dry and different in the wet and optimal root medium water content treatments In the Spring 2009 experiment, tissue Ca was similar between the three supplemental calcium treatments in the saturated treatment; 500 $mg \cdot L^{-1}$ and 1000 $mg \cdot L^{-1}$ supplemental Ca treatments were similar in the wet and optimal treatments but different from the 0 $mg \cdot L^{-1}$ Ca in those two water content treatments. Tissue Mg were similar

between the 0 mg$\cdot$ L^{-1} and 500 mg$\cdot$ L^{-1} Ca and different from the 1000 mg$\cdot$ L^{-1} supplemental Ca treatment in the saturated root medium water content, and all were different in the wet and optimal root medium water content treatments.

All of the abovementioned interactions may be explained by the frequency of the supplemental Ca application between root medium water content treatments. In Fall 2008, the Ca drenches were applied at similar intervals between the dry (about every 1.5 weeks) and saturated treatments (weekly), whereas the wet treatment received the calcium drench almost daily and the optimal treatment was watered at irregular intervals between 5 and 9 days. The interaction in the Spring 2009 experiment is similar. The interval in which the wet root medium water content treatment received supplemental Ca (every 1 to 3 days) was right in the middle of the intervals in which the saturated (weekly) and optimal (every 3 to 5 days) treatments received drenches. This explains why the more frequently watered treatments had higher Ca levels in their tissue.

A three-way interaction between VPD environment, root medium water content treatment and supplemental Ca occurred in Fall 2008 for height (Table 2.4b). Generally, height followed a similar pattern to the two-way interaction with VPD x water. However, supplemental Ca often resulted in the same height or smaller than plants not receiving supplemental Ca, with the exception of the saturated water treatment in the high VPD environment where plants were larger with the addition of supplemental Ca. This is a curious anomaly because across these experiments supplemental Ca either had no effect on growth or tended to result in smaller plants if applied at high levels.

Effect of environmental conditions

As discussed earlier, the experiment (Fall 2008) to test the effect of two vapor pressure deficits on edema occurrence did not produce environments that were sufficiently different to adequately evaluate the question (Table 2.1). However, we did observe different transpiration rates in the high VPD environment between the dry treatment and the wetter treatments (Fig. 2.6A), but stomatal conductance of optimal, wet, and saturated treatments was similar between the two environments (Fig. 2.6 B and C).Temperature had a role in our growth results between experiments. High temperatures lead to leaf bleaching (Dhir, 2008) in the Summer 2008 experiment, which affected the edema scanning procedure (Appendix B) and required modification by individually masking all of the bleached areas on affected leaves to correct

inaccuracies in leaf area. Low temperatures in the Fall 2008 experiment may have contributed to the lack of growth differences between treatments, since temperatures were kept cool to prevent heat build up, and thus more leaf bleaching, in our VPD cages.

Summary

While environment (Table 2.1) and plant nutrient status were different across the experiments and should be factored into the interpretation of these results, major findings were similar across experiments. Supplemental Ca did not have any effect on edema in any of the experiments; therefore, we found no evidence to encourage growers to provide supplemental Ca drenches as a means to abate edema. Similarly, a range of root medium water contents did not have a marked effect on differences in edema occurrence or severity, which is also contrary to previous convention. Different transpiration rates resulting from dry versus optimal to saturated root medium water contents also had no affect on edema occurrence. Finally, the idea that edema results in epidermal cells bursting needs to be re-evaluated based on evidence from scanning electron and dissection scope micrographs. Further research could focus on manipulating humidity and VPD in well-controlled experimental environments to determine environment's affect on the occurrence and severity of edema. In addition, the effect on edema when rapid, soft growth is being pushed may provide answers to plant status that result in greatest susceptibility. Finally, selecting ivy geranium cultivars more resistant to this disorder offers perhaps the best option for growers to manage it.

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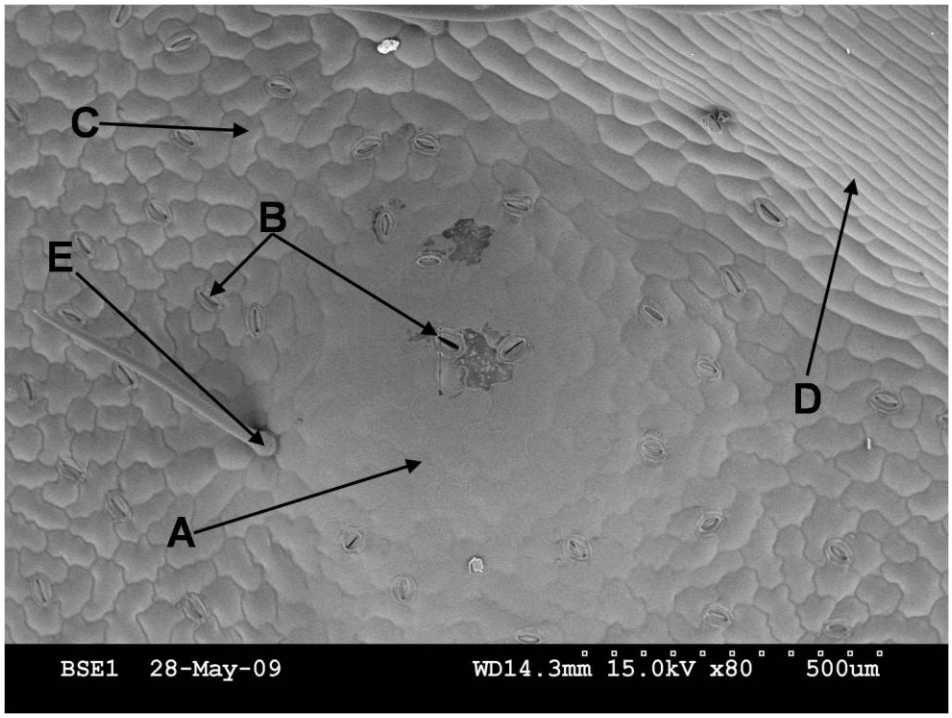


Figure 2.1 Scanning electron micrograph of a single edemata on the abaxial surface of a leaf of the ivy geranium cultivar ‘Amethyst 96.’ A: Cells being affected by the edemata; B: Stomata; C: Normal, unaffected cell; D: Primary vein; E: Trichome

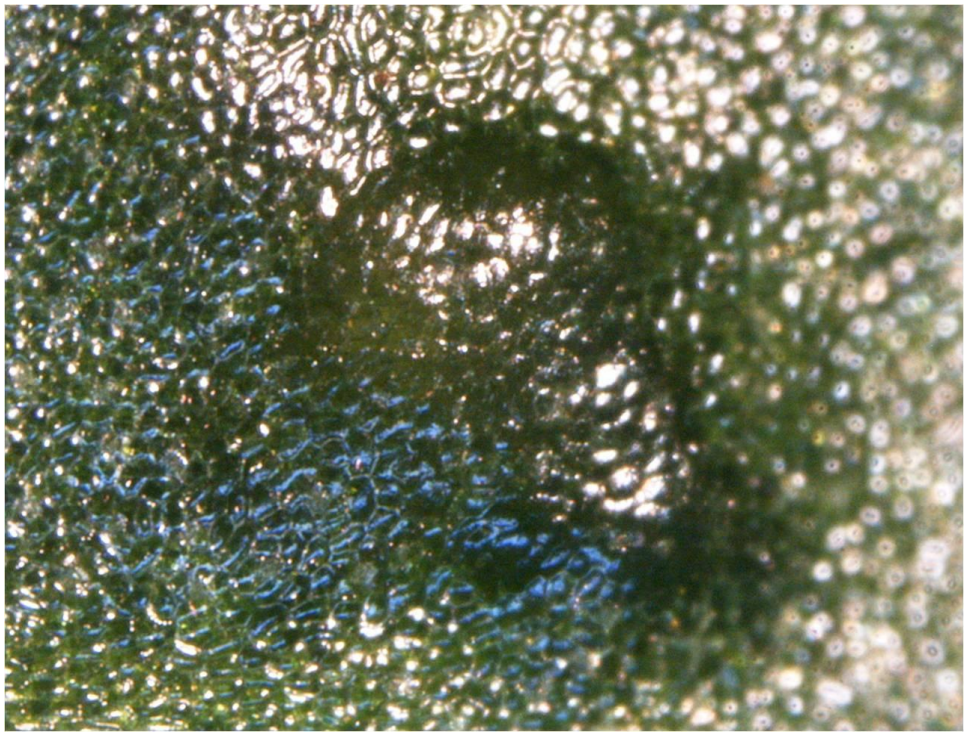


Figure 2.2 Dissection micrograph of a single edemata on the abaxial surface of the ivy geranium cultivar ‘Amethyst 96.’

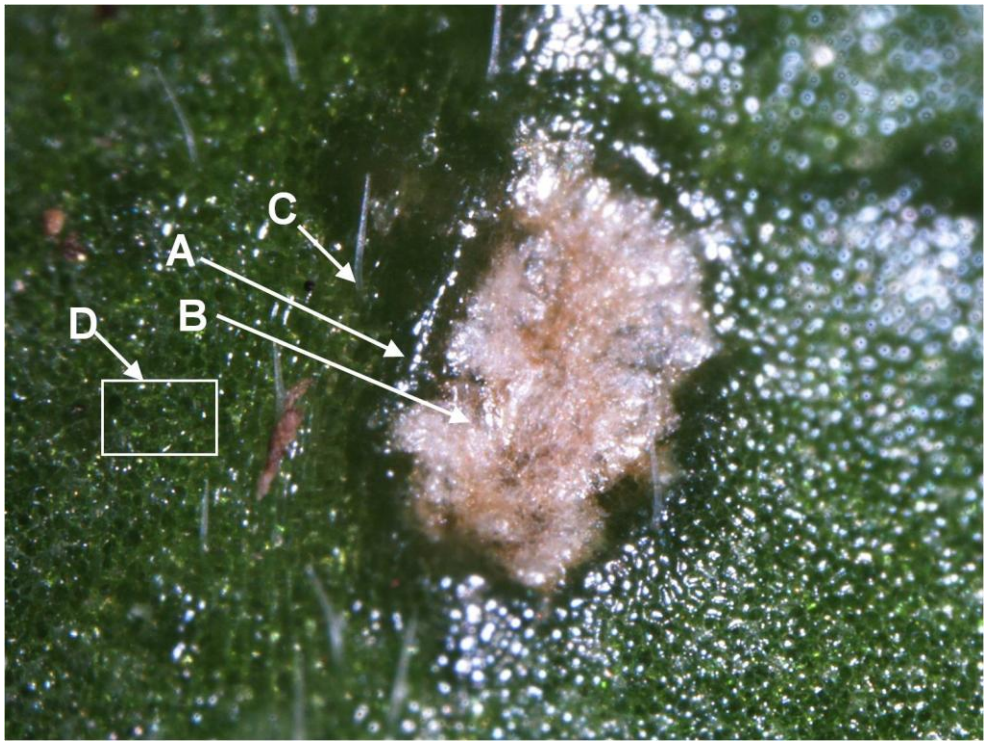


Figure 2.3 Dissection micrograph of a collapsed and necrotic edemata on the abaxial surface of a leaf of the ivy geranium cultivar ‘Amethyst 96.’ A: Edemata cells still exhibiting the swelling characteristic; B: Mature edemata cells that have collapsed and turned necrotic; C: Trichome; D: Unaffected cells

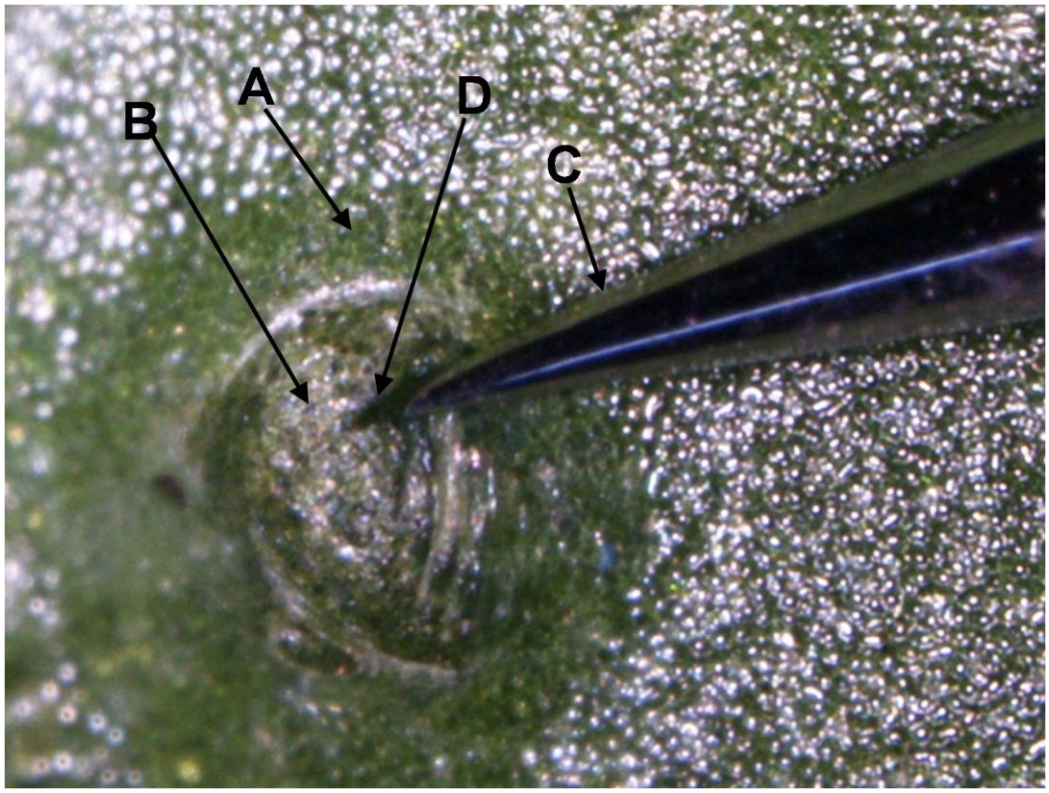


Figure 2.4 Dissection micrograph of a collapsed and edemata on the abaxial surface of a leaf of the ivy geranium cultivar ‘Amethyst 96.’ A: Edemata cells still exhibiting the swelling characteristic; B: Mature edemata cells that have collapsed and turned necrotic; C: Dissection needle; D: Shadow of dissection needle showing collapse of edemata.

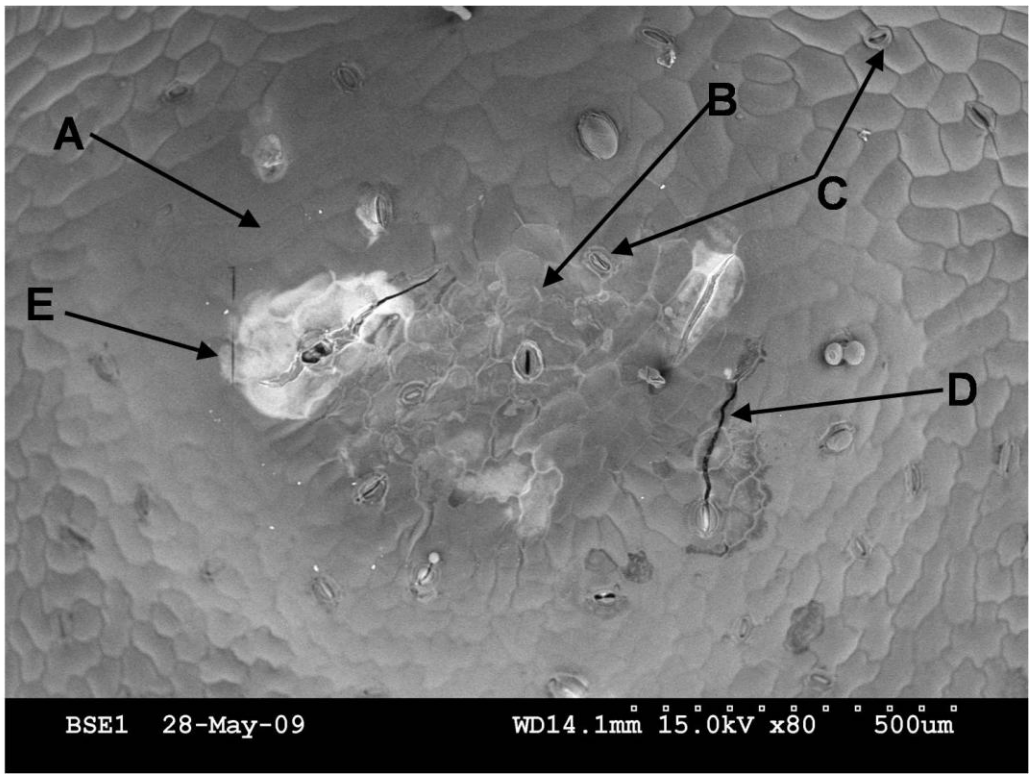
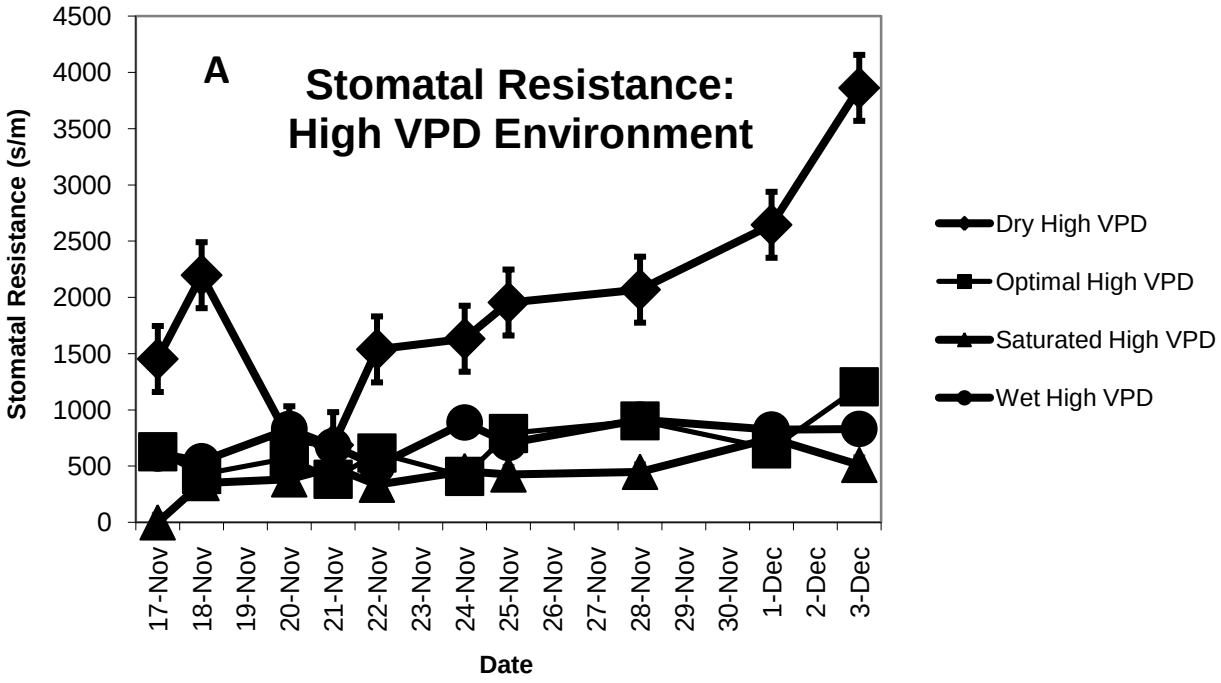


Figure 2.5 Scanning electron micrograph of a maturing edemata on the abaxial surface of a leaf of the ivy geranium cultivar 'Amethyst 96.' A: Edemata cells still exhibiting the swelling characteristic; B: Maturing edema-affected cells collapsing; C: Stomata; D: Cracking of cells affected by edemata likely caused by the rapid freezing of the tissues prior to scanning; E: Regions on edemata appear white on SEM which suggests accumulation and higher concentration of heavy metals.


A
Stomatal Resistance:
High VPD Environment
Stomatal Resistance (s/m)
4500
4000
3500
3000
2500
2000
1500
1000
500
0
17-Nov
18-Nov
19-Nov
20-Nov
21-Nov
22-Nov
23-Nov
24-Nov
25-Nov
26-Nov
27-Nov
28-Nov
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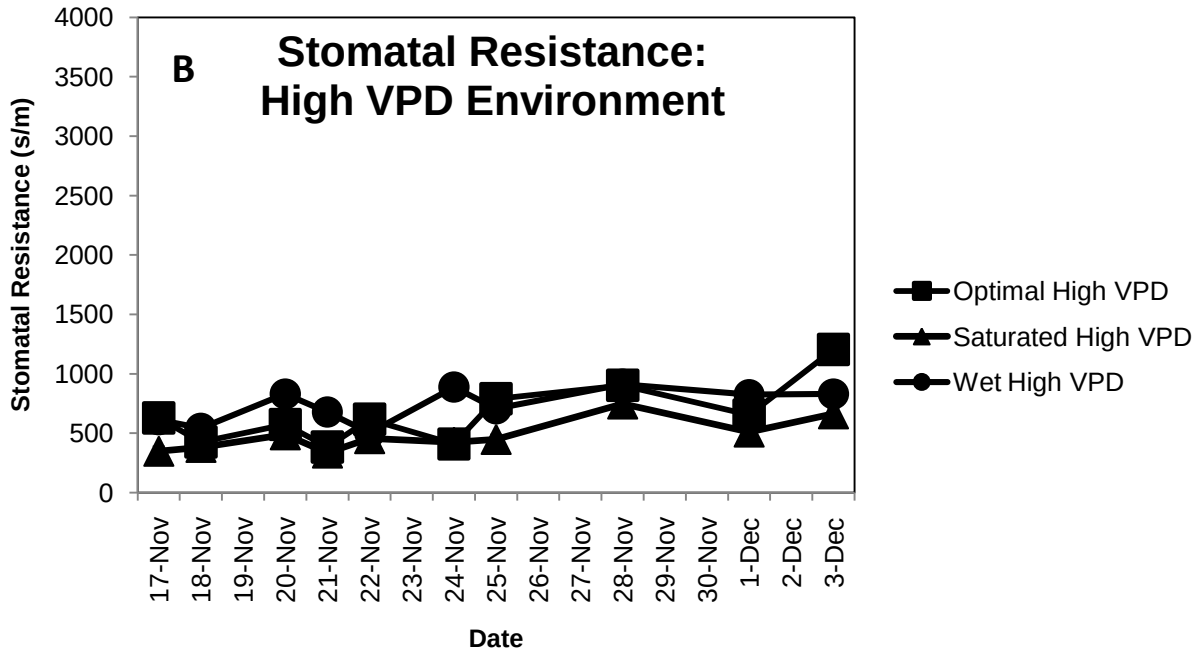
Date
Dry High VPD
Optimal High VPD
Saturated High VPD
Wet High VPD


B
Stomatal Resistance:
High VPD Environment
Stomatal Resistance (s/m)
4000
3500
3000
2500
2000
1500
1000
500
0
17-Nov
18-Nov
19-Nov
20-Nov
21-Nov
22-Nov
23-Nov
24-Nov
25-Nov
26-Nov
27-Nov
28-Nov
29-Nov
30-Nov
1-Dec
2-Dec
3-Dec
Date
Optimal High VPD
Saturated High VPD
Wet High VPD

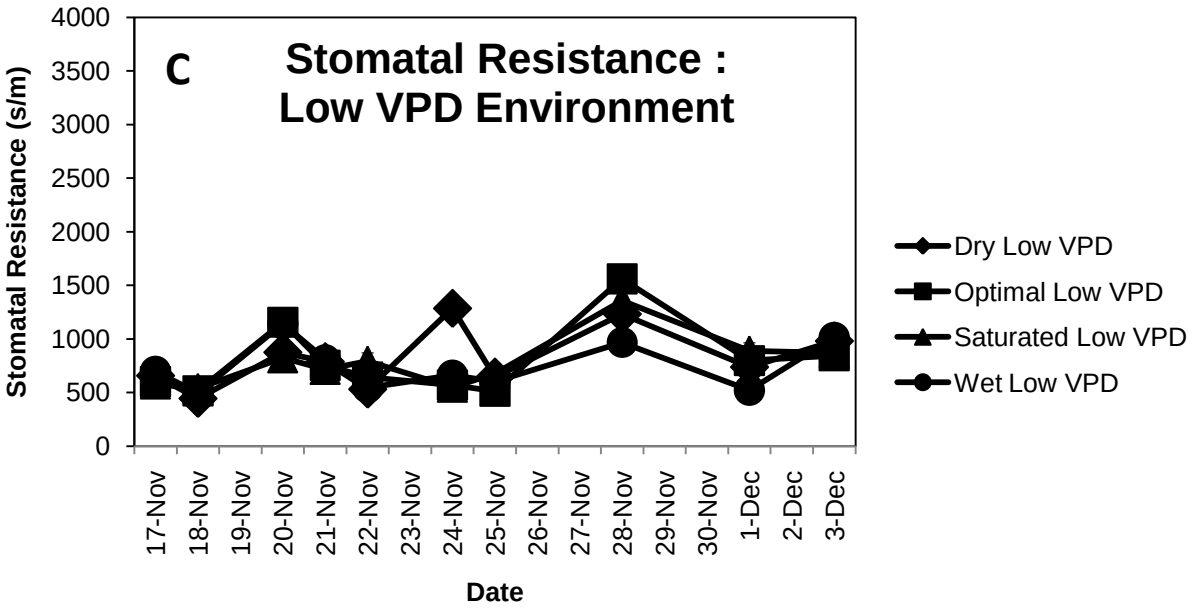


Figure 2.6 Stomatal resistances over the course of one full irrigation cycle of the dry root medium water content treatment. Higher stomatal resistances indicate lower transpiration rates via stomatal closure. Standard error bars are included at every data point, but may be masked by markers. A: Stomatal resistances of all root medium water content treatments in the high VPD environment; B: Stomatal resistance in the high VPD environment without the dry water treatment included; C: Stomatal resistances of all root medium water contents in the low VPD environment.

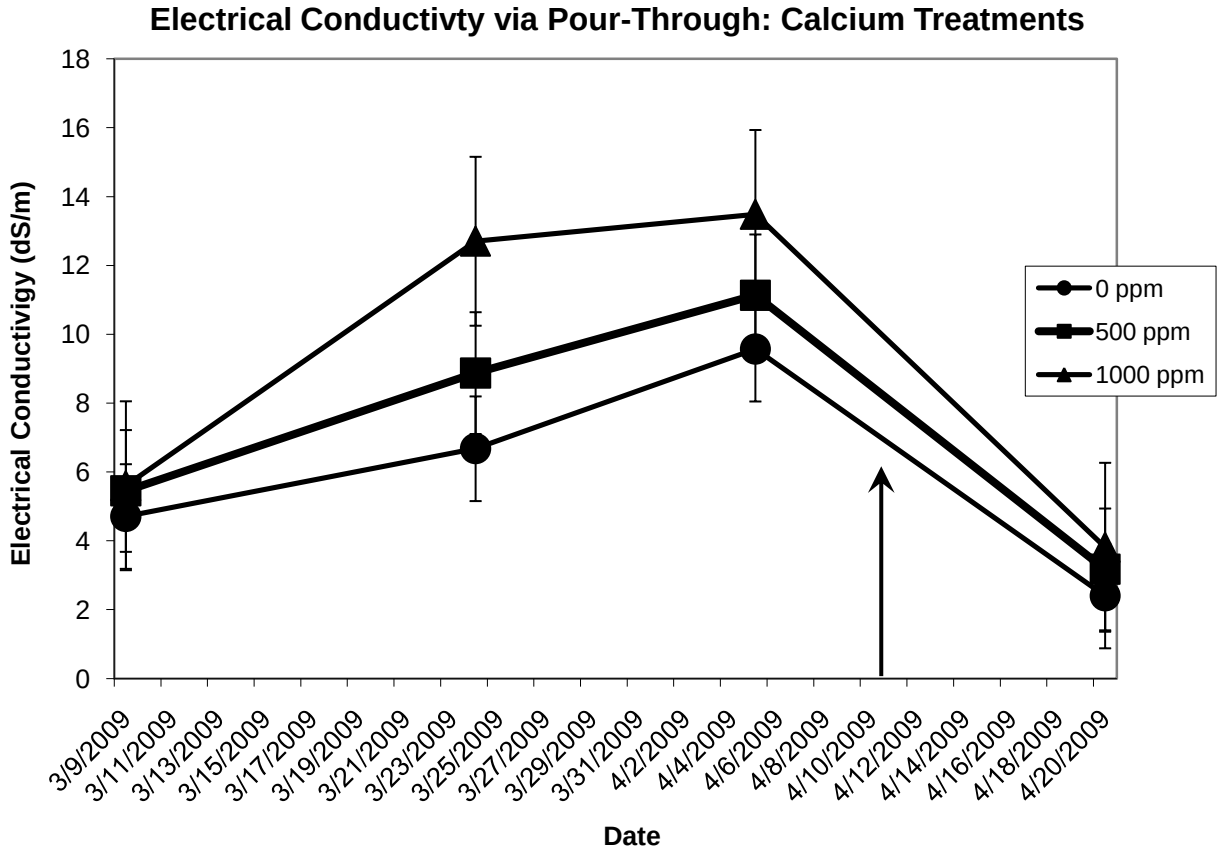


Figure 2.7 Electrical conductivities (EC), analyzed by the pour-through method, on four dates during the Spring 2009 experiment. A full experiment, root media leach took place on 10 April 2009 (Day 52) indicated by the arrow, dropping EC levels back down to tolerable levels.

Table 2.1 Plant growth characteristics and subjective edema ratings from the Spring 2008 experiment taken at the end of the production period (Day 70) of three ivy geranium cultivars grown under three root medium water content regimens: saturated, wet or dry.

		Fresh Weight (g)	Dry Weight (g)	Edema Rating[z]
Cultivar		NS[y]	NS	NS
Water		NS	*	NS
Water*Cultivar		NS	NS	NS
Water * Cultivar				
Cultivar - 'Amethyst 96'				
	Saturated	153.8	15.4	2.0
	Wet	132.3	13.2	3.3
	Dry	112.8	10.7	2.4
Cultivar - 'Lambada'				
	Saturated	150.8	14.3	2.9
	Wet	127.5	12.1	3.0
	Dry	115.8	10.0	2.1
Cultivar - 'Sybil Holmes'				
	Saturated	117.8	11.3	2.1
	Wet	156.3	15.3	2.1
	Dry	98.5	8.5	1.5
Cultivar	'Amethyst 96'	133	13.1	2.5
	'Lambada'	131	12.1	2.7
	'Sybil Holmes'	124	11.7	1.9
Water	Saturated	109	13.7a[x]	2.3
	Wet	139	13.5a	2.8
	Dry	141	9.7b	2.0

[z] The whole-plant edema rating system, based on the incidence and severity of edema, is as follows: 0 = no edema; 1 = a few edemata beginning to form on 3 or fewer leaves; 2 = a few edemata forming on 4 or more leaves or numerous edemata on 3 or fewer leaves; 3 = numerous edemata on 4 or more leaves or callusing of edemata on 3 or fewer leaves; 4 = edema on all fully expanded leaves with some callusing on multiple leaves; 5 = edema on all fully expanded leaves and callusing on almost all leaves with some lesions forming on adaxial surface.

[y] NS, *, **, ***, **** Nonsignificant or significant at P=0.05, 0.01, 0.001, and 0.0001, respectively.

[x] If no $LSD_{0.05}$ is listed, different letters indicate that means are significantly different based on pair-wise comparisons at α=0.05.

Table 2.2 Plant growth characteristics from the Summer 2008 experiment taken at the end of the production period (Day 74) of three ivy geranium cultivars grown under three root medium water content regimens: saturated, wet or dry, with either 0 or 200 $mg \cdot L^{-1}$ supplemental calcium.

		Width (cm)	Fresh Weight (g)	Dry Weight (g)	Leaf Area (cm^2)
Block		NS[z]	NS	NS	NS
Cultivar		**	****	****	****
Water		**	***	****	****
Calcium		NS	NS	NS	*
Cultivar*Water		NS	NS	NS	NS
Cultivar*Calcium		NS	NS	NS	NS
Water*Calcium		NS	NS	NS	NS
Cultivar*Water*Calcium		NS	NS	NS	*
Cultivar*Water*Calcium					
Cultivar - 'Amethyst 96'					
Saturated	Ca	20.4	34.3	3.4	407
	No Ca	21.6	42.0	2.9	525
Wet	Ca	19.4	26.8	2.7	384
	No Ca	23.5	41.8	3.6	717
Dry	Ca	16.8	26.0	2.5	352
	No Ca	18.1	24.3	2.4	342
Cultivar - 'Lambada'					
Saturated	Ca	18.6	36.5	3.3	512
	No Ca	16.7	36.3	2.9	478
Wet	Ca	18.9	34.0	2.9	532
	No Ca	24.6	41.3	5.4	914
Dry	Ca	16.5	26.8	2.3	382
	No Ca	16.7	28.3	2.4	414
Cultivar - 'Sybil Holmes'					
Saturated	Ca	20.5	55.3	4.5	733
	No Ca	28.8	81.0	6.4	1165
Wet	Ca	26.5	84.5	6.9	1144
	No Ca	25.9	70.8	6.2	994
Dry	Ca	20.1	41.8	3.5	521
	No Ca	22.4	43.3	3.3	585
Cultivar*Water					
Amethyst-96	Saturated	21.0	38.1	3.1	466
	Wet	21.5	34.3	3.1	551
	Dry	17.5	25.1	2.4	347

	‘Lambada’	Saturated	17.6	36.4	3.1	495
		Wet	21.8	37.6	4.1	723
		Dry	16.6	27.5	2.3	398
	‘Sybil Holmes’	Saturated	24.7	68.1	5.4	949
		Wet	26.2	77.6	6.5	1069
		Dry	21.3	42.5	3.4	553
Cultivar*Calcium						
	‘Amethyst-96’	Ca	18.9	29.0	2.8	381
		No Ca	21.1	36.0	2.9	528
	‘Lambada’	Ca	18.0	32.4	2.8	476
		No Ca	19.3	35.3	3.6	602
	‘Sybil Holmes’	Ca	22.7	60.5	4.9	799
		No Ca	25.7	65.0	5.3	915
Water*Calcium						
	Saturated	Ca	19.8	42.0	3.8	551
		No Ca	22.4	53.1	4.0	723
	Wet	Ca	21.6	48.4	4.1	687
		No Ca	24.7	53.1	5.1	875
	Dry	Ca	17.8	31.5	2.8	418
		No Ca	19.1	31.9	2.7	447
Cultivar						
	‘Amethyst-96’		19.9b[y]	32.5b	2.9b	454b
	‘Lambada’		18.7b	33.8b	3.2b	539b
	‘Sybil Holmes’		24.1a	62.8a	5.1a	857a
Water		Saturated	21.1a	47.5a	3.9a	637a
		Wet	23.1a	49.8a	4.6a	781a
		Dry	18.4b	31.7b	2.7b	433b
Calcium		Ca	19.7	40.6	3.6	552b
		No Ca	22.0	45.4	3.9	682a

[z] NS, *, **, ***, **** Nonsignificant or significant at P=0.05, 0.01, 0.001, and 0.0001, respectively.
[y] If no $LSD_{0.05}$ is listed, different letters indicate that means are significantly different based on pair-wise comparisons at α=0.05.

Table 2.3 a and b. Plant growth characteristics from the high VPD treatment of the Fall 2008[z] experiment taken at the end of the production period (Day 77) of the ivy geranium cultivar 'Amethyst 96' grown under four root medium water content regimens: saturated, wet , optimal, or dry with either 0 or 300 $mg \cdot L^{-1}$ supplemental calcium.

			Height (cm)	Width (cm)	Fresh Weight (g)	Dry Weight (g)	Leaf Area (cm^2)
Block			NS	NS	NS	NS	NS
Water			NS	NS	NS	NS	NS
Calcium			NS	NS	NS	NS	NS
Water*Calcium			NS	NS	NS	NS	NS
Water*Calcium							
	Saturated	Ca	18.9	26.5	97.0	8.67	1331
		No Ca	17.1	24.6	82.8	6.67	1114
	Wet	Ca	17.8	23.8	76.0	6.53	878
		No Ca	18.4	23.2	82.7	7.00	1073
	Optimal	Ca	18.8	25.7	90.8	8.26	1265
		No Ca	21.6	23.3	82.4	7.43	1003
	Dry	Ca	18.9	20.7	63.4	5.26	779
		No Ca	16.3	21.7	67.0	5.88	888
Water		Saturated	18.0	25.5	89.9	7.76	1222
		Wet	18.1	23.5	79.4	6.77	976
		Optimal	20.2	24.5	86.6	7.84	1134
		Dry	17.6	21.2	65.2	5.57	834
Calcium		Ca	18.9	24.1	81.8	7.18	1063
		No Ca	18.3	23.2	78.7	6.74	1020

[z] Statistical analysis does not include the low VPD environment treatment
[y] NS, *, **, ***, **** Non-significant or significant at P=0.05, 0.01, 0.001, and 0.0001, respectively.

Table 2.3b Plant growth characteristics from the Fall 2008[z] experiment taken at the end of the production period (Day 77) of the ivy geranium cultivar 'Amethyst 96' grown under four root medium water content regimens: saturated, wet , optimal, or dry with either 0 or 300 $mg \cdot L^{-1}$ supplemental calcium.

		Height (cm)	Width (cm)	Fresh Weight (g)	Dry Weight (g)	Leaf Area (cm^2)
Block		**	NS	NS	NS	NS
VPD		****	NS	NS	NS	NS
Water		NS	NS	NS	NS	NS
Calcium		NS	NS	NS	NS	NS
VPD*Water		****	*	***	*	**
VPD*Calcium		NS	NS	NS	NS	NS
Water*Calcium		NS	NS	NS	NS	NS
VPD*Water*Calcium		*	NS	NS	NS	NS
Water * Ca *VPD						
VPD - High						
Saturated	Ca	20.9b	27.3	118.3	10.5	1522
	No Ca	16.0c	26.0	92.0	7.2	1210
Wet	Ca	10.4e	19.7	32.0	3.3	354
	No Ca	13.9d	19.6	56.3	5.4	647
Optimal	Ca	17.4bc	24.5	99.3	9.3	1137
	No Ca	20.9b	21.8	66.3	5.7	805
VPD - Low						
Saturated	Ca	17.0c	25.6	75.8	6.8	1140
	No Ca	18.1b	23.1	73.5	6.2	1019
Wet	Ca	25.1a	27.8	104.5	8.6	1191
	No Ca	23.0a	26.8	93.0	7.4	1254
Optimal	Ca	20.1ab	26.9	82.3	7.3	1393
	No Ca	22.4a	24.9	98.5	9.2	1268
	$LSD_{0.05}$[y]	**3.6**	**NS**	**NS**	**NS**	**NS**
VPD*Water						
VPD-High	Saturated	18.4bc	26.7a	105.1a	8.9a	1366a
	Wet	12.1d	19.6b	44.1b	4.4b	501b
	Optimal	19.1bc	23.2a	82.8a	7.5ab	971a

VPD-Low	Saturated	17.6c	24.4a	74.6a	6.5ab	1079a
	Wet	24.1a	27.3a	98.8a	8.0a	1223a
	Optimal	21.3ab	25.9a	90.4a	8.2a	1330a
	$LSD_{0.05}$	**3.6**	**6.4**	**33.3**	**3.3**	**523**
VPD*Calcium						
VPD-High	Ca	16.2	23.8	83.2	7.7	1004
	No Ca	16.9	22.5	82.4	7.4	887
VPD-Low	Ca	20.8	26.8	87.5	7.6	1241
	No Ca	21.2	24.9	88.3	7.6	1180
Water*Calcium						
Saturated	Ca	18.9	26.5	97.0	8.7	1331
	No Ca	17.1	24.6	82.8	6.7	1114
Wet	Ca	17.8	23.8	68.3	6.0	772
	No Ca	18.4	23.2	74.6	6.4	951
Optimal	Ca	18.8	25.7	90.8	8.3	1265
	No Ca	21.6	23.3	82.4	7.4	1036
VPD	High	16.6	23.1	77.3	6.9	946
	Low	21.0	25.9	87.9	7.6	1211
Water	Saturated	18.0	25.5	89.9	7.7	1222
	Wet	18.1	23.5	71.4	6.2	862
	Optimal	20.2	24.5	86.6	7.8	1151
Calcium	Ca	18.5	25.3	85.3	7.6	1223
	No Ca	19.0	23.7	79.9	6.8	1034

[z] Statistical analysis does not include the dry root medium water content treatment

[y] NS, *, **, ***, **** Non-significant or significant at P=0.05, 0.01, 0.001, and 0.0001, respectively.

[x] LSD calculated as: $LSD=t\sqrt{(2(EMS)/\#\ obs.\ per\ mean)}$ where t is determined at $\alpha=0.05$ and df of error term. LSD is used to compare within a row and within a column of the factorial treatment structure.

Table 2.4 Plant growth characteristics from the Spring 2009 experiment taken at the end of the production period (Day 90) of two ivy geranium cultivars grown under three root medium water content regimens: saturated, wet , or optimal with either 0, 500, or 1000 $mg \cdot L^{-1}$ supplemental calcium.

		Height (cm)	Width (cm)	Fresh Weight (g)	Dry Weight (g)	Leaf Area (cm^2)
Block		NS[z]	NS	NS	NS	NS
Cultivar		***	*	NS	NS	NS
Water		NS	****	****	****	****
Calcium		NS	***	**	NS	**
Cultivar*Water		NS	*	NS	NS	NS
Cultivar*Calcium		NS	NS	NS	NS	NS
Water*Calcium		NS	NS	NS	NS	NS
Cultivar*Water*Calcium		NS	NS	NS	NS	NS
Cultivar*Water*Calcium						
Cultivar - 'Amethyst 96'						
Saturated	0	25.0	48.0	301	24.0	2935
	500	26.0	55.4	320	27.6	3225
	1000	23.3	44.3	274	25.3	2737
Wet	0	24.5	46.4	222	19.8	2141
	500	24.3	43.0	218	18.4	2018
	1000	22.8	34.8	190	14.7	1749
Optimal	0	27.3	36.4	202	16.6	1872
	500	24.3	34.8	187	16.6	1810
	1000	24.3	30.8	163	14.3	1511
Cultivar - 'Sybil Holmes'						
Saturated	0	24.7	48.4	313	22.2	1889
	500	21.5	50.8	289	22.3	3079
	1000	23.0	41.3	276	23.7	1021
Wet	0	22.5	100.2	148	11.3	2481
	500	19.0	45.2	230	13.8	3075
	1000	20.0	37.5	135	16.7	1703
Optimal	0	24.0	39.0	228	19.4	2343
	500	19.8	36.4	184	13.4	1903
	1000	18.8	38.8	142	11.0	1451
Cultivar*Water						
Amethyst-96	Saturated	24.8	49.2b	298	25.6	2966
	Wet	23.8	41.2bc	210	17.6	1969

		Optimal	25.3	34.0c	184	15.8	1731
	‘Sybil Holmes’	Saturated	23.1	46.8b	293	22.7	1996
		Wet	20.5	61.0a	171	13.9	2420
		Optimal	20.8	38.0c	185	14.6	1899
		$LSD_{0.05}$[y]	**NS**	**8.0**	**NS**	**NS**	**NS**
Cultivar*Calcium							
	‘Amethyst-96’	0	25.6	43.6	241	20.1	2316
		500	24.8	44.4	242	20.9	2351
		1000	23.4	36.6	209	18.1	1999
	‘Sybil Holmes’	0	23.7	62.5	229	17.6	2237
		500	20.1	44.1	235	17.5	2686
		1000	20.6	39.2	185	16.2	1392
Water*Calcium							
	Saturated	0	24.8	48.2	307	23.1	2412
		500	23.8	53.1	305	24.9	3152
		1000	23.1	42.8	275	24.5	1879
	Wet	0	23.5	73.3	185	15.6	2311
		500	21.6	44.1	224	17.6	2547
		1000	21.4	36.1	163	14.2	1726
	Optimal	0	25.6	37.7	215	18.0	2107
		500	22.0	35.6	186	15.0	1857
		1000	21.5	34.8	153	12.7	1481
Cultivar							
	‘Amethyst-96’		24.6a[x]	41.5b	231	19.7	2222
	‘Sybil Holmes’		21.5b	48.6a	216	17.1	2105
Water		Saturated	23.9	48.0b	296a	24.2a	2481a
		Wet	22.2	51.2a	191b	15.8b	2194b
		Optimal	23.0	36.0c	184b	15.2b	1815b
Calcium		0	24.7a	53.1	235a	18.9	2277a
		500	22.5b	44.2	238a	19.2	2519a
		1000	22.0b	37.9	197b	17.1	1695b

[z] NS, *, **, ***, **** Non-significant or significant at P=0.05, 0.01, 0.001, and 0.0001, respectively.
[y] LSD calculated as: $LSD=t\sqrt{(2(EMS)/\#\ obs.\ per\ mean)}$ where t is determined at $\alpha=0.05$ and df of error term. LSD is used to compare within a row and within a column of the factorial treatment structure.
[z] If no $LSD_{0.05}$ is listed, different letters indicate that means are significantly different based on pair-wise comparisons at $\alpha=0.05$.

Table 2.5 Tissue concentrations of calcium and magnesium taken at the termination of the Summer 2008 experiment (Day 74) of whole shoots and root medium pH and electrical conductivity (EC) via saturated media extracts on three cultivars of ivy geranium grown under three root medium water contents saturated, wet, or dry with either 0 or 200 $mg \cdot L^{-1}$ supplemental calcium.

		Ca Concentration (%)	Mg Concentration (%)	pH	EC (dS/m)
Block		NS[z]	NS	NS[z]	NS
Cultivar		****	****	**	NS
Water		****	****	****	*
Calcium		****	NS	NS	NS
Cultivar*Water		NS	NS	NS	NS
Cultivar*Calcium		NS	NS	NS	NS
Water*Calcium		*	*	NS	NS
Cultivar*Water*Calcium		NS	NS	NS	NS
Cultivar*Water*Calcium					
Cultivar - 'Amethyst 96'					
Saturated	Ca	3.81	0.34	4.9	1.18
	No Ca	2.95	0.33	4.61	1.3
Wet	Ca	3.91	0.33	4.74	1.33
	No Ca	5.21	0.39	4.55	1.54
Dry	Ca	3.53	0.47	5.32	1.34
	No Ca	2.64	0.42	5.09	1.24
Cultivar - 'Lambada'					
Saturated	Ca	4.19	0.43	5.19	1.19
	No Ca	3.58	0.44	4.97	1.2
Wet	Ca	5.75	0.45	4.65	1.46
	No Ca	8.65	0.5	4.85	1.34

Dry	Ca	3.43	0.51	5.11	1.37
	No Ca	3.15	0.51	5.33	1.2
Cultivar - 'Sybil Holmes'					
Saturated	Ca	5.04	0.4	5.3	1.23
	No Ca	7.17	0.42	4.96	1.06
Wet	Ca	12.4	0.44	4.74	1.49
	No Ca	8.55	0.5	5.39	1.42
Dry	Ca	5.09	0.63	5.65	1.49
	No Ca	4.22	0.56	5.81	1.2
Cultivar*Water					
‘Amethyst-96’	Saturated	3.38	0.34	4.76	1.24
	Wet	4.56	0.36	4.64	1.43
	Dry	3.09	0.45	5.2	1.29
‘Lambada’	Saturated	3.88	0.39	5.08	1.2
	Wet	7.2	0.47	4.75	1.4
	Dry	3.29	0.51	5.22	1.29
‘Sybil Holmes’	Saturated	6.1	0.41	5.13	1.14
	Wet	10.47	0.47	50.7	1.45
	Dry	4.66	0.6	5.73	1.34
Cultivar*Calcium					
‘Amethyst-96’	Ca	3.75	0.38	4.99	1.29
	No Ca	3.6	0.38	5.06	1.36
‘Lambada’	Ca	4.45	0.46	4.99	1.35
	No Ca	5.13	0.48	5.05	1.25
‘Sybil Holmes’	Ca	7.51	0.49	5.23	1.4
	No Ca	6.65	0.5	5.38	1.22

Water * Calcium						
	Saturated	Ca	4.35b	0.39c	5.13	1.2
		No Ca	4.57b	0.40bc	4.85	1.18
	Wet	Ca	7.35a	0.40bc	4.71	1.43
		No Ca	7.47a	0.46b	4.93	1.43
	Dry	Ca	4.02b	0.54a	5.36	1.4
		No Ca	3.34b	0.50ab	5.41	1.21
		$LSD_{0.05}$[y]	**1.34**	**0.06**	**NS**	**NS**
Cultivar						
	‘Amethyst-96’		3.68c[y]	0.38b	4.87b[y]	1.32
	‘Lambada’		4.79b	0.47a	5.01b	1.29
	‘Sybil Holmes’		7.08a	0.49a	5.30a	1.31
Water		Saturated	4.46b	0.39c	4.99b	1.19ab
		Wet	7.41a	0.43b	4.82b	1.43a
		Dry	3.68c	0.52a	5.38a	1.30a
Calcium		Ca	5.24a	0.44	5.07	1.34
		No Ca	5.12b	0.45	5.06	1.28

[z] NS, *, **, ***, **** Non-significant or significant at P=0.05, 0.01, 0.001, and 0.0001, respectively.
[y] LSD calculated as: LSD=t√(2(EMS)/# obs. per mean) where t is determined at α=0.05 and degrees of freedom of error term. LSD is used to compare within a row and within a column of the factorial treatment structure.
[x] If no $LSD_{0.05}$ is listed, different letters indicate that means are significantly different based on pair-wise comparisons at α=0.05.

Table 2.6 a and b. Tissue concentrations of calcium and magnesium taken at the termination of the Fall 2008[z] (Day 77) experiment in the youngest, fully-expanded leaves and root medium pH and electrical conductivity (EC) via saturated media extracts of ivy geranium grown under four root medium water contents saturated, wet, optimal, or dry with either 0 or 300 $mg \cdot L^{-1}$ supplemental calcium.

		Ca Concentration %	Mg Concentration %	pH	EC (dS/m)
Block		NS[y]	NS	NS	NS
Water		NS	NS	NS	*
Calcium		*	**	NS	*
Water*Calcium		NS	*	NS	NS
Water*Calcium					
Saturated	Ca	1.13	0.35c	4.54	0.77
	No Ca	1.18	0.37c	4.65	0.56
Wet	Ca	1.68	0.24d	4.52	0.59
	No Ca	0.86	0.63a	3.38	0.3
Optimal	Ca	1.41	0.31c	4.77	0.76
	No Ca	1.26	0.43b	4.79	0.76
Dry	Ca	1.36	0.38bc	4.67	0.86
	No Ca	1.3	0.42b	4.71	0.55
	$LSD_{0.05}$[x]	**NS**	**0.05**	**NS**	**NS**
Water					
	Saturated	1.15	0.36	4.59	0.67a
	Wet	1.27	0.43	3.95	0.45b
	Optimal	1.34	0.37	4.78	0.76a
	Dry	1.33	0.4	4.69	0.71a
Calcium					
	Ca	1.40a[w]	0.32b	4.62	0.75a
	No Ca	1.15b	0.46a	4.38	0.54b

[z] Data means do not include the low VPD environment treatment

[y] NS, *, **, ***, **** Non-significant or significant at P=0.05, 0.01, 0.001, and 0.0001, respectively.

[x] LSD calculated as: LSD=t√(2(EMS)/# obs. per mean) where t is determined at α=0.05 and degrees of freedom of error term. LSD is used to compare within a row and within a column of the factorial treatment structure.
[w] If no $LSD_{0.05}$ is listed, different letters indicate that means are significantly different based on pair-wise comparisons at α=0.05.

Table 2.6b Tissue concentrations of calcium and magnesium taken at the termination of the Fall 2008[z] experiment (Day 77) in the youngest, fully-expanded leaves and root medium pH and electrical conductivity (EC) via saturated media extracts of ivy geranium grown under four root medium water contents saturated, wet, optimal, or dry with either 0 or 300 $mg \cdot L^{-1}$ supplemental calcium.

		Ca Concentration %	Mg Concentration %	pH	EC (dS/m)
Block		NS[y]	NS	NS	NS
VPD		NS	NS	NS	NS
Water		**	*	NS	NS
Calcium		*	***	NS	**
VPD*Water		NS	NS	NS	NS
VPD*Calcium		NS	NS	NS	NS
Water*Calcium		NS	NS	NS	NS
Water*Ca*VPD		NS	NS	NS	NS
Water * Ca * VPD					
VPD - High					
Saturated	Ca	1.13	0.36	4.54	0.77
	No Ca	1.18	0.37	4.65	0.56
Wet	Ca	1.68	0.24	4.52	0.59
	No Ca	1.13	0.34	4.79	0.3
Optimal	Ca	1.41	0.31	4.77	0.76
	No Ca	1.26	0.43	4.79	0.76
VPD - Low					
Saturated	Ca	1.09	0.31	4.78	0.93
	No Ca	0.94	0.3	5.21	0.38
Wet	Ca	1.32	0.31	4.64	0.78
	No Ca	1.23	0.39	4.67	0.49
Optimal	Ca	1.48	0.36	4.69	1.03
	No Ca	1.25	0.42	4.54	0.79
VPD*Water					

	VPD-High	Saturated	1.15	0.36	4.59	0.67
		Wet	1.4	0.29	4.65	0.45
		Optimal	1.34	0.37	4.78	0.76
	VPD-Low	Saturated	1.01	0.31	4.99	0.66
		Wet	1.28	0.35	4.65	0.64
		Optimal	1.37	0.39	4.61	0.91
VPD*Calcium						
	VPD-High	Ca	1.41	0.3	4.61	0.71
		No Ca	1.19	0.38	4.74	0.54
	VPD-Low	Ca	1.3	0.33	4.7	0.91
		No Ca	1.14	0.37	4.8	0.55
Water*Calcium						
	Saturated	Ca	1.11	0.33	4.66	0.85
		No Ca	1.06	0.34	4.93	0.47
	Wet	Ca	1.5	0.28	4.58	0.69
		No Ca	1.18	0.36	4.72	0.4
	Optimal	Ca	1.45	0.33	4.73	0.89
		No Ca	1.26	0.42	4.66	0.77
VPD		High	1.3	0.34	4.67	0.62
		Low	1.22	0.35	4.75	0.73
Water		Saturated	1.08b[x]	0.34b	4.79	0.66
		Wet	1.34a	0.32b	4.65	0.54
		Optimal	1.35a	0.38a	4.69	0.83
Calcium		Ca	1.35a	0.31b	4.65	0.81a
		No Ca	1.17b	0.38a	4.77	0.55b

[z] Data means do not include the low VPD environment treatment

[y] NS, *, **, ***, **** Non-significant or significant at P=0.05, 0.01, 0.001, and 0.0001, respectively.

[x] If no $LSD_{0.05}$ is listed, different letters indicate that means are significantly different based on pair-wise comparisons at α=0.05.

Table 2.7 Averages of temperature and relative humidity as recorded with a HOBO data sensor (Onset Computer Corp., Bourne, MA) every 30 min and vapor pressure deficits (VPD) calculated using Murray's equation (Murray, 1967).

	Average Temperature (°C)		Average Relative Humidity (%)		Average VPD (kPa)	
	Day[z]	Night[y]	Day	Night	Day	Night
Spring 2008[x]	28.6	20.1	40	52.7	3.06	1.37
Summer 2008[w]	28.4	25.6	67.4	66.5	1.42	1.13
Fall 2008[v]						
Low VPD Environment	23.3	15.1	60.4	76.7	1.14	0.39
High VPD Environment	20.6	15.7	45.4	56.4	1.35	0.77
Spring 2009[u]	25.5	18.6	38.2	56.8	2.03	0.92

[z] Average of measurements from 0800 HR to 1730 HR
[y] Average of measurements from 1800 HR to 0730 HR
[x] From 1 April to 30 May 2008
[w] From 28 June to 16 August 2008
[v] From 17 November to 18 December 2008
[u] From 5 March to 18 May 2009

Table 2.8 Tissue concentrations of calcium and magnesium taken at the termination of the Spring 2009 experiment (Day 90) of whole shoots and root medium pH and electrical conductivity (EC) via saturated media extracts on three cultivars of ivy geranium grown under three root medium water contents saturated, wet, or dry with either 0 or 200 $mg \cdot L^{-1}$ supplemental calcium.

			Ca Concentration (%)	Mg Concentration (%)	pH	EC (dS/m)
Block			NS[z]	NS	NS	NS
Cultivar			NS	*	*	NS
Water			****	*	****	**
Calcium			****	****	NS	****
Cultivar*Water			NS	NS	NS	NS
Cultivar*Ca			NS	NS	NS	NS
Water*Ca			*	**	NS	NS
Cultivar*Water*Ca			NS	NS	NS	NS
Cultivar*Water*Ca						
Cultivar - 'Amethyst 96'						
	Saturated	0	1.03	0.33	4.94	2.18
		500	1.25	0.32	4.89	3.62
		1000	1.45	0.25	4.88	3.74
	Wet	0	1.2	0.37	4.23	2.47
		500	1.77	0.27	4.43	3.53
		1000	2.11	0.24	4.44	3.81
	Optimal	0	1.24	0.39	4.24	1.92
		500	2.14	0.24	4.41	2.52
		1000	2.45	0.17	4.33	3.45
Cultivar - 'Sybil Holmes'						
	Saturated	0	0.84	0.33	5.14	2.31
		500	1.39	0.28	5.13	3.43

	1000	0.41	0.19	5.07	4.87
Wet	0	0.75	0.29	3.94	2.43
	500	2.06	0.24	4.33	2.94
	1000	1.78	0.19	4.34	4.13
Optimal	0	1.2	0.33	4.66	1.79
	500	1.85	0.22	4.51	2.47
	1000	2.43	0.19	4.66	2.46

Cultivar*Water

‘Amethyst-96’					
	Saturated	1.24	0.3	4.9	3.18
	Wet	1.69	0.3	4.36	3.27
	Optimal	1.94	0.27	4.32	2.63
‘Sybil Holmes’					
	Saturated	0.88	0.26	5.11	3.54
	Wet	1.53	0.24	4.2	3.16
	Optimal	1.83	0.24	4.61	2.24

Cultivar*Ca

‘Amethyst-96’	0	1.15	0.36	4.47	2.19
	500	1.72	0.27	4.58	3.22
	1000	2.01	0.22	4.55	3.66
‘Sybil Holmes’	0	0.93	0.32	4.59	2.18
	500	1.67	0.25	4.66	2.94
	1000	1.63	0.19	4.69	3.82

Water*Ca

Saturated	0	0.93d	0.33ab	5.04	2.24
	500	1.32c	0.30b	5.01	3.52
	1000	0.99cd	0.22d	4.97	4.3
Wet	0	0.98d	0.33ab	4.08	2.45
	500	1.78b	0.26bcd	4.38	3.23

		1000	2.08b	0.21c	4.39	3.97
	Optimal	0	1.22c	0.36a	4.45	1.85
		500	1.99b	0.23cd	4.46	2.49
		1000	2.44a	0.18d	4.5	2.95
		$LSD_{0.05}$[y]	**0.33**	**0.05**	**NS**	**NS**
Cultivar	‘Amethyst-96’		1.63	0.29a[x]	4.53b	3.02
	‘Sybil Holmes’		1.41	0.25b	4.64a	2.98
Water	Saturated		1.06c	0.28a	5.01a	3.36a
	Wet		1.61b	0.27ab	4.28b	3.21b
	Optimal		1.88a	0.26b	4.47b	2.43b
Calcium	0		1.04c	0.34a	4.52	2.18c
	500		1.70b	0.26b	4.62	3.08b
	1000		1.82a	0.20c	4.62	3.74a

[z] NS, *, **, ***, **** Non-significant or significant at P=0.05, 0.01, 0.001, and 0.0001, respectively.
[y] LSD calculated as: $LSD=t\sqrt{(2(EMS)/\#\ obs.\ per\ mean)}$ where t is determined at $\alpha=0.05$ and degrees of freedom of error term. LSD is used to compare within a row and within a column of the factorial treatment structure.
[x] If no $LSD_{0.05}$ is listed, different letters indicate that means are significantly different based on pair-wise comparisons at $\alpha=0.05$.

Table 2.9 Edema development in the Summer 2008 experiment measured at the end of the production period (Day 74) of three ivy geranium cultivars grown under three root medium water content regimens; saturated, wet , or dry with either 0 or 200 $mg \cdot L^{-1}$ supplemental calcium.

			Edema Ratings	Affected Area (cm^2)	% Damage
Block			NS	NS	NS
Cultivar			NS	NS	***
Water			NS	NS	**
Calcium			NS	NS	NS
Cultivar*Water			NS	NS	*
Cultivar*Ca			NS	NS	NS
Water*Ca			NS	NS	NS
Cultivar*Water*Ca			NS	NS	NS
Cultivar*Water*Ca					
Cultivar - 'Amethyst 96'					
	Saturated	Ca	3.5	8.0	1.18
		No Ca	2.4	4.7	0.79
	Wet	Ca	2.8	5.0	1.28
		No Ca	2.9	6.4	1.09
	Dry	Ca	3.3	9.5	2.76
		No Ca	2.8	7.5	2.04
Cultivar - 'Lambada'					
	Saturated	Ca	3.5	6.6	1.16
		No Ca	2.5	5.6	0.90
	Wet	Ca	3.8	6.9	1.26
		No Ca	3.1	9.2	1.18
	Dry	Ca	2.9	4.0	1.05
		No Ca	3.0	3.9	1.16
Cultivar - 'Sybil Holmes'					
	Saturated	Ca	2.9	4.1	0.63
		No Ca	2.4	5.8	0.45
	Wet	Ca	2.9	8.1	0.69
		No Ca	3.4	6.2	0.61
	Dry	Ca	3.1	6.3	1.28
		No Ca	3.0	4.2	0.71
Cultivar*Water					

	‘Amethyst-96’	Saturated	2.9	6.3	0.50b
		Wet	2.8	5.7	0.70b
		Dry	3.0	8.5	1.91a
	‘Lambada’	Saturated	3.0	6.1	0.55b
		Wet	3.4	8.1	0.73b
		Dry	2.9	5.3	0.45b
	‘Sybil Holmes’	Saturated	2.6	4.9	0.06b
		Wet	3.1	7.1	0.16b
		Dry	3.1	5.3	0.50b
		$LSD_{0.05}$[y]	**NS**	**NS**	**0.75**
Cultivar*Ca					
	‘Amethyst-96’	Ca	3.2	7.5	1.74
		No Ca	2.7	6.2	1.31
	‘Lambada’	Ca	3.4	5.8	1.16
		No Ca	2.9	6.3	0.97
	‘Sybil Holmes’	Ca	2.9	6.2	0.86
		No Ca	2.9	5.4	0.59
Water*Ca					
	Saturated	Ca	3.3	6.2	0.99
		No Ca	2.4	5.4	0.71
	Wet	Ca	3.1	6.7	1.08
		No Ca	2.4	7.2	0.96
	Dry	Ca	3.1	6.6	1.69
		No Ca	2.9	5.2	1.19
Cultivar		‘Amethyst-96’	2.9	6.8	1.52a[y]
		‘Lambada’	3.1	6.1	1.06a
		‘Sybil Holmes’	2.9	5.8	0.73b
Water		Saturated	2.9	5.8	0.55b
		Wet	3.1	6.9	0.72b
		Dry	3.0	5.9	1.14a
Calcium		Ca	3.2	6.5	1.25
		No Ca	2.8	6.0	0.96

[z] NS, *, **, ***, **** Non-significant or significant at P=0.05, 0.01, 0.001, and 0.0001, respectively.
[y] LSD calculated as: LSD=t√(2(EMS)/# obs. per mean) where t is determined at α=0.05 and df of error term. LSD is used to compare within a row and within a column of the factorial treatment structure.

[z] If no $LSD_{0.05}$ is listed, different letters indicate that means are significantly different based on pair-wise comparisons at α=0.05.

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Table 2.10 a and b Edema development in the Fall 2008[z] experiment measured at the end of the production period (Day 77) of the ivy geranium cultivar 'Amethyst 96' grown in a high VPD environment under four root medium water content regimens: saturated, wet, optimal or dry with either 0 or 300 $mg \cdot L^{-1}$ supplemental calcium.

		Edema Ratings	Affected Area (cm^2)	% Damage
Block		NS[y]	NS	NS
Water		NS	NS	NS
Calcium		NS	NS	NS
Water*Calcium		NS	NS	NS
Water* Calcium				
Saturated	Ca	1.6	4.6	0.32
	No Ca	1.6	5.2	0.54
Wet	Ca	1.3	6.1	1.61
	No Ca	1.3	7.3	0.42
Optimal	Ca	1.8	8.5	0.72
	No Ca	1.9	6.9	0.84
Dry	Ca	1.6	7.9	1.25
	No Ca	2.4	8.5	0.72
Water	Saturated	1.6	4.9	0.43
	Wet	1.3	6.7	1.01
	Optimal	1.8	7.7	0.78
	Dry	2.0	8.2	0.98
Calcium	Ca	1.6	6.8	0.97
	No Ca	1.8	7.0	0.63

[z] Statistical analysis does not include the low VPD environment treatment
[y] NS, *, **, ***, **** Non-significant or significant at P=0.05, 0.01, 0.001, and 0.0001, respectively.

Table 2.10b Edema development in the Fall 2008[z] experiment measured at the end of the production period (day 77) of the ivy geranium cultivar 'Amethyst 96' grown under three root medium water content regimens: saturated, wet, or optimal in a high VPD or low VPD environment with either 0 or 300 $mg \cdot L^{-1}$ supplemental calcium.

		Edema Ratings	Affected Area (cm^2)	% Damage
Block		NS[y]	NS	NS
VPD		**	NS	NS
Water		NS	NS	NS
Calcium		NS	NS	NS
VPD*Water		NS	NS	NS
VPD*Calcium		NS	NS	NS
Water*Calcium		NS	NS	NS
VPD*Water*Calcium		NS	NS	NS
VPD*Water*Calcium				
VPD - High				
Saturated	Ca	1.6	4.6	0.32
	No Ca	1.6	5.2	0.54
Wet	Ca	1.3	6.1	1.61
	No Ca	1.3	7.3	0.42
Optimal	Ca	1.8	8.5	0.72
	No Ca	1.9	6.9	0.84
VPD - Low				
Saturated	Ca	1.6	5.6	0.66
	No Ca	2.6	7.0	0.63
Wet	Ca	2.6	7.6	0.72
	No Ca	2.3	7.2	0.56
Optimal	Ca	2.3	7.5	0.54
	No Ca	2.0	5.3	0.45
VPD*Water				
VPD-High	Saturated	1.6	4.9	0.43
	Wet	1.3	6.7	1.01
	Optimal	1.8	7.7	0.78

	VPD-Low	Saturated	2.1	6.3	0.65
		Wet	2.4	7.4	0.64
		Optimal	2.1	6.4	0.50
VPD*Calcium					
	VPD-High	Ca	1.5	6.4	0.88
		No Ca	1.6	6.5	0.60
	VPD-Low	Ca	2.2	6.9	0.64
		No Ca	2.3	6.5	0.55
Water* Calcium					
	Saturated	Ca	1.6	5.1	0.49
		No Ca	2.1	6.1	0.58
	Wet	Ca	1.9	6.8	1.16
		No Ca	1.8	7.2	0.49
	Optimal	Ca	2.0	8.0	0.63
		No Ca	1.9	6.1	0.65
VPD		High	1.6b[x]	6.5	0.74
		Low	2.2a	6.7	0.60
Water		Saturated	1.9	5.6	0.54
		Wet	1.8	7.0	0.83
		Optimal	2.0	7.1	0.64
Calcium		Ca	1.9	6.7	0.76
		No Ca	1.9	6.5	0.57

[z] Statistical analysis does not include the dry root medium water content treatment
[y] NS, *, **, ***, **** Non-significant or significant at P=0.05, 0.01, 0.001, and 0.0001, respectively.
[x] If no $LSD_{0.05}$ is listed, different letters indicate that means are significantly different based on pair-wise comparisons at α=0.05.

Table 2.11 Edema development in the Spring 2009 experiment measured at the end of the production period (Day 90) of two ivy geranium cultivars grown under three root medium water content regimens: saturated, wet, or optimal with either 0, 500, or 1000 $mg \cdot L^{-1}$ supplemental calcium.

			Affected Area (cm^2)	% Damage
Block			NS[z]	NS
Cultivar			NS	NS
Water			**	NS
Calcium			NS	NS
Cultivar*Water			NS	NS
Cultivar*Ca			NS	NS
Water*Ca			NS	NS
Cultivar*Water*Ca			NS	NS
Cultivar*Water*Ca				
Cultivar - 'Amethyst 96'				
	Saturated	0	153	4.92
		500	395	11.81
		1000	238	9.71
	Wet	0	232	11.59
		500	152	8.00
		1000	174	10.28
	Optimal	0	117	5.68
		500	95	5.19
		1000	100	6.24
Cultivar - 'Sybil Holmes'				
	Saturated	0	413	13.55
		500	150	5.19
		1000	125	4.54
	Wet	0	316	12.60
		500	99	4.50
		1000	107	6.20
	Optimal	0	103	4.31
		500	118	5.40
		1000	93	7.54

Cultivar*Water				
	‘Amethyst-96’	Saturated	262	8.81
		Wet	186	9.96
		Optimal	104	5.70
	‘Sybil Holmes’	Saturated	229	7.76
		Wet	174	7.77
		Optimal	105	5.75
Cultivar*Ca				
	‘Amethyst-96’	0	167	7.40
		500	214	6.68
		1000	170	8.74
	‘Sybil Holmes’	0	277	10.15
		500	122	5.03
		1000	109	6.09
Water*Ca				
	Saturated	0	283	9.24
		500	273	8.50
		1000	181	7.12
	Wet	0	274	12.09
		500	125	6.25
		1000	141	8.24
	Optimal	0	110	4.99
		500	107	5.29
		1000	96	6.89
Cultivar		‘Amethyst-96’	184	8.16
		‘Sybil Holmes’	169	7.09
Water		Saturated	246a[y]	8.29
		Wet	180ab	8.86
		Optimal	104b	5.74
Calcium		0	222	8.77
		500	168	6.68
		1000	139	7.42

[z] NS, *, **, ***, **** Non-significant or significant at P=0.05, 0.01, 0.001, and 0.0001, respectively.
[y] If no $LSD_{0.05}$ is listed, different letters indicate that means are significantly different based on pair-wise comparisons at α=0.05.

CHAPTER 3 - Root Medium Water Content and Supplemental UVB Light on Intumescence Development of Tomato

Introduction

Intumescence development is a physiological disorder of several greenhouse-grown crops characterized by gall-like or protuberant growths typically found on leaf tissues. This disorder has been known by several different names that are often used interchangeably: oedema (edema), enations, genetic tumors, intumescences, neoplasms, and non-pathogenic galls. Two of these terms, oedema and intumescences, are most commonly found in literature. Based on our research, we believe there are two different disorders being described by these terms. We are assigning the term intumescence development to the physiological disorder resulting in individual cells swelling and bursting.

While mild symptoms of intumescence development may be tolerated, moderate to severe symptoms include leaf chlorosis, leaf necrosis and leaf drop. This, along with the finding that intumescence development lowers the number of chloroplasts in palisade cells (Eliza and Dobrenz, 1971) may cause plants to grow slowly and have lower yields than unaffected plants and will be severely affected aesthetically, resulting in economic loss in plant sales to consumers.

The exact environmental triggers of intumescence development are unknown. Previous authors have suggested many possible factors that can lead to the development of intumescences. Eliza and Dobrenz (1971) observed that oedema or intumescences of eggplant (*Solanum melongena* L.) were induced in hot, humid environments within a plastic greenhouse and were caused by hypertrophy (cellular swelling) of palisade cells which then ruptured the epidermal layer. Air pollution was a probable cause in the development of enations on potatoes (*Solanum tuberosum* L. 'Russet Burbank') grown in a growth chamber in according to Kirkham and Keeney (1974). Petitte and Ormrod also found that air contaminants SO_2 and NO_2 had an effect on intumescence development on potato, as well as propagation method. In this case, potatoes ('Russet Burbank,' 'Norchip,' and 'Kennebec') propagated from a high carbohydrate source

(tuber) generated more intumescences than potatoes propagated via stem cutting. However, Lang and Tibbitts (1983) found air pollution to be an inconclusive cause on tomato plants (*Solanum lycopersicum* var. *esculentum* L. 'Oxheart'). In that same paper, relative humidity and radiance levels did not have an effect on intumescence development, but temperature and ultraviolet wavelengths did. Lang et al. (1983) performed an anatomical study and found similar results in tomato as Eliza and Dobrenz (1971) in that palisade cells were hypertrophic, swelling to almost double in size, and rupturing through the leaf epidermis. In sweet potato (*Ipomoea batatas* L. Lam), a different anatomy of the disorder was described by Wetzstein and Frett (1984), where both hypertrophy (cell swelling) and hyperplasia (cell proliferation) were evident within mesophyll cells.

Jaworski et al. (1988) observed that intumescence development was highly dependent on cultivar in *Cuphea* species and was not caused by insect predation. The removal of blue-green light with a yellow filter was found to reduce the formation of intumescences of potato in a study performed by Seabrook and Douglass (1998). This is contrary to a finding by Morrow and Tibbitts (1988), where the addition of red light promoted intumescence development on potato, while blue and green lights had no effect. Finally, a study by Pinkard et al. (2006) found that in eucalyptus (*Eucalyptus nitens* Libill. and *Eucalyptus globulus* Deane and Maiden), intumescence growth was actually the formation of lenticel-like structures on plant leaves as a result of high relative humidity, which is a way to facilitate gas exchange.

When root medium water content resulted in no effects on intumescence formation, the literature was re-evaluated and a possible link between ultraviolet (UV) wavelengths and intumescence prevention was found. Several studies mentioned that this disorder occurred only in controlled environments, such as greenhouses and growth chambers (Jaworski et al., 1987; Kirkham and Keeney, 1974; Lang and Tibbitts, 1983; Lang et al., 1983; Petitte and Ormrod, 1986). Further, Lang and Tibbitts (1983) exposed tomato plants to the UV emissions of cool while fluorescent lights in a growth chamber, effectively preventing intumescence development.

These reports lead to our study of the effects of UVB (280-320 nm) wavelengths on the prevention of intumescences on two tomato cultivars, *Solanum lycopersicum* L. var. *hirsutum* 'Maxifort' and *Solanum lycopersicum* L. var. *esculentum* 'Trust.' 'Maxifort,' is used as a rootstock in grafting tomatoes for disease resistance and was chosen for its extreme susceptibility (Xin Zhao, University of Florida, Gainesville, personal communication, May 2008). 'Trust' was

a used because it is a commercial variety that is indeterminate, like 'Maxifort,' and was reported to develop intumescences in high tunnels (Lewis Jett, West Virginia University, Morgantown WV, personal communication, June 2008). Wavelengths in the B range of UV light were chosen for this study because they are almost completely blocked by most greenhouse glazing materials, unlike UVA (315-400 nm) which has some transmittance (Kittas and Baille, 1998) and is often completely absent in growth chambers. We also investigated the effect of root medium water content to see if it affected intumescence formation in tomato. For this work we again used 'Maxifort,' the susceptible cultivar, but the resistant cultivar was 'Florida-47' (Xin Zhao, University of Florida, Gainesville, personal communication, May 2008).

Materials and Methods

Two experiments were conducted to evaluate environmental effects on the occurrence and development of intumescences. The first experiment evaluated the effects of root media water content and was carried out from 26 March 2008 to 11 May 2008 on the cultivars 'Maxifort' and 'Florida-47'. The second experiment evaluated the effects of ultra violet (UV) light in the B wavelengths on the cultivars 'Maxifort' and 'Trust' from 28 February 2009 to 27 March 2009 and included two runs (runs A and B) each containing a UV blocked and a UV supplemented environment.

Plants and Growing Environment

In each experiment, two tomato (*Solanum lycopersicum* L.) cultivars were used: 'Maxifort' (var. *hirsutum* Humb. & Bonpl), 'Florida-47' and 'Trust' (var. *esculentum* Mill.). Seeds were sown as plugs in soilless media (Fafard 2: Conrad Fafard, Inc., Agawam, MA) and germinated under mist. Seedlings were fertilized with 50 mg $\cdot$ L^{-1} N from 20N-0.43P-16.6K fertilizer (Peter's 20-10-20 Peat-Lite Special, Scotts Co., Marysville, OH). For the first experiment, seedlings were transplanted on 7 March 2008 (day 1) into nursery pots with dimensions of 23 cm diameter x 24.5 cm depth and a volume of 10.2 L. In the second experiment, plants were transplanted on 10 February 2009 (day 1) into pots with dimensions of 16.5 cm diameter x 11.5 cm depth and a volume of 730 ml. In both experiments, pots were filled with peat-based, soil-less rooting medium (Fafard 2 Mix; Conrad Fafard, Inc., Agawam, MA).

The plants were then grown in a 7.6 m x 7.6 m glass greenhouse in the Throckmorton Plant Sciences range at Kansas State University, Manhattan, KS for the duration of the experiments.

In the first experiment, plants were fertilized with a controlled release fertilizer, 19N-2.6P-9.9K (Osmocote 19-6-12, Scotts Co., Marysville, OH). A surfactant (Suffusion Granular; OHP Inc., Mainland, PA) was applied to allow the peat-based media to re-wet. The fertilizer and the surfactant were top-dressed on each pot at rates of 84 g/pot and 40 g/pot, respectively, on 25 March 2008 (day 19). The watering regime for the first experiment is described in the next section. Plants were watered according to the root medium water status experiment described below. In the second experiment, plants were fertilized with a pre-plant incorporation of triple superphosphate (0N-19.7P-0K) at a rate of 0.6 $g \cdot L^{-1}$. During the experiment, runs A and B were fertilized 150 $mg \cdot L^{-1}$ N from 15N-0P-12.5K (Jack's Professional 15-0-15 Water Soluble Fertilizer; JR Peters Inc., Allentown, Pa) applied at every watering.

In the first experiment, pest management consisted of releasing biological control agents on 25 March 2008, which were *Ambiyseius swirskii* ((Athias-Henriot) Acarina: Phytoseiidae) and *Hypoaspis miles* ((Berlese) Acarina: Hypoaspidae) to control thrips (*Frankliniella occidentalis* (Pergande) Thysanoptera: Thripidae) and whiteflies (*Bemisia argentifolii* Bellows & Perring), respectively. In the second experiment, plants in runs A and B were sprayed prior to the experiment with a mixture of Azatin XL (Azadirachtin) (OHP Inc., Mainland, PA) and Pylon (4-bromo-2-(4-chlorophenyl)-1-(ethoxymethyl)-5-(trifluoromethyl)-1H-pyrrole-3-carbonitrile) (OHP Inc., Mainland, PA) on the first spray date, 24 February 2009, and Conserve SC (Spinosad) (Dow AgroSciences, Indianapolis, IN) on the second spray date, 27 February 2009. They were applied to control thrips and whiteflies.

Temperature was monitored using HOBO Environmental Monitors (Onset Comptuer Corp., Bourne, MA). In the first experiment, air temperatures averaged 28.6°C during the day and 20.1°C at night. In the second experiment, there were different air temperatures depending upon whether or not UV light was blocked. In runs A and B air temperatures averaged 27.3°C during the day and 22.9°C at night in the UV-blocked half of the UV apparatus and 27.8°C (day) and 22.9°C (night) under the supplemented UV half of the apparatus.

Scanning Electron and Dissection Microscopy Procedure

Scanning electron micrographs were taken on the abaxial surface of 'Maxifort' tomato leaves in the Spring of 2009. Small pieces of leaf, approximately 75 mm^2 were glued onto a scanning electron microscope (SEM) slide using a graphite emulsion. The slides were placed into the scanning electron microscope (S-3500N Hitachi Science Systems Ltd., Hitachinaka, Japan) and were rapidly cooled using liquid nitrogen to fix the samples. Micrographs were taken under high vacuum using a backscatter detector (Robinson Detector ETP-USA/Electron Detectors Inc., Rocklin, CA)

A dissection microscope (Nikon SMZ1500; Nikon Corp., Kawasaki, Japan) with a digital camera attachment (Nikon Digital Sight DS-5m; Nikon Instruments Inc., Melville, NY) was used to photograph intumescences on the abaxial surface of tomato leaves at a magnification of 3X.

Root Medium Water Content Treatments

For the first experiment, two watering regimes, wet and dry, were established on the tomato cultivars 'Maxifort' and 'Florida-47'. In the wet treatment, plants were watered daily to keep pots at container capacity, which in terms of weight was an average of 7.01 kg/pot, while the air-dry weight of the container with media was 1.77 kg. In the dry treatment, pots were weighed at the beginning of the experiment at container capacity to get a starting weight, which was then used to determine the percent dry down between irrigations. The target percent dry down was 40% water loss by weight which equaled an average mass of 2.72 kg. A 15% leaching fraction was maintained for both water treatments. Leaching fractions were determined by watering three, randomly chosen pots within the experiment with a known quantity of water. Saucers were placed under those pots to collect the leachate. Once the leachate totaled 15% of the total water applied, no more water was applied until the next irrigation and all other pots in the experiment received the quantity of water determined by those three randomly assigned pots.

Water loss was measured by weighing representative pots in each treatment on a regular basis, in conjunction with ECH_2O EC-5 probes (Decagon Devices Inc., Pullman WA). During the first two dry down cycles, pots were weighed and probe readings were taken to correlate the % dry down by weight to the probe readouts; the probes were then used to provide a rough estimate of the % dry down. Once the probes began to give readings similar to those correlated

with the pot weights, pots were weighed to determine the exact dry down percentages in grams. The probes gave the most exact watering dates without the need for daily weighing.

Establishing UVB Treatments

For the second experiment, two UV structures were built out of metal conduit arches attached to an expanded metal greenhouse bench, each divided in half (Fig. 3.1). One half in each UV structure blocked UV transmittance through the use of UV blocking greenhouse plastic (DuraGreen EM 3 Years OF D7/11 by DuraGreen Marketing USA, Mount Dora, FL) attached to the metal conduit structure (UV blocked treatment). The other half did not have the UV blocking plastic between the plants and the UV light source (UV supplemented treatment).

For this experiment, we decided to focus on UVB (280-320 nm) as opposed to UVA (320-400 nm) and UVC (100-280 nm) because UVB is often blocked in greenhouse glazing materials, unlike UVA, and is less harmful to living cells than UVC. The UVB light source in both UV structures consisted of six UV transmitting fluorescent light tubes (UVB-313 lamps from Q-Lab Westlake, OH). These lights emitted wavelengths from 250 nm to 400 nm, but had the highest irradiance in the 290-340 nm range – fulfilling our requirements for testing UVB (280-320nm) specifically. Protective equipment was worn to protect researchers from exposure to UV-light, which included UV-resistant goggles and face masks, nitrile gloves, and lab coats. These tubes were placed in three fluorescent light fixtures (122 cm long) which were then hung from metal conduit using chain 87.6 cm above the expanded metal bench upon which plants were placed. Plastic light diffusers (Styrene Prismatic Clear 2’x 4’ by Plaskolite, Columbus, OH) with dimensions of 61 cm x 122 cm were installed 9 cm below the lights (78.8 cm above the bench surface). The diffusers served to prevent sunburn of plant tissues by diffusing and scattering the light, while still transmitting UVB wavelengths. The UV light source was shared by both halves of each structure– the area directly beneath the lights and diffusers was separated in half, with one half covered with the UV block plastic, the other left open to receive the UV wavelengths. This gave both halves of the UV structure an effective treatment area of 123.2 cm (width of the bench) by 61 cm (one half of a 48’ (122 cm) long fluorescent light tube) or 7515.2 cm2.

The UV blocked half of each structure was constructed of UV blocked plastic attached to the metal conduit. The plastic was stretched to make a surface covering the width of the bench

(123.2 cm) with a length of 121.9 cm and was held 68 cm above the bench surface. The effective area of the UV blocked treatment, where the plants were placed in the structure, ran from the middle of the fluorescent light tubes out to a distance of 61 cm. The UV supplemented side had nothing except the light diffusers between the plants and the UV light source, with the effective area of the UV supplemented treatment running from the middle of the light tubes again to a distance of 61 cm away. The treatments were separated by a piece of UV blocking plastic connected to the top of the UV blocking plastic surface from the UV blocked treatment down to the bench surface along the full bench width and was situated directly under the midpoint of the fluorescent light tubes. All other sides of the treatments were left open to allow air movement through the apparatus.

Additionally, a baffle made of the UV block plastic 48.3 cm wide was run along the perimeter of the UV apparatus. It served to keep air movement similar between the two halves of the UV apparatus and as a safety precaution for the researchers. It was attached perpendicular to the UV blocking surface at the height of the surface and was run along the whole perimeter, which allowed the UV supplemented treatment to get similar air movement to the UV blocked treatment. The baffle was wide enough that the UV lights could not directly transmit wavelengths to researchers, unless directly under the lights in the UV supplemented treatment, acting as a safety mechanism.

The UV lights were on 12 hrs each day, from 0700 HR to 1900 HR daily. UV light was monitored on a weekly basis in all sections of the UV structure using a Field Scout 3414 Ultraviolet Light Meter (Spectrum Technologies Inc., Plainfield, IL) (Fig. 3.2). Another UV-instrument (Mannix UV-340 UV Light Meter (General Tools and Instruments Co. New York, NY) was also used to monitor UV light, with data given in Appendix D.

Intumescence Characterization

Intumescences in the first experiment were characterized several times per week by taking ratings of three pre-chosen leaves at three different stages of tomato development. In addition, percent leaf area damaged was estimated after studying a software program (Disease.Pro; written by Nutter, F.W., O. Worawitlikit, and D. Litwiller; Dept of Plant Pathology, Iowa State University, Ames, IA; Available for purchase by contacting F.W. Nutter 315 Bessey Hall, Iowa State University, Ames, IA 50011) which allows researchers to train

themselves in assessing leaf area damaged. The program is designed to train plant pathology students in recognizing foliar area damaged of peanut (*Arachis hypogaea* L.) crops. Leaf ratings were on a scale of zero to ten, with a score of zero representing no intumescence development, a five representing widespread intumescence development over the entire leaf with little to no necrosis, and a ten representing the necrosis of the entire leaf due to succumbing to intumescences.

In the second experiment, intumescences were tracked on a leaflet basis. Each leaflet was looked at several times per week. Whether a leaflet had a single intumescence or several, it was noted as being affected by intumescences and the total number of leaflets affected for each leaf was recorded.

Growth Data at Harvest

Plants in the first experiment were harvested on 11 May 2008 (day 47). Plants in runs A and B of the second experiment were harvested on 27 March 2009 (day 28). In both experiments, plant heights, shoot fresh weights, and shoot dry weights were determined at harvest. Plant heights were measured from the root medium surface to the top of the foliage canopy. Dry weights were measured after plants were dried at 70°C in a forced-air oven for several days.

Statistical Procedures

The first experiment was a completely randomized design with two water status treatments (wet and dry) and two cultivars. The second experiment was a split plot design with the whole plot being the two UV light treatments (blocked and not blocked) with cultivar as the subplot.

Analysis of variance was conducted on both experiments using PROC GLM and PROC MIXED in SAS version 9.1.3 (SAS Institute Inc., Cary, NC).

Results and Discussion

Scanning Electron Microscope (SEM)

Because the tomato ‘Maxifort’ showed intumescence damage, and ‘Trust’ and ‘Florida-47’ did not, dissection and scanning electron micrographs were taken on the leaves of

'Maxifort.' In Fig 3.3, epidermal cells are swollen and protruding resulting in the intumescence found on the leaf surface.The pictures showed epidermal cells swelling to extreme sizes in non-necrotic intumescences (Fig 3.4). In the micrograph, the normal, unaffected cells (A) look like puzzle pieces and are approximately 25-50 µm in diameter. The cells involved in the intumescence development (B) are much larger, with diameters approximately 50-75 µm and are no longer shaped as puzzle pieces. As the intumescences mature over time, the swollen protoplasts burst, leaving large areas of cell wall structures which remain and form the necrotic regions associated with mature intumescences (Fig. 3.5). As noted in the Introduction, intumescences have been stated to be the result of hypertrophy or hyperplasia. Hypertrophy is the abnormal enlargement of a cell, while hyperplasia is an abnormal multiplication of cells. The finding that the Maxifort tomato cells burst agrees with those who state that intumescences are caused by hypertrophy (Lang et al., 1983; Wetzstein and Frett, 1984), but it does not agree with those who state that it is caused by hyperplasia (La Rue, 1933).

Effects of Root Medium Water Content Treatments

As noted in Materials and Methods, the effect of water on the development of intumescence development was studied on 'Maxifort' and 'Florida-47' during the spring of 2008. The data showing intumescence damage are given in Table 3.1. This damage, as determined by the subjective visual percentage affected described above, was significant at the 0.05 level (Table 3.1) for the water factor, suggesting that root medium water content may have a role in intumescence development based on visual observation. These data must be interpreted with caution because the method of determining leaf area that was damaged by intumescences was qualitative. It was impossible to distinguish damage due to intumescences and damage due to drought (i.e., chlorosis) Lang et al. (1983) found no evidence that water-soaked tissues were the cause of intumescences on tomato.

The data for vegetative growth from the spring 2008 experiment are shown in Table 3.2, and the data for yield from this experiment are shown in Table 3.3. For an unknown reason, root medium water content did not have an effect on plant growth in this experiment, measured in terms of plant height, fresh weight and dry weight (Table 3.2), or on tomato yields as determined by overall fruit counts and fresh weights (Table 3.3). Plant growth and fruiting were significantly different between 'Maxifort' and 'Florida-47' with 'Florida-47'producing an average of 16 fruits

per plant with a total fresh yield of 533.5g per plant and with an average height of 63.5 cm while 'Maxifort' produced an average 7 fruits with a total fresh yield of 2.2 g per plant and with an average plant height of 164.3 cm. Despite these differences, the total fresh and dry weights of the two cultivars were not significantly different. 'Maxifort' is an indeterminate disease-resistant rootstock. 'Florida-47' is a determinant cultivar commercial fruiting cultivar. It is because of these traits that 'Maxifort' was much greater in height, but not in fresh or dry weights, because of its open, sparse, growth and small, underdeveloped fruits compared to 'Florida-47' which had bushy, lush growth and full, ripening fruits (Fig. 3.6).

Effects of UVB Light Treatments

As noted in Materials and Methods, experiments to study the effect of UV light on intumescence formation on the cultivars 'Maxifort' and 'Trust' were done in the spring of 2009. Growth results (Table 3.4) show 'Maxifort' was taller than 'Trust,' at 65.3 cm and 52.1 cm, respectively, and had more above-ground dry weight with 16.7 g compared to 'Trust' at 13.8 g. Trust did not develop intumescences with UV light or when it was filtered out. UVB light supplementation had a significant effect in the prevention of intumescence development on 'Maxifort' (Table 3.4). The 'Maxifort' plants receiving the supplemented UV had a very low rate of intumescence development with an average of 11.9% of the total number of leaves being affected in the UV supplemented treatment compared with 60.5% leaves affected by intumescence development in the UV blocked treatment (Table 3.5).

A dimension of the tomato UVB experiment was to determine when intumescences developed on the susceptible 'Maxifort' plants. To determine this, the percentage of leaflets affected with intumescence development out of all leaflets on true-leaf 7 was tracked over the course of the experiment.

As seen in Figures 3.7 and 3.8, intumescence development on leaf 7 began on 11 March 2009 (day 12) for Run A and 13 March 2009 (day 14) for Run B. Development leveled off from 17 Mar. 2009 (day 18) to 20 Mar. 2009 (day 21) for Run A and 13 Mar. 2009 (day 14) to 20 Mar. 2009 (day 21). Development increased again for both run from 23 Mar. 2009 (day 24) until the termination of the experiment on 27 Mar. 2009 (day 28). The second increase in intumescence development could be the result of cloudy days observed around 20 Mar 2009, decreasing overall ambient UV levels. The leaves that did develop intumescences were low in

the canopy and were likely being shaded from the full UV effect from leaves in the upper canopy. Leaves in the UV supplemented treatment did not develop intumescences until 24 days after the experiment began.

Plants in the UV-supplemented treatment had some intumescence development at the end of the experiment. This could be due to shading of the leaves by the upper canopy, reducing the UVB light to the leaves. It could also be due to the cloudy days recorded around 20 March 2009. While the UV levels were statistically different on this day between the UV-supplemented and UV-blocked treatments, the UV-supplemented levels of UV light were closer to those normally found in the UV-blocked treatment on brighter days. The differences in intumescence development between runs might be explained by the age of the UV-emitting lights. Run B had lights that were not used for three months while the lights in run A were running for preliminary studies.

As an area for further study, we believe that there might be a threshold mechanism at work where, if a plant susceptible to the development of intumescences receives a certain amount or intensity of UV light, intumescence development may be prevented. This could explain why leaves in the lower canopy began developing intumescences as the plants aged, as leaves in the upper canopy were shading the lower leaves, preventing the optimal levels of UV light from getting to the leaf tissues. Further study of this proposed mechanism should be undertaken and the threshold intensity of UV light for intumescence-prevention needs to be determined.

These results concur with those of Lang et al. (1983). They added UV radiation to a growth chamber by the use of cool-white fluorescent lighting. This also lends credence to those researchers who have noted that intumescence development occurred only in controlled environments (Jaworski et al., 1987; Lang and Tibbitts, 1983; Petitte and Ormrod, 1986; Pinkard et al., 2006; Wetzstein and Frett, 1984). This result suggests that cultivars susceptible to this disorder would likely have better yields due to an increase in photosynthetic area if grown outdoors or in environments were UV light can be supplemented economically.

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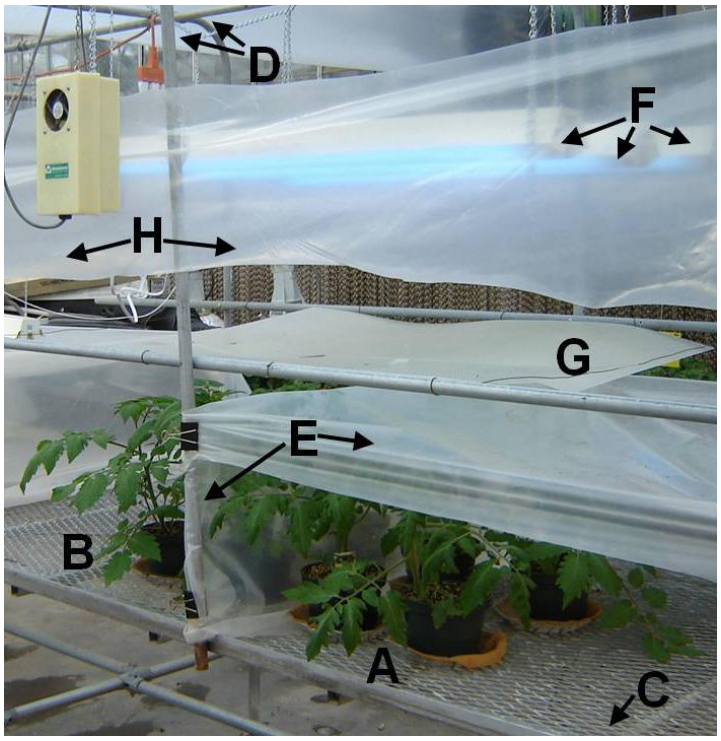

Figure 3.1 Photograph of the UV structure containing the UVB Blocked treatment (A) and the UVB supplemented treatment (B). Plants were placed on an expanded metal bench (C) with metal conduit (D) allowing attachment of UV-absorbing plastic (E) to create a barrier between the two treatments and between the blocked treatment and the UV-emitting lights (F). Plastic light diffusers (G) were placed between the UV-emitting lights and both treatments to prevent

sunburn and a baffle (H) made out of the UV-absorbing plastic to equalize air-flow between the treatments and to provide added safety to researchers. For the UV experiment discussed in this chapter, the UV-absorbing plastic and plastic light diffusers were raised up to a position directly under the UV-emitting lights to provide room for the tomatoes' heights. In the preliminary UV experiments in Appendix C the UV-absorbing plastic was at the height shown and no plastic diffusers were used.

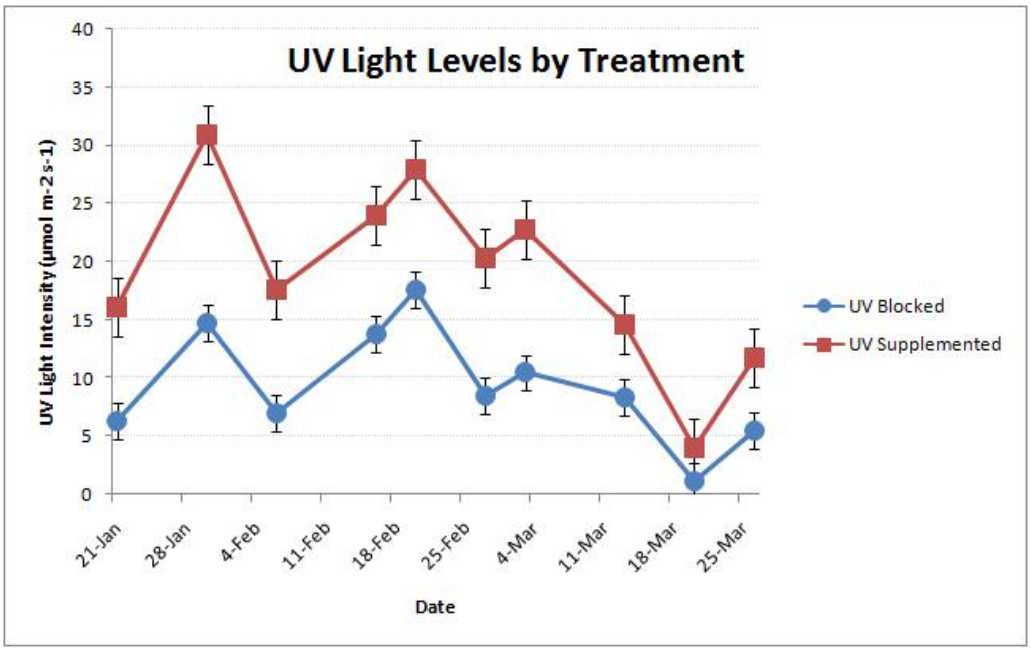

Figure 3.2 Graph of UV intensity as determined using a Field Scout 3414 Ultraviolet Light Meter (Spectrum Technologies Inc., Plainfield, IL) taken weekly from 6 weeks prior to the start through the termination of the Spring 2009 UV light experiment. The wavelengths measured with this meter ranged from 250-400 nm, reporting a broad spectrum of UV wavelengths.

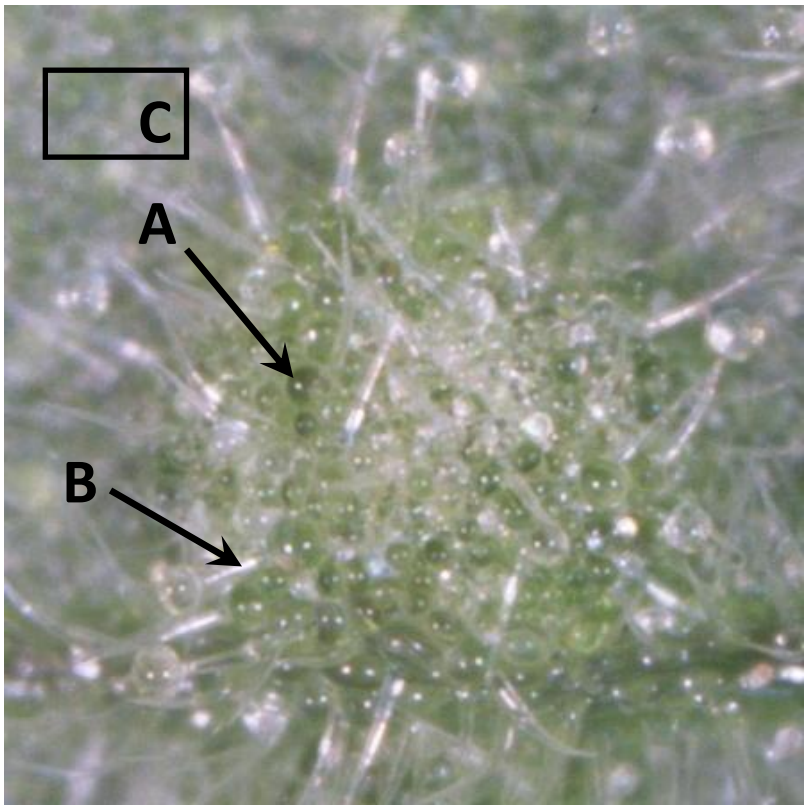

Figure 3.3 Dissection micrograph of an intumescence on the abaxial surface of a ‘Maxifort’ tomato leaf. A: Intumescence cell exhibiting extreme swelling; B: Trichome; C: Unaffected cells.

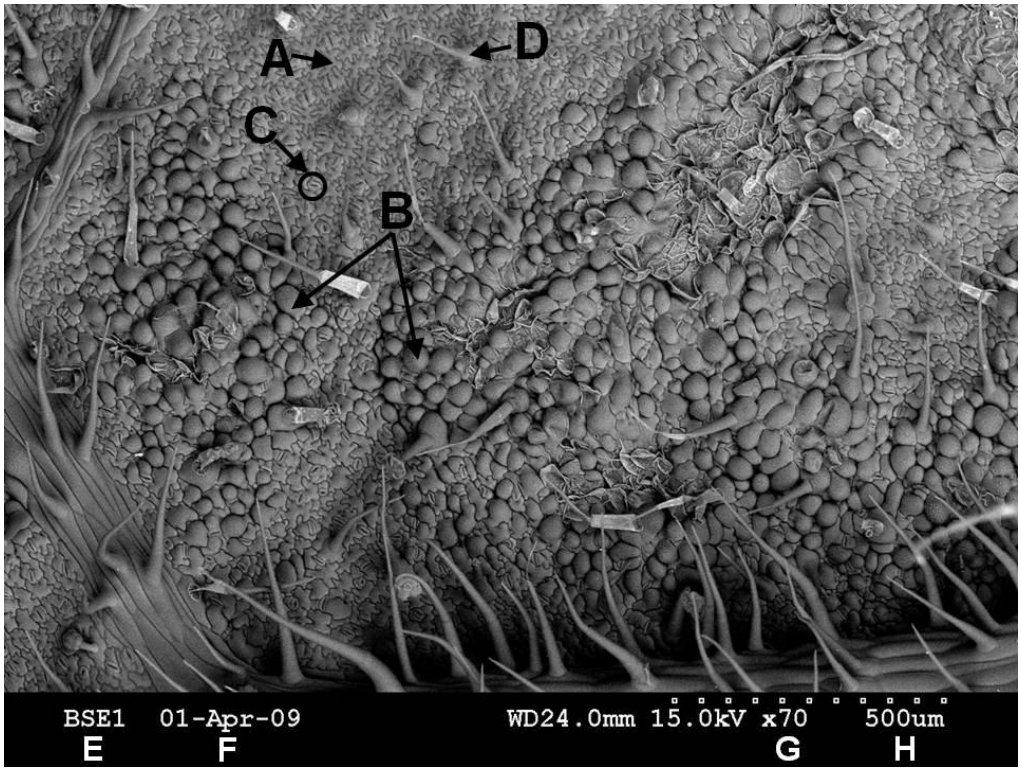

Figure 3.4 Scanning electron micrograph of the abaxial surface of a tomato leaf showing area affected by intumescence development. A: Unaffected cell; B: Intumescence cells exhibiting extreme swelling; C: Stomate; D: Trichome; E: Detector type backscatter electron; F: Date taken; G: Magnification 70X; H: Scale: 500 µm from the first square to the last square on the scale bar

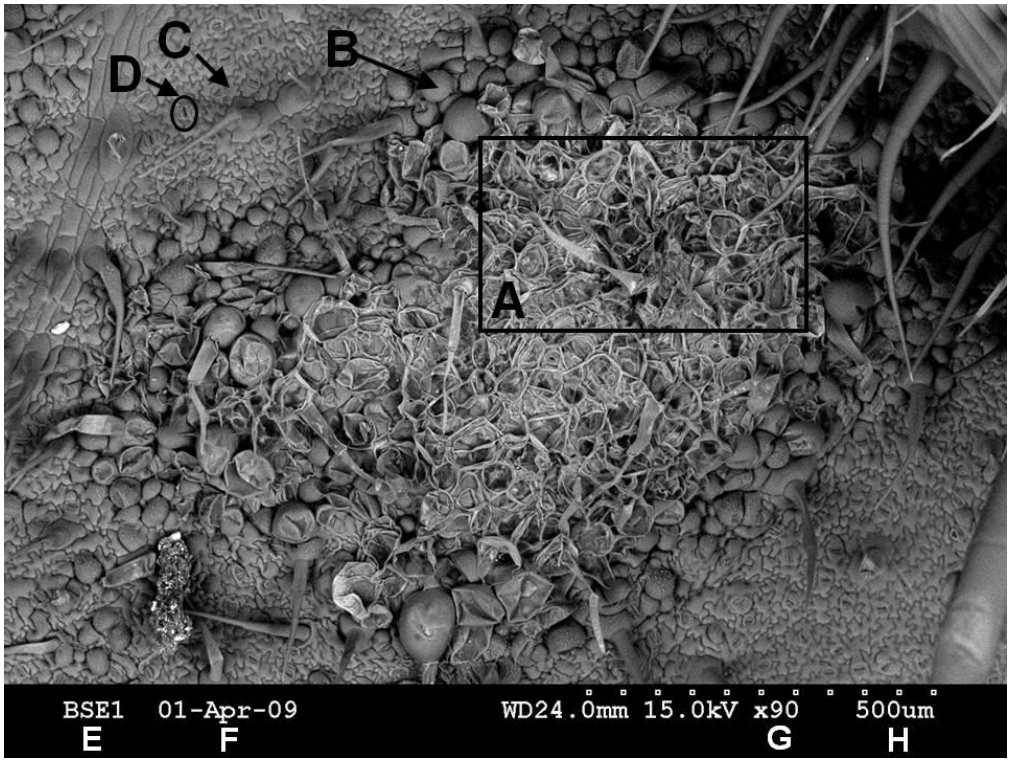

Figure 3.5 Scanning electron micrograph of the abaxial surface of a tomato leaf showing necrotic area affected by intumescence development. A: This area depicts the empty cell walls left after intumescence cells burst; B: Intumescence cell prior to bursting; C: Unaffected cell; D: Stomate; E: Type of scan: backscatter electron; F: Date taken; G: Magnification 90X; H: Scale: 500 µm from the first square to the last square on the scale bar

Figure 3.6 Comparison of ‘Maxifort’ (foreground) and ‘Florida-47’ (background) growth characteristics. ‘Maxifort’ has an open, tall growth habit as seen on the left, while ‘Florida-47’ has a compact growth habit, resulting in differences in growth. Also seen in this photo, ‘Maxifort’ was severely affected by intumescence development, while ‘Trust’ was not affected at all.

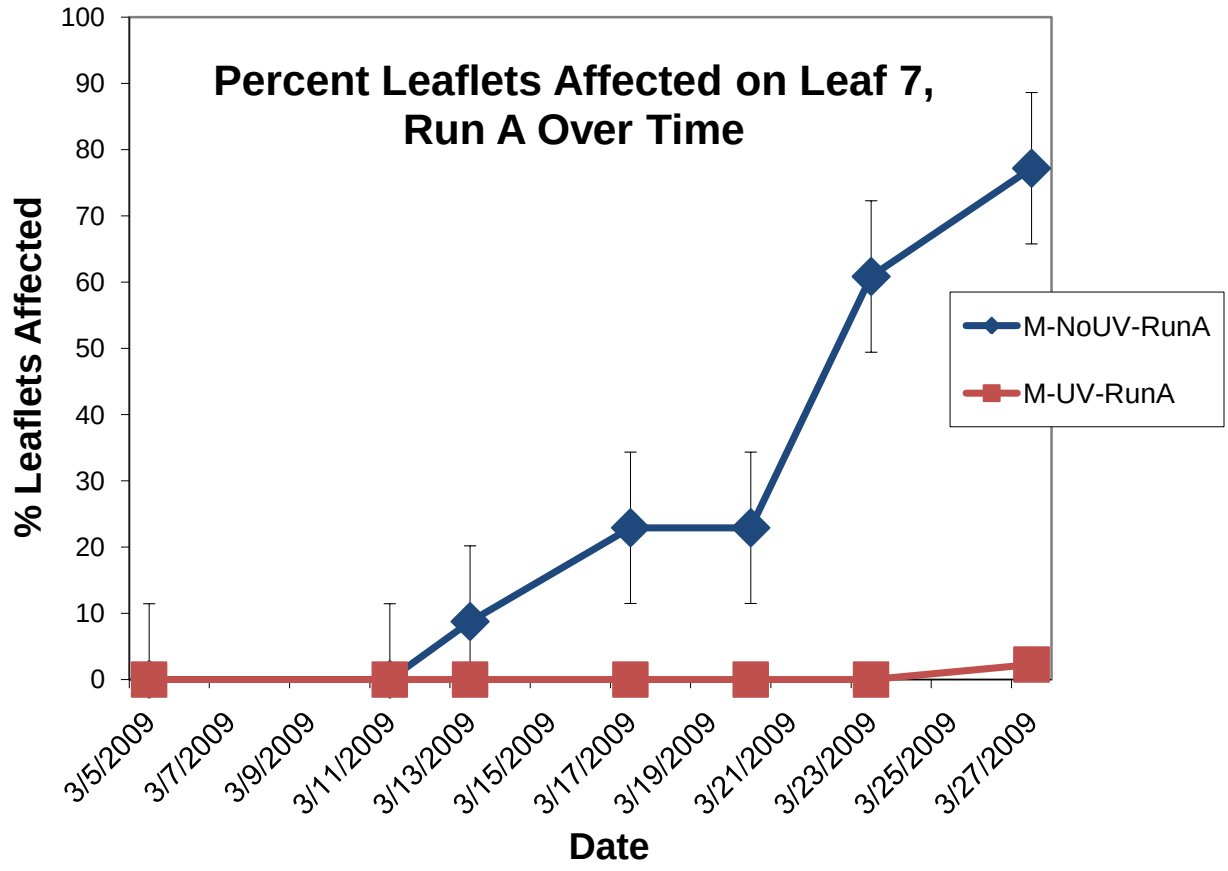

Figure 3.7 Percent leaflets affected by intumescences on 'Maxifort' tomato plants in the Spring 2009 UVB experiment. Percent leaflets affected were obtained from all 'Maxifort' plants in run A on true leaf number 7 over the course of the experiment.

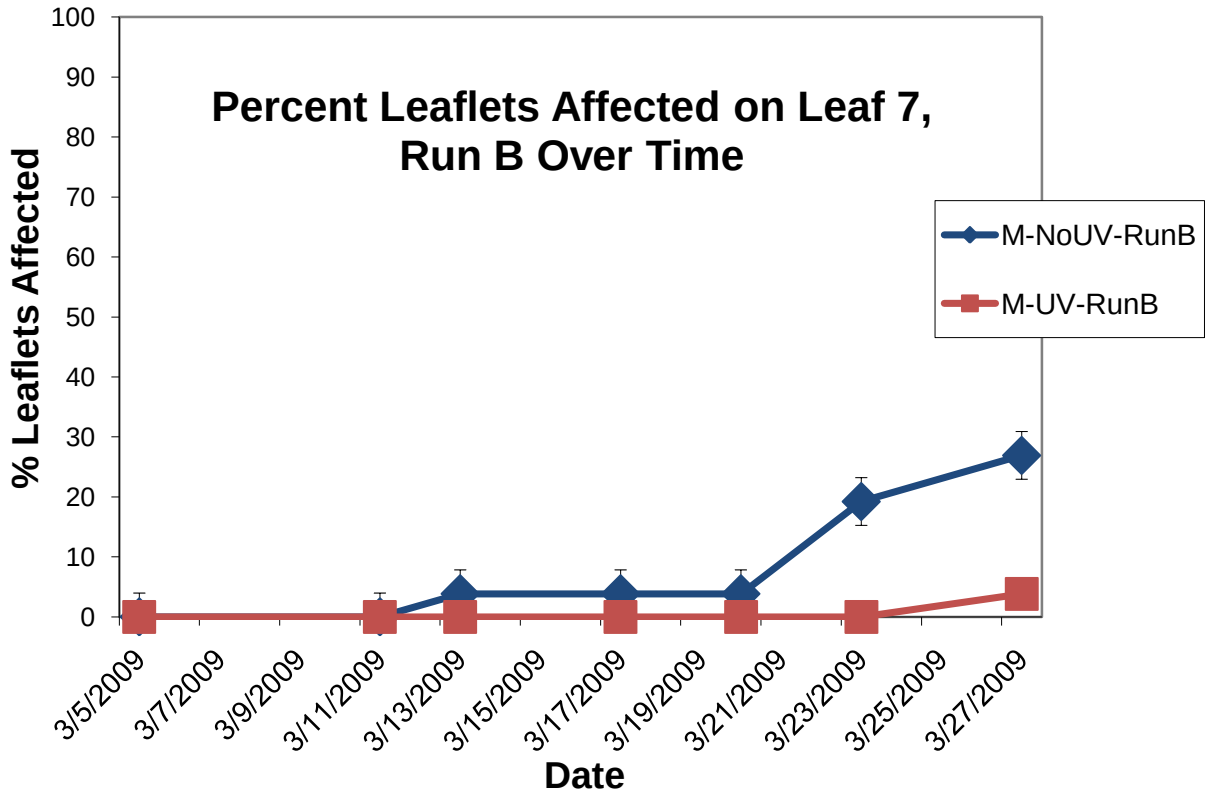

Figure 3.8 Percent leaflets affected by intumescences on 'Maxifort' tomato plants in the Spring 2009 UVB experiment. Percent leaflets affected were obtained from all 'Maxifort' plants in run B on true leaf number 7 over the course of the experiment.

Table 3.1 Intumescence damage at the end of the production period (day 67) of two tomato varieties, var. *esculentum* 'Florida 47' and var. *hirsutum* 'Maxifort' grown in either a wet or dry root medium water content environment in the Spring 2008 root medium water content experiment.

		% Damage (by sight)
Cultivar		****[z]
Water		*
Cultivar*Water		*
Cultivar*Water		
Florida 47	Dry	1.0c
	Wet	2.0c
Maxifort	Dry	28.0b
	Wet	44.0a
	$LSD_{0.05}$	7.0
Cultivar		
	Florida 47	1.0b[y]
	Maxifort	36.0a
Water		
	Dry	14.0b
	Wet	23.0a

[z] NS, *, **, ***, **** Non-significant or significant at P=0.05, 0.01, 0.001, and 0.0001, respectively.
[y] If no $LSD_{0.05}$ is listed, different letters indicate that means are significantly different based on pair-wise comparisons at α=0.05.

Table 3.2 Growth characteristics at the end of the production period (day 67) of two tomato varieties, var. *esculentum* 'Florida 47,' and var. *hirsutum* 'Maxifort,' grown in either a wet or dry root medium water content environment in the Spring 2008 root medium water content experiment.

		Height (cm)	Fresh Weight (g)	Dry Weight (g)
Cultivar		****	NS	NS
Water		NS	NS	NS
Cultivar*Water		NS	NS	NS
Cultivar * Water				
Florida 47	Dry	62.0	402.5	46.9
	Wet	65.0	570.8	56.2
Maxifort	Dry	153.3	494.0	68.7
	Wet	175.3	719.8	82.7
Cultivar				
Florida 47		63.5b[y]	486.6	51.5
Maxifort		164.3a	606.9	75.7
Water				
	Dry	107.6	448.3	57.8
	Wet	120.1	645.3	69.5

[z] NS, *, **, ***, **** Non-significant or significant at P=0.05, 0.01, 0.001, and 0.0001, respectively.
[y] If no $LSD_{0.05}$ is listed, different letters indicate that means are significantly different based on pair-wise comparisons at α=0.05.

Table 3.3 Fruit yield at the end of the production period (day 67) of two tomato varieties, var. *esculentum* 'Florida 47' and var. *hirsutum* 'Maxifort,' grown in either a wet or dry root medium content environment in the Spring 2008 root medium water content experiment.

		Total Fruit Number	Fruit Fresh Weight (g)
Cultivar		*[z]	****
Water		NS	NS
Cultivar*Water		NS	NS
Cultivar*Water			
Florida 47	Dry	15	355.0
	Wet	17	712.0
Maxifort	Dry	10	2.4
	Wet	5	2.0
Cultivar			
	Florida 47	16a[y]	533.5a
	Maxifort	7b	2.2b
Water			
	Dry	13	178.7
	Wet	11	357.0

[z] NS, *, **, ***, **** Non-significant or significant at P=0.05, 0.01, 0.001, and 0.0001, respectively.
[y] If no $LSD_{0.05}$ is listed, different letters indicate that means are significantly different based on pair-wise comparisons at α=0.05.

Table 3.4 Growth characteristics at the end of the production period (day 46) of two tomato varieties, var. *hirsutum* 'Maxifort,' and var. *esculentum* 'Trust,' grown in either a wet or dry root medium water content environment in the Spring 2009 UVB light experiment.

			Height (cm)	Fresh Weight (g)	Dry Weight (g)
Run			NS[z]	*	NS
Cultivar			**	NS	**
UV Treatment			NS	NS	NS
Cultivar*UV			NS	NS	NS
Cultivar*UV	Maxifort	No UV	63.3	156	16.5
		UV	67.4	154	16.8
	Trust	No UV	52.8	137	12.6
		UV	51.4	157	15.1
UV Treatment		No UV	58.0	147	14.6
		UV	59.4	156	15.9
Cultivar		Maxifort	65.3a[y]	155	16.7a
		Trust	52.1b	147	13.8b

[z] NS, *, **, ***, **** Non-significant or significant at P=0.05, 0.01, 0.001, and 0.0001, respectively.
[y] If no $LSD_{0.05}$ is listed, different letters indicate that means are significantly different based on pair-wise comparisons at α=0.05.

Table 3.5 Intumescence damage at the end of the production period (day 46) on two tomato varieties, var. *hirsutum* 'Maxifort' and var. *esculentum* 'Trust' grown in either a wet or dry root medium water content environment in the Spring 2009 UVB light experiment.

			Total Leaves Affected	% Leaves Affected	Total Leaflets Affected	% Leaflets Affected
Run			NS[z]	NS	NS	*
Cultivar			****	****	****	****
UV Treatment			****	****	****	****
Cultivar*UV			****	****	****	****
Cultivar*UV	Maxifort	No UV	8.3	60.5	39.5	23.4
		UV	1.5	11.9	3.6	2.2
	Trust	No UV	0	0	0	0
		UV	0	0	0	0
UV Treatment		No UV	4.1a[y]	30.3a	19.8a	11.7a
		UV	0.8b	6.0b	1.8b	1.1b
Cultivar		Maxifort	4.9a	36.2a	21.6a	12.8a
		Trust	0b	0b	0b	0b

[z] NS, *, **, ***, **** Non-significant or significant at P=0.05, 0.01, 0.001, and 0.0001, respectively.
[y] If no $LSD_{0.05}$ is listed, different letters indicate that means are significantly different based on pair-wise comparisons at α=0.05.

Appendix A - Root Medium Nutrition over Time

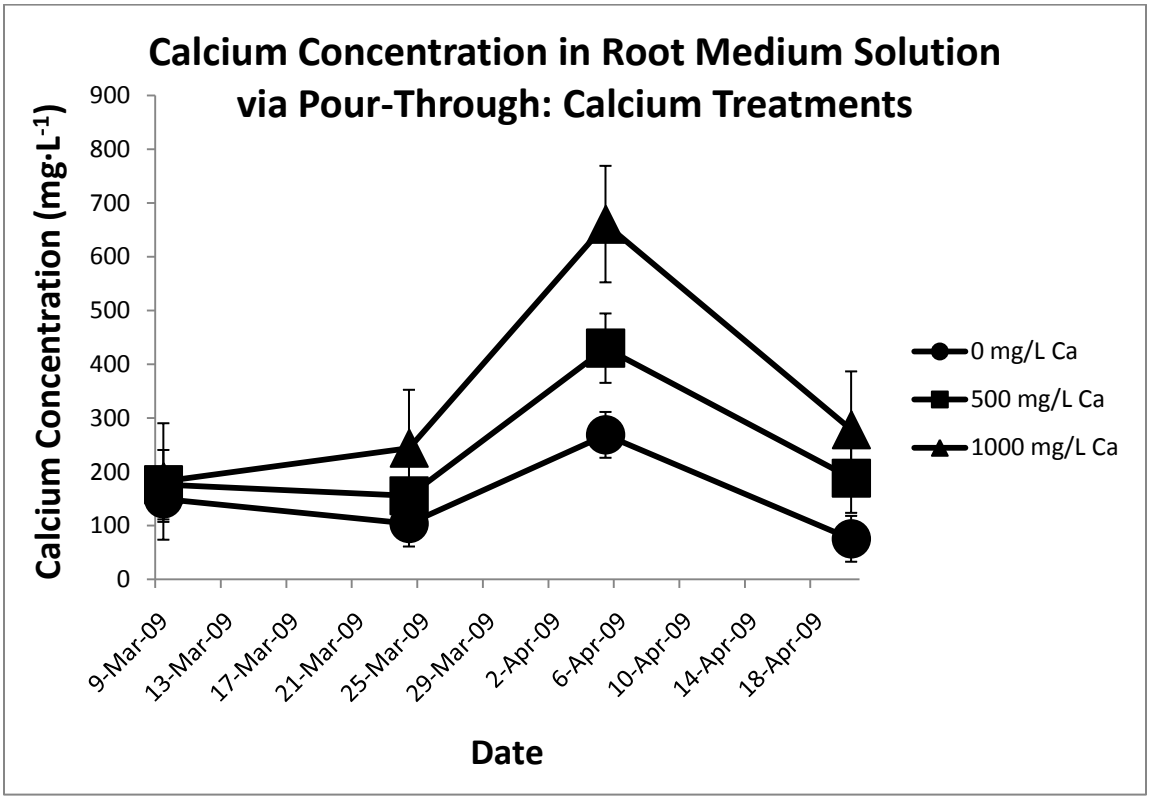

Figure A.1 Average calcium concentration in the supplemental calcium treatments' root media solutions taken using the pour-through method in the Spring 2009 experiment. A full experiment root media leach occurred on 10 April 2009 (Day 52).

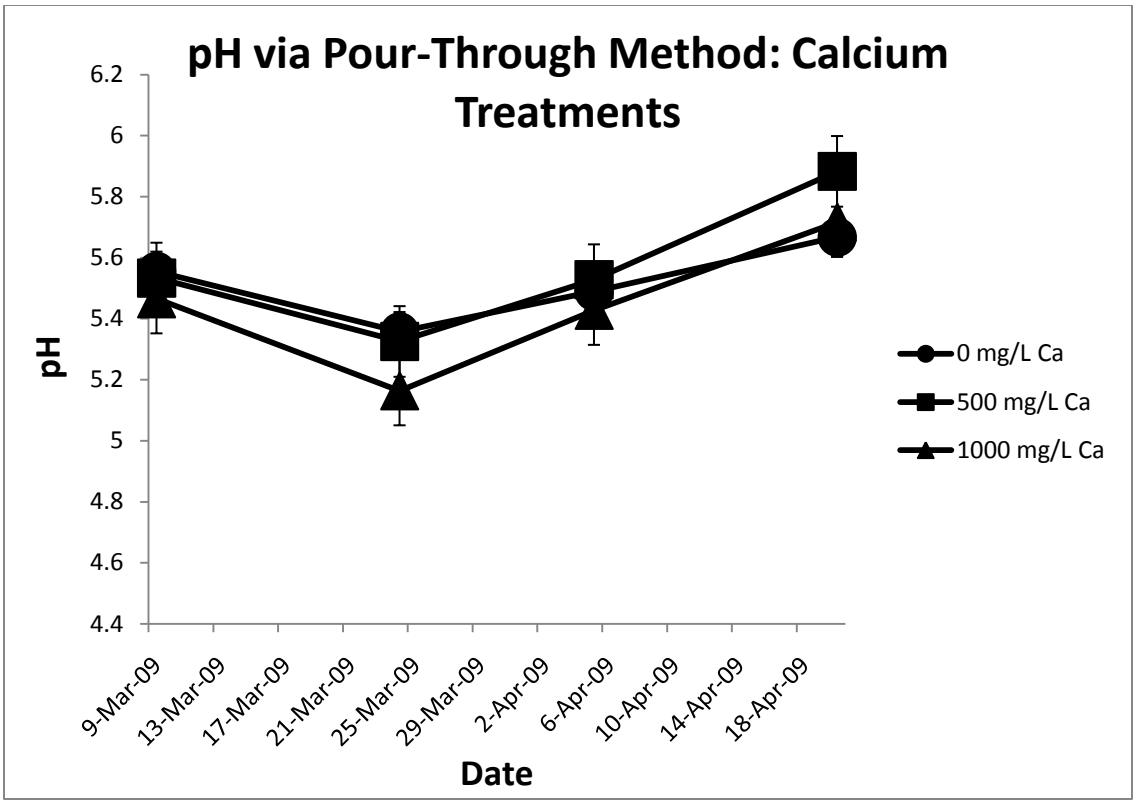

Figure A.2 Average pH in the supplemental calcium treatments' root media solutions taken using the pour-through method in the Spring 2009 experiment. A full experiment root media leach occurred on 10 April 2009 (Day 52).

Appendix B - Details of Scanning Procedures used to Quantify Edema

All leaves from every plant in the Summer 2008, Fall 2008 and Spring 2009 experiments with ivy geraniums were scanned into bitmap (.bmp) files (example: "treatment.bmp") at a high resolution of 600 dots per inch (dpi) against a white background. The scanner used was an Epson Perfection 4490 Photo (Seiko Epson Corp., Nagano, Japan) flatbed scanner. Two different methods were used to quantify edema damage on the leaves depending on whether the edemata had formed necrotic callused lesions (Summer and Fall 2008; 'Color Range Method') or whether the edemata were similar in color to the leaf tissue (Spring 2009; 'Lasso Method'). The 'Color Range Method' used the color range feature of Adobe Photoshop 7.0 (Adobe Systems Inc., San Jose, Ca). The 'Lasso Method' used the Lasso tool in Photoshop to outline and remove areas of damaged oedema that the color range was unable to pick up. Both are useful, but have drawbacks. The Color Range Method is not useful if the damage is the same color as other leaf tissues, but allows for overall damage to be quantified. The Lasso Method does not rely on specific colors, but on human recognition of damage and gives an analysis of area affected but requires significantly more time to process.

Color Range Method

A color range to outline and remove damaged areas was created by opening a test scan with oedema damage in Adobe Photoshop. The steps are as follows:

1. Select a Color Range
    a. Open the scan in Adobe Photoshop
    b. Zoom in on a damaged area close enough to see individual pixels
    c. Select "Select" > "Color Range" in the upper toolbar
        i. The Color Range box will appear; to begin selecting the damaged areas, select the box with the eyedropper and the + sign
        ii. Run the eyedropper tool over damaged areas

1. Select pixels either by left clicking on individual pixels or by holding down the left mouse button and running the eyedropper over a damaged area

iii. To reduce the amount of unintended pixels being selected, decrease the "Fuzziness" which is located in the Color Range box, near the top

iv. As pixels are selected from the damaged area, check the black-and-white image in the color range box as it is a good indicator of the areas being selected for ultimate removal

2. Once the color range is set, save it by using the "Save" button in the color range box
    a. It usually requires multiple color ranges and two to three test scans to optimize the range for the treatment scans; however, once chosen, only one range should be used for an entire experiment

Once an appropriate color range has been selected, the oedema on the treatment scans can be eliminated via whiting the areas out, leaving whitespace which can then be analyzed by software. Procedures to remove the damaged tissue are as follows:

1. Open the treatment scan in Adobe Photoshop
2. Apply the Color Range saved from the last steps
    a. To open a Color Range, go to "Select" > "Color Range;" in the color range box, click the "Load" button and find the color range previously saved and tested; hit OK
    b. This will cause areas on the leaves to be surrounded by moving dotted lines; these lines should be enclosing the damaged areas. If they are not and if a significant amount of undamaged tissue is included, a new color range should be established
3. White out the enclosed areas by selecting "Edit" > "Fill." A Fill box will appear; in the drop down "Use:" menu at the top, select "White", and hit the OK button on the side
    a. All of the areas selected from the color range will now be white and the scan will look like the damage has been cut out
4. Save the new white-holed image as a Tiff file (.tif) – example filename: "treatment-white.tif"

With the damage now removed and white holes in its place, the scans can now be opened into Scion Image, where analysis on leaf area and damaged area can be performed. However, our scans were too big for Scion to handle which resulted in the program crashing, so the white-holed images' resolution was reduced to 75 dpi in Photoshop prior to opening them in Scion. To reduce the resolution (and thus the file size) in Photoshop:

1. Open the white-holed image
2. Select "Image" > "Image Size;" the Image Size box will pop up; in the "Resolution" numeral box, reduce the resolution to 75 dpi or other appropriate level
    a. This step resulted in the added advantage of reducing the amount of "noise" (pixels of undamaged tissue) from the color range as individual pixels from the high resolution are eliminated and are condensed together when the scan is saved at the lower resolution
3. Save the reduced image as a .tif file (example: "treatment-white-resize.tif")

Now Scion Image can be used to analyze the scans for leaf area and damaged area.

1. Open Scion Image
2. Increase the "Undo and Clipboard Buffer" size by selecting "Options" > "Preferences" from the upper toolbar and putting 99999 in the numeral box
    a. This allows for larger file sizes to be used
    b. The program will indicate that Scion Image must be restarted for the changes to take place; however, this is neither necessary nor recommended. In our experience, Scion did not save this preference, so it was lost every time Scion was closed.
3. Open the white-holed treatment file in Scion Image
    a. Two copies of the image will come up, one color and one grayscale; close the color image, as the grayscale image will be used for analysis
4. Threshold the image
    a. In the upper toolbar: "Options" > "Threshold" the threshold image will be black and white with the damaged areas showing as white and the normal tissue showing as black – this is necessary for the program to run the analyses
5. Set the scale to a useful unit

a. In the upper toolbar: “Analyze” > “Set Scale” then choose the units you wish to use in the drop down “Units” menu
    i. Pixels were used in our work because it is the smallest unit possible, reducing rounding errors, and could later be converted to cm^2 using the resolution (example: 75 dpi = 75 pixels/inch; so (total pixels/75^2)*2.54^2 = cm^2)
b. This “Set Scale” step must be repeated every time a new image is opened

6. Analyze the total leaf area
    a. In the upper toolbar: “Analyze” > “Analyze Particles” -- the “Particles Analysis Options” box will appear; check the boxes labeled “Outline Particles,” “Include Interior Holes,” and “Reset Measurement Counter”
        i. This often changes the image; undo the changes by selecting “Undo Editing” in the “Edit” menu of the upper toolbar and re-threshold the image
    b. Go back to “Analyze” in the upper toolbar and select “Show Results”
        i. A box will pop up giving the total pixels in each ‘particle;’ copy results in this box and paste them into a spreadsheet
            1. Along with the total pixels, mean density is also reported; this feature was not used and can be removed from the area box by going to “Analyze”> “Options” and un-checking the box marked “Mean Density” – you only have to do this once each time Scion is opened
7. Analyze the leaf area without the damaged areas
    a. This is almost exactly the same as step 6, only this time in the “Analyze Particles” box, uncheck “Include Interior Holes”
    b. Again, copy results in this box into a spreadsheet for further analysis
8. Analyzing the results
    a. With the information now in the spreadsheet, getting the percent damaged area is as simple as adding up the pixels from both versions of the scans (the column with the interior holes counted and the column with the interior holes not counted)

then dividing the sum of those without interior holes by the sum of those with interior holes, subtracting the number from one and multiplying by 100:

i. % Damage = 1-(area with damage removed/area without damage removed)*100

Lasso Method

1. Open the scan to be analyzed in Adobe Photoshop
    a. Select the Lasso tool on the left-hand tool bar
        i. The tool looks like a little lasso, or a comic text bubble
    b. Use the Lasso tool by clicking and dragging the mouse around the affected area
        i. To select multiple areas, hold down the shift key before and during each selection
    c. Once all of the damaged areas on all of the leaves on the scan have been selected, copy them by using Ctrl C (the control key plus the C key on your keyboard)
    d. Open a new project
        i. Click on "File" > "New" in the upper tool bar
        ii. Name the project something like "treatment-cutout"
    e. Copy the cutouts of the affected area onto the new project by using Ctrl V (the control key plus the V key on your keyboard)
    f. Save the project
        i. Save the file using "Save As" function
            1. The file type should be a TIFF (.tif)
            2. Use a slightly different name than the original scan; example: treatment-cutout.tif
    g. As with the Color Range Method, the image size will need to be reduced to work in Scion
        i. In this method, *both* the original and the "cutout" files will need to be reduced following the aforementioned method
            1. Remember, if the originals will be kept as they were, do a "Save As" and save with a different name (treatment-resize.tif for

example), otherwise your originals will be reduced in size and it might be difficult to use in the future

2. Analyze the scan using Scion
    a. Open the original scan (reduced in size) in Scion
        i. Set the scale to pixels or other units of choice
            1. In the upper toolbar: “Analyze” > “Set Scale” then choose the units using the drop down “Units” menu
        ii. Set up the analysis the program will run by following “Analyze”> “Options” and un-checking the box marked “Mean Density;” you only have to do this once each time Scion is openedThreshold the image: “Options” > “Threshold”
        iii. Run the analysis: “Analyze” > “Analyze Particles”
            1. Make sure “Include Interior Holes” and “Reset Measurement Counter” are checked and click OK
                a. This will cause the image to look like the leaves disappeared and are outlined in grey, which is supposed to happen
        iv. Access the analysis by “Analyze” > “Show Results”
            1. This will bring up a window with a column of numbers; use Ctrl C to copy this column and paste it into a spreadsheet
3. Analyze the “cutout” scan following these exact same directions
    a. Instead of Scion running an analysis with and without the white space within the leaves being counted and analyzed, the “cutout” version of the original scan replaces the scan with white space from the ‘Color Range Method’

Appendix C - Preliminary UVB Experiments and Intumescence Age Effects on 'Maxifort' Tomato

Preliminary UVB Experiments

Spring 2008 Geranium UVB Experiment

UVB fluorescent lighting was set 33.5 cm above the height of the bench with the UV-absorbing plastic of the UV-blocked treatment set directly underneath. Otherwise, the apparatus set-up was the same as mentioned in the Materials and Methods in Chapter 2 of this thesis. The plants included three cultivars of ivy geranium (*Pelargonium peltatum* L'Herr ex. Ait.), 'Amethyst 96,' 'Lambada' and 'Sybil Holmes.'

This experiment began on 21 April 2008 (day 1). Within five days (25 April 2008, day 5), plants in the UV-supplemented treatment were turning "tan" (sunburn) with the effects of the UV lights. On 30 April 2008 (day 9), leaves of geraniums in the UV supplemented treatment were necrotic and falling off and whole plants were senescing. Edema of ivy geranium leaves was occurring despite the supplementation of UVB light.

Summer 2008 Geranium UVB Experiment

Lights in the UV apparatus were raised to 49 cm above the bench surface to prevent sunburn of leaves. The same three cultivars of ivy geranium were used as in the Spring 2009 geranium UVB experiment were used in this experiment. This experiment began 13 May 2008 (day 1) when the plants were placed in the UV apparatus. Sunburn began three days later (day 4, 16 May 2008) when leaves of plants in the UV-supplemented treatment began turning brown. By day 8, most of the plants in the UV-supplemented treatment were necrotic, if not fully senescent and the experiment was terminated. Again, despite the UV-supplementation, all leaves tracked in this experiment had similar amounts of edema damage. The faster occurrence of sunburn on this crop may be due to ambient levels of UV-light in the greenhouse being higher as a result of the season.

Summer 2008 Tomato UVB Experiment I

A trial experiment looking at the effect of UVB on tomato intumescence development was attempted. Two cultivars of tomato (*Solanum lycopersium* L.), 'Maxifort' (var. *hirsutum*) and 'Florida-47' (var. *esculentum*), were placed in the UV-apparatus with the same dimensions as the Summer 2008 geranium experiment. The experiment started 30 June 2008 (day 1). On day 3, the tomato plants in the UV-supplemented treatment began turning brown because of sunburn. By day 4, the plants in the UV-supplemented treatment were necrotic and senescing and the experiment was terminated.

Summer 2008 Tomato UVB Experiment II

Concurrently with Summer 2008 tomato experiment I, a study of possible ways to prevent sunburn of plants was attempted. In this experiment, the UVB-emitting lights were raised to 87.6 cm above the bench surface and plastic light diffusers were placed over half the UV-apparatus; so the diffusers covered one half of the plants in the UV-supplemented treatment and one half of those in the UV-blocked treatment. Over the other half, plastic film without UV-absorbing properties was placed between the lights and the tops of the plants.

Plants in the UV-supplemented treatment with the light diffusers and those in the UV-blocked treatment did not show signs of sunburn throughout the experiment with the termination of the experiment on 18 July 2008. Plants in the UV-supplemented treatment with the plastic film without UV-absorbing properties between them and the UVB-emitting lights began turning brown on day 12 of the experiment and were necrotic and senescing by the termination of the experiment.

Fall 2008 Tomato UVB Experiment

In this experiment, lights in both UV-apparatuses were raised to 87.6 cm above the bench surface and the plastic light diffusers were placed between the plants and the UVB-emitting lights in all treatments. This preliminary study began on 26 August 2008 and included the tomato cultivars 'Maxifort' and 'Trust.' By 7 November 2008, 'Maxifort' plants in the UV-blocked treatment had intumescences on their leaves ('Trust' was resistant to intumescence development), while plants in the UV-supplemented treatment showed no signs of intumescence development or sunburn. This preliminary study was terminated on 14 November 2008.

The diffusers proved to be vital to this experiment. It is our thought that the UV-emitting lights were providing UVB light at too direct of an angle to the plant surfaces. The use of the diffusers diffused this light, enabling it to pass through, but hitting the plant surfaces at an angle the plant could withstand, acting almost as if the ozone layer to the sun's rays would in the wild. This set-up is what the Spring 2009 tomato UVB experiment used, as explained in Chapter 2 of this thesis.

This system was also what was used in run C in the Summer of 2009. However, due to degradation of our plastic diffusers and the age of the UVB lights, the UV system was no longer effective in preventing intumescence development. As a result, similar levels of intumescence development were seen between those in the UV-blocked and UV-supplemented experiments. Data from this experiment has been included in Appendix D (Table D.3).

Appendix D - Raw Data All Tomato Experiments

Tomato Root Medium Water Content Spring 2008

Table D.1 Raw data from the Spring 2008 root medium water content experiment. Cultivars included 'Maxifort' (M) and 'Florida-47' (F). There were four replications of two water content treatments, dry (D) and wet (W). Data collected included percent foliage damaged determined using visual analysis, plant heights (cm), fruit number, fruit weight (g), total fresh weight (g), total dry weight (g) and tissue calcium levels.

Cultivar	Water Status	Rep	% Damage	Plant Height	Fruit #	Fruit Wt (g)	Total FWt (g)	Total Dry Wt (g)	µg/g Ca	Ca Uptake
M	D	1	0.25	158	15	3	470	68.01	12036	818568.36
M	D	2	0.2	165	17	6	456	63.8	15743	1004403.4
M	D	3	0.25	161	8	0.5	588	82.46	14448	1191382.08
M	D	4	0.4	129	0	0	462	60.61	16281	986791.41
M	W	1	0.5	180	0	0	517	35.06	20661	724374.66
M	W	2	0.35	197	13	7	1100	155.13	22342	3465914.46
M	W	3	0.4	197	5	1	955	104.27	18221	1899903.67
M	W	4	0.5	127	0	0	307	36.45	8412	306617.4
F	D	1	0.01	45	10	355	352	37.37	23363	873075.31
F	D	2	0.01	77	20	320	493	56.34	18324	1032374.16
F	D	3	0.02	67	18	457	337	42.21	22763	960826.23
F	D	4	0.01	59	13	288	428	51.5	28769	1481603.5
F	W	1	0.01	61	27	1181	725	76.57	20556	1573972.92
F	W	2	0.03	71	10	605	566	53.01	28353	1502992.53
F	W	3	0.01	47	14	570	535	55.05	23171	1275563.55
F	W	4	0.01	81	16	492	457	40.25	.	.

Tomato UVB Experiment Spring 2009 Raw Data

Table D.2 Raw data from the termination of the Spring 2009 tomato UVB experiment. Two cultivars, 'Maxifort' (M) and 'Trust' (T) in two UV treatments with four replications in each of two runs. Growth data included height, fresh weight, dry weight and total leaf and leaflet numbers. Intumescence data included total number of leaves affected per plant and total number of leaflets affected per plant.

CV.	UV	Run	Rep	Height (cm)	Height AVE	Height STDEV	Fresh Wt (g)	Fresh Wt AVE	Fresh Wt STDEV	Dry Wt	Dry Wt AVE	Dry Wt STDEV	Total # of Leaves	Tot Leaf AVE	Tot Leaf STDEV	Total # Leaflets	Tot Lflt AVE	Tot Lflt STDEV	#Leaves Affected	Lfs Affected AVE	Lfs Affect STDEV	# Leaflets Affected	Lflts Affected AVE	Lflts Affected STDEV
M	NoUV	A	1	66	67.5	7.3258	156	154	7.1647	16.88	16.158	3.799	13	13.25	0.95743	165	162.75	9.7425	8	8.5	2.0817	54	49	14.8997
M	NoUV	A	2	75			161			16.81			14			172			11			60		
M	NoUV	A	3	58			155			20.02			14			165			9			55		
M	NoUV	A	4	71			144			10.92			12			149			6			27		
M	NoUV	B	1	24	59	25.443	166	158.5	16.299	16.35	16.878	1.6374	13	14	1.1547	184	176	17.493	9	8	1.8257	28	30	6.68331
M	NoUV	B	2	63			151			19.24			15			178			6			23		
M	NoUV	B	3	85			177			16.46			15			191			10			39		
M	NoUV	B	4	64			140			15.46			13			151			7			30		
M	UV	A	1	59	64.75	4.9244	136	137.5	9.1469	17.24	15.243	3.7815	13	11.5	1.29099	158	153	7.3937	1	1.25	1.2583	1	2.5	2.38048
M	UV	A	2	71			126			10.5			10			144			0			0		
M	UV	A	3	64			140			14.1			11			160			3			5		
M	UV	A	4	65			148			19.13			12			150			1			4		
M	UV	B	1	73	70	6.6833	159	170.8	9.6047	16.18	18.41	2.7569	12	14	1.41421	162	184	15.427	3	1.75	0.9574	12	4.75	4.99166
M	UV	B	2	64			167			20.79			15			189			2			4		
M	UV	B	3	78			180			15.87			15			187			1			1		
M	UV	B	4	65			177			20.8			14			198			1			2		
T	NoUV	A	1	48	51	4.761	145	140.8	16.112	13.37	14.1	1.1117	11	11.5	0.57735	127	127	8.2865	0	0	0	0	0	0
T	NoUV	A	2	54			144			15.07			11			116			0			0		
T	NoUV	A	3	56			156			15.03			12			129			0			0		
T	NoUV	A	4	46			118			12.93			12			136			0			0		
T	NoUV	B	1	57	54.5	5	127	133	26.242	10.18	11.175	2.8947	9	10	1.82574	127	120.25	19.822	0	0	0	0	0	0
T	NoUV	B	2	57			143			9.61			11			135			0			0		
T	NoUV	B	3	47			162			15.49			12			128			0			0		
T	NoUV	B	4	57			100			9.42			8			91			0			0		
T	UV	A	1	39	47.75	5.909	151	140.8	29.341	15.2	13.388	3.4354	11	11	0.8165	133	115	20.801	0	0	0	0	0	0
T	UV	A	2	50			108			8.75			10			96			0			0		
T	UV	A	3	52			128			12.98			11			98			0			0		
T	UV	A	4	50			176			16.62			12			133			0			0		
T	UV	B	1	53	55	1.8257	176	173	5.2915	19.49	16.733	2.1975	12	11.5	1	152	139	22.106	0	0	0	0	0	0
T	UV	B	2	56			166			14.84			12			147			0			0		
T	UV	B	3	57			178			17.51			12			151			0			0		
T	UV	B	4	54			172			15.09			10			106			0			0		

Tomato UVB Experiment Summer 2009 Raw Data

Table D.3 Raw data taken at the termination of the Summer 2009 UVB experiment "run C." Two cultivars, 'Maxifort' (M) and 'Trust' (T) in two UV treatments with four replications. Growth data included heights, fresh weights, dry weights, total number of leaves per plant and total number of leaflets per plant. Intumescence data includes total leaves affected per plant and total leaflets affected per plant.

Cult	UV	Run	Rep	Height (cm)	Height AVE	Height STDEV	Fresh Wt (g)	Fresh Wt AVE	Fresh Wt STDEV	Dry Wt (g)	Dry Wt AVE	Dry Wt STDEV	Total Lf #	Total Lfs AVE	Total Lfs STDEV	Total Lflts	Total Lflts AVE	Total Lflts STDEV	Lfs Affected	Lfs Aff AVE	Lfs Aff STDEV	Lflts Affected	Lflts Aff AVE	Lflts Aff STDEV
M	NoUV	C	1	60	60.5	4.5092	10	66	38.288	9.44	9.565	2.1038	11	11.5	1.290994	172	187.5	15.438	10	9.75	1.2583	117	135	18.44813
M	NoUV	C	2	66			73			7.17			10			178			8			123		
M	NoUV	C	3	61			88			9.35			13			194			11			140		
M	NoUV	C	4	55			93			12.3			12			206			10			158		
M	UV	C	1	69	74.75	5.058	131	128.3	12.093	12.1	12.555	0.6191	13	12	0.816497	206	199.3	7.2744	8	7.25	0.9574	95	102	15.02221
M	UV	C	2	73			114			13.3			11			204			8			124		
M	UV	C	3	76			143			12			12			190			6			93		
M	UV	C	4	81			125			12.8			12			197			7			94		
T	NoUV	C	1	68	72.25	4.6458	203	180	39.48	24.3	19.343	4.1998	17	16.3	0.5	196	179.3	11.758	0	0	0	0	0	0
T	NoUV	C	2	74			201			20.3			16			178			0			0		
T	NoUV	C	3	78			195			18.6			16			169			0			0		
T	NoUV	C	4	69			121			14.2			16			174			0			0		
T	UV	C	1	75	73.5	1.7321	190	185.3	7.8049	17.4	19.275	2.9449	18	17.5	1	191	183.8	10.243	0	0	0	0	0	0
T	UV	C	2	71			186			20.6			18			193			0			0		
T	UV	C	3	74			191			22.8			18			180			0			0		
T	UV	C	4	74			174			16.4			16			171			0			0		

Tomato UVB Experiment Spring 2009 UV Meter Raw Data

Table D.4 Raw UV-meter data from two instruments, one from Spectrum (Field Scout 3414 Ultraviolet Light Meter; Spectrum Technologies Inc., Plainfield, IL), the other from Mannix (Mannix UV-340 UV Light Meter; General Tools and Instruments Co. New York, NY). Readings were taken over the course of the Spring 2009 Tomato UVB experiment.

Spectum Units: $\mu mol \cdot m^{-2} \cdot s^{-1}$

	21-Jan	30-Jan	6-Feb	16-Feb	20-Feb	27-Feb	3-Mar	13-Mar	20-Mar	26-Mar
No UV 1	4.975	11.125	5.775	9.9	13.65	6.725	8.975	9	1.175	5.275
UV 1	11.8	20.15	13.4	15.55	34.1	15.75	22.925	14.275	3.25	12.075
No UV 2	7.55	18.475	8.1	17.7	21.65	10.15	11.95	7.575	1.2	5.725
UV 2	20.425	41.8	21.925	32.3	21.65	24.8	22.675	14.925	4.65	11.475
Ambient	.	.	.	.	.	.	23.8	37.875	11.9	37.6

Mannix Units: $\mu W/cm^2$

	21-Jan	30-Jan	6-Feb	16-Feb	20-Feb	27-Feb	3-Mar	13-Mar	20-Mar	26-Mar
No UV 1	142.5	123.75	148.75	289.75	384.25	205.5	275.5	194.25	76.5	235.25
UV1	304	238.5	277	389	613.25	356.5	498	327	134	363.25
No UV 2	200.75	106.5	182.25	345.75	479	262.25	364.75	211	71.5	232.75
UV 2	404.5	276.25	415	572	713.5	481.25	599.75	332.5	158.75	361.5
Ambient	.	.	.	.	.	.	335	528.75	258	732