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Causes of whitening of ivy geraniums (Pelargonium peltatum)

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CAUSES OF WHITENING OF IVY GERANIUMS (*Pelargonium peltatum)*

By

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The development of whitening of the youngest leaves of actively growing ivy geranium (*Pelargonium peltatum* L.) has been observed as the season changes from late spring to summer. This study was conducted to determine the specific environmental causes of whitening, if micronutrients deficiencies cause similar whitening, whether low night temperatures can reverse whitening, and whether salicylic acid affects growth and whitening in ivy geraniums. Two cultivars, 'Beach' and 'Butterfly', with different susceptibility to whitening were chosen for this study. Elevated air temperature, but not elevated root-zone temperature, was found to be the environmental cause of whitening in ivy geranium. Elevated air temperatures severely reduced plant growth, leaf area, fresh weight, and dry weight in both cultivars. Elevated air temperature reduced photosynthetic pigments and their ratios in ivy geranium. Carotenoids and pheophytins decreased in 'Butterfly' at elevated air temperature. Foliar total Fe levels indicated no inhibition of Fe-uptake at elevated temperatures. Applications of Fe-chelate at elevated

temperatures helped chlorophyll synthesis in ivy geraniums. Deficiency treatments of all micronutrients, Fe, Mn, Zn, S or Mg did not result in whitening in either cultivar of ivy geraniums. Salicylic acid did not affect whitening of ivy geraniums. It did not affect growth, leaf area, fresh or dry (stem, leaf or total) weight, fresh: dry weight ratio, leaf area ratio, specific leaf area or foliar nutrient (Fe, Mn, Zn, Mg and S) content in either cultivar. Cultivars varied in their response to low night temperature. 'Beach' reduced its plant growth and fresh to dry weight ratio whereas 'Butterfly' did not. Fe-chelate application did not reduce growth, leaf area, fresh weight, dry weight or fresh:dry weight ratio of either cultivar. Although Fe-application did not reduce whitening in ivy geraniums, it helped to preserve chlorophyll, particularly chlorophyll b as indicated by Chl a:b ratio in 'Beach'. Whitening in ivy geranium is a heat stress response initially exhibited by young, developing leaves and is caused by elevated air temperatures. Whitening is the result of impaired photosynthetic pigments synthesis and/or degradation.

DEDICATION

I would like to dedicate this dissertation to my father, Mr Jugal Kishore Dhir and my mother, Mrs Shanti Devi. Without their inspiration, encouragement and sacrifices, this chapter of my life has never been possible.

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CHAPTER I

INTRODUCTION

Geranium is an important floriculture crop worth $32 million wholesale and retail in 2001 (Economic Research Service, 2002). Ivy geranium, *Pelargonium peltatum* L. belonging to the Geraniaceae, is native to South Africa. In the United States, it is mainly grown in hanging baskets, window boxes and as potted plants. Ivy geranium is a very popular plant in the northern states of the U.S. Ivy geranium does not perform well in southeastern U.S. summers. The leaves turn white and expand less, having upward cupping as the season changes from late spring to summer. This physiological disorder is referred to as 'whitening'.

High growing temperature induced iron deficiency has been suspected as the cause of this whitening disorder. There have been reports that treating ivy geranium with iron chelate prior to summer prevents whitening (Personal communication, Harvey Lang, Fischer, USA). In addition, plants exhibiting this disorder under high temperatures resume normal growth as the temperature moderates. Thus, there is observational evidence that high temperature and iron interact to cause this extreme chlorosis (i.e. whitening). Neither the actual cause for this problem nor the role of iron in preventing whitening is known.

Iron (Fe) plays an important role in plant nutrition. Fe is involved in numerous physiological processes, such as photosynthesis, respiration, DNA synthesis, hormone formation and nitrogenase activity (Rivero *et al*, 2003; Havlin *et al*, 2003). Iron is an immobile element in plants, so its deficiency symptoms first appear in the youngest expanding leaves. Those leaves become chlorotic (yellow) initially, and then completely white under severe Fe deficiency. Temperature stress has been reported to cause Fe deficiency in many plants. Lettuce plants grown under hot ambient root-zone temperatures normally show symptoms of iron deficiency (He and Lee, 1998). Concentration of leaf N, P, Fe and Mn of cucumber plants decreased when root zone temperature was raised to 35^{o}C (Du and Tachibana, 1994). Research on Fe nutrition in relation to temperature has been done on many crops. However, there appears to be no literature available on this disorder in ivy geraniums. Objectives of this study were:

- to determine the environmental cause of whitening in ivy geraniums;
- to determine the effects of Fe-chelate applications on growth of ivy geranium;
- to determine whether whitening is heat stress related to iron deficiency or just heat stress;
- to determine if other micronutrients and their deficiencies cause similar whitening in ivy geraniums;
- to determine whether low night temperatures can reverse whitening in ivy geraniums;
- to determine whether salicylic acid affects growth and whitening in ivy geraniums.

CHAPTER II

LITERATURE REVIEW

Species and cultivar description

Pelargonium peltatum L., the ivy geranium, was first discovered in Southern Africa in the Cape Province growing in sheltered locations (Holcomb and O'Donovan, 1993). Ivy geraniums have been cultivated since 1701. Hundreds of cultivar exist which differ in foliage color, leaf size, leaf shape, leaf variegation, bloom color, types, and growth habit (Holcomb and O'Donovan, 1993). Ivy geranium 'Butterfly' is characterized by the combined features of large, light-violet double flowers, very early flower response, medium green foliage with strong zonation, medium growth characteristics and fast rooting ability (Schumann, 1993). 'Beach' is characterized by the combined features of brilliant-red, double flowers, compact inflorescence borne well-above the foliage, fresh-green, slightly crenated foliage without zonation, vigorous growth and well-branched, bushy and uniform plant habit (Utecht, 2002).

Breeding background

Ivy geranium 'Butterfly' is a product of a planned breeding program with the objective of creating new geranium cultivars with violet flower color, good cultivation habit, and possibly with other unique characteristics in order to provide a possible

replacement for the well known commercial cultivar ‘Amethyst’. ‘Butterfly’ originated from a hybridization made by the inventor Ingeborg Schumann in a controlled breeding program in Spain in 1985. The female parent was an unnamed hybrid, originating from crossing a hybrid resulting from a cross of ‘Salmon Queen’ and ‘Amethyst’, with an inbred line of ‘Rouletta’. The male parent of ‘Butterfly’ was ‘Bardo Lolli’, characterized by large dark rose semi-double flowers. ‘Butterfly’ was selected in 1986 as one flowering plant within the progeny of the stated cross (Schumann, 1993).

Ivy geranium ‘Beach’ is also a product of a planned breeding program that had the objective of creating new ivy geranium cultivars with red flower color, vigorous growth, and bushy and well-branched plant habit. ‘Beach’ originated from a hybridization made by the inventor Angelika Utecht in a controlled breeding program in Spain in 1992. The female parent was a hybrid seedling, designated No. 361-7 (unpatented) characterized by dark-red, double flowers, medium-green, slightly zoned foliage and vigorous growth but poorly-branched habit. The male parent was the cultivar ‘Guimo’ (unpatented), commercially known as ‘Momo’, characterized by scarlet, single-type flowers, large foliage without zonation, good branching ability and relatively vigorous growth. The zonation of young leaves is very weak. ‘Beach’ was selected as one flowering plant within the progeny of the stated cross in 1993 (Utecht, 2002).

Whitening

During high summer temperatures, newly developing leaves of ivy geranium turn white or bleach, fail to expand fully, and have an upward cupped appearance. They resume normal growth as the temperature moderates in fall. This physiological disorder is

referred to as “whitening”. Iron deficiency has been suspected to play a role in this whitening disorder. Neither the cause or trigger for this problem nor the role of iron in preventing whitening is known.

Bleaching, the destruction of photosynthetic pigment, can occur in both higher plants and algae (Asada, 1996; Ledford and Niyogi, 2005). Bleaching is triggered by a range of environmental stressors, including temperature extremes and high irradiance (Brown, 1997; Venn *et al*, 2006). Heat-induced bleaching (i.e whitening) was observed in *Euglena gracilis* when exposed to slightly elevated (33^{o}C) temperatures (Ortiz and Wilson, 1988). Bleaching of reef corals is a phenomenon linked to temperature stress (Warner *et al*, 1996). The primary mechanism underlying temperature/irradiance triggered bleaching is thought to involve photoinhibition of *Symbiodinium* photosystems, perhaps arising from depressed levels of the D1 protein in photosystem II (Warner *et al*, 1999) or impairment in carboxylation within the Calvin cycle (Jones *et al* 1998), leading to reduced rates of electron transport. Photooxidation of protochlorophyll(ide) or chlorophyll is responsible for the chlorosis of rye leaves at 32^{o}C (Feierabend, 1977).

Temperature

High temperature is a major factor limiting growth of cool-season grasses (Xu and Huang, 2002). Different species show differences in the genetically determined range of environmentally induced photosynthetic adaptation to temperature. Photosynthetic response to high temperature can vary significantly within a species (Reynolds *et al*, 1990). For any plant in a particular environmental situation, there is a critical temperature above which heat injury occurs and only slight increases beyond this critical temperature

will result in irreversible damage (Bilger *et al*, 1984). *Solanum phureja* which is found at low altitudes on the eastern slopes of the Andes, showed a tolerance to heat comparable to that of the warm adapted clones of the common potato, the two most heat tolerant of which contained some *S. phureja* in their parentage (Hetherington *et al*, 1983).

The optimum temperature for shoot and root growth for cool season plants is between 15-24^{o}C. Severe heat injury and death of cool season grasses often occurs as temperature increase above 30^{o}C (Huang and Gao, 2000). Temperature stress can lead to inhibition of photosynthesis (Haldimann and Feller, 2004; Karim *et* al., 1999; Laekey *et al*, 2003), alterations in membrane fluidity and permeability (Alfonso *et al*, 2001; Sangwan *et al*, 2002) and inactivation of enzymes due to protein denaturation (Feierabend, 1977; Kampinga *et al*, 1995; Vierling, 1991).

Temperature and photosynthesis

Photosynthesis is the foundation of life for all plants and animals. Photosynthesis is the conversion of solar energy to chemical energy. Photosynthesis consists of light and dark (or carbon) reactions (Taiz and Zeiger, 2002). In the light reaction, photosynthetic pigments first absorb the energy of sunlight and a strong reductant is generated using electrons that have been dissociated from water during its oxidation. This reductive power is further used for electron transfer reactions which eventually generate ATP and NADPH for carbon reactions of photosynthesis. In Calvin cycle, CO_2 is reduced in the dark reactions, reacting with ribulose 1, 5-bisphosphate (RuBP) catalyzed by ribulose, 1, 5-bisphosphate carboxylase (Rubisco). Through this cycle, CO_2 is transformed into carbohydrates (Taiz and Zeiger, 2002). Photosynthesis is highly sensitive to high

temperature stress (Berry and Bjorkmann, 1980; Sharkey, 2005). Temperatures above 30^{o}C are quite common during summers in the southeastern states of the U.S (Weather Channel, 2008). Photosystem II (PSII) is the most thermo-labile aspect of photosynthesis (Berry and Bjorkmann, 1980; McDonald and Paulsen, 1997). Temperatures above 30^{o}C reduced photosynthetic rate as a result of a reduction in PSII efficiency (Kadir *et al*, 2006).

High temperature inhibits photosynthesis by several mechanisms including ionic permeability of thylakoid membranes (Havaux *et al*, 1996) or deactivation of Rubisco (Sharkey *et al*, 2001). Both increased thylakoid membrane ionic conductance and ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) deactivation have been suggested as primary causes of inhibition of photosynthesis at elevated temperatures (Schrader *et al*, 2004). At moderately high temperatures, reversible deactivation of Rubisco has been observed (Weis, 1980; Feller *et al*, 1998). Photosynthesis can be inhibited at high temperature by a reversible increase in thylakoid leakiness to protons (Pastens and Horton, 1996; Bukhov *et al*, 1999). At higher temperatures, PSII function can be affected (Berry and Bjorkman, 1980), but these effects are not rapidly reversible (Seeman *et al*, 1984). High temperatures strongly promote the process by which carbamylated Rubisco sites become inhibited or inactivated (Sharkey *et al*, 2001). The reversible decarbamylation of Rubisco at moderately high temperature may be a protective mechanism by which the plant avoids more serious effects on Rubisco and the rest of the photosynthetic apparatus (Sharkey *et al*, 2001).

In PSII, the key photosynthetic reaction consists of the transfer of an electron from the primary donor called P680 to a nearby pheophytin molecule (Groot *et al*, 1997).

Pheophytin (Pheo) was considered as a product of chlorophyll degradation in plant cells, but the photoreducible Pheo is really the natural primary electron acceptor of PSII in the early steps of photosynthetic solar energy conversion, acting between P680 and plastoquinone (Klimov, 2003). So, estimating the amount of pheophytins acts as an indication of impairment of the electron transport chain that inhibits photosynthesis.

At high temperatures, there is a decrease in the strength of hydrogen bonds and electrostatic interactions between polar groups of proteins within the aqueous phase of the membrane. Membrane disruption also causes the inhibition of processes such as photosynthesis and respiration that depend on the activity of membrane associated carriers and enzymes (Taiz and Zeiger, 2002).

Temperature and photosynthetic pigments

The most active photosynthetic tissue in higher plants is the mesophyll of the leaves. Mesophyll cells have many chloroplasts, which contain the specialized light-absorbing green pigments, the chlorophylls (Chl). Chlorophyll a (Chl a) and chlorophyll b (Chl b) are abundant in green plants. Carotenoids are also integral constituents of thylakoid membranes of chloroplasts. The light absorbed by the carotenoids is transferred to chlorophyll for photosynthesis, they are known as accessory pigments. They protect chlorophyll molecules from photo- or thermo-damage (Taiz and Zeiger, 2002). Heat-stresed chlorotic rye plants had lower levels of carotenoids. Lower carotenoids could not give sufficient protection and thus they facilitate photooxidation of chlorophyll (Feierabend, 1977).

Pigment concentrations are often used as an indirect measure of photosynthetic biomass (Hurley and Watras, 1991). Chlorophyll (Chl) stability index acts as an aid in screening heat tolerance of *Cocoa* genotypes (Ravindran and Menon, 1981). Temperatures influence chloroplast development, chlorophyll biosynthesis and the greening process (Mohanty *et al*, 2006). Chl a is more sensitive to heat than Chl b (Weemaes *et al*, 1999; Loey *et al*, 1998). A shift in the ratio of chlorophyll a and chlorophyll b is an indicator of heat bleaching in *Euglena gracilis* (Thomas and Ortiz, 1995). Chl a to b and Chl to carotenoids ratios decreased in heat-stressed plants of tomato (Camejo *et al*, 2005). Chl biosynthesis of cucumber seedlings was reduced 60% upon heat-stress due to impairment of Chl biosynthetic enzymes, in comparison to normal growth conditions (Tewari and Tripathy, 1998). Chl accumulation reduction in heat-stressed plants may be due to its impaired synthesis, its faster degradation or both. Reduced synthesis of Chl in heat-stressed plants could account for the reduced presence of light-harvesting complex photosystem II (LHCP II) in cucumber seedlings exposed to heat stress (Mohanty *et al*, 2006). Chlorosis was induced in tree fern (*Cyathea cooperi*) by 41.2^{o}C air temperature, which also resulted in reduced Chl concentration, reduced Chl a/Chl b ratio, fewer number of grana and almost completely absent stroma thylakoids (Doley, 1983). Rye plants growing at 32^{o}C were deficient in chlorophyll and chloroplastic 70-S ribosomes (Feierabend, 1977). There was a heat-induced block of photosystem II reaction centers and heat-induced block of Chl b to Chl a energy transfer, resulting in a functional disconnection of the light-harvesting complex from the reaction center complexes (Schreiber and Armond, 1978). Chl acts as an intrinsic fluorescence

probe of the thylakoid membrane and responds to the same changes which cause irreversible denaturation of photosynthetic enzymes (Schreiber and Berry, 1977).

Root-zone temperature

The ability of plants to obtain water and mineral nutrients from the soil is related to their capacity to develop an extensive root system (Taiz and Zeiger, 2002). Hood and Mills (1994) stated that maintaining root-zone temperature near 22^{o}C maximizes growth and nutrient uptake of snapdragons. Shoot and root weight and root volumes of peach seedlings increased linearly with increasing root-zone temperature to 22^{o}C (Tagliavini and Looney, 1991). High root-zone temperatures profoundly affect plant growth by reducing root growth at temperatures above 30^{o}C (Mathers, 2003). Roots are more sensitive to high temperature than shoots and a decline in root growth has been found to precede that of shoots under heat stress (Carrow, 1996; Xu and Huang, 2000a, b). At high root zone temperatures, the membrane integrity of the root is lost and the roots are injured or killed. High root-zone temperatures can result in reduced photosynthesis (Mathers, 2003). High root zone temperature (36^{o}C) reduced the leaf area, shoot dry weight and shoot water potential of red maple plants (Graves *et al*, 1989). Nutrient uptake and interaction is decreased by increased root zone temperature (Sartaz and Barthakur, 1995; Tagliavini *et al*, 1991).

Root thickening increased and mineral accumulation in lettuce plant parts decreased at root zone temperatures above 20^{o}C (Tan *et al*, 2002). Greatest nutrient uptake temperature varied with nutrient, but ranged from 15-29^{o}C in snapdragons (Hood and Mills, 1994). Du and Tachibana (1994) reported a decrease in the concentration of

Fe, Mn, Mg, and Ca in cucumber plants when root temperature was raised from 25°C to 35°C. Lettuce plants grown under high ambient root-zone temperatures normally show symptoms of iron deficiency (He and Lee, 1998a; He and Lee, 1998b). The effect of increased root-zone temperature on Fe-uptake and translocation was decreased by increases in the levels of Fe in the pretreatment cultures (Riekels and Lingle, 1966). The growth depression in peach seedlings at high temperature was related to increased young leaf chlorosis reduced shoot Fe and a possible P to Zn imbalance (Tagliavini *et al*, 1991). Translocation of iron within plant is dependent both on root temperature and presence of iron (Schmidt and Steinbach, 2000). *Plantago lanceolata* L. plants grown at 24°C decreased the rates of root-mediated Fe reduction with increasing Fe levels in the external growth solution. Such control suggests a dependence of Fe acquisition on changes in (either external or internal) Fe concentration (Schmidt and Steinbach, 2000).

Air temperature

Increased leaf temperature during summers is also a potential danger in greenhouses where low air speed and high humidity decrease the rate of leaf cooling (Taiz and Zeiger, 2002). Maintenance of transpirational cooling was an important factor associated with better summer stress performance of Kentucky bentgrass (Bonos and Murphy, 1999). Air temperature could be the cause of heat stress. Root temperatures appreciably lower than those of the shoot occur frequently both diurnally and seasonally (Schmidt and Steinbach, 2000). Different species show differences in the genetically determined range of environmentally induced photosynthetic adaptation to temperature. *Solanum* species screened for heat tolerance indicated considerable variation in the

degree of chlorosis developing as a stress response to high temperature (Reynolds *et al*, 1990). Pansy plants showed nutrient deficiencies when grown during warm periods (>18^{o}C) of the growing season. These deficiencies typically are not seen when production temperatures are optimal (<18^{o}C) even though fertility regimes may remain the same (Hamlin *et al*, 1999). Chlorosis was induced in tree fern (*Cyathea cooperi*) by 41.2^{o}C air temperature (Doley, 1983). Ferrochelatase gene expression operating at the branch site of the Fe- and Mg-porphyrin biosynthetic pathway was partially reduced suggesting reduced heme and cytochrome synthesis in temperature-stressed wheat seedlings (Tewari and Tripathy, 1998). Net photosynthesis of pea plants decreased with increasing leaf temperature up to 45^{o}C (Haldimann and Feller, 2005). Leaf temperatures up to 45^{o}C not only enhance respiratory rates and exceed optimum temperature for photosynthesis, but also lead to large vapor pressure differences between leaves and air (Jifon and Syvertsen, 2003). Heat sensitivity of photosynthetic oxygen evolution of thylakoids isolated from spinach increases by increasing the pH above a neutral value (Weis, 1982). Liu and Huang (2003) stated that high soil temperature in combination with high air temperature decreased chlorophyll (Chl) content and accelerated leaf senescence in creeping bentgrass. In contrast, reducing soil temperature at high air temperature improves shoot and root growth (Liu *et al*, 2002).

Iron

Iron (Fe) is an essential element in plant nutrition. Fe is involved in numerous physiological processes, such as photosynthesis, respiration, DNA synthesis, hormone formation and nitrogenase activity (Briat and Lobreaux, 1997; Havlin *et al*, 2003). Fe has

an important role in the formation of thylakoid membranes in higher plants. Fe deficiency reduced the number of granal and stromal lamellae per chloroplast. This is accompanied by a decrease in all membrane components, including the electron carriers in the photosynthetic electron transport chain (Spiller and Terry, 1980) and light harvesting pigments, chlorophylls and carotenoids (Morales *et al*, 1990; Morales *et al*, 1994). Fe-deficiency was found to markedly enhance thermal energy dissipation in photosystem II (PSII) antenna that was associated with the de-epoxidation of violaxanthin to zeananthin and anthrocyanin. The changes within the xanthophylls cycle (i.e. carotenoids) induced by Fe-deficiency may protect the PSII reaction centers against the excess light (Morales *et al*, 1998a). Elevated levels of Fe applied in the nutrient solution increased tissue chlorophyll content of *Pelargonium* X *hortorum* (Lee *et al*, 1996). Similar findings have been documented that high Fe (Fe-EDDHA) levels in the nutrient solution were associated with a higher concentration of leaf pigments of chile peppers (Anchondo *et al*, 2001). Fe deficiency not only reduced photosynthetic rate but also the actual PSII efficiency at steady state photosynthesis (Morales *et al*, 1998a).

Although Fe is the fourth most abundant element in the lithosphere, its presence in the soil solution is negligible due to low solubility dependent on pH. Low solubility of Fe further affects uptake and use of Fe (Briat and Lobreaux, 1997; Chen *et al*, 1998; Havlin *et al*, 2003; Lucena, 2003). Fe is often unavailable due to its presence in its oxidized form (Fe^{3+}) at higher pH. Low pH (5.5-6.0) maximizes Fe-uptake, whereas higher pH (>6.0) starts causing Fe-deficiency. The greatest influence of pH on mineral element concentration was for Fe. The concentration of Fe at a pH of 3.0 was almost three times the concentration at higher pHs (Marler, 1998). Interveinal chlorosis was

observed in seed geraniums at a pH above 6.3. Fe and Mn concentration in tissues declined at pHs above 6.3 (Smith *et al*, 2004). Fe-deficiency is most often observed in plants growing in high pHs, calcareous soils, but it may also occur on acidic soils that are very low in total Fe (Havlin *et al*, 2003). Fe-deficiency is also associated with high soil moisture and poor soil aeration (Lucena, 2003; Havlin *et al*, 2003). High soil moisture and poor soil aeration affects the reduction of Fe^{2+} and ferrous soluble species. Moreover, the dissolution rate of Fe from the solid phase, which is governed by crystallinity (reticular energy) of Fe solids and the presence of complex agents in the soil solution, is normally very slow (Lucena, 2003). NO_3^- nutrition is known to increase the pH around the roots due to efflux of HCO_3^- or OH^- from the roots. The pH increase outside the root can considerably reduce Fe availability (Lucena, 2000; Havlin *et al*, 2003).

Chelated Fe sources improved *Ixora* chlorosis when applied to the soil (Broschat, 2003). Fe-EDDHA has been considered the most effective chelate for correcting Fe deficiency at high soil pH. Fe-chelates have the capacity to maintain soluble iron in the soil solution over time (Cantera *et al*, 2002). Only 20% of $FeSO_4.7H_2O$ was DTPA extractable after just one week compared with 70% Fe-chelate after 7 weeks and 26% after 14 weeks. Chelated-Fe is protected from usual soil reactions that result in formation of insoluble Fe (III) oxides (Havlin *et al*, 2003). Adsorption of the chelating agents occurred mainly in the first day and ranged from about 10 to 75% of the total (Alvarez-Fernandez *et al*, 1997). Fe deficiencies are mainly corrected by foliar application of Fe at 7–14 day intervals (Havlin, *et al* 2003). Soil application of Fe-chelate increased concentration and uptake of Fe in soybean genotypes (Ghasemi-Fasaei *et al*, 2003). Foliar spray of Fe-chelate corrected the chlorosis of peanut leaves. Peanut productivity and Fe-

nutrition improved significantly both with soil and foliar treatment. Retention of Fe in the medium was greater with water-soluble fertilizer than with granular incorporated fertilizer. Application of micronutrients in the form of water-soluble fertilizer was recommended for reducing micronutrient deficiencies (Frost *et al*, 2003).

Iron uptake by plants

All plant species, except the grasses, have developed a mechanism of improved Fe acquisition when soluble Fe concentration is low in the media. This mechanism is referred as Strategy I Fe-efficiency mechanism. The reactions of Strategy I plants mainly include an increased capacity to release protons (H^+) from their roots, increased capacity of Fe (III)-chelate reductases to reduce Fe (III)-chelates at the root surface, and an increased tendency to form root hairs (Bienfait, 1996; Guerinot and Yi, 1994; Marschner and Romheld, 1994; Romheld, 1987; Vizzotto *et al*, 1999). The roots of Strategy I plants release more protons when they are iron-deficient, lowering the rhizosphere pH and thereby increasing the solubility of Fe^{3+} (Vizzotto *et al*, 1999). Strategy I plants respond to Fe deprivation by inducing the activity of membrane-bound Fe^{3+} chelate reductases that reduce Fe^{3+} to the more soluble Fe^{2+} form (Vizzotto *et al*, 1997). The Fe^{2+} product is then taken into the roots by a Fe^{2+} transport system that is induced by iron-limiting growth conditions (Fox *et al*, 1996). Low bioavailability of Fe induces morphogenetic changes in roots that lead to a higher surface-to-volume ratio (Schmidt and Schikora, 2001).

Fe-efficiency reactions do not occur when Fe is sufficient (Albano and Miller, 1996). Regreening of young growth is a typical response in Fe-efficient plants to

increased Fe supply as a result of Fe-stress induced secretion of protons and/or reductants from the roots (Raju and Marschner, 1973). Roots of Fe-deficient *Actinidia* showed an increased capacity of net H^+ extrusion and higher ferric ethylenediaminetetracetate [Fe^{3+}EDTA] reductase activity compared to the plants grown in the presence of Fe^{3+}EDTA (Vizzotto *et al*, 1997). Romheld (1987) classified *Pelargonium* as possessing Strategy I Fe-efficiency mechanism. Different species or genotypes have different capacities to release H^+. Fe-stressed plants of *Actinidia* genotype D1 showed a higher capability to lower the pH of the nutrient solution as compared to plants of sel.2084 (Vizzotto *et al*, 1999).

Iron translocation in plants

Fe uptake from the soil is not the only limiting step in Fe acquisition. Fe must be transported from roots to the sites of use in the plant. About 80% of the leaf Fe is located in the chloroplast. To arrive at its final destination, most of the Fe must cross several biological membrane systems (Graziano *et al*, 2002). Fe is probably transported in the xylem as Fe^{3+}-citrate (Guerinot and Yi, 1994), and reduction of Fe^{3+} to Fe^{2+} is an essential step to cross the plasma membrane. The enzyme involved in this reaction is the plasma-membrane-bound Fe^{3+}-chelate reductase, whose activity seems to depend on the apoplastic pH (Kosegarten *et al*, 1999; Gonzalez-Vallejo *et* al, 2000). It has been suggested that some steps of the internal transport system may be impaired by the iron deficiency itself (Gonzalez-Vallejo *et al*, 2000). Chlorosis in the leaves is caused not only by insufficient uptake or translocation of Fe from roots to aerial parts, but it can also result from the failure of the leaves to reduce Fe^{3+}, a process that is affected by the

presence of the nitrate ions. The activity of chelate reductase located in the plasma-lemma appears to be depressed at alkaline pH under these conditions (Mengel *et al*, 1994). In many Fe-deficient plants, total Fe concentrations were found to be similar to those found in Fe-sufficient plants (Morales *et al,* 1998b). The concentration of total iron in green leaves was not significantly different from that of chlorotic ones, but the concentration of ferrous iron was much lower in chlorotic leaves than that in green leaves of peach (Gharsalli and Hajji, 2002). Average Fe retranslocation from leaves of Fe-sufficient plants was not large enough to meet the Fe demand of the growing shoot. This was not due to a limitation in Fe availability for transport as an excess amount of Fe was supplied which was not biologically bound, but a limitation due to transport facilities (Huve *et al*, 2003). Singh and Sahu (1993) suggested that Fe, despite absorption and uptake, was subject to inactivation and the mobility of Fe was impaired in the chlorotic plants of groundnut. Zohlen (2002) reported leaves that were chlorotic at the onset of spraying ($FeSO_4$, 0.5%) did not totally recover. As total leaf Fe on acid and calcareous soils was similar, it was concluded to be a physiological Fe deficiency caused by an immobilization of Fe to a metabolically inactive form in the plant tissues. Similar findings that iron could be immobilized and accumulated as inactive forms in peach leaves have been documented (Morales *et al*, 1998b).

Iron and its interactions

The antagonistic interaction among the different elements is well known. The interactions between elements occur when the supply of one element affects the absorption, distribution and functioning of another element (Zhang *et al*, 1996). It could

be due to competition between ions with similar properties. It has been documented that Fe has that kind of competition specifically with Mn (Perez-Sanz *et al*, 1996; Hauck *et al*, 2002) and Zn (Bucher and Schenk, 2000). Fe and Mn are intimately interdependent in their effects upon the plant and that the nature of the effects of the one is determined by the proportionate presence of the other (Somers and Shive, 1942). Foliar application of Fe reduced the Zn toxicity in tomato plants (Kaya *et al*, 1999; Kaya and Higgs, 2002). Acidification of the medium caused by Fe-stress is a major factor in mobilizing toxic levels of Mn in soilless media of zonal geraniums (Handreck, 1997). Rates of Fe were positively affected by the presence of Zn and Mn in the medium (Schmidt and Steinbach, 2000). As the Mn in the nutrient solution was increased, Fe uptake and traslocation was increased to a point and then decreased (Riekels and Lingle, 1966). Somers and Shive (1942) reported that Mn appeared to oxidize ferrous Fe to the more insoluble ferric ion. The high concentration of soluble Mn in the tissues is invariably associated with low concentration of soluble Fe and vice-versa. Mn-stressed plants of barley showed symptoms of Fe-deficiency (Alam *et al*, 2001). Combined deficiency of iron and Zn or Mn mitigated the symptoms of iron deficiency in tobacco plants (Kobayashi *et al*, 2003). Collier and Grossman (1992) concluded that although bleaching appears to be a general response to nutrient deprivation, it is not the same under all nutrient-limited conditions and is probably composed of independently controlled subprocesses.

Night temperature

In the southern states of the U.S., day and night temperatures are high during summers. Plants respire by using the metabolites made during photosynthesis. Both

photosynthesis and respiration are inhibited at high temperatures, but as temperature increases, photosynthetic rates drop before respiratory rates. At temperatures above the temperature compensation point, photosynthesis cannot replace the carbon used as a substrate for respiration. This imbalance between photosynthesis and respiration is one of the main reasons for deleterious effects of high temperatures (Taiz and Zeiger, 2002). Growth was favored by high day temperature but not by high night temperature (McDonald and Paulsen, 1997). The decline of carbohydrates in shoots as well as roots during summer may be due to the imbalance between carbon production by photosynthesis and consumption by respiration (Carrow, 1996; Liu and Huang, 2001; Xu and Huang, 2000b). High night temperature reduced specific leaf weight without affecting leaf area, which may be related to the depletion of reserve starch and other carbohydrates during dark period by respiratory losses (McDonald and Paulsen, 1997). Fe deficiency affected dark respiration much less than photosynthesis in leaves of sugarbeet (Morales *et al*, 1998a). Reynolds *et al* (1990) concluded that Chl loss at 40/30^{o}C (day/night) undoubtedly had a major impact on biomass accumulation in *Solanum chacoense*. Total Chl loss probably accounted for most of the decreased CO_2 fixation. Decline in the total Chl per cm^2 leaf area and a preferential loss of Chl b in the heat sensitive accession supports a heat-induced loss of light-harvesting complexes.

The effects of heat stress on the function of the acceptor site of PSII were reversible after the heated cyanobacterium cells were cooled down to the growth temperature (Wen *et al*, 2005). Feierabend (1977**)** suggested that deficiency in chlorophyll accumulation at 32^{o}C was not due to the temperature sensitivity of some step in the biochemical pathway of chlorophyll biosynthesis, since large amounts of

chlorophyll accumulated at 32°C when the leaves were allowed to form chloroplastic ribosomes by short intermittent transfers to 22°C. Furthermore, the rather short exposure times at 22°C, which were sufficient to restore the formation of a significant amount of chloroplastic rRNA at 32°C, suggests that the block in the formation of chloroplastic ribosomes observed at 32°C might be due not to the complete absence of some enzymes needed for their biosynthesis but rather to a failure of proper activation of enzymes.

Salicylic acid

Production of plants with tolerance to environmental stresses is one of the priorities in plant science research. Certain chemicals have been demonstrated to regulate the expression of stress tolerance (Fletcher *et al*, 2000; Senaratna *et al*, 2000). Plants have the capability to increase heat tolerance when ambient temperatures increase within non-lethal levels (Wang and Li, 2006). Salicylic acid (SA) is a natural signal molecule, which plays an important role in regulating a number of physiological processes in plants (Singh and Usha, 2003). Heat acclimation induced changes in endogenous SA and antioxidants concentration in relation to induced thermotolerance (Dat *et al*, 1998a). SA has been classed as a new potential plant hormone (Raskin, 1992). Currently, exogenous application of SA is of great interest because of its ability to induce thermotolerance. SA improved tolerance of mustard seedlings (Dat *et al*, 1998b) and *Arabidopsis* mutants (Larkindale *et al*, 2005) to heat stress. Similar findings have been documented for creeping bentgrass. Pre-treatment with SA resulted in increased tolerance to prolonged heat stress (1 month) and showed more green leaves, decreased membrane leakage and reduced oxidative damage (Larkindale and Huang, 2005). SA application enhanced heat

tolerance in Kentucky bluegrass possibly by scavenging active oxygen species and increasing the activity of antioxidant enzymes under heat stress (He *et al,* 2005). Salicylic acid induced thermotolerance was related to changes of antioxidant enzyme activities and antioxidant concentration in grape leaves (Wang and Li, 2006).

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CHAPTER III

EFFECT OF ELEVATED ROOT-ZONE TEMPERATURE ON GROWTH OF IVY GERANIUM

Abstract

Ivy geranium (*Pelargonium peltatum* L.) does not grow well in hot, humid weather. The development of whitening of the youngest leaves of actively growing ivy geranium has been observed as the season changes from late spring to summer in the southeastern United States. Iron deficiency has been suspected as causing this whitening disorder. A study was conducted to determine the response of ivy geraniums to elevated root-zone temperatures and Fe-chelate applications. Ivy geranium 'Beach' and 'Butterfly' were grown in media containing sphagnum peat and perlite (70:30 v/v) for one month to develop a substantial root system before elevating the root-zone temperature. The root-zone temperature treatments were 24^{o}C or 31^{o}C average. Iron was applied at 0mg Fe (control), 0.54mg Fe foliar spray, 1.08mg Fe foliar spray, 54mg Fe drench, or 108mg Fe drench per plant using Sprint 138, Fe-EDDHA, 6% Fe, applied twice during the experiment. Plants were grown at elevated root-zone temperatures for 8 weeks. Relative growth rate, chlorophyll index, leachate pH, soluble salts (EC), and Fe levels were measured at bi-weekly intervals throughout the experiment. Fresh and dry weights were determined at harvest. Plant relative growth rate, chlorophyll, pH, EC and leachate Fe

levels were not affected by temperature after 8 weeks of elevated root-zone temperatures. No whitening was observed due to elevated root-zone temperatures. In addition, high levels of Fe-chelate, which have been observed to prevent whitening, actually suppressed growth reducing fresh weight, dry weight and fresh to dry weight ratio in 'Butterfly'.

Introduction

Ivy geranium (*Pelargonium peltatum* L.) is an important floriculture crop, mainly used for hanging baskets. Ivy geranium is a very popular plant, but in the southeastern U.S. it does not grow well in hot weather. The newly developing leaves of ivy geranium turn white, expand less, and cup upward. This physiological disorder is called 'whitening'. Plants may resume normal growth as the temperature moderates. There has been observational evidence that applying Fe-chelate before whitening starts helped to reduce the problem (Personal communication, Harvey Lang, Fischer, USA). Whitening of the young foliage is thought to be caused by elevated root zone temperatures resulting in an iron deficiency.

The ability of plants to obtain water and mineral nutrients from the soil is related to their capacity to develop an extensive root system (Taiz and Zeiger, 2002). High temperature is a major factor limiting growth of cool-season grasses (Xu and Huang, 2002). High root-zone temperatures above 30^{o}C profoundly affect plant growth by reducing root growth (Mathers, 2003). Roots are more sensitive to high temperatures than shoots and a decline in root growth has been found to precede that of shoots under heat stress (Carrow, 1996; Xu and Huang, 2000a, 2000b). At high root temperatures, the membrane integrity of the root is lost and roots are injured or killed. High root-zone

temperatures can result in reduced photosynthesis (Mathers, 2003). In red maple, high root zone temperatures (36^{o}C) reduced leaf area, shoot dry weight, and shoot water potential (Graves *et al*, 1989). Root thermotolerance could be related to the maintenance of positive whole-plant carbon balance, and down-regulation of whole-plant and root respiration rates in response to increasing soil temperatures (Lyons *et al*, 2007).

Temperature stress can lead to inhibition of photosynthesis (Sharkey *et al*, 2001). Chlorophyll biosynthesis was reduced by 60% in cucumber seedlings upon heat-stress due to impairment of chlorophyll biosynthetic enzymes, in comparison to normal growth conditions (Tewari and Tripathy, 1998). High root-zone and air temperatures caused a significant reduction in photosynthetic rate, leaf chlorophyll content and ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) activity of creeping bentgrass (Xu *et al*, 2002). High root-zone temperature significantly decreased chlorophyll content (SPAD) of wheat plants grown at 26^{o}C/38^{o}C (air/soil) temperature (Tahir *et al*, 2005). Lettuce plants grown at root-zone temperatures 23-40^{o}C showed lower photosynthetic carbon assimilation, stomatal conductance and midday leaf water content than 20^{o}C root-zone temperature (He *et al*, 2001). Photosynthetic response to high temperature can vary significantly within a species (Reynolds *et al*, 1990). Photosynthesis decreased and respiration rate increased with increasing soil temperature from 20^{o}C to 37^{o}C for *Agrostis stilonifera* while *A. scabra* maintained its photosynthetic and respiration rate when exposed to 37^{o}C for 17 days (Lyons *et al*, 2007).

Although iron is the fourth most abundant nutrient in the lithosphere, its presence in the soil solution is negligible due to low solubility (Lucena, 2003). Fe-chelates have the capacity to maintain soluble iron in the soil solution over time (Cantera *et al*, 2002). Iron

uptake is decreased by increased root zone temperatures (Raeini-Sartaz and Barthakur, 1995; Tagliavini *et al*, 1991). Hood and Mills (1994) stated that maintaining root-zone temperature near 22^{o}C maximizes growth and nutrient uptake of snapdragons. Lettuce plants grown under hot ambient root-zone temperatures normally show symptoms of iron deficiency (He and Lee, 1998a; 1998b). Concentration of leaf nutrient elements such as N, P, Fe, and Mn of cucumber plants decreased when root zone temperature was raised to 35^{o}C (Du and Tachibana, 1994). The effect of increased root-zone temperature on Fe-uptake and translocation was decreased by increases in the levels of Fe in the pretreatment cultures (Riekels and Lingle, 1966). Translocation of iron within the plant is dependent both on root temperature and presence of iron (Schmidt and Steinbach, 2000). *Plantago lanceolata* plants grown at 24^{o}C decreased the rates of root-mediated Fe reduction with increasing Fe levels in the external growth solution. Such control suggests a dependence of Fe acquisition on changes in (either external or internal) Fe concentration (Schmidt and Steinbach, 2000). Elevated levels of iron increased tissue chlorophyll content of *Pelargonium* X *hortorum* plants (Lee *et al*, 1996). Similar findings have been documented that high iron (Fe-EDDHA) levels in the nutrient solution were associated with higher concentration of leaf pigments of chile peppers (Anchondo *et al*, 2001).

Much work has been done on Fe nutrition in relation to root-zone temperature. However little is known about whitening disorder in ivy geranium. The objectives of this study were to determine the effect of root zone temperature and Fe-chelate application on foliar growth of ivy geraniums.

Materials and Methods

Two cultivars of ivy geraniums with different susceptibility to whitening were chosen. 'Beach' has low susceptibility and 'Butterfly' has high susceptibility (Personal Communication, Harvey Lang, Fischer, USA). Rooted cuttings of ivy geraniums 'Beach' and 'Butterfly' (Fischer Horticulture, Boulder, CO) were potted on 23 Sept. 2005 into 15-cm diameter containers. They were potted in sphagnum peat and perlite (70:30 by volume) with 0.96 Kg gypsum$\cdot m^{-3}$, 7.7 Kg limestone$\cdot m^{-3}$, and 0.32 Kg wetting agent (SaturAid, Debco Pty. Ltd., Tyabb, Australia) $\cdot m^{-3}$ added. Plants were fertigated with 250mg $N \cdot L^{-1}$ from 20N-4.4P-16.6K (Peters Peat-lite fertilizer, Scotts Company, Marysville, Ohio) at every irrigation. The potted cuttings were grown in a double layer inflated polyethylene greenhouse with 23°C day and 22°C night venting temperatures until they developed a substantial root system. Styrofoam boxes were constructed with heating cables (Gro-Quick Cables, Wrap-On Company, Bedford Park, IL) running on the bottom. The tops of the boxes had 15cm holes to set the pots in so the foliage was isolated from the heat. The root zone temperature treatments averaged 24 or 31°C (Table 3.1) with 3 replications of each temperature. Plants were placed in the boxes on 29 Oct. 2005 and root-zone temperature treatments commenced.

Iron (Sprint 138, Fe-EDDHA, 6% Fe, Becker Underwood Inc, Ames, IA) was applied at 0mg Fe (control), 0.54mg Fe foliar spray, 1.08mg Fe foliar spray, 54mg Fe soil drench or 108mg Fe drench per pot on 3 Nov and 3 Dec 2005. 0.54mg Fe foliar spray and 54mg Fe soil drench are calculated as labeled rates of Sprint 138 for ornamentals. A surfactant (X-77 at 1/2 tsp/gallon, Loveland Industries Ltd. Greeley, CO) was added to the Fe-chelate foliar spray.

Data on plant growth was collected at bi-weekly intervals starting from 27 Oct 2005 for 8 weeks. Relative growth rate (RGR) was measured as the difference in growth index at two consecutive biweekly measurements. Growth index was calculated as: GI (cm^3) = 3.14*(width/2)^2*height, where plant height was measured from the rim of the container and width as an average of two widths measured, one at the widest point and another at 90^o. Chlorophyll (SPAD-meter, Minolta Co. Ltd., Japan), leachate pH, leachate soluble salts (EC) and leachate iron concentrations were also measured at bi-weekly intervals. Chlorophyll was measured on three fully-expanded mature leaves of each plant and their average represented the chlorophyll index for that plant. Leachate was collected using the Virginia Tech pour-through technique to determine pH and soluble salts (Wright, 1986). Leachate samples were filtered, using Whatman no.1 filters (Whatman International Ltd, Maidstone, England). Samples were then refrigerated at 4.4^{o}C until analysis for Fe. Iron concentration in the filtered leachate was determined by inductively coupled argon-plasma emission spectrometry.

Plants were harvested by cutting at the media line at the end of the experiment (22 Dec 2005) to determine fresh weights. The plants were then dried at 60^{o}C and dry weight was measured. The experiment was a split plot design split by temperature. Plant size indices, SPAD-chlorophyll, plant fresh weight, plant dry weight, pH, soluble salts and leachate iron concentrations were analyzed by analysis of variance using Proc-mixed (SAS software, SAS Institute, Cary, N.C.). Fisher's protected least significance difference (LSD) at P= 0.05 was used to indicate significant differences between treatment means.

Results and Discussion

There was no interactive effect of temperature and Fe-chelate application on relative growth rate (RGR). No differences were observed in RGR for either cultivar due to elevated root-zone temperature after 2, 4, 6, or 8 weeks (Table 3.2). 'Beach' and 'Butterfly' continued growing under higher root zone temperatures without affecting RGR. Elevated root-zone temperature did not suppress the growth of ivy geraniums.

Fe-chelate application affected the RGR of both cultivars after 2 weeks (Table 3.3). In 'Beach', RGR was the greatest with 0.54mg Fe foliar spray, which was similar to RGR obtained with 1.08mg Fe foliar spray or 54 mg Fe soil drench. Control was only different from 0.54mg Fe foliar spray in its RGR. Soil drench at 108mg Fe resulted in a RGR similar to the control. In 'Butterfly', the plants receiving no Fe-chelate had the maximum RGR 2 weeks after starting treatments (Table 3.3). The control plants were similar in RGR to the plants receiving foliar sprays of Fe-chelate at 0.54mg or 1.08mg Fe foliar spray. These results are consistent with the findings documented for holiday cactus where plant growth decreased but marginal chlorosis increased as the Fe-chelate levels increased (Ramirez and Lang, 1997). There were no differences in RGR in either cultivar after 4 (Table 3.3), 6 or 8 (Table 3.4) weeks. These findings indicate that higher applications of Fe reduced growth in 'Butterfly' after 2 weeks but effect did not persist. As the experiment progressed to 8 weeks, there were no differences in RGR in either cultivar due to Fe-chelate application. Schmidt and Steinbach (2000) also documented no effect of Fe availability on growth of *Plantago lanceolata*.

No interactive effect for SPAD chlorophyll index was observed between temperature and Fe-chelate application in either cultivar, except for 'Beach' after 2

weeks. Chlorophyll indices (measured with SPAD-meter) of plants under all treatments were similar (Tables 3.5) before elevating the root-zone temperature. Elevated root-zone temperature did not result in a reduction of chlorophyll after 4, 6 or 8 weeks of elevated root-zone temperatures in 'Beach' or 2, 6, or 8 weeks in 'Butterfly' (Tables 3.5). There was a slight decrease in chlorophyll due to heat stress in 'Butterfly' at week 4 but plants recovered in week 6 and 8. Similar results had been documented for pawpaw (*Asimina triloba*) seedlings which had higher leaf chlorophyll concentrations with bottom heating (32^{o}C) than the seedlings grown at ambient temperature (Pomper *et al*, 2002). There was no whitening observed under either root-zone temperature, indicating that elevated root-zone temperature was not the cause of whitening of ivy geraniums.

There was an interaction effect of temperature and Fe-chelate application on chlorophyll index in 'Beach' 2 weeks after start of elevated root-zone temperatures (Table 3.6). The maximum chlorophyll index was obtained in plants receiving 1.08mg Fe foliar spray at 31^{o}C which was similar to those observed with drench (54mg or 108mg Fe) application at 31^{o}C, control, 1.08mg Fe foliar, and 108mg Fe drench at 24^{o}C. The least chlorophyll index resulted with control at 31^{o}C, which was similar to 24^{o} and 31^{o}C at 0.54mg Fe foliar, and drench of 54 or 108mg Fe at 24^{o}C in its SPAD chlorophyll index. These results imply that applying Fe-chelate does not matter at low root-zone (24^{o}C) temperature but it helps accumulate chlorophyll at higher root zone (31^{o}C) temperatures. Schmidt and Steinbach (2000) stated that temperature regime did not affect Fe accumulation by root cells of *Plantago lanceolata*, but it decreased translocation of Fe to the shoot and chlorosis was observed at suboptimal root-zone temperature. Translocation of Fe to shoots was dependent on both root-zone temperature and presence

of Fe. Increased root-zone temperature decreased its effect on Fe-uptake and translocation in tomato plants with an increase in the levels of Fe in the pretreatment cultures (Riekels and Lingle, 1966). This discussion indicates that elevated root-zone temperature in the presence of Fe in the nutrient solution might helped 'Beach' to accumulate Fe and so the chlorophyll index.

Before applying Fe-chelate, chlorophyll indices (measured with SPAD-meter) of plants of 'Beach' and 'Butterfly' under all application were similar (Table 3.7). Fe-chelate alone did not affect chlorophyll 2 weeks after treatment in 'Butterfly'. In 'Beach', chlorophyll index increased as Fe-application increased after 4 weeks (Table 3.7). The maximum chlorophyll index was obtained with 108mg Fe drench/pot and the least with the control (0mg Fe). Chlorophyll index was not different from remaining Fe-chelate applications. Four weeks after treating with Fe, the Fe-chelate also helped to preserve chlorophyll content of 'Butterfly' compared to the control (Table 3.7). These findings suggest that iron-chelate increased chlorophyll content in ivy geraniums after 4 weeks. These results are consistent with the findings documented for chile peppers and zonal geraniums (Anchondo *et al*, 2001; Lee *et al*, 1996). After 6 and 8 weeks of starting the experiment, Fe-application did not affect chlorophyll index of either cultivar, even when a second application of Fe-chelate was made 12 days before week 6 (Table 3.7). Fe-toxicity symptoms were observed as a bronzing of the leaves in both cultivars receiving Fe-chelate drench (observational data not measured). Fe toxicity may be the reason no differences in chlorophyll retention were observed with the second application of Fe-chelate. Toxicity symptoms observed in 'Beach' and 'Butterfly' with application of Fe-

chelate drench might implicate the statement ‘external Fe as a possible control factor in Fe uptake’ given by Schmidt and Steinbach (2000).

There was no interactive effect of temperature and Fe-chelate application on leachate pH, EC (soluble salts) or Fe concentration. There were no differences in leachate pH, EC (soluble salts) or Fe concentration due to root-zone temperature at any of the measurement times (Tables 3.8) in either cultivar. Elevated root-zone temperature did not cause whitening by changing the soil solution chemistry.

Leachate pH, EC and Fe levels were not different between treatments due to addition of Fe-chelate in either of the cultivars (Table 3.9) at the start of experiment. After 2 weeks, pH was unaffected by Fe application in cultivars ‘Beach’ or ‘Butterfly’. The leachate EC after 2 weeks was the greatest with 108mg drench dose of Fe in ‘Beach’ and ‘Butterfly’ (Table 3.9). Leachate Fe levels were the greatest at 108mg Fe drench/pot, followed by 54mg Fe drench/pot (Table 3.9) in ‘Beach’ and ‘Butterfly’. These results indicate that drench applications of Fe increased soluble salts (EC) and leachate Fe levels. This may be the reason for toxicity symptoms in ivy geraniums receiving a soil drench of Fe-chelate.

There were no differences in leachate pH due to Fe-chelate application for both cultivars after 4 weeks (Table 3.10). In ‘Beach’, leachate EC was greatest in the 108mg Fe drench application (Table 3.10). In ‘Butterfly’, Fe-application did not affect leachate pH or EC (Table 3.10). Fe-chelate drench applications affected leachate Fe levels in ‘Beach’ and ‘Butterfly’ (Table 3.10). Fe-chelate at 108mg Fe drench increased leachate Fe levels the most, followed by 54 mg Fe drench in both cultivars. After 6 weeks, pH was not different due to Fe application in ‘Beach’ whereas the soluble salts (EC)

concentration was the greatest with the greatest drench dose (Table 3.10). All the remaining Fe-treatments resulted in no difference in EC (Table 3.10). In 'Butterfly', leachate pH nor EC were affected by Fe-application (Table 3.10) after 6 weeks. After 8 weeks, leachate pH and EC were not affected by Fe-chelate treatments in either cultivar (Table 3.10). At 8 weeks, leachate Fe levels were the greatest at 108mg Fe drench/pot, followed by 54mg Fe drench/pot. There was no difference in leachate Fe concentrations between the control and foliar Fe applications. These results indicate that drench applications affected the leachate Fe in both cultivars due to the direct addition of Fe-chelate to the soil. Foliar Fe-chelate applications did not affect leachate Fe concentration as no Fe chelate was applied to the soil in these treatments.

No interactive effect was observed between temperature and Fe-chelate application for plant fresh weight, dry weight or fresh-dry weight ratio in either cultivar. Plant biomass (fresh and dry weight) and plant water content (fresh/dry weight ratio) of ivy geraniums were unaffected by root-zone temperature (Table 3.11). Plant biomass of wheat genotypes 'Fang' and 'Siete Cerros' were not affected by elevated root-zone temperature (Tahir *et al*, 2005). These findings are not consistent with the results documented for rep maple (Graves *et al*, 1989), where elevated (36^{o}C) root-zone temperature reduced leaf area and shoot dry weight.

Fe-chelate treatments did not affect plant fresh weight, dry weight or fresh-dry weight ratio in 'Beach' (Table 3.12). However, in 'Butterfly', fresh weight decreased as Fe-chelate concentration in the medium increased (Table 3.12). The drench applications of Fe-chelate resulted in a greater decrease in fresh weight. Dry weight in 'Butterfly' followed a similar pattern to fresh weight. Control plants gained the most dry weight,

while drench application of Fe suppressed dry weight (Table 3.12). These results are consistent with the findings documented for chickpea (Ghasemi-Fasaei *et al*, 2005). Fresh-dry weight ratio is an index of plant water content (Hunt, 1982). The maximum fresh-dry weight ratio was obtained under control and foliar Fe treatments. Drench Fe applications resulted in the lowest fresh-dry weight ratios. These findings suggest that high Fe applications can inhibit plant biomass and water content.

No suppressing effect of Fe-chelate applications were observed on RGR in ivy geraniums even though there were visible Fe-toxicity symptoms. There was suppressing effect of Fe on plant biomass and fresh to dry weight ratio in 'Butterfly' but not in 'Beach' which implies that 'Beach' may tolerate higher levels of Fe in nutrient solution. Increased leachate EC and Fe levels were obtained with the drench application at 108mg Fe which may have caused reduced plant biomass in ivy geraniums.

Conclusion

The results of this study indicate that elevated root-zone temperature is not the cause of whitening of ivy geraniums. Elevated root-zone temperatures did not affect relative growth rate, SPAD-chlorophyll index, fresh weight, dry weight nor fresh/dry weight ratio. Ivy geraniums grew well under elevated root-zone temperatures without whitening or a reduction in growth. High levels of Fe-chelate drenches (108mg Fe drench/pot at monthly interval) caused visible toxicity symptoms in ivy geraniums and suppressed plant biomass (fresh and dry weight), and water content (fresh/dry weight ratio) of 'Butterfly' ivy geranium.

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Table 3.1. Daily average root-zone temperature treatments 24°C and 31°C throughout the experiment

Day	24°C loggers	31°C loggers	Day	24°C loggers	31°C loggers
1	29.4	32.3	30	23.9	18.6
2	37.6	33.1	31	27.9	25.4
3	37.4	33.5	32	32.5	34.9
4	33.5	33.1	33	24.5	34.7
5	24.9	32.3	34	23.9	35.4
6	23.7	32.7	35	23.4	35.5
7	23.7	33.6	36	23.5	35.3
8	23.9	33.1	37	23.7	35.4
9	24.1	33.9	38	23.3	36.0
10	24.2	33.5	39	22.7	35.3
11	24.2	33.7	40	22.6	35.6
12	24.0	33.7	41	23.7	36.6
13	22.7	35.0	42	24.6	35.1
14	22.3	29.9	43	24.1	39.7
15	22.8	28.7	44	24.8	40.1
16	21.3	29.8	45	24.8	40.2
17	20.6	30.1	46	24.3	40.5
18	22.3	30.4	47	24.6	40.5
19	19.2	29.4	48	24.2	39.9
20	16.7	29.3	49	24.4	41.0
21	16.9	28.8	50	24.7	33.4
22	16.9	28.4	51	24.9	27.6
23	18.1	28.6	52	24.5	26.9
24	20.2	28.5	53	24.6	26.4
25	20.8	28.7	54	24.3	25.5
26	21.9	16.8	55	23.3	24.6
27	23.2	17.3			
28	23.5	15.9			
29	24.6	16.3	Average	24.04°C	31.46°C

Note- Values from day 26 to day 31 were low due to faulty sensor readings which shut down heating cables during these days.

Table 3.2. Effect of root-zone temperature on relative growth rate (RGR) in ivy geranium 'Beach' and 'Butterfly' measured biweekly throughout the experiment

Temperature	Beach	Butterfly
2 weeks		
24°C	2983 a[x]	1334 a
31°C	5561 a	1850 a
4 weeks		
24°C	8440 a	5626 a
31°C	10496 a	4057 a
6 weeks		
24°C	10096 a	8463 a
31°C	6810 a	3267 a
8 weeks		
24°C	8419 a	7323 a
31°C	4771 a	5506 a

[x]Means followed by same letters within column and within week are not different according to Fisher's protected LSD test (*P*=0.05).

Table 3.3. Effect of iron chelate on relative growth rate (RGR) in ivy geranium 'Beach' and 'Butterfly' at week 2 and week 4

Fe treatment	Beach	Butterfly
2 weeks		
0mg	3545 b[x]	1982 a
0.54mg foliar	5662 a	1868 ab
1.08mg foliar	4004 ab	1630 abc
54mg drench/pot	4371 ab	1296 bc
108mg drench/pot	3434 b	1183 c
4 weeks		
0mg	11684 a	5586 a
0.54mg foliar	9953 a	4879 a
1.08mg foliar	11718 a	5956 a
54mg drench/pot	6686 a	3445 a
108mg drench/pot	7300 a	4341 a

[x]Means followed by same letters within column and within week are not different according to Fisher's protected LSD test (*P*=0.05).

Table 3.4. Effect of iron chelate on relative growth rate (RGR) in ivy geranium 'Beach' and 'Butterfly' at week 6 and week 8

Fe treatment	Beach	Butterfly
6 weeks		
0mg	7526 a[x]	7945 a
0.54mg foliar	9823 a	7400 a
1.08mg foliar	9128 a	4367 a
54mg drench/pot	9074 a	4678 a
108mg drench/pot	6716 a	4934 a
8 weeks		
0mg	7057 a	8095 a
0.54mg foliar	8768 a	7219 a
1.08mg foliar	5512 a	7369 a
54mg drench/pot	6297 a	5162 a
108mg drench/pot	3904 a	3488 a

[x]Means followed by same letters within column and within week are not different according to Fisher's protected LSD test (*P*=0.05).

Table 3.5. Effect of root-zone temperature on SPAD chlorophyll index in ivy geranium 'Beach' and 'Butterfly' measured biweekly throughout the experiment

	SPAD chlorophyll index				
Temperature	Week 0	Week 2	Week 4	Week 6	Week 8
Beach					
24°C	54.97 a[x]	*z	54.45 a	53.16 a	52.96 a
31°C	54.55 a	*z	53.77 a	55.30 a	54.32 a
Butterfly					
24°C	47.97 a	53.33 a	52.85 a	49.68 a	50.70 a
31°C	48.63 a	52.25 a	50.68 b	50.37 a	49.69 a

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).

*z indicates the interaction between temperature and Fe-chelate application for SPAD chlorophyll index.

Table 3.6. Interactive effects of temperature and Fe-chelate application on SPAD chlorophyll index in 'Beach' at 2 weeks

Temperature	Fe-application	SPAD chlorophyll
24°C	0mg	56.24 abc[x]
24°C	0.54mg foliar	53.04 bcd
24°C	1.08mg foliar	55.27 abc
24°C	54mg drench/pot	53.28 bcd
24°C	108mg drench/pot	53.80 abcd
31°C	0mg	51.18 d
31°C	0.54mg foliar	52.21 cd
31°C	1.08mg foliar	57.55 a
31°C	54mg drench/pot	55.86 abc
31°C	108mg drench/pot	56.36 ab

[x]Means followed by same letters within column are not different according to Fisher's protected LSD test (*P*=0.05).

Table 3.7. Effect of iron chelate on SPAD chlorophyll index in ivy geranium 'Beach' and 'Butterfly' measured biweekly throughout the experiment

	SPAD chlorophyll index				
Fe treatment	Week 0*	Week 2	Week 4**	Week 6	Week 8
Beach					
0mg	55.07 a[x]	*z	51.84 c	53.35 a	52.76 a
0.54mg foliar	53.22 a	*z	54.96 ab	52.71 a	53.63 a
1.08mg foliar	55.64 a	*z	54.22 abc	53.42 a	52.18 a
54mg drench/pot	56.32 a	*z	53.08 bc	53.72 a	54.59 a
108mg drench/pot	53.56 a	*z	56.46 a	57.96 a	55.05 a
Butterfly					
0mg	47.33 a	53.04 a	49.25 b	48.84 a	49.21 a
0.54mg foliar	49.17 a	51.02 a	52.40 a	48.38 a	50.10 a
1.08mg foliar	49.87 a	52.99 a	52.69 a	50.98 a	51.49 a
54mg drench/pot	47.67 a	52.23 a	51.15 ab	51.27 a	49.45 a
108mg drench/pot	47.45 a	54.68 a	53.33 a	50.65 a	50.72 a

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).
* is the week of first application and **is the week of second application of Fe-chelate
*z indicates the interaction between temperature and Fe-chelate application for SPAD chlorophyll index.

Table 3.8. Effect of root-zone temperature on leachate pH, EC and Fe in ivy geranium 'Beach' and 'Butterfly' measured biweekly throughout the experiment

Temperature	Beach			Butterfly		
	pH	EC (dS/m)	Fe (mg $\cdot l^{-1}$)	pH	EC (dS/m)	Fe (mg $\cdot l^{-1}$)
Start of experiment						
24°C	6.41 a[x]	1.96 a	1.52 a	6.25 a	2.59 a	1.52 a
31°C	6.39 a	1.96 a	1.54 a	6.30 a	2.84 a	1.49 a
2 weeks						
24°C	6.28 a	2.60 a	20.01 a	6.12 a	3.15 a	16.97 a
31°C	6.17 a	3.26 a	17.03 a	6.04 a	3.97 a	15.99 a
4 weeks						
24°C	5.57 a	4.31 a	17.15a	5.58 a	4.50 a	15.42 a
31°C	5.97 a	4.38 a	17.60 a	5.81 a	4.99 a	16.56 a
6 weeks						
24°C	5.93 a	3.74 a	31.30 a	5.76 a	3.89 a	31.57 a
31°C	6.04 a	4.01 a	30.68 a	5.75 a	4.78 a	32.73 a
8 weeks						
24°C	5.75 a	4.05 a	30.80 a	5.73 a	3.87 a	20.57 a
31°C	5.98 a	4.27 a	21.20 a	5.92 a	4.33 a	19.67 a

[x]Means followed by same letters within column and within week are not different according to Fisher's protected LSD test (P=0.05).

Table 3.9. Effect of iron chelate on leachate pH, EC and Fe in ivy geranium 'Beach' and 'Butterfly' at the start of experiment and after 2 weeks

	Beach			Butterfly		
Fe Treatment	pH	EC (dS/m)	Fe (mg $\cdot l^{-1}$)	pH	EC (dS/m)	Fe (mg $\cdot l^{-1}$)
Start of experiment						
0mg	6.40 a[x]	2.02 a	1.54 a	6.31 a	2.69 a	1.44 a
0.54mg foliar	6.45 a	1.87 a	1.58 a	6.25 a	2.82 a	1.56 a
1.08mg foliar	6.51 a	1.97 a	1.46 a	6.24 a	2.77 a	1.51 a
54mg drench/pot	6.43 a	2.09 a	1.51 a	6.25 a	2.75 a	1.51 a
108mg drench/pot	6.23 a	1.84 a	1.56 a	6.35 a	2.56 a	1.51 a
2 weeks						
0mg	6.28 a	2.97 b	1.62 c	6.14 a	3.14 b	1.44 c
0.54mg foliar	6.24 a	2.61 b	1.42 c	6.08 a	3.31 b	1.32 c
1.08mg foliar	6.25 a	2.44 b	1.64 c	6.10 a	3.33 b	1.42 c
54mg drench/pot	6.18 a	2.75 b	26.77 b	6.03 a	3.79 ab	25.34 b
108mg drench/pot	6.20 a	3.89 a	61.15 a	6.03 a	4.24 a	52.88 a

[x]Means followed by same letters within column and within week are not different according to Fisher's protected LSD test (P=0.05).

Table 3.10.Effect of iron chelate on leachate pH, EC and Fe in ivy geranium 'Beach' and 'Butterfly' after 4, 6 and 8 weeks

	Beach			Butterfly		
Fe Treatment	pH	EC (dS/m)	Fe (mg $\cdot l^{-1}$)	pH	EC (dS/m)	Fe (mg $\cdot l^{-1}$)
4 weeks						
0mg	5.70 a[x]	4.89 ab	2.75 c	5.68 a	4.42 a	1.87 c
0.54mg foliar	5.73 a	3.77 b	3.08 c	5.69 a	4.94 a	2.63 c
1.08mg foliar	5.75 a	3.49 b	2.26 c	5.66 a	4.76 a	2.55 c
54mg drench/pot	5.89 a	3.82 b	18.82 b	5.70 a	4.76 a	22.46 b
108mg drench/pot	5.78 a	5.76 a	59.95 a	5.74 a	4.83 a	50.43 a
6 weeks						
0mg	5.95 a	3.54 b	3.12 c	5.74 a	4.19 a	3.17 c
0.54mg foliar	6.00 a	3.44 b	3.24 c	5.71 a	4.42 a	2.75 c
1.08mg foliar	5.90 a	3.79 b	3.57 c	5.76 a	3.92 a	3.06 c
54mg drench/pot	6.06 a	3.84 b	39.29 b	5.74 a	4.69 a	58.52 b
108mg drench/pot	6.02 a	4.77 a	105.73 a	5.82 a	4.46 a	93.24 a
8 weeks						
0mg	5.83 a	4.08 a	4.64 c	5.79 a	3.41 a	3.78 c
0.54mg foliar	5.83 a	3.94 a	4.08 c	5.66 a	4.32 a	3.26 c
1.08mg foliar	5.76 a	4.23 a	4.73 c	5.84 a	4.66 a	4.23 c
54mg drench/pot	5.94 a	3.96 a	37.79 b	5.87 a	4.26 a	34.63 b
108mg drench/pot	5.98 a	4.61 a	80.78 a	5.97 a	3.87 a	54.69 a

[x]Means followed by same letters within column and within week are not different according to Fisher's protected LSD test (*P*=0.05).

Table 3.11.Effect of root-zone temperature on fresh weight, dry weight and fresh to dry wt ratio in ivy geranium 'Beach' and 'Butterfly' at the end of experiment

	Beach			Butterfly		
Temperature	Fresh wt. (g)	Dry wt. (g)	Fresh/dry wt. ratio	Fresh wt. (g)	Dry wt. (g)	Fresh/dry wt. ratio
24°C	251.6 a[x]	26.3 a	7.60 a	157.5 a	15.7 a	7.00 a
31°C	222.5 a	24.0 a	7.31 a	116.0 a	11.8 a	6.50 a

[x]Means followed by same letters within column are not different according to Fisher's protected LSD test (*P*=0.05).

Table 3.12.Effect of iron chelate on fresh weight, dry weight and fresh to dry wt ratio in ivy geranium 'Beach' and 'Butterfly' at the end of experiment

	Beach			Butterfly		
Fe Treatment	Fresh wt (g)	Dry wt. (g)	Fresh/dry wt. ratio	Fresh wt (g)	Dry wt. (g)	Fresh/dry wt. ratio
0mg	232.6 a[x]	24.8 a	7.31 a	173.3 a	17.0 a	7.35 a
0.54mg foliar	255.8 a	27.9 a	7.61 a	149.0 a	14.7 ab	7.10 a
1.08mg foliar	231.3 a	24.8 a	7.46 a	143.1 ab	14.2 ab	7.00 ab
54mg drench/pot	249.4 a	26.4 a	7.64 a	113.0 bc	11.7 bc	5.95 c
108mg drench/pot	216.2 a	22.8 a	7.26 a	105.1 c	10.9 c	6.19 bc

[x]Means followed by same letters within column are not different according to Fisher's protected LSD test (*P*=0.05).

CHAPTER IV

EFFECT OF ELEVATED AIR TEMPERATURE ON GROWTH OF IVY GERANIUM

Abstract

Ivy geranium (*Pelargonium peltatum* L.) does not perform well in southeastern U.S. summers. Whitening of the youngest leaves of actively growing ivy geranium has been observed as the season changes from late spring to summer. High temperatures and iron deficiency have been suspected as causes of this whitening disorder. A study was conducted to determine the specific environmental cause of whitening of ivy geranium and to determine the effectiveness of Fe-chelate on preventing whitening. Ivy geranium 'Beach' and 'Butterfly' plants were grown for 6 weeks in modified greenhouse chambers (1.27m x 1.20m x 0.91m), with air temperature averaging 28°C-day/16°C-night or 36°C-day/22°C-night. Iron chelate was applied at 0mg (control) or 27mg Fe (Fe-EDDHA, 6% Fe) drench per pot 3 times during the experiment. Elevated air temperature severely reduced plant growth, leaf area, fresh and dry weights of the shoot, leaf, and total plant and fresh:dry weight ratios in both cultivars. Elevated air temperature was determined to be the cause of whitening of ivy geranium. 'Beach' increased its size lesser due to heat stress, whereas 'Butterfly' did not. Elevated air temperature reduced chlorophyll a,

carotenoids and pheophytins in ‘Butterfly’ but not in ‘Beach’. Fe-chelate application had no effect at the control temperature, but it increased chlorophyll to carotenoids ratio (Chl:Caro) at elevated air temperature in ‘Butterfly’, which is more susceptible to whitening. Increased Chl:Caro ratio may be the reason Fe-chelate application helps in prevention of whitening of ivy geranium. There was greater accumulation of Mn and Zn in foliar tissues of ‘Beach’ at elevated temperature, but their concentrations were not affected by temperature in ‘Butterfly’. Foliar S content increased in ‘Butterfly’ but not in ‘Beach’ at elevated air temperature. Fe-chelate application reduced foliar Mn content and its ratio with Fe and Zn. These results suggest that carotenoids, pheophytins, and foliar Mn, Zn and S content play a role in decreasing susceptility of ivy geraniums to whitening.

Introduction

Ivy geranium (*Pelargonium peltatum* L.) does not grow well in southeastern U.S. summers. The newly developing leaves of ivy geranium turn white, enlarge less and have an upward cupped appearance. This physiological disorder is referred to as “whitening” (Fig. 4.1). Plants resume normal growth as the temperature moderates in fall. There has been observational evidence that applying Fe-chelate before whitening helped to prevent the disorder. Neither the direct cause of this problem nor the role of iron in whitening is known. Whitening of the young foliage is thought to be caused by elevated air temperature and iron deficiency.

Increased leaf temperatures during the summer are a potential hazard in greenhouses where low air speed and high humidity decrease the rate of leaf cooling

(Taiz and Zeiger, 2002). Maintenance of transpirational (leaf) cooling was an important factor associated with better summer stress performance of Kentucky bluegrass (Bonos and Murphy, 1999). Pansy plants showed nutritional problems when grown during warm periods (>18^{o}C) of the growing season. These problems typically were not seen when production temperatures were optimal (<18^{o}C) even though fertility regimes remained the same (Hamlin *et al*, 1999). *Solanum* species screened for heat tolerance indicated considerable variation in the degree of chlorosis developing as a stress response to high temperature (Reynolds *et al*, 1990).

High temperature inhibits photosynthesis (Sharkey *et al*, 2001). Temperatures above 30^{o}C reduced photosynthetic rate as a result of a reduction in photosystem II (PSII) efficiency (Kadir *et al*, 2006). Net photosynthesis of pea plants decreased with increasing leaf temperature up to 45^{o}C (Haldimann and Feller, 2005). Leaf temperatures up to 45^{o}C enhanced respiratory rates as well as exceeded optimum temperature for photosynthesis (Jifon and Syvertsen, 2003). High temperature influenced chloroplast development, chlorophyll biosynthesis and greening process (Mohanty *et al*, 2006). Chlorophyll a (Chl a) is more heat sensitive than chlorophyll b (Chl b) (Weemaes *et al*, 1999; Loey *et al*, 1998). A shift in the ratio of Chl a and Chl b is an indicator of heat bleaching in *Euglena gracilis* (Thomas and Ortiz, 1995). Chlorophyll a to b ratio (Chl a:b) and total chlorophyll to carotenoids ratio decreased in heat-stressed tomato plants (Camejo *et al*, 2005). Chlorophyll biosynthesis of cucumber seedlings was reduced 60% upon heat-stress due to impairment of chlorophyll biosynthetic enzymes compared to normal growth conditions (Tewari and Tripathy, 1998). Chlorosis was induced in tree fern (*Cyathea cooperi*) by 41.2^{o}C air temperature which resulted in reduced chlorophyll concentration,

reduced Chl a:b ratio, fewer number of grana and almost completely absent stroma thylakoids (Doley, 1983). There was a heat-induced block of PSII reaction centers and a heat-induced block of Chl b to Chl a energy transfer resulting in a functional disconnection of the light-harvesting complex from the reaction center complexes (Schreiber and Armond, 1978). The photo reducible pheophytin is the natural primary electron acceptor of PSII in the early steps of photosynthetic solar energy conversion, acting between P680 and plastoquinone (Klimov, 2003). In PSII of green plants, the key photosynthetic reaction consists of the transfer of an electron from the primary donor called P680 to a nearby pheophytin molecule (Groot *et al*, 1997).

Iron (Fe) is involved in photosynthesis, respiration, DNA synthesis, hormone formation and nitrogenase activity (Briat and Lobreaux, 1997). Fe has an important role in the formation of thylakoid membranes in higher plants. Fe deficiency is accompanied by a decrease in all membrane components, including the electron carriers in the photosynthetic electron transport chain (Spiller and Terry, 1980) and light harvesting pigments, chlorophylls and carotenoids (Morales *et al*, 1990; Morales *et al*, 1994). High iron levels in *Pelargonium* X *hortorum* were associated with dark green leaves (Lee *et al*, 1996). Fe uptake from soil is not the only limiting step in Fe acquisition. Fe must be transported from roots to the sites of use in the plant. About 80% of the leaf Fe is located in the chloroplast. To arrive at its final destination, most of the Fe must cross several biological membrane systems (Graziano *et al*, 2002). Fe is probably transported in the xylem as Fe^{3+}-citrate (Guerinot and Yi, 1994), and reduction of Fe^{3+} to Fe^{2+} is an essential step to cross the plasma membrane. Average Fe retranslocation from leaves of Fe-sufficient plants was not large enough to meet the Fe demand of the growing shoot.

This was not due to a limitation in Fe availability for transport as an excess amount of Fe was supplied which was not biologically bound, but a limitation due to transport facilities (Huve *et al*, 2003). The concentration of total iron in green leaves was not significantly different from that of chlorotic leaves, but the concentration of ferrous iron was much lower in chlorotic leaves than in green leaves of peach (Gharsalli and Hajji, 2002). Singh and Sahu (1993) suggested that Fe, despite absorption and uptake, was subjected to inactivation and the mobility of Fe was impaired in the chlorotic plants of groundnut.

The objectives of the present study were to determine if elevated air temperature was the specific environmental cause of whitening of ivy geranium and to determine the effectiveness of Fe-chelate on preventing whitening of ivy geraniums.

Materials and Methods

Rooted cuttings of ivy geranium, 'Beach' and 'Butterfly' (Fischer Horticulture LLC, Boulder, CO) were potted on 15 Dec 2006 into 15-cm (1L) diameter pots. They were potted in sphagnum peat and perlite (70:30 by volume) with 0.96 kg gypsum$\cdot m^{-3}$, 7.7kg lime$\cdot m^{-3}$, and 0.32kg wetting agent (SaturAid, Debco Pty. Ltd., Tyabb, Australia) $\cdot m^{-3}$ added. Plants were fertilized with 250 mg N$\cdot L^{-1}$ from 20N-4.4P-16.6K (Peters peat-lite fertilizer, Scotts Company, Marysville, Ohio) as a continuous liquid feed. The cuttings were then grown for 6 weeks to develop a substantial root system before beginning experimental treatments. 'Beach' is known to be more resistant to whitening than 'Butterfly' (Personal communication, Harvey Lang, Fischer, USA).

Six growth chambers (1.27m width x 1.20m length x 0.91m height) were constructed of a single layer (4mm clear) polyethylene over PVC tubing inside a double

layer polyethylene covered Quonset greenhouse to maintain the set temperatures. Heating cables (Gro-Quick Cables, Wrap-On Company, Bedford Park, IL) were run at three levels below, on top of, and 10 cm above the lath bench top, to heat the growth chambers. Sidewalls were raised or lowered to regulate temperature (Figure 4.2). Air temperature was recorded at hourly intervals with WatchDog data loggers (Model 125, Spectrum Technologies, Plainfield, IL). The temperature sensors were kept at canopy level and shielded from the sun. There were three replications for each temperature. Average day/night temperatures were $28^{o}C/16^{o}C$ for unheated chambers and $36^{o}C/22^{o}C$ for heated chambers (Table 4.1). Light intensity ranged between 400-800 $\mu mol \cdot s^{-1} \cdot m^{-2}$ PPF inside the growth chambers at canopy level when measured between 10:00am to 2:00pm from 2 Feb to 16 March.

Fe (Sprint 138, Fe-EDDHA, 6% Fe, Becker Underwood Inc, Ames, IA) was applied at 0mg Fe (60ml distilled water application) or 27mg Fe (i.e. 0.45g Fe-chelate dissolved in 60ml of distilled water) drench per container at 15-day intervals starting from 1 Feb 2007. Fe-chelate was always applied between 16:00-17:30hr to prevent sun scorch. Plants were placed in growth chambers on 2 Feb. 2007 and grown for 6 weeks.

Plant Growth

Data was collected for plant height (from the rim of the container) and width (an average of two widths measured, one at the widest point and another at 90^{o}) at bi-weekly intervals. Growth index was calculated as: GI (cm^3) = 3.14*(width/2)^2*height. At the end of experiment, extent of whitening was determined as visual rating on a scale of 1 to 7, where 1 = 0%, 2= 17%, 3= 34%, 4 =50%, 5= 67%, 6= 84% and 7 = 100% whitening.

Five recently mature leaves were harvested from each plant and one 38.5mm^2 leaf disk cut from each leaf of that plant was used for pigment analysis. Each leaf disk was cut into 5-6 small pieces for easy extraction. The five leaf disks from a plant were placed in a vial with 10 ml of 80% acetone. These vials were incubated at room temperature (20°C) in the dark to allow complete extraction of pigment concentrations [i.e. chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids (Caro)]. The absorptions of the extracts were determined at wavelengths 663, 645, and 470 nm using Bio-Rad UV/VIS spectrophotometer (Bio-Rad Laboratories, Hercules, CA, USA). A vial of 10 ml 80% acetone was used as a control blank. The following equations were used to calculate the pigment concentrations:

$$Chl\,a = 12.7A_{663\,nm} - 2.69A_{645\,nm} \qquad \text{(Hill, 1963)}$$

$$Chl\,b = 22.9A_{645\,nm} - 4.80A_{663\,nm} \qquad \text{(Hill 1963)}$$

$$Caro = \frac{\left(1000A_{470\,nm} - 1.82\,Chl\,a_c - 85.02\,Chl\,b_c\right)}{198} \qquad \text{(Lichtenthaler, 1987)}$$

where A = absorbance

Pigment concentrations were in μg·ml^{-1}. Pigment concentrations were expressed on a leaf area basis (μg·cm^{-2}) by using the conversion factor

$$Chlorophyll\,Concentration\ \ (\mu g \cdot cm^{-2}) = \frac{Chlorophyll\,Concentration\ \ (\mu g \cdot ml^{-1}) \times 10}{1.925}$$

where, 10 is the volume of acetone (ml) used for a sample and 1.925 is the total area (cm^2) of five leaf disks of a sample.

Chlorophyll extracts were acidified with 50μl of 1N HCl to measure total pheophytin content of leaves. 80% acetone plus 50μl of 1N HCl was used as the control

blank. Pheophtytin content was determined spectrophotometrically and calculated using the following equation (Vernon, 1960):

$$Pheophytins(\mu g \cdot ml^{-1}) = 6.75A_{666\,nm} + 26.03A_{655\,nm}$$

where A = absorbance, and pheophytins concentration was expressed in μg·cm^{-2} by using the following equation:

$$PheophytinsConcentration\ (\mu g \cdot cm^{-2}) = \frac{PheophytinsConcentration\ (\mu g \cdot ml^{-1}) \times 10}{1.925}$$

where, 10 is the volume of acetone (ml) used for a sample and 1.925 is the total area (cm^2) of five leaf disks of a sample.

The total phenolics content of leaves was also determined spectrophotometrically using five 38.5mm^2 (or 0.385 cm^2) leaf discs placed in 10 ml of extractant, a mixture of methanol, distilled water, and hydrochloric acid (79:20:1 v/v/v). The vials were incubated at 20°C for 24 hours in the dark to allow complete extraction of phenolic compounds. The absorption of ivy geranium leaf disks extract showed peak absorbance at 320 nm when scanned between 200 and 900 nm using the Bio-Rad UV/VIS spectrophotometer. The phenolics content was calculated using the equation (Kakani *et al*, 2004)

$$Phenolics\ (\mu g \cdot ml^{-1}) = 16.05 \times A_{320\ nm}$$

where A = absorbance. Results were converted to μg cm^{-2} by using the following equation:

$$Phenolics\,Concentration\ (\mu g \cdot cm^{-2}) = \frac{Phenolics\,Concentration\ (\mu g \cdot ml^{-1}) \times 10}{1.925}$$

where, 10 represents the volume of extractant (ml) used for a sample and 1.925 is the total area (cm^2) of five leaf disks of a sample.

Plants were harvested at the media line at the end of experiment (16 March 2007). Three plants per treatment (one from each replication) were harvested to determine leaf area, stem fresh weight and leaf fresh weight. Total leaf area was determined using a portable leaf area meter (LI-3000, LI-COR, Lincoln, NE). Plant tissues were dried in an oven at 60^{o}C until dry to measure stem dry weight and leaf dry weight. Total dry weight was calculated by adding leaf and stem dry weights. Leaf area ratio (LAR) was calculated as the ratio of total leaf area to total plant dry weight (Hunt, 1982). LAR represents the ratio of photosynthesizing to respiring material within the plant. Leaf area index (LAI) is the leaf area per unit area of land and was calculated by the following equation:

$$LAI = \frac{Leafarea}{3.14(r)2}$$, where r = radius of plant canopy. (Hunt, 1982)

Specific leaf area (SLA) is the mean area of leaf displayed per unit of leaf weight, a measure of leaf density or relative thickness (Hunt, 1982). It was calculated as the ratio of leaf area to leaf dry weight. Leaf weight ratio (LWR) is an indicator of leafiness of the plant on a weight basis (Hunt, 1982). It was calculated as the ratio of leaf dry weight to total dry weight. Shoot weight ratio (SWR) was calculated as the ratio of shoot dry weight to total dry weight (Hunt, 1982).

The remaining plants were harvested (one from each replication), fresh weight was measured and plants were then rinsed with 0.1N HCl and rinsed 3 times with deionized water. Plants were then dried at 60^{o}C until dry. Dry weight was measured. Dry leaves were ground through a 0.5 mm screen (20 mesh) using a cyclotec sample mill

(UDY corporation, Fort Collins, Colorado). Ground tissue samples were used to determine total iron (Fe), manganese (Mn), zinc (Zn), magnesium (Mg) and sulfur (S) using dry ash method (Crouse, 2001) and inductively-coupled plasma (ICP) atomic emission spectrometry (Optima 4300DV, PerkinElmer Instruments, Norwalk, Connecticut).

The experiment was a split plot design, split by temperature with three replications for each treatment. Each replication had 2 sub-samples. Plant size indices, fresh weights, dry weights, pigments, pheophytins, phenolic contents and foliar tissue nutrient concentrations were analyzed by analysis of variance, using Proc-Glimmix (SAS software, SAS Institute, Cary, N.C.). Fischer's protected least significant difference, (LSD) at P=0.05 was used to indicate significant differences between treatment means.

Results and Discussion

There was no interaction effects of elevated air temperature and Fe-chelate application observed for plant height, width or growth index in either cultivar throughout the experiment. There were no differences in plant height, width or growth index in either cultivar before starting the experimental treatments (Table 4.2). Elevated air temperature did not affect plant height, width or growth index in either cultivar after 2 or 4 weeks (Table 4.2). Elevated air temperature did not affect plant height, but it did cause reduced plant width in both cultivars after 6 weeks (Table 4.2). Growth index was smaller at elevated air temperature in 'Beach' but it was not different from the control (28^{o}C-day/16^{o}C-night) in 'Butterfly'. Elevated (36^{o}C-day/22^{o}C-night) temperature suppressed size of 'Beach' but not of 'Butterfly'. These results were consistent with other studies

showing elevated temperature reduced growth of creeping bentgrass and strawberry plants (Fu and Huang, 2003; Kadir *et al*, 2006 respectively).

There were no differences in plant height, width or growth index in either cultivar before starting the Fe-chelate applications (Table 4.3). Fe-chelate application suppressed plant height in 'Beach', but not in 'Butterfly' after 2 weeks (Table 4.3). Plant width and growth index were not different due to Fe-chelate application in either cultivar 2 weeks after the start of treatments. Fe chelate did not affect plant height, width or growth index in either cultivar 4 or 6 weeks after treatments began (Table 4.3). Even though there were differences in plant height in 'Beach' after 2 weeks, at the end of experiment there were no differences in any of the growth parameters due to Fe-chelate application.

There were no interactive effects of elevated air temperature and Fe-chelate application on extent of whitening in either cultivar. 'Butterfly' started showing whitening symptoms earlier than 'Beach' after 7 days of elevated air temperature (observational data). At the end of the experiment, 'Beach' and 'Butterfly' both showed whitening of leaves at elevated temperature (Table 4.4), confirming elevated air temperature causes whitening in ivy geranium. Similar findings have been documented that heat stress (56^{o}C) caused bleaching of *Pelargonium* X *hortorum* Bailey (Senaratna *et al*, 2002). These results were also similar to those reported for tree fern (*Cyathea cooperi*), *Euglena gracilis* and reef corals which showed chlorosis or bleaching at elevated air temperatures (Doley, 1983; Ortiz and Wilson, 1988; Warner *et al*, 1996). Fe-chelate application did not help to prevent or reduce whitening in either cultivar (Table 4.4).

No interactive effect between elevated air temperature and Fe-chelate application was observed for Chl a, Chl b, Chl a+b, Caro, chlorophyll a to b ratio (Chl a:b), Chl a:Caro, Chl b:Caro, and total chlorophyll:Caro in 'Beach'. Elevated air temperature did not reduce Chl a, Chl b, Chl a+b, Caro nor Chl a:b in 'Beach'. However, elevated temperature did decrease Chl a:Caro, Chl b:Caro and Chl (a+b):Caro ratios in 'Beach' (Table 4.5), implying that the chlorophyll degradation is greater than that of carotenoids in 'Beach'. These results are similar to those documented for tomato, where chlorophyll to carotenoids ratios decreased in heat-stressed plants (Camejo *et al*, 2005). The concentration of Chl a, Caro and Chl (a+b) were less in the elevated air temperature treatment in 'Butterfly' (Table 4.5), whereas no differences were observed for Chl b and Chl a:b ratio. These findings suggest more sensitivity in 'Butterfly' of Chl a to high temperature compared to Chl b, which is consistent with results from other studies (Weemaes *et al*, 1999; Loey *et al*, 1998). Fu and Huang (2003) also reported a reduction in leaf chlorophyll due to heat stress in creeping bentgrass.

No differences were observed in carotenoids concentration in 'Beach' due to elevated air temperature, but there was a reduction in carotenoids concentration in 'Butterfly' at elevated air temperature (Table 4.5). As a difference in carotenoids concentration was observed in the susceptible cultivar, 'Butterfly', it is possible that carotenoids may play a role in preventing whitening of ivy geranium. Chl a:b ratio did not show any difference in either cultivar indicating temperature reduced concentrations proportionally.

Fe-chelate applications did not affect Chl a, Chl b, Caro, or Chl a:b in 'Beach' or 'Butterfly' (Table 4.6). The pigment ratios were unaffected by temperature in 'Beach',

however, there was an interaction effect of temperature and Fe-application on Chl a:Caro, Chl b:Caro and Chl (a+b):Caro ratios in 'Butterfly' (Table 4.7). All pigment ratios were the lowest in 'Butterfly' plants grown under elevated air temperatures with no Fe-chelate application. None of the other combinations of temperature and Fe-chelate showed any difference in Chl a:Caro, Chl b:Caro and Chl (a+b):Caro ratios. These results imply that Fe-chelate application helped to preserve or accumulate chlorophyll in 'Butterfly' in the elevated temperature treatment. Further, this interaction of temperature and Fe-chelate application suggests that ivy geranium growers may adjust the Fe-fertility regime based on production temperature to maximize chlorophyll retention of plants.

Temperature did not affect the pheophytin concentration in 'Beach', but elevated temperature did reduce pheophytin concentration in 'Butterfly' (Table 4.8), indicating that there may be a heat-induced functional disconnection in electron transfer as the key photosynthetic reaction consists of the transfer of an electron from the primary donor called P680 to a nearby pheophytin molecule (Groot *et al*, 1997). These results indicate a reduction in electron carrier in photosynthetic electron transport chain at elevated air temperature. Fe-chelate application did not affect pheophytins content in either cultivar (Table 4.8).

There were no differences observed in total phenolic contents due to temperature in either cultivar (Table 4.8). These results were not consistent with the findings that documented elevated temperature reduced the concentration of several phenolic compounds in the leaves of *Salix myrsinifolia* (Veteli *et al*, 2002). Fe-chelate application did not affect total phenolic content in either cultivar (Table 4.8). Pigment concentrations are often used as indirect measure of photosynthetic biomass (Hurley and Watras, 1991).

The results indicate that there is a reduction in photosynthesis pigments and biomass in heat-stressed plants of ivy geraniums, so there would be a resulting reduction in photosynthesis. The whitening in ivy geranium may be thermal-inhibition as indicated by reduced pheophytins in 'Butterfly', followed by impaired photosynthetic pigments synthesis or their degradation or both, which further leads to whitening (i.e. bleaching) of chlorophyll. It would be of interest to look for reactive oxygen species, antioxidants, or heat-shock proteins during whitening of ivy geraniums to answer the photo-inhibition question. It would also be of interest to examine other electron carriers of the photosynthetic electron transport chain, especially ferredoxins (Fe-S proteins) that play an important role in photosynthetic electron transport (Abdel-Ghany *et al*, 2005).

No interactive effect of elevated temperature and Fe-chelate application was observed for stem fresh weight, leaf fresh weight, total fresh weight, stem dry weight, leaf dry weight, total dry weight, fresh to dry weight ratios in either cultivar. Stem, leaf, and total fresh weight, and stem, leaf, and total dry weight were reduced at elevated air temperature in 'Beach' and 'Butterfly' (Table 4.9). Elevated temperature reduced growth in ivy geraniums, which is in agreement with the similar work for pansy and strawberry plants (Hamlin *et al*, 1999; Kadir *et al*, 2006). In this study, there were no differences in total fresh to dry weight ratio, SWR and LWR due to temperature in 'Beach' or 'Butterfly (Table 4.9). Elevated temperature reduced all growth parameters proportionally, so no differences were observed in the ratios between them.

Fe-chelate applications did not affect stem, leaf, and total fresh weight, and stem, leaf, and total dry weight, total fresh to dry weight ratio, SWR or LWR in 'Beach' or 'Butterfly' (Table 4.10). Fe-chelate applications did not aid or hinder growth of plants.

Leaf area was reduced at elevated air temperatures in 'Beach' and 'Butterfly' (Table 4.11) indicating a reduced leaf surface area for transpiration, and therefore reduced transpirational cooling capacity. Similarly, high temperature has been documented to reduce leaf expansion in strawberry plants (Kadir *et al*, 2006). No differences were found in LAI (i.e. leaf area: area of plant canopy) or LAR (i.e. leaf area: whole plant dry weight). This may be due to a proportional reduction of area of plant canopy and whole plant dry mass with respect to whole plant leaf area in both cultivars. Specific leaf area (SLA) was not different in 'Beach', but it was reduced in 'Butterfly' plants grown at elevated temperature (Table 4.11) indicating leaf thickness or leaf density increased in 'Butterfly' at elevated temperature. SLA is a link between vegetation water and carbon cycles and describes the allocation of leaf biomass per unit leaf area (Pierce *et al*, 1994). SLA is also correlated with water use efficiency (WUE) of peanut (Craufurd *et al*, 1999). WUE decreased significantly at high temperature (Kadir *et al*, 2006). SLA could be used as an indirect measure of WUE (Craufurd *et al*, 1999), suggesting that in ivy geranium, reduced SLA may also be an indicator of increased WUE or a response to reduced WUE. Reduced WUE may cause 'Butterfly' to increase leaf thickness in addition to reducing leaf expansion at elevated temperature. 'Beach' reduced its overall growth at elevated temperature whereas 'Butterfly' increased leaf thickness as indicated by reduced SLA thereby increasing WUE.

Fe-chelate applications did not affect leaf area, LAI, LAR, and SLA in either cultivar (Table 4.12). These results indicate that Fe-chelate applications did not affect leaf morphology in ivy geranium.

There were no interaction effects of temperature and Fe-chelate application on foliar nutrient composition of either cultivar, except for total foliar Fe in 'Beach' (Table 4.13). Elevated air temperature and Fe-chelate at 27mg Fe drench application resulted the maximum total foliar Fe content compared to that at elevated temperature with no Fe-chelate and at ambient temperature with or without Fe-chelate. Higher level of total Fe at elevated air temperature with Fe-chelate application indicates that the Fe was taken up by plants from the soil-solution and uptake of Fe was not inhibited during whitening of ivy geranium at elevated temperature. Fe-chelate application at ambient temperature did not increase uptake. Similar concentrations of Fe at ambient temperature with or without Fe-chelate application and at elevated temperature without Fe-chelate may be due to less demand by plants at ambient temperature or it may be due to a dilution factor contributed by more growth at ambient temperature.

Despite the differences in severity of whitening of leaves, foliar total Fe content in 'Butterfly' was similar at ambient and elevated temperatures (Table 4.14) indicating that the absorption of Fe was unaffected by temperature in 'Butterfly'. Similar foliar tissue concentration of total Fe in normal healthy green plants of 'Butterfly' grown at ambient temperature and in heat-stressed plants of 'Butterfly' grown at elevated temperature may be supported by the 'Chlorosis paradox' when chlorotic plants had higher total Fe than green ones (Romheld, 2000). Chlorosis in leaves is caused not only by insufficient uptake or translocation of Fe from roots to aerial parts, but also from the failure of the leaves to reduce Fe^{3+}, a process that is affected by the presence of nitrate ions, since the activity of chelate reductase located in the plasma-lemma appears to be depressed at alkaline pH under these conditions (Mengel *et al*, 1994). Zohlen (2002) concluded chlorosis to be a

physiological Fe deficiency caused by an immobilization of Fe to a metabolically inactive form in the plant tissues. There may be an inactivation or immobilization of Fe in heat stressed plants of ivy geraniums. Another possible explanation for similar total Fe content at ambient and elevated air temperature could be the sampling material. Tissue analysis was done on a whole plant leaf basis, where white and green leaves were not separated for analysis. Fe may not be distributed evenly among older and new leaves. Fe may be more concentrated in older green leaves and was not re-translocated to the upper, newly developing, white leaves. There may be a difference in total Fe content between green and white leaves of 'Butterfly'. It would be of interest to examine ferrous (active) Fe in leaves at different levels on the plants to answer the inactivation or immobilization question.

Mn and Zn contents of foliar tissue of 'Beach' were greater at elevated temperature compared to at ambient temperature, whereas Mg and S contents of 'Beach' were not affected by temperature (Table 4.14). A reduced nutrient dilution due to dimished growth at elevated temperature may cause accumulation of nutrients in foliar tissues of 'Beach'. These results are consistent with the findings that Fe and Mn levels in the leaves and roots of tomato plants were greater during summer than in winter (Darawsheh *et al*, 2006). Foliar tissue contents of total Fe, Mn, Zn and Mg in 'Butterfly' were not affected by temperature (Table 4.14), despite severity of whitening of its leaves.

The higher uptake rates of Mn and Zn in 'Beach' plants grown at elevated temperature (irrespective of Fe-chelate application) supports the view of Kochian (1993) that divalent cations (Fe, Mn, Zn) are taken up through the same channels in the plasma membrane of the root cell. Romheld (1987) classified *Pelargonium* as a Strategy-I plant,

having Fe-efficiency mechanisms. Fe-efficiency mechanisms may also enhance the availability of Mn (Welch *et al*, 1993). It may be due to greater need of nutrients, especially Fe, at elevated temperature.

Chelate (EDTA) liquid feed may act as an extractant and thus elevate the water-soluble levels of Mn and Zn recovered from the substrate (Kreij, 1998). Higher mobilization of Zn from soil to maize plants was observed with an application of EDTA (Chen *et al*, 2004). This mobilization or extracting effect may be greater at elevated temperature, which may have contributed to increased concentrations of Mn and Zn in plant tissue, even though none were applied beyond that present in liquid feed

Foliar S content increased in 'Butterfly' at elevated air temperature (Table 4.14). Sulfur is taken up as SO_4^{2-} by the roots and is transported via xylem to the leaves. The uptake and subsequent distribution of SO_4^{2-} to the leaves is closely regulated in response to demand (Sunarpi and Anderson, 1996). All enzymatic and most non-enzymatic antioxidants contain Sulfur (S). Glutathione, as the major non-protein thiol, plays a central role in reactive oxygen species (ROS) defense. Antioxidant defense systems are often constitutive multifactorial elements of plant cell metabolism that are up regulated under the impact of biotic and abiotic stressors (Kandlbinder *et al* 2004). Temperature had a dominant effect on growth, shoot and root sulfur contents (Gadallah, 1996). 'Butterfly' plants may need more S at elevated temperatures to synthesize heat-shock proteins or antioxidants in response to temperature-stress, which may explain higher concentration of S in leaves at elevated air temperature. The developing leaves are strong sinks of S, but show a net loss of S after full expansion (Sunarpi and Anderson, 1996). During whitening of 'Butterfly', leaf expansion reduced in 'Butterfly' as indicated by its

leaf area and SLA. The younger white leaves with an upward cupped appearance at elevated temperature did not fully expand, this may explain the higher concentrations of foliar S in 'Butterfly' at elevated air temperature.

Fe-chelate application increased foliar total Fe concentration in 'Beach' and 'Butterfly' (Table 4.13 and Table 4.15). Foliar Mn content of 'Beach' and 'Butterfly' decreased with application of Fe-chelate (Table 4.15). Foliar concentration of Zn, Mg and S did not change with application of Fe-chelate in either cultivar (Table 4.15). Concentration of Mn in foliar tissues was greater in both cultivars receiving no Fe-chelate application. Additional-Fe inhibited the uptake and/or translocation of Mn, which is consistent with the findings, documented for bean and barley plants (Zhang *et al*, 1996; Alam *et al*, 2001). Somers and Shive (1942) reported that Mn appeared to oxidize ferrous Fe to the more insoluble ferric ion. The high concentration of soluble Mn in the tissues is invariably associated with low concentration of soluble Fe and vice-versa.

Foliar tissue Fe:Mn ratio decreased in 'Beach' at elevated temperature, whereas Fe:Mn ratio remained the same in 'Butterfly' under both temperatures (Table 4.16). Fe: Zn ratio was not affected by temperature in either cultivar. Fe concentration was reduced with respect to Mn concentration at elevated temperature as indicated by Fe:Mn ratio in 'Beach'. An effective balance between Fe and Mn concentrations may not exist for normal growth of plants at elevated temperature and may contribute to the cause of whitening in ivy geranium 'Beach'. Fe and Mn are intimately interdependent in their effects upon the plant and the nature of the effects of one is determined by the proportionate presence of the other (Somers and Shive, 1942). The oxidation of ferrous

ions to ferric ions and then precipitation of Fe in the form of ferric organic complexes is determined by the relative quantity of manganese ions present in the plant system (Somers and Shive, 1942). An increase in manganese brings about a decrease in the soluble Fe and an increase in the percentage of insoluble Fe in the plant indicating that manganese, with its high oxidizing potential, is responsible for the decrease of soluble iron by oxidation of the ferrous ions to ferric ions ultimately resulting in the precipitation of Fe (Somers and Shive, 1942). The optimum ratio of Fe:Mn is probably determined by factors other than nutritional such as temperature, light intensity or day length (Twyman, 1950). In this study, temperature clearly affected Fe and Mn homeostasis in 'Beach' but not in 'Butterfly'. These results further imply that this change in nutrient homoestasis at elevated temperature was only observed in the cultivar that is less susceptible to whitening. 'Beach' adjusted its uptake or absorption of nutrients required at elevated temperature to reduce temperature injury at elevated temperatures whereas 'Butterfly' did not.

Elevated temperature did not affect tissue Fe:Mn and Fe:Zn ratios in 'Butterfly' (Table 4.16). Elevated temperature increased the extent of whitening, but decreased biomass and leaf area of 'Butterfly'. These results suggest that even if these ratios were not different at elevated temperature, there may be a difference in soluble and insoluble fractions of these nutrients in plants at elevated temperature.

Foliar tissue Fe:Mn and Fe:Zn ratios increased significantly in 'Beach' as well as in 'Butterfly' (Table 4.16) when plants were supplied with Fe-chelate. These results suggest Fe from chelate was readily taken up and was not a limiting factor. This is consistent with the findings that increasing external supply of Fe to plants receiving

constant Mn in soil solution leads to the progressive depression of Mn concentration in the tissues (Twyman, 1950).

The accumulation of Fe, Mn, Zn and reduction in Fe: Mn ratio at elevated air temperature was obtained in 'Beach' that is resistant to whitening, but not in 'Butterfly' that is susceptible to whitening. Only S increased in 'Butterfly' at elevated temperature. These nutritional analyses indicate that there may be a cultivar difference in nutrients requirements at elevated air temperature.

Conclusion

Elevated air temperature causes whitening of ivy geranium. Elevated air temperature severely reduced the growth, leaf area, fresh (shoot, leaf and total) weight and dry (shoot, leaf and total) weight of ivy geraniums. 'Beach' responded to heat stress by increasing less in size whereas 'Butterfly' did not. Specific leaf area (SLA) was reduced in 'Butterfly' but not in 'Beach'.

Elevated air temperature reduced photosynthetic pigments and their ratios in ivy geranium. Degradation of chlorophyll was greater than carotenoids. Reduction in carotenoids concentration in 'Butterfly' indicated the possible role of carotenoids in preventing whitening of ivy geraniums. Elevated temperatures reduced total pheophytins concentration and may be an indication of heat-induced functional disconnection in electron transfer resulting in photo-inhibition.

Higher levels of total foliar Fe indicated no inhibition of Fe-uptake in 'Beach' at elevated temperature, whereas similar levels of foliar Fe at both temperatures in 'Butterfly' suggest a possibility of inactivation or immobility of Fe within plants at

elevated air temperature. ‘Beach’ accumulated higher foliar Mn and Zn contents at elevated temperature whereas ‘Butterfly’ had similar concentrations of foliar Mn and Zn at elevated temperature. Higher levels of foliar S observed in ‘Butterfly’ at elevated temperature suggested the greater demand of S in stressed plants at elevated temperature.

Fe-chelate application did not reduce growth, leaf area, fresh (stem, leaf and total) weight, dry (stem, leaf and total) weight, fresh: dry weight ratio. Even though Fe-application did not help to reduce whitening in ivy geraniums, it helped to preserve chlorophyll in ‘Butterfly’. Fe-chelate application was of no benefit at low ambient temperature, but it increased Chl:Caro ratio at elevated air temperature in ‘Butterfly’.

Whitening in ivy geranium is a symptom of heat stress first expressed in young developing leaves. It is caused by elevated air temperature, followed by impaired photosynthetic biomass synthesis and/or degradation leading to bleaching of chlorophyll. Fe-uptake was not inhibited due to elevated air temperature during whitening. Accumulation of total Fe, Mn, Zn and S in foliar tissues and no breakdown or reduction in carotenoids and pheophytins concentration reduces susceptibility of ivy geranium to whitening.

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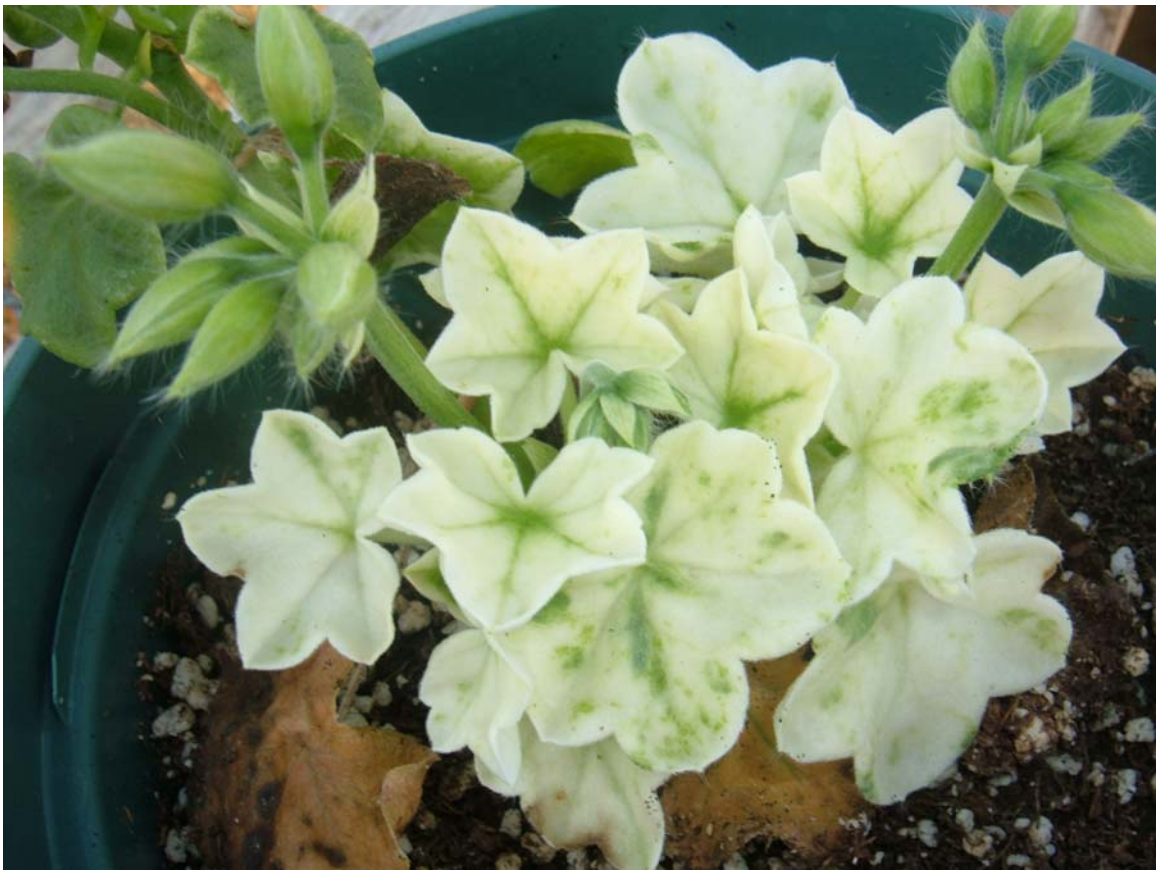

Figure 4.1 Symptoms of whitening disorder in ivy geraniums

Figure 4.2 Growth chambers with sidewalls raised or lowered to regulate temperature

Table 4.1. Average daily growth chamber temperature from 2 Feb to 16 March 2007, Day= 7am -5pm, and night = 6pm- 6am.

	Unheated Temperature (^{o}C)		Heated Temperature (^{o}C)	
Day	Day-time	Night-time	Day-time	Night-time
1	28.5	13.8	35.2	19.9
2	25.1	14.4	32.8	20.5
3	26.6	14.5	35.4	20.8
4	27.0	15.9	36.6	21.8
5	27.1	16.3	36.5	23.1
6	19.1	16.3	25.6	23.1
7	18.6	15.2	25.3	22.4
8	29.3	13.7	37.8	21.4
9	30.0	14.8	38.3	21.8
10	27.0	16.9	34.7	23.3
11	24.4	16.9	31.7	23.7
12	24.4	14.7	32.6	21.9
13	25.3	14.4	33.7	20.9
14	28.7	13.7	37.8	20.4
15	24.0	14.4	32.3	20.6
16	29.3	13.8	38.2	20.3
17	28.1	14.8	35.5	21.0
18	23.9	17.7	30.2	23.8
19	28.9	18.1	36.7	25.1
20	32.2	16.2	40.5	23.7
21	28.4	15.8	36.9	22.7
22	23.8	17.8	31.2	23.8
23	31.8	16.4	40.1	22.3
24	31.8	15.2	39.5	19.9
25	32.1	15.5	40.3	20.0
26	29.4	16.5	36.4	20.7
27	23.3	17.5	28.0	21.6
28	29.9	15.1	38.9	20.3
29	27.0	14.5	32.9	19.2
30	30.9	13.8	38.9	18.4
31	30.7	14.5	38.6	18.6
32	27.4	15.5	35.4	20.2
33	29.6	16.1	39.0	22.3
34	29.4	16.3	38.8	22.8
35	29.2	16.8	38.2	23.5
36	29.0	18.1	38.1	24.4

Table 4.1. (Continued)

	Unheated Temperature (°C)		Heated Temperature (°C)	
Day	Day-time	Night-time	Day-time	Night-time
37	29.5	18.1	38.0	25.0
38	28.5	17.8	36.4	24.9
39	27.1	18.9	35.5	25.6
40	28.6	19.1	36.0	26.0
41	26.6	19.8	35.7	26.5
42	28.1	17.1	38.1	25.0
43	28.3	15.9	38.4	23.1
44	30.2	17.8	38.1	23.9
Average	27.7	16.1	35.8	22.3

Table 4.2. Effect of elevated air temperature on plant height, width and growth index (GI) in ivy geranium

	Beach			Butterfly		
Temperature	Height (cm)	Width (cm)	GI (cm^3)	Height (cm)	Width (cm)	GI (cm^3)
At the start of experiment						
28°C	16.3 a[x]	30.4 a	11843 a	12.9 a	24.9 a	6484 a
36°C	15.8 a	29.2 a	10737 a	13.8 a	25.1 a	6817 a
2 weeks						
28°C	16.3 a	36.9 a	17301 a	14.3 a	32.2 a	12047 a
36°C	16.1 a	32.6 a	13727 a	13.8 a	29.0 a	9104 a
4weeks						
28°C	18.3 a	41.9 a	25290 a	15.6 a	37.5 a	17481 a
36°C	16.5 a	34.0 a	15334 a	15.3 a	32.0 a	12386 a
6weeks						
28°C	18.1 a	47.4 a	31934 a	15.1 a	42.8 a	21996 a
36°C	17.8 a	35.0 b	17446 b	16.8 a	33.4 b	14968 a

[x]Means followed by same letters within column and within 2 weeks period are not different according to Fisher's protected LSD test (P=0.05).

Table 4.3. Effect of Fe-chelate application on plant height, width and growth index (GI) in ivy geranium

Treatment	Beach Height (cm)	Beach Width (cm)	Beach GI (cm^3)	Butterfly Height (cm)	Butterfly Width (cm)	Butterfly GI (cm^3)
At the start of experiment						
0mg Fe	16.3 a[x]	28.8 a	10772 a	13.4 a	24.9 a	6610 a
27mg Fe	15.8 a	30.7 a	11809 a	13.2 a	25.1 a	6691 a
2 weeks						
0mg Fe	17.4 a	33.5 a	15575 a	14.5 a	30.4 a	10963 a
27mg Fe	15.0 b	36.0 a	15453 a	13.6 a	30.8 a	10188 a
4weeks						
0mg Fe	18.5 a	38.0 a	21862 a	15.9 a	35.1 a	15678 a
27mg Fe	16.2 a	37.8 a	18762 a	15.0 a	34.4 a	14189 a
6weeks						
0mg Fe	18.7 a	40.5 a	24909 a	16.2 a	38.1 a	18337 a
27mg Fe	17.1 a	41.9 a	24471 a	15.8 a	38.1 a	18627 a

[x]Means followed by same letters within column and within 2 weeks period are not different according to Fisher's protected LSD test (*P*=0.05).

Table 4.4. Main effects of elevated air temperature and Fe-chelate applications on extent of whitening (on scale of 1 to 7, 1= 0%, 2= 17%, 3= 34%, 4=50%, 5= 67%, 6= 84% and 7= 100% whitening) of ivy geranium 'Beach' and 'Butterfly' grown at 28°C or 36°C and supplied with Fe-chelate at 0 or 27 mg Fe drench per plant

	Beach	Butterfly
Temperature		
28°C	1.02 b[x]	1.08 b
36°C	2.08 a	4.17 a
Fe-application		
0mg (control)	1.63 a	2.71 a
27mg drench	1.48 a	2.54 a

[x]Means followed by same letters within column and within treatment are not different according to Fisher's protected LSD test (*P*=0.05).

Table 4.5. Effect of elevated air temperature on pigment concentrations ($\mu g \cdot cm^{2-}$) and their ratios in ivy geranium 'Beach' and 'Butterfly'

Temperature	Chl a	Chl b	Caro	Chl a+b	Chl a:b	Chl a:Caro	Chl b:Caro	Total Chl:Caro
Beach								
28°C	22.82 a[x]	13.41 a	7.76 a	36.23 a	1.70 a	2.95 a	1.73 a	4.68 a
36°C	17.16 a	10.41 a	7.42 a	27.58 a	1.64 a	2.34 b	1.43 b	3.78 b
Butterfly								
28°C	19.63 a	11.24 a	6.89 a	30.87 a	1.75 a	*z	*z	*z
36°C	10.94 b	6.46 a	4.57 b	17.39 b	1.87 a	*z	*z	*z

[x]Means followed by same letters within column and cultivar are not different according to Fisher's protected LSD test (*P*=0.05).
*Chlorophyll a represented as Chl a, Chlorophyll b as Chl b, Carotenoids as Caro, Total Chlorophyll as Chl a+b
*z =interaction effects of temperature and Fe-chelate (See Table 4.7)

Table 4.6. Effect of Fe-chelate application on pigment concentrations ($\mu g \cdot cm^{2-}$) and their ratios in ivy geranium 'Beach' and 'Butterfly'

Temperature	Chl a	Chl b	Caro	Chl a+b	Chl a:b	Chl a:Caro	Chl b:Caro	Total Chl:Caro
Beach								
0mg Fe	18.76 a[x]	11.06 a	7.12 a	29.82 a	1.69 a	2.66 a	1.57 a	4.23 a
27mg Fe	21.22 a	12.76 a	8.06 a	33.99 a	1.65 a	2.64 a	1.59 a	4.23 a
Butterfly								
0mg Fe	14.92 a	8.28 a	5.75 a	23.21 a	1.98 a	*z	*z	*z
27mg Fe	15.64 a	9.41 a	5.71 a	25.06 a	1.64 a	*z	*z	*z

[x]Means followed by same letters within column and cultivar are not different according to Fisher's protected LSD test (*P*=0.05).
*Chlorophyll a represented as Chl a, Chlorophyll b as Chl b, Carotenoids as Caro, Total Chlorophyll as Chl a+b
*z =interaction effects of temperature and Fe-chelate (See Table 4.7)

Table 4.7. Interaction effect of elevated air temperature and Fe-chelate application on pigment ratios* in ivy geranium 'Butterfly'

Interaction		Chl a:Caro	Chl b:Caro	Total Chl:Caro
28°C	0mg Fe	2.87 a	1.67 a	4.54 a
28°C	27mg Fe	2.83 a	1.59 a	4.42 a
36°C	0mg Fe	2.13 b	1.01 b	3.14 b
36°C	27mg Fe	2.60 a	1.73 a	4.33 a

Means within column separated using LSD P=0.05
*Chlorophyll a represented as Chl a, Chlorophyll b as Chl b, Carotenoids as Caro, Total Chlorophyll as Chl a+b

Table 4.8. Main effects of elevated air temperature and Fe-chelate application on total pheophytins and phenolic contents in ivy geranium 'Beach' and 'Butterfly' grown at temperature 28°C or 36°C and supplied with Fe-chelate at 0 or 27 mg Fe drench per plant at biweekly intervals throughout the experiment

	Beach		Butterfly	
	Pheophytins	Total phenolics	Pheophytins	Total phenolics
Temperature				
28°C	25.69 a	97.66 a	18.51 a	118.21 a
36°C	17.40 a	94.17 a	3.56 b	96.97 a
Fe-application				
0mg	18.93 a	100.93 a	12.64 a	114.55 a
27mg drench	24.16 a	90.91 a	9.23 a	100.64 a

Means within column and with treatment separated using LSD P=0.05.

Table 4.9. Effect of elevated air temperature on fresh (shoot, leaf and total) weight, dry (shoot, leaf and total) weight, fresh to dry weight ratio, shoot weight ratio* (SWR) and leaf weight ratio** (LWR) in ivy geranium 'Beach' and 'Butterfly'

Temperature	Stem fresh wt (g)	Leaf fresh wt (g)	Total fresh wt (g)	Stem dry wt (g)	Leaf dry wt (g)	Total dry wt (g)	Fresh: dry wt ratio	SWR	LWR
Beach									
28°C	49.4 a[x]	244.8 a	284.3 a	9.6 a	22.0 a	29.6 a	9.62 a	0.30 a	0.70 a
36°C	26.7 b	126.7 b	169.2 b	4.3 b	12.2 b	17.5 b	9.76 a	0.25 a	0.75 a
Butterfly									
28°C	44.3 a	191.6 a	229.1 a	7.5 a	16.6 a	22.7 a	10.10 a	0.31 a	0.69 a
36°C	23.6 b	90.4 b	113.0 b	3.8 b	8.7 b	12.1 b	9.37 a	0.30 a	0.69 a

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).

* is the ratio of stem dry weight to total dry weight and ** is the ratio of leaf dry weight to total dry weight.

Table 4.10. Effect of Fe-chelate application on fresh (stem, leaf and total) weight, dry (stem, leaf and total) weight, fresh to dry weight ratio, shoot weight ratio* (SWR) and leaf weight ratio** (LWR) in ivy geranium 'Beach' and 'Butterfly'

Treatments	Stem fresh wt (g)	Leaf fresh wt (g)	Total fresh wt (g)	Stem dry wt (g)	Leaf dry wt (g)	Total dry wt (g)	Fresh: dry wt ratio	SWR	LWR
Beach									
0mg Fe	36.8 a[x]	188.4 a	221.5 a	7.3 a	17.4 a	23.3 a	9.59 a	0.28 a	0.72 a
27mg Fe	39.3 a	183.0 a	231.9 a	6.6 a	16.8 a	23.8 a	9.79 a	0.27 a	0.73 a
Butterfly									
0mg Fe	35.7 a	144.6 a	169.4 a	6.0 a	13.3 a	17.2 a	9.80 a	0.31 a	0.69 a
27mg Fe	32.5 a	137.4 a	172.7 a	5.3 a	12.3 a	17.6 a	9.70 a	0.29 a	0.69 a

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).

* is the ratio of stem dry weight to total dry weight and ** is the ratio of leaf dry weight to total dry weight.

Table 4.11. Effect of elevated air temperature on leaf area (LA), leaf area index (LAI), leaf area ratio (LAR) and specific leaf area (SLA) in ivy geranium 'Beach' and 'Butterfly'

Temperature	LA (cm^2)	LAI	LAR	SLA
Beach				
28°C	3111 a[x]	1.82 a	98.6 a	141.5 a
36°C	1643 b	1.91 a	101.5 a	135.8 a
Butterfly				
28°C	2579 a	1.90 a	107.2 a	155.9 a
36°C	1192 b	1.55 a	94.5 a	136.5 b

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).

Table 4.12. Effect of Fe-chelate application on leaf area (LA), leaf area index (LAI), leaf area ratio (LAR) and specific leaf area (SLA) in ivy geranium 'Beach' and 'Butterfly'

Treatments	LA (cm^2)	LAI	LAR	SLA
Beach				
0mg Fe	2417 a[x]	1.95 a	99.0 a	138.2 a
27mg Fe	2337 a	1.77 a	101.1 a	139.1 a
Butterfly				
0mg Fe	1945 a	1.64 a	101.5 a	147.8 a
27mg Fe	1826 a	1.81 a	100.0 a	144.6 a

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).

Table 4.13. Effect of elevated air temperature and Fe-chelate application on foliar total Fe content of 'Beach'

Interaction		
Temperature	Treatments (drench per pot)	Total Fe (ppm)
28°C	0mg Fe	58.52 b[x]
28°C	27mg Fe	64.12 b
36°C	0mg Fe	59.99 b
36°C	27mg Fe	99.15 a

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).

Table 4.14. Effect of elevated air temperature on foliar nutrient (total Fe, Mn, Zn, Mg and S) contents of ivy geranium 'Beach' and 'Butterfly'

Temperature	Fe (ppm)	Mn (ppm)	Zn (ppm)	Mg (%)	S (%)
Beach					
28°C	*z	39.61 b[x]	21.04 b	0.24 a	0.10 a
36°C	*z	78.59 a	34.95 a	0.34 a	0.16 a
Butterfly					
28°C	67.43 a	27.83 a	18.72 a	0.25 a	0.10 b
36°C	72.28 a	40.01 a	23.90 a	0.25 a	0.16 a

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).
*z indicates the interaction between temperature and Fe-chelate application for Fe (ppm).

Table 4.15. Effect of Fe-chelate application on foliar nutrient (Fe, Mn, Zn, Mg and S) contents of ivy geranium 'Beach' and 'Butterfly'

Treatments	Fe (ppm)	Mn (ppm)	Zn (ppm)	Mg (%)	S (%)
Beach					
0mg Fe	*z	69.59 a[x]	29.68 a	0.27 a	0.13 a
27mg Fe	*z	48.61 b	26.31 a	0.31 a	0.12 a
Butterfly					
0mg Fe	55.09 b	40.68 a	21.12 a	0.27 a	0.13 a
27mg Fe	80.66 a	27.17 b	21.49 a	0.23 a	0.13 a

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).
*z indicates the interaction between temperature and Fe-chelate application for Fe (ppm).

Table 4.16. Main effects of elevated air temperature or Fe-chelate application on foliar total Fe:Mn and total Fe:Zn ratios in 'Beach' and 'Butterfly' grown at temperature 28°C or 36°C and supplied with Fe-chelate at 0 or 27 mg Fe drench per plant

	Beach		Butterfly	
Temperature	Fe:Mn	Fe:Zn	Fe:Mn	Fe:Zn
28°C	1.59 a[x]	2.98 a	2.50 a	3.50 a
36°C	1.13 b	2.35 a	1.98 a	3.04 a
Fe-application				
0mg Fe	0.97 b	2.13 b	1.47 b	2.70 b
27mg Fe	1.75 a	3.20 a	3.00 a	3.84 a

[x]Means followed by same letters within column and within treatment are not different according to Fisher's protected LSD test (*P*=0.05).

CHAPTER V

CREATING NUTRIENT DEFICIENCIES TO INDUCE WHITENING IN IVY GERANIUM

Abstract

Ivy geranium (*Pelargonium peltatum* L.) develops whitening when grown in warm southeastern U.S. summers. The development of whitening of the youngest leaves of actively growing ivy geranium has been observed as the season changes from late spring to summer. Iron (Fe) deficiency has been suspected to cause this whitening disorder. A study was conducted to determine if Fe or other nutrient (Mn, Zn, S or Mg) deficiencies could cause whitening of ivy geraniums. Ivy geranium 'Beach' and 'Butterfly' were supplied with a complete Hoagland's solution (Control) or solution deficient in one of the following: all micronutrients (–Micro), iron (–Fe), zinc (–Zn), manganese (–Mn), sulfur (–S) or magnesium (–Mg). Results showed that –Micro and –S treatments severely reduced plant width, growth index, shoot fresh weight, and shoot dry weight of ivy geranium. Root fresh and dry weights were not affected by deficiency treatments in either cultivar. Fresh shoot: root ratio in 'Beach' declined under –Micro and –S treatments, whereas it did not in 'Butterfly'. 'Beach' did not show nutrient deficiency symptoms while 'Butterfly' plants grown under –Micro, –Fe, –S and –Mg treatments

developed symptoms of their deficiencies respectively. However, they did not result in symptoms similar to whitening. None of the deficiency treatments resulted in whitening in either cultivar of ivy geraniums. Fe deficiency stimulated the accumulation of Mn in both cultivars of ivy geranium. Plants of both cultivars grown under –Zn treatment had lower Zn and Mg concentration in their leaves. Mn deficiency did not stimulate the accumulation of Fe or Zn in ivy geraniums. S deficiency reduced the accumulation of S and Mg in both cultivars of ivy geranium. Mg deficiency reduced the accumulation of Mg, but stimulated the accumulation of Mn in both cultivars of ivy geranium.

Introduction

Ivy geranium (*Pelargonium peltatum* L.) grows poorly in the heat of southeastern U.S. summers. The leaves of ivy geranium turn white, become smaller in size and develop an upward cupped appearance. This physiological disorder is referred to as "whitening". There has been observational evidence that applying Fe-chelate before whitening occurs helped to reduce whitening.

Although bleaching of *Synchoccus sp.* appears to be a general response to nutrient deprivation, it is not the same under all nutrient-limited conditions (Collier and Grossman, 1992). Iron (Fe) has an important role in the formation of thylakoid membranes in higher plants. It functions to accept and donate electrons and plays important roles in the electron-transport chains of photosynthesis and respiration (Connolly and Guerinot, 2002). Typical symptoms of Fe-deficiency are interveinal chlorosis, and a pale, washed-out appearance of new leaves (Abadia, 1992; Havlin *et al*, 2003). Fe deficiency leads to defects in photosynthesis and bleaching in young leaves,

phenomena that are closely related to a defect in chloroplastic photosystem-I (PS-I) accumulation, a major Fe-S cluster protein complex in plants (Pilon *et al*, 2006). Fe deficiency is accompanied by a decrease in all membrane components, including the electron carriers in the photosynthetic electron transport chain (Spiller and Terry, 1980) and light harvesting pigments, chlorophylls and carotenoids (Morales *et* al, 1990; Morales *et* al, 1994). High iron levels in the nutrient solution were associated with dark green leaves of *Pelargonium* X *hortorum* (Lee *et al*, 1996). Fe-efficiency mechanisms may also enhance the availability of Mn (Welch *et al*, 1993). Acidification from Fe-stress is a major factor in mobilizing toxic levels of Mn in *Pelargonium* X *hortorum* from soilless media (Handreck, 1997). Excess Mn or Zn can cause Fe deficiency (Havlin *et al*, 2003).

Zinc (Zn) is an essential component of various proteins in plants. Zn is acquired from the soil solution primarily as Zn^{2+}, but also potentially complexed with organic ligands, by roots, which feed the shoots via the xylem (Broadley *et al*, 2007). Zn deficiency symptoms were characterized by interveinal mottling, followed by interveinal chlorosis, interveinal necrosis, and marginal curling in pecans (Kim *et al*, 2002). Zn deficiency developed auxin deficiency-like symptoms such as rosetting i.e. shortening of internodes, epinasty, inward curling of leaf blades called goblet leaves and reductions in leaf size known as little leaf (Broadley *et al*, 2007). Adequate supply of Zn significantly enhanced leaf area, leaf to stem ratio, biomass production, and succulence of alfalfa plants (Grewal and Williams, 2000). Zn not only reduced the uptake of Fe by the roots but, to a greater extent, interfered with translocation of Fe to the tops (Lingle *et al*, 1962). It is well established that plants grown with high Zn produce symptoms of Fe-deficiency

(Kaya *et al*, 1999). Under Zn toxicity, Fe and Zn translocation from roots to shoots increased in soybean as the S supply to the plants was increased (Fontes and Cox, 1998).

Manganese (Mn) is involved in photosynthesis, particularly in the evolution of O_2 (Havlin *et al*, 2003). Mn is involved as tightly bound Mn in photoreaction II of photosynthesis and in certain superoxide dismutases. Loosely bound Mn^{2+} is a unique activator of some enzymes and is an alternative to Mg^{2+} in activating many other enzymes (Raven, 1990). Mn deficiency symptoms first appear in younger leaves as interveinal chlorosis (Havlin *et al*, 2003). Visual symptoms of Mn deficiency, such as pale green and irregular chlorotic areas between the main veins of leaves of soybean, were first observed in the first trifoliate leaf of two-week-old plants grown in media deficient in Mn. The yellowing of the leaves continued until the new leaves were nearly white (Izaguirre-Mayoral and Sinclair, 2005). Mn competes with the available Fe for reaction with the active Fe acceptors forming stable, inactive metabolites. This would intensify chlorosis while both Fe and Mn accumulate in plant tissues (Twyman, 1950). Deficient or normal Mn antagonized Fe absorption, but at toxic values Mn was always antagonistic to Fe transport (Alvarez-Tinaut *et al*, 1980). Fe and Mn are interdependent in their effects on the plant. The nature of the effects of one is determined by the proportionate presence of the other (Somers and Shive, 1942).

Sulfur (S) is required for synthesis of S-containing amino acids, which are essential components of protein. S is a vital part of ferredoxin, an Fe-S protein occurring in the chloroplasts (Havlin *et al*, 2003). All enzymatic and most non-enzymatic antioxidants contain S. Glutathione, as the major non-protein thiol, plays a central role in reactive oxygen species (ROS) defense. Antioxidant defense systems are often

constitutive, multifactorial elements of plant cell metabolism that are up regulated under the impact of biotic and abiotic stressors (Kandlbinder *et al* 2004). S is taken up as SO_4^{2-} by the roots and is transported via the xylem to the leaves. The uptake and subsequent distribution of SO_4^{2-} to the leaves is closely regulated in response to demand (Sunarpi and Anderson, 1996). S deficiency symptoms included overall yellowing of new leaves, reddening on the leaf abaxial starting from older leaves and moving acropetally in cut chrysanthemum (Huang *et al*, 1997).

Magnesium (Mg) is a central element of chlorophyll, which is required for photosynthesis (Beale, 1999; Havlin *et al*, 2003). Chlorophyll usually accounts for about 15-20% of the total Mg^{2+} content of plants (Havlin *et al*, 2003). Mg is required for light-induced electron transport and for CO_2 assimilation (Alam *et al*, 2001). Mg also serves as a structural component in ribosomes, stabilizing them in the configuration necessary for protein synthesis (Beale, 1999; Havlin *et al*, 2003). Mg is essential for the function of many enzymes, including adenosine triphosphatases (ATPases), RNA polymerases, phosphatases, protein kinases, glutathione synthase, and carboxylases (Shaul, 2002). Mg deficiency increased the sugar concentration and altered sucrose export from young source leaves before any noticeable effect on photosynthetic activity (Hermans and Verbruggen, 2005). Shortage of Mg resulted in interveinal chlorosis of older leaves (Ding *et al*, 2006; Havlin *et al*, 2003).

The objectives of the present study were to determine if deficiency of all micronutrients, Fe, Mn, Zn, S or Mg result in symptoms similar to whitening in ivy geraniums and to determine the effects of their deficiencies on plant growth and foliar

nutrient composition of ivy geraniums. All micronutrients were omitted as one of the treatments to check if multi-nutrient deficiency caused whitening in ivy geraniums.

Materials and Methods

Rooted cuttings of ivy geranium, 'Beach' and 'Butterfly' (Fischer Horticulture LLC, Boulder, CO) were received on 15 Nov. 2006. They were fertilized with 250 mg $N \cdot l^{-1}$ from 20N-4.4P-16.6K (Peters Peat-lite fertilizer, Scotts Company, Marysville, OH) as a continuous liquid feed for 1 week. After 1 week, rooted cuttings were irrigated with clear tap water to leach out all nutrients from the plugs before transplanting. The plugs were then transplanted into 15 cm pots (1L) containing acid-washed sand on 27 Nov. 2006. Sand was washed with 0.1 N HCl followed by deionized, distilled water three times. Plants were grown in a glass greenhouse with temperatures at 23/21^{o}C (day/night). Light intensity ranged from 300-800$\mu mol \cdot s^{-1} \cdot m^{-2}$ PPF at canopy level when measured from 27 Nov 2006 to 8 Feb 2007.

There were 7 different nutrient solutions (complete, deficient in all micronutrients, deficient in Fe, Mn, Zn, Mg, or S). Stock solutions were made for macronutrients (Table 5.1) and micronutrients (Table 5.2) as explained by Baqir (1998). Stock solution for Fe was kept separate. Fe-EDDHA (Sprint 138, Fe-EDDHA, 6% Fe, Becker Underwood Inc, Ames, IA) was used at rate of 56g per liter distilled water. Both the stock solutions were mixed at the time of dilution. Stock solutions were diluted to provide fertilization on the basis of 250mg $N \cdot l^{-1}$. 1ml of each of micronutrient stock solution and Fe-EDDHA solution was used per liter of diluted macronutrient solution.

Plants were fertilized with 250mg $N \cdot l^{-1}$ from diluted stock solutions as a continuous liquid feed. Plants were supplied with clear deionized water after 10 days to leach excess salts.

Data was collected for plant height (from the rim of the container) and width (an average of two widths measured, one at the widest point and another at 90^{o}) at bi-weekly intervals. Relative growth rate (RGR) was measured as a difference in growth index [GI (cm^3) = 3.14*(width/2)^2*height] of two consecutive biweekly measurements.

Deficiency symptoms were recorded photographically before ending the experiment (8 Feb 2007). At completion of the experiment (9 Feb 2007) plants were harvested by cutting at the media line to determine total shoot (stems and leaves) and root fresh weight. After measuring total fresh weight, harvested plants were rinsed with 0.1N HCl and rinsed thrice with deionized, distilled water, and dried in an oven at 60^{o}C until dry to measure shoot (stems and leaves) and root dry weights. Dried leaf samples were separated from stems. The leaves were ground through a 0.5 mm screen (20 mesh) using a Cyclotec sample mill (UDY corporation, Fort Collins, CO). Ground tissue samples were used to determine total Fe, Zn, Mn, Mg and S (Crouse, 2001) using inductively-coupled plasma (ICP) atomic emission spectrometry (Optima 4300DV, PerkinElmer Instruments, Norwalk, CT).

The experiment consisted of 42 plants of each cultivar, including 7 treatments with 3 replications and 2-sub samples. Nutrient treatments were placed in a randomized complete block design with three replications. Plant size indices, fresh weights, dry weights, and foliar tissue nutrient concentrations were analyzed by analysis of variance, using PROC-GLM (SAS software, SAS Institute, Cary, N.C.). Fisher's protected least

significant difference means separation test (LSD) at P=0.05 was used to indicate significant differences between treatment means.

Results and Discussion

Relative growth rate (RGR) of 'Beach' was unaffected by the deficiencies after 2 weeks (Table 5.3). In 'Butterfly' at 2 weeks, the greatest RGR was obtained under –Zn treatment, which was not different from that under Control, –Fe, –Mn and –S treatments. The RGR in 'Butterfly' was the least under –Micro treatment, which was not different from the –Mg treatment. Omission of all micronutrients from the nutrient solution for 2 weeks reduced overall plant growth of 'Butterfly', but not of 'Beach'. 'Butterfly' was more sensitive to micronutrient omission than 'Beach'.

The RGR of 'Beach' but not 'Butterfly' was affected by deficiency treatments after 4 weeks (Table 5.3). RGR of 'Beach' was the least with –Micro treatment which was similar to –Zn and –S treatments. There was a difference in RGR between the –Micro and the control treatments. Omission of all micronutrients from the nutrient solution for 4 weeks reduced growth rate of 'Beach'.

There were no difference due to treatments in RGR of 'Beach' and 'Butterfly' after 6 weeks (Table 5.4). The RGR of 'Butterfly' was unaffected by deficiency treatments after 8 weeks (Table 5.4). RGR of 'Beach' after 8 weeks was significantly reduced in the –Micro and –S treatments compared to Control, –Fe, –Zn, –Mn, and –Mg treatments. The –Mn treatment had a greater RGR than the Control, –Micro, and –S treatments (Table 5.4). Mn is a cofactor of auxin oxidase (Krajncic and Nernek, 2002).

Mn influences auxin levels as high Mn favors the breakdown of indoleacetic acid (IAA) to decarboxylated products (Havlin *et al*, 2003; Srivastava, 2002). IAA is responsible for stem elongation and apical dominance in plants (Srivastava, 2002). In Mn deficient nutrient solution, there may be a reduced breakdown of IAA and subsequently more stem elongation.

RGR was affected in both cultivars after 10 weeks (Table 5.5). The RGR of 'Beach' in the Control treatment was similar to –Zn, –Fe, –Mn and –Mg treatments. The –S and –Micro treatments resulted in the least RGR in 'Beach'. RGRs in 'Butterfly' under –Zn, –Fe and –Mg treatments were similar after 10 weeks. The least RGR was obtained with –Micro and –S treatments. –Micro and –S treatments inhibited ivy geranium growth the most. Micronutrients are as important in plant nutrition as the major nutrients (Havlin *et al*, 2003).

Deficiency treatments of all micronutrients, Fe, S and Mg (Figure 5.1-5.4 respectively) produced clear symptoms in 'Butterfly' but not in 'Beach', indicating 'Beach' may have a higher capacity to tolerate deficiencies. –Zn and –Mn treatments did not show visible symptoms of deficiency in either of the cultivars after 10 weeks.

'Butterfly' in the –Micro treatment showed symptoms after 15 days of starting –Micro treatment. The younger leaves of the plants in –Micro treatment had interveinal chlorosis and a rossetted appearance (Figure 5.1). These symptoms most resemble Zn deficiency symptoms, i.e. shortening of internodes, epinasty, inward curling of leaf blades called goblet leaves and reductions in leaf size known as little leaf (Broadley *et al*, 2007). This suggests that elimination of all micronutrients emphasized the symptoms of

Zn deficiency in 'Butterfly'. Interestingly, chlorosis of 'Butterfly' under –Micro treatment was not as severe as under –Fe and –Mg treatments, but plant growth reduction was the most severe by omitting all micronutrients in comparison to omitting one nutrient from nutrient solution (Table 5.5).

Leaves of 'Butterfly' under –Fe treatment started showing clear symptoms after 23 days of omission of Fe from the nutrient solution. –Fe nutrient solution resulted in interveinal chlorosis of younger leaves of 'Butterfly' and eventually leaves became necrotic (Figure 5.2). These symptoms are similar to those described in other studies (Abadia, 1992; Kobayashi *et al*, 2003).

Plants of 'Butterfly' in the –S treatment began uniform yellowing of younger leaves (Figure 5.3) after 28 days of omission of S from the nutrient solution. S is a vital part of several proteins and enzymes, especially ferredoxin, an Fe-S protein occurring in the chloroplasts (Havlin *et al*, 2003). Production of ferredoxin may have been reduced resulting in overall yellowing of leaves due to inhibition of electron transport and photosynthesis. There may not have been enough S to support the plant system and to compensate for the breakdown of enzymes.

Leaves of 'Butterfly' in the –Mg treatment started showing symptoms after 35 days of omission of Mg from the nutrient solution. Older, lower leaves of 'Butterfly' showed interveinal mottling and became necrotic (Figure 5.4). Leaves with interveinal chlorosis were drooping. These findings are consistent with the results documented for rice in Mg free culture (Ding *et al*, 2006). As Mg is an important component of chlorophyll (Beale, 1999; Havlin *et al*, 2003), so its omission from the nutrient solution

could reduce chlorophyll formation. Mg is a mobile nutrient in plants, which would explain why the earliest signs of Mg deficiency appeared on older leaves (Havlin *et al*, 2003).

Chlorosis under Fe deficiency was the most severe in comparison with –Micro, –Mg and –S treatments, which was in agreement with the findings documented for tobacco (Kobayashi *et al*, 2003). However, none of the deficiency treatments produced whitening symptoms in either cultivar even after 10 weeks of omission of these nutrients from the nutrient solution. These results indicate that whitening in ivy geranium is not due to a deficiency of micronutrients, Fe, Mn, Zn, S or Mg when plants are grown under optimal temperatures.

Shoot fresh weight, dry weight, and fresh: dry weight ratios of 'Beach' was similar to the Control, –Fe, –Zn, –Mn, and –Mg treatments and were greater than in the –Micro and –S treatments (Table 5.6). These results are not consistent with the findings documented for sugar beet (Hermans *et al*, 2004) or *Arabidopsis thaliana* (Hermans and Verbruggen, 2005), where omitting Mg from the media reduced plant biomass. –Fe treatment had similar shoot fresh wt. as the Control, which is similar to the findings documented for *Dioscorea alata* (Sullivan and Jenner, 2006). –S treatment resulting in smaller plants is also in agreement with the findings documented in *Dioscorea alata* (Sullivan and Jenner, 2006). Root fresh weight, root dry weight, and root fresh: dry weight ratios were not affected by deficiency treatments. No reduction in root growth in 'Beach' due to deficiencies is in agreement with studies on sugar beet (Hermans *et al*, 2004). The fresh shoot:root weight ratio followed a pattern similar to the fresh shoot

weight where the –Micro and –S treatments had the lowest ratios. The absence of micronutrients and sulfur inhibited shoot growth but not root growth.

Shoot fresh and dry weight of 'Butterfly' followed a pattern similar to ''Beach' in that there were few differences in weight due to nutrient deficiencies, except –Micro and –S treatments (Table 5.7). There were no differences in shoot fresh to dry weight ratio due to deficiency treatments in 'Butterfly'. Deficiency treatments reduced shoot fresh and dry weight proportionally, so no differences were observed in the ratios. Root fresh weight, root dry weight, root fresh: dry weight ratio and fresh shoot: root ratio were not affected by deficiency treatments (Table 5.6). Omission of all the micronutrients from the nutrient solution reduced plant shoot biomass of both cultivars. Root biomass was not affected by nutrient deficiencies in either cultivars of ivy geranium.

Total foliar Fe of 'Beach' was reduced to similar levels when Fe, Zn, S, or Mg was deficient (Table 5.8). Total foliar Fe of 'Beach' was the greatest under –Micro treatment and Control treatments. These findings are similar to the findings in tobacco where foliar Fe concentrations were similar in Fe deficient, and Fe plus Zn deficient media (Kobayashi *et al*, 2003). In 'Butterfly' total foliar Fe content was the greatest under the –Micro treatment. There were no differences in foliar Fe content between the control and the other deficiency treatments. The greatest levels of total Fe in foliar tissues of both cultivars in the –Micro treatment may have been due to diminished growth and diminished dilution of nutrients. These results may be supported by the chlorosis paradox, where chlorotic plants had higher total Fe than green plants due to a reduction in growth (Romheld, 2000). The same concept of the chlorosis paradox may also be applicable to increased concentration of all other nutrients (Zn, Mn, S and Mg) under –

Micro treatment. Broscht and Elliot (2004) also found that iron concentration in the leaves of St. Augustinegrass showed no relationship to fertilizer treatment. Total foliar Fe content in 'Butterfly' was similar in the control and –Fe treatments despite differences in severity of chlorosis of leaves (Figure 5.2). These results are consistent with the findings that the concentration of total iron in green leaves was not significantly different from that of chlorotic ones, but the concentration of ferrous iron was much lower in chlorotic leaves than that in green leaves of peach (Gharsalli and Hajji, 2002). Due to no reduction in growth and biomass of 'Butterfly', similar levels of total Fe under control and –Fe treatment would not be supported by the chlorosis paradox, where chlorotic plants had even higher total Fe than green plants (Romheld, 2000). Leaf chlorosis in plants under –Fe treatment could be due to inability to produce and/or stabilize new chlorophyll molecules in the thylakoid membrane (Belkhodja *et al,* 1998). Opposed to total Fe, green plants always contain more Fe^{2+} (active Fe) than chlorotic plants (Katyal and Sharma, 1980). Singh and Sahu (1993) suggested that Fe, despite absorption and uptake, was subjected to inactivation and the mobility of Fe was impaired in the chlorotic plants of groundnut. As the amount of total Fe present in plants of –Fe treatment was simiar as in the Control treatment in 'Butterfly', there may be an inactivation of Fe or inhibition of Fe retranslocation to newly developing leaves in Fe-deficient plants, which suggests a difference in concentration of active Fe between the treatments. Another possible explanation could be the sampling material. Tissue analysis was done on a whole plant leaf basis, chlorotic and green leaves were not separated for analysis. It is not known if the foliar Fe was more concentrated in the green leaves or distributed equally between green and chlorotic leaves. If Fe levels were determined from chlorotic and green leaves

of each treatment separately, the chlorotic leaves may have less total Fe content than the green leaves.

The greatest foliar Zn content in 'Beach' and 'Butterfly' was obtained under –Micro treatment. It could be due to the severely reduced growth and the resulting diminished dilution of Zn in these plants under –Micro treatment. The least foliar Zn content resulted under –Zn and –Mg treatments. Control resulted similar Zn content as –Fe, –Mn, –S and –Mg treatments in 'Beach', indicating the presence of Fe, Mn, S or Mg in the nutrient solution had no effect on Zn uptake by plants of 'Beach'. Foliar Zn contents of 'Butterfly' followed almost the same pattern as in 'Beach' with the exception of –Mg and –S which were similar in Zn content to –Zn treatment. Zn content in 'Butterfly' in the Control treatment was not different from –Fe, –Mn, –S or –Mg treatments. –Zn treatment reduced Zn content of ivy geraniums, which is consistent with the findings documented in lupins (Yu and Rengel, 1999). –Micro treatment had Zn contents greater than the Control treatment in both cultivars, but it had reduced growth rate and plant biomass, indicating that the higher concentration may be due to a diminished dilution of nutrients in the plant tissues of ivy geranium.

The highest Mn content in foliar tissues of 'Beach' was obtained in the –Fe, –S, –Mg, and –Micro treatments. These results are consistent with the findings that Fe deficiency stimulated the accumulation of Mn in leaves of tobacco (Kobayashi *et al*, 2003). –Fe treatment had higher foliar Mn content than Control treatment had, which suggests that Fe-efficiency mechanisms may also enhance the uptake of Mn (Welch *et al*, 1993). These findings indicate that not only the absence of Fe in the nutrient solution but

also of S and Mg increase the uptake of Mn in 'Beach'. The least Mn content was obtained under –Mn, –Zn, and Control treatments. In 'Butterfly', –Fe, and –Mg treatments increased foliar Mn content. The least Mn content was obtained under –Mn, Control, –Micro and –Zn treatments. The control treatment was only different from the –Fe and –Mg treatments in its Mn content, which indicates that Fe and Mg interfere with Mn uptake by roots. Somers and Shive (1942) documented that Fe and Mn are intimately interdependent in their effects upon the plant and that the nature of the effects of the one is determined by the proportionate presence of the other. No difference in foliar Mn contents under Control and –Mn treatments could be due to the existence of divalent cations such as Fe and Mg in the complete solution, which interfere with Mn uptake when present. The higher uptake rates of other divalent cations support the view of Kochian (1993) that divalent cations are taken up through the same channels in the plasma membrane of the root cell. These results imply that Fe and Mn moderate each other's uptake, explaining reason why foliar Mn contents are highest when there is no Fe in the nutrient solution. Acidification of the medium caused by Fe-stress was a major factor in mobilizing toxic levels of Mn from soilless media (Handreck, 1997). Results also suggest that S and Mg omission from the nutrient solution facilitates the uptake of Mn to the plant tissues. –Micro treatment had Mn contents similar to Control treatment in both cultivars, but it reduced growth rate, which indicates that similar concentration was due to diminished dilution of nutrients in plant tissues.

Foliar S content in 'Beach' was least in the –S treatment (Table 5.8) and the greatest in the –Micro treatment. –Mg had almost as low a S content as the –S treatment. –Fe, –Zn, and –Mn treatments had similar foliar S content. A very similar pattern of S

content based on nutrient deficiency treatment was also observed in 'Butterfly'. These results indicate that the greatest levels of S in foliar tissue of both cultivars in the –Micro treatment may be explained by the diminished growth and diminished dilution of nutrients. These results also suggest that omission of Fe and Mn from the nutrient solution did not affect the uptake of S in either cultivar as they were similar to the Control. Omission of Mg decreased S content in 'Beach'.

Control, –Micro and –Fe treatments resulted in the greatest foliar Mg content in 'Beach'. The least foliar Mg was obtained in the –Mg treatment, which was different from all other treatments. Similar results were found in *Arabidopsis thaliana* (Hermans and Verbruggen, 2005). The results were almost identical in 'Butterfly' where the omission of Zn, Mn or S from the nutrient solution reduced foliar Mg contents. The presence of Zn, Mn and S in the nutrient solution appears to aid Mg uptake. Similar levels of Mg in foliar tissues of both cultivars under Control and –Micro treatments may be due to the diminished growth of plants in the –Micro treatment. Results also suggest no effect of omission of Fe from nutrient solution on foliar Mg content in either cultivar. Zn is involved in many enzymatic activities (Havlin *et al,* 2003). S is a vital part of many proteins and enzymes (Havlin *et al,* 2003). Uptake of Mg by root cells may need a certain transporter to be active. So omission of S or Zn from the nutrient solution may reduce the enzymes or proteins which act as transporters for Mg from the media or roots to shoots.

Conclusion

This experiment was conducted to determine if all micronutrients, Fe, Mn, Zn, S or Mg deficiencies could result in symptoms similar to whitening in ivy geraniums and to

determine the effects of their deficiencies on plant growth and nutrient composition of ivy geraniums. None of the given deficiency (–Micro, –Fe, –Zn, –Mn, –S or –Mg) treatments caused whitening in ivy geraniums after 10 weeks when plants were grown at 23/21^{o}C. Whitening in ivy geraniums is not simply a micronutrient deficiency. 'Beach' did not show any deficiency symptoms while 'Butterfly' plants grown under –Micro, –Fe, –S and –Mg treatments developed symptoms of the respective nutrient. 'Beach' had greater capacity to tolerate total micronutrient, S and Mg deficiencies.

–Micro and –S treatments reduced relative growth rate, shoot fresh weight and shoot dry weight of ivy geranium. Root fresh and dry weights of ivy geraniums were not affected by deficiency treatments. Fresh shoot: root ratio in 'Beach' declined under –Micro and –S treatments, whereas it did not in 'Butterfly'. In 'Butterfly', there was proportional reduction in fresh and dry weights and no differences were observed.

Fe deficiency stimulated the accumulation of Mn in both cultivars of ivy geranium. Plants of both cultivars grown under –Zn treatment had lower Zn and Mg concentrations in their leaves. Mn deficiency did not stimulate the accumulation of Fe or Zn in ivy geraniums. S deficiency reduced the accumulation of S and Mg in both cultivars of ivy geranium. Mg deficiency reduced the accumulation of Mg, but stimulated the accumulation of Mn in both cultivars of ivy geranium.

Deficiency treatments of all micronutrients and S reduced plant growth the most. Deficiency of all micronutrients, Fe, Zn, Mn, S or Mg did not cause whitening in ivy geranium plants grown at 23/21^{o}C.

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Figure 5.1 Symptoms obtained under –Micro treatment compared to Control

Figure 5.2 Symptoms obtained under –Fe treatment compared to Control

Figure 5.3 Symptoms obtained under –S treatment compared to Control

Figure 5.4 Symptoms obtained under –Mg treatment compared to Control

Table 5.1. Composition of Macronutrient stock solution

Macronutrient stock solution ($g \cdot L^{-1}$) for induced nutrients deficiencies							
Salt	Control	-Micro	-Fe	-Zn	-Mn	-S	-Mg
KNO_3	5	5	5	5	5	5	5
$Ca(NO_3)_2.H_2O$	5	5	5	5	5	5	5
$MgSO_4.7H_2O$	2	2	2	2	2	2	0
KH_2HPO_4	1	1	1	1	1	1	1
$MgCl_2$	0	0	0	0	0	1.65	0
Elemental S	0	0	0	0	0.29	0	0.26
Total Content (mg per liter) in above mentioned stock solutions							
Nitrogen	1285.0	1285.0	1285.0	1285.0	1285.0	1285.0	1285.0
Phosphorus	227.5	227.5	227.5	227.5	227.5	227.5	227.5
Potassium	1930.0	1930.0	1930.0	1930.0	1930.0	1930.0	1930.0
Calcium	1982.0	1982.0	1982.0	1982.0	1982.0	1982.0	1982.0
Magnesium	197.2	197.2	197.2	197.2	197.2	197.2	0
Sulfur	562.6	562.6	562.6	562.6	562.6	0	562.6

Table 5.2. Composition of Micronutrient stock solution

Micronutrient stock solution ($g \cdot L^{-1}$) for induced nutrients deficiencies							
Salt	Control	-Micro	-Fe	-Zn	-Mn	-S	-Mg
$ZnCl_2$	0.46	0	0.46	0	0.46	0.46	0.46
$MnSO_4$	1.54	0	1.54	1.54	0	0	1.54
$MnCl_2$	0	0	0	0	0	1.80	0
H_3BO_3	2.86	0	2.86	2.86	2.86	2.86	2.86
$CuSO_4.5H_2O$	0.08	0	0.08	0.08	0.08	0	0.08
$CuCO_3.5H_2O$	0	0	0	0	0	0.03	0
$H_2MoO_4.H_2O$	0.09	0	0.09	0.09	0.09	0.09	0.09
Total Content (mg per liter) in above mentioned micronutrients stock solutions							
Zinc	220	0	220	0	220	220	220
Manganese	500.65	0	500.65	500.65	0	500.65	500.65
Boron	499.90	0	499.90	499.90	499.90	499.90	499.90
Copper	20.36	0	20.36	20.36	20.36	20.36	20.36
Molybdenum	48	48	48	48	48	48	48

Table 5.3. Relative growth rate (RGR) of ivy geranium 'Beach' and 'Butterfly' at 2 and 4 weeks as affected by nutrient deficiencies

Treatment	Beach	Butterfly
Week 2		
Control	181.4 a[x]	371.8 ab
-Micro	303.1 a	118.3 c
-Fe	323.6 a	384.4 ab
-Zn	332.4 a	460.3 a
-Mn	430.5 a	340.6 ab
-S	447.2 a	376.4 ab
-Mg	394.0 a	254.3 bc
Week 4		
Control	1497.3 a	556.9 a
-Micro	361.0 b	330.6 a
-Fe	1336.5 a	800.7 a
-Zn	989.6 ab	1037.2 a
-Mn	1159.5 a	863.0 a
-S	850.2 ab	763.8 a
-Mg	1297.5 a	910.9 a

[x]Means followed by same letters within column and within weeks are not different according to Fisher's protected LSD test (P=0.05).

Table 5.4. Relative growth rate (RGR) of ivy geranium 'Beach' and 'Butterfly' at 6 and 8 weeks as affected by nutrient deficiencies

Treatment	Beach	Butterfly
Week 6		
Control	2148.5 a[x]	911.8 a
-Micro	1208.2 a	499.2 a
-Fe	2311.3 a	569.4 a
-Zn	2545.2 a	974.0 a
-Mn	3015.0 a	1047.0 a
-S	1465.2 a	538.8 a
-Mg	2578.3 a	952.2 a
Week 8		
Control	2255.2 bc	1262.9 a
-Micro	1211.6 c	668.9 a
-Fe	3535.7 ab	1104.7 a
-Zn	3813.2 ab	2202.6 a
-Mn	5017.5 a	1499.8 a
-S	384.0 c	539.1 a
-Mg	4047.9 ab	1735.8 a

[x]Means followed by same letters within column and within weeks are not different according to Fisher's protected LSD test (*P*=0.05).

Table 5.5. Relative growth rate (RGR) of ivy geranium 'Beach' and 'Butterfly' at 10 weeks as affected by nutrient deficiencies

Treatment	Beach	Butterfly
Control	3771 a[x]	1796.3 bc
-Micro	828 b	161.8 d
-Fe	3630 a	2159.3 ab
-Zn	3765 a	3531.3 a
-Mn	3476 a	1331.8 bcd
-S	109 b	328.4 cd
-Mg	3322 a	2069.0 ab

[x]Means followed by same letters within column are not different according to Fisher's protected LSD test (*P*=0.05).

Table 5.6. Effect of deficiency treatments on shoot fresh weight (wt), shoot dry wt., shoot fresh to dry wt. ratio, root fresh weight (wt), root dry wt., root fresh to dry wt. ratio and fresh shoot: root ratio of ivy geranium 'Beach' after 10 weeks of growth

Treatment	Shoot			Root			Fresh shoot: root ratio
	Fresh wt. (g)	Dry wt. (g)	Fresh: dry wt	Fresh wt (g)	Dry wt (g)	Fresh: dry wt	
Control	79.0 a[x]	8.7 a	9.0 a	10.0 a	1.4 a	7.0 a	9.4 a
-Micro	29.7 b	3.9 b	7.7 b	5.3 a	0.8 a	6.9 a	7.3 b
-Fe	76.7 a	8.7 a	8.8 a	8.8 a	1.5 a	5.8 a	9.8 a
-Zn	81.9 a	8.9 a	9.2 a	8.2 a	1.4 a	5.9 a	9.4 a
-Mn	83.2 a	9.0 a	9.2 a	7.4 a	1.2 a	6.4 a	10.6 a
-S	38.3 b	4.8 b	8.0 b	8.8 a	1.1 a	8.3 a	4.6 b
-Mg	83.3 a	9.2 a	9.1 a	8.9 a	1.2 a	7.4 a	9.4 a

[x]Means followed by same letters within column are not different according to Fisher's protected LSD test (*P*=0.05).

Table 5.7. Effect of deficiency treatments on shoot fresh weight (wt), shoot dry wt., shoot fresh to dry wt. ratio, root fresh weight (wt), root dry wt., root fresh to dry wt. ratio and fresh shoot: root ratio of ivy geranium 'Butterfly' after 10 weeks of growth

Treatment	Shoot			Root			Fresh shoot: root ratio
	Fresh wt. (g)	Dry wt. (g)	Fresh: dry wt	Fresh wt (g)	Dry wt (g)	Fresh: dry wt	
Control	47.2 ab[x]	5.3 ab	8.4 a	5.6 a	1.0 a	5.6 a	8.3 a
-Micro	23.8 c	2.8 c	8.2 a	2.5 a	0.5 a	5.1 a	9.0 a
-Fe	42.0 bc	4.8 b	8.3 a	6.0 a	0.8 a	7.7 a	5.4 a
-Zn	56.8 ab	6.2 ab	9.0 a	6.4 a	1.1 a	6.4 a	7.3 a
-Mn	47.2 ab	5.4 ab	8.6 a	6.0 a	0.9 a	6.4 a	8.2 a
-S	40.3 bc	4.4 bc	9.2 a	6.6 a	0.8 a	8.4 a	5.8 a
-Mg	64.9 a	6.8 a	9.5 a	6.9 a	0.8 a	9.1 a	8.2 a

[x]Means followed by same letters within column are not different according to Fisher's protected LSD test (*P*=0.05).

Table 5.8. Effect of induced nutrient deficiencies on foliar tissue total Fe, Mn, Zn, S and Mg contents of ivy geranium 'Beach' and 'Butterfly'

Treatment	Beach					Butterfly				
	Fe (ppm)	Zn (ppm)	Mn (ppm)	S (%)	Mg (%)	Fe (ppm)	Zn (ppm)	Mn (ppm)	S (%)	Mg (%)
Control	71.1 ab[x]	15.9 b	107.9 bc	0.152 b	0.39 a	77.4 b	14.0 bc	106.3 bc	0.132 bc	0.32 ab
-Micro	80.9 a	20.9 a	133.0 ab	0.169 a	0.40 a	178.7 a	21.8 a	99.3 c	0.155 a	0.35 a
-Fe	56.3 d	14.5 b	143.3 a	0.146 bc	0.39 a	72.6 b	15.3 b	140.3 a	0.134 b	0.32 ab
-Zn	60.6 bcd	8.6 c	93.7 c	0.142 c	0.29 c	75.6 b	6.6 d	95.3 c	0.121 bc	0.25 c
-Mn	68.8 bc	16.0 b	93.3 c	0.147 bc	0.36 b	95.1 b	16.0 b	93.6 c	0.131 bc	0.31 b
-S	64.4 bcd	13.8 b	135.3 a	0.056 e	0.34 b	91.5 b	10.2 cd	128.0 ab	0.055 d	0.27 c
-Mg	57.6 cd	12.2 bc	142.0 a	0.108 d	0.08 d	60.6 b	10.5 cd	142.0 a	0.112 c	0.06 d

[x]Means followed by same letters within column and within weeks are not different according to Fisher's protected LSD test (*P*=0.05).

CHAPTER VI

EFFECT OF SALICYLIC ACID AND ELEVATED TEMPERATURES ON GROWTH AND WHITENING OF IVY GERANIUM

Abstract

Ivy geranium (*Pelargonium peltatum* L.) does not grow well in southeastern U.S. summers. The development of whitening of the youngest leaves of actively growing ivy geranium has been observed as the season changes from late spring to summer. A study was conducted to determine the effect of salicylic acid and elevated temperatures on ivy geraniums growth and whitening. Ivy geranium 'Beach' and 'Butterfly' plants were grown for 6 weeks in modified greenhouse chambers (1.27m x 1.20m x 0.91m) with air temperature averaging 28°C/16°C or 36°C/22°C (day/night). Salicylic acid (SA) was applied at 0 (control) or 100 μmol l^{-1} as a foliar spray at bi-weekly intervals (3 times). It was confirmed that elevated air temperature caused whitening in ivy geraniums. 'Butterfly' is more susceptible to whitening than 'Beach'. Elevated temperature severely reduced plant growth, leaf area, fresh (shoot, leaf and total) weight, dry (shoot, leaf and total) weight, and leaf area ratio in both cultivars. 'Beach' responded to heat stress by increasing its size less, whereas 'Butterfly' did not. Salicylic acid did not affect plant

growth, leaf area, and plant biomass in either cultivar. Elevated temperature reduced carotenoids in 'Butterfly' but not in 'Beach'. Total pheophytins concentration reduced in both cultivars at elevated air temperature. The decrease in carotenoids and pheophytins due to increased temperature may increase susceptibility to whitening. Total Fe in foliar tissues was not affected by temperature in either cultivar. Results also indicate that there was a higher accumulation of Mn, Mg and S in foliar tissues of 'Beach' grown at the elevated temperature. Mn contents were not affected by temperature in 'Butterfly' but foliar Zn and S content increased with temperature. Salicylic acid did not affect carotenoids, pheophytins and foliar nutrient contents in either cultivar. These results imply that carotenoids, pheophytins and foliar Mn, Zn, Mg and S content play a role in susceptibility of ivy geraniums to whitening.

Introduction

Ivy geranium does not grow well in southeastern U.S. summers. The young developing leaves of ivy geranium turn white, expand less and have an upward cupped appearance. This physiological disorder is referred to as 'whitening'. Plants resume normal growth as the temperature moderates in fall.

Increased leaf temperature during summers is a potential danger in greenhouses, where low air speed and high humidity decrease the rate of leaf cooling (Taiz and Zeiger, 2002). Maintenance of transpirational (leaf) cooling was an important factor associated with better summer stress performance of Kentucky bluegrass (Bonos and Murphy, 1999). Heat induced bleaching was observed in *Euglena gracilis* when exposed to slightly elevated (33^{o}C) temperature (Ortiz and Wilson, 1988). Chlorosis was induced in

tree fern (*Cyathea cooperi*) by 41.2^{o}C air temperature (Doley, 1983). *Solanum* species screened for heat tolerance indicated considerable variation in the degree of chlorosis developing as a stress response to high temperature (Reynolds *et al*, 1990).

Temperatures above 30^{o}C reduced photosynthetic rate as a result of a reduction in photosystem II (PSII) efficiency (Kadir *et al*, 2006). Net photosynthesis of pea plants decreased with increasing leaf temperature up to 45^{o}C (Haldimann and Feller, 2005). Leaf temperatures up to 45^{o}C enhance respiratory rates while exceeding the optimum temperature for photosynthesis (Jifon and Syvertsen, 2003). Temperature also influences chloroplast development, chlorophyll biosynthesis, and greening process (Mohanty *et al*, 2006). Chlorophyll biosynthesis of cucumber seedlings was reduced 60% upon heat-stress due to impairment of chlorophyll biosynthetic enzymes (Tewari and Tripathy, 1998). Chlorosis induced in tree fern (*Cyathea cooperi*) by 41.2^{o}C air temperature also resulted in reduced chlorophyll concentration, reduced Chl a: b ratio, fewer numbers of grana, and almost completely absent stroma thylakoids (Doley, 1983). Chlorophyll a is more heat sensitive than chlorophyll b (Weemaes *et al*, 1999; Loey *et al*, 1998). Chlorophyll a to b (Chl a: b) and chlorophyll to carotenoids (Chl: Caro) ratios decreased in heat-stressed tomato plants (Camejo *et al*, 2005). There was an heat-induced block of PSII reaction centers and an heat-induced block of Chl b to Chl a energy transfer, resulting in a functional disconnection of the light-harvesting complex from the reaction center complexes (Schreiber and Armond, 1978). The photoreducible pheophytin is the natural primary electron acceptor of PSII in early steps of photosynthetic solar energy conversion, acting between P680 and plastoquinone (Klimov, 2003). In PSII of green plants, the key photosynthetic reaction consists of the transfer of an electron from the

primary donor called P680 to a nearby pheophytin molecule (Groot *et al*, 1997).

Salicylic Acid (SA)

Production of plants with tolerance to environmental stresses is one of the priorities in plant science research. Certain chemicals have been demonstrated to regulate the expression of stress tolerance (Fletcher *et al*, 2000; Senaratna *et al*, 2000). Plants have the capability to increase heat tolerance when ambient temperatures increase within non-lethal levels (Wang and Li, 2006). Salicylic acid is a natural signal molecule, which plays an important role in regulating a number of physiological processes in plants (Singh and Usha, 2003). Heat acclimation induced changes in endogenous SA and antioxidant concentrations compared to induced thermotolerance (Dat *et al*, 1998a). Salicylic acid has been described as a new potential plant hormone (Raskin, 1992). Currently, exogenous application of salicylic acid is of great interest because of its ability to induce thermotolerance. Salicylic acid improved tolerance of mustard seedlings (Dat *et al*, 1998b) and *Arabidopsis* mutants (Larkindale *et al*, 2005) to heat stress. Similar findings have been documented for creeping bentgrass. Pre-treatment with SA resulted in increased tolerance to prolonged heat stress (1 month) and showed more green leaves, decreased membrane leakage, and reduced oxidative damage (Larkindale and Huang, 2005). Salicylic acid application enhanced heat tolerance in Kentucky bluegrass and could be involved in scavenging active oxygen species and increasing the activity of antioxidant enzymes under heat stress (He *et al*, 2005). Salicylic acid induced thermotolerance was related to changes in antioxidant enzyme activities and antioxidant concentration in grape leaves (Wang and Li, 2006).

The objectives in the present study were to determine the effect of salicylic acid and elevated temperatures on ivy geraniums growth and whitening and to determine the effect of elevated temperature and salicylic acid on chlorophyll concentration, chlorophyll a: b ratio, pheophytins, total phenolic content and foliar nutrient (Fe, Mn, Zn, Mg and S) composition of ivy geranium plants.

Materials and Methods

Rooted cuttings of ivy geranium 'Beach' and 'Butterfly' (Fischer Horticulture, LLC, Boulder, CO) were potted on 15 Dec 2006 into 15 cm diameter pots (1L). They were potted in sphagnum peat and perlite (70:30 by volume) with 0.96 kg gypsum/m^3, 7.7kg lime/m^3, and 0.32kg wetting agent (SaturAid, Debco Pty. Ltd., Tyabb, Australia) /m^3 media. Plants were fertilized with 250mg $N \cdot l^{-1}$ from 20N-4.4P-16.6K (Peters Peat-lite fertilizer, Scotts Company, Marysville, OH) as a continuous liquid feed. The potted cuttings were grown for 6 weeks and had developed a substantial root system before beginning experimental treatments. 'Beach' is known to be less susceptible to whitening than 'Butterfly' (Personal communication, Harvey Lang, Fischer, USA).

Growth chambers (1.27m width x 1.20m length x 0.91m height) were constructed of a single layer (4mm clear) polyethylene over PVC tubing inside a double layer polyethylene covered Quonset greenhouse to maintain the set temperatures. Heating cables (Gro-Quick Cables, Wrap-On Company, Bedford Park, IL) were run at three levels below, on top of, and 10 cm above the lath bench top, to heat the growth chambers. Sidewalls were raised or lowered to regulate temperature. Data loggers (Model 125, Spectrum Technologies, Plainfield, IL) recorded air temperature at hourly intervals

throughout the experiment. The thermocouples were kept at canopy level and shielded from the sun. There were three replications for each temperature. Average day/night temperatures were 28°C/16°C for unheated chambers and 36°C/22°C for heated chambers (Table 6.1). Light intensity ranged between 400-800 $\mu mol \cdot s^{-1} \cdot m^{-2}$ inside the growth chambers at canopy level when measured between 9:00am and 2:00pm from 2 February to 16 March 2007.

Salicylic acid was applied at 100μmol $SA \cdot l^{-1}$ (0.0138 g SA dissolved per liter of distilled water) adjusted to pH 7.0 with 0.01N NaOH. 20 ml of the SA solution was sprayed per plant to cover the leaf surfaces. Control plants were sprayed with 20ml distilled water adjusted to pH 7.0 with NaOH. Treatments were applied between 4:00-5:30pm at 15-day intervals throughout the experiment starting from 1 Feb. 2007. There were 3 replications for each treatment with 2 sub-samples per treatment. Plants were placed in the growth chambers on 2 Feb. 2007 and were grown for 6 weeks.

Data was collected for plant height (from the rim of the container) and width (an average of two widths measured one at the widest point and another at 90°) at bi-weekly intervals. Growth index was calculated as: GI (cm^3) = 3.14*(width/2)^2*height. At the end of the experiment, extent of whitening was measued as a visual rating on a scale of 1 to 7, where 1 = no, 2 =17%, 3 =34%, 4 =50%, 5 =67%, 6 = 84%, and 7 = 100% whitening.

On 15 March 2007, five recently mature leaves were harvested from each plant to get one 38.5 mm^2 leaf disk from each leaf of that plant for pigment analysis. Each leaf disk was cut into 5-6 small pieces to aid pigment extraction. Five 38.5mm^2 leaf disks were placed in a vial with 10 ml of 80% acetone. The vials were incubated at 20°C in the dark to allow complete pigment extraction [i.e. chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl

b) and carotenoids (Caro)]. The pigment concentrations were determined at 663, 645, and 470 nm using a Bio-Rad UV/VIS spectrophotometer (Bio-Rad Laboratories, Hercules, CA, USA). 80% acetone was used as blank. Equations described in chapter 4 were used to calculate Chl a, Chl b, Caro (Hill, 1963) and pheophytins (Vernon, 1960).

The total phenolic content of leaves was also determined spectrophotometrically using five 38.5mm^2 leaf discs placed in 10 ml of extractant. Extractant was a mixture of methanol, distilled water, and hydrochloric acid in 79:20:1 ratio by volume. The vials were incubated at 20^{o}C for 24 hours in the dark to allow complete extraction of phenolic compounds. The absorption of ivy geranium leaf disks extract had peak absorbance at 320 nm when scanned using the spectrophotometer between 200 and 900 nm. The phenolics content was calculated using the equation (Kakani *et al*, 2004).

Plants were harvested at the substrate line at the end of the experiment (16 March 2007). One subsample from each replication was harvested to determine leaf area, shoot fresh weight, and leaf fresh weight. Total leaf area was determined using a portable leaf area meter (LI-3000, LI-COR. Lincoln, NE.). Leaves and stems were weighed separately to give leaf fresh weight and stem fresh weight respectively. Plants tissues were dried in an oven at 60^{o}C until dry to measure stem dry weight, leaf dry weight, and total dry weight. Leaf area ratio (LAR) was calculated as the ratio of total leaf area to whole plant dry weight (Hunt, 1982). LAR represents the ratio of photosynthesizing to respiring material within the plant. Leaf area index (LAI) is the leaf area per unit area of land and it was calculated as

$LAI = \frac{Leafarea}{3.14(r)^2}$, where r = radius of plant canopy (Hunt, 1982).

Specific leaf area (SLA) is the mean area of leaf displayed per unit of leaf weight, a measure of leaf density or relative thickness (Hunt, 1982). It was calculated as the ratio of leaf area to leaf dry weight. Leaf weight ratio (LWR) is also an indicator of leafiness of the plant on a weight basis (Hunt, 1982). It was calculated as the ratio of leaf dry weight to total dry weight. One subsample from each replication was rinsed with 0.1N HCl then rinsed 3-times with deionized water. Plants were dried at 60^{o}C until dry. Dry weight was measured and dry leaf samples were separated from stems. Dry leaves were ground through a 0.5 mm screen (20 mesh) using a Cyclotec sample mill (UDY corporation, Fort Collins, CO). Ground tissue samples were used to determine total Fe, Zn, Mn, Mg, and S (Crouse, 2001) using inductively coupled plasma (ICP) atomic emission spectrometry (Optima 4300DV, PerkinElmer Instruments, Norwalk, CT).

The experiment was a split plot design, split by temperature with three replications for each treatment and two sub-samples per replication. Plant growth indices, fresh weights, dry weights, pigments, pheophytins, phenolics, and foliar tissue nutrient concentrations were analyzed by analysis of variance using Proc-Glimmix (SAS software, SAS Institute, Cary, N.C.). Fischer's protected least significant difference (LSD) at P=0.05 was used to indicate significant differences between treatment means.

Results and Discussion

There were no interactive effects of temperature and SA on plant height, width, or growth index of either 'Beach' or 'Butterfly'. There were no differences in plant height, plant width or growth index in either cultivar before starting the experiment (Table 6.2). Elevated air temperature did not affect plant height, width or growth index in either

cultivar at 2 or 4 weeks (Table 6.2) after start of the treatments. Elevated air temperature did not affect plant height in either cultivar. Plant width and growth index decreased at elevated air temperature in 'Beach' after 6 weeks, whereas they were not affected in 'Butterfly' (Table 6.2). 'Beach' increased its size less due to heat stress whereas 'Butterfly' did not. The reduced size of 'Beach' after 6 weeks of elevated temperatures may be an adjustment of metabolism to minimize heat injury, which may also reduce its susceptibility to whitening. These results were consistent with the other studies showing elevated temperature reduced growth of strawberry and *Viola* X *wittrockiana* (Kadir *et al*, 2006; Warner and Erwin, 2006).

There were no differences in plant height, plant width or growth index in either cultivar before starting SA applications (Table 6.3). SA did not affect plant height, width or growth index in either cultivar at 2 weeks (Table 6.3). Plant height and width of neither cultivar were affected by SA application at 4 weeks (Table 6.3). However, plant growth index of 'Beach' was reduced with application of SA at 4 weeks, whereas there was no change in growth index of 'Butterfly' (Table 6.3). Growth index of 'Beach' as well as 'Butterfly' was not different due to SA application at the end of experiment (Table 6.3) indicating that SA applications did not affect overall growth in ivy geranium. Salicylic acid increased growth in maize when applied exogenously (Gunes *et al*, 2007) but there was no improvement in growth or grain yield with SA application in wheat cv M-97 (Arfan *et al*, 2007).

'Butterfly' plants started showing whitening symptoms within 7 days of elevating air temperature (observational data), however, at the end of experiment, both cultivars had significant whitening of leaves in the elevated temperature treatment (Table 6.4)

confirming elevated air temperatures cause whitening in ivy geranium. This was also observed by Senaratna *et al* (2002) where heat stress (56^{o}C) caused bleaching of zonal geraniums, *Pelargonium* X *hortorum* Bailey. These results were also similar to those reported for tree fern (*Cyathea cooperi*), *Euglena gracilis*, and reef corals which all showed chlorosis or bleaching at elevated temperatures (Doley, 1983; Ortiz and Wilson, 1988; Warner *et al*, 1996). SA application did not prevent or reduce whitening in either cultivar (Table 6.4).

There were no interactive effects of temperature and SA on photosynthetic pigments or total phenolics. Elevated air temperature reduced Chl a, Chl b, total chlorophyll (Chl a+b), Chl a:Caro, Chl b:Caro and Chl a+b:Caro ratios in 'Beach' (Table 6.5), however, carotenoids and Chl a:b ratio were not affected by temperature. These results are similar to those found in tomato, which showed chlorophyll to carotenoids ratios decreased in heat-stressed plants (Camejo *et al*, 2005). The concentration of Chl a, Chl b, Caro, Chl a+b, Chl a:Caro and Chl (a+b):Caro ratios were decreased at elevated temperature in 'Butterfly' (Table 6.5), whereas no differences were observed for Chl a:b and Chl b:Caro ratios. Chl a:b ratio was not different in either cultivar which indicated temperature reduced them proportionally. Leaf chlorophyll content of wheat and creeping bentgrass was also reduced at elevated temperatures (Xu *et al*, 2002; Tahir *et al*, 2005)

No differences were observed in carotenoids concentration in 'Beach' due to elevated temperature, but there was reduction in carotenoids concentration in 'Butterfly' at elevated temperature (Table 6.5). Carotenoids serve as accessory light-harvesting pigments and as antioxidants that quench tissue-damaging free radicals such as singlet oxygen species (Guerinot, 2000). As the differences in carotenoid concentration were

only observed in the most susceptible cultivar, i.e. 'Butterfly', it is possible that carotenoids play a role in preventing whitening of ivy geranium.

SA applications did not affect pigment concentrations and ratios in 'Beach' (Table 6.6). However, SA applications decreased Chl a, Chl b and total Chl in 'Butterfly' (Table 6.6). Caro concentration, Chl a:b, Chl a:Caro, Chl b:Caro, and total Chl:Caro ratios did not decrease due to application of SA in 'Butterfly (Table 6.6). Improvements in photosynthetic rate due to SA application were associated with metabolic factors other than photosynthetic pigments and leaf carotenoids (Arfan *et al*, 2007).

Elevated temperature decreased total pheophytins in 'Beach' as well as in 'Butterfly' (Table 6.7). This suggests a heat-induced functional disconnection in electron transfer due to reduced total pheophytins at elevated temperatures. Photoreducible pheophytin is the natural primary electron acceptor of PSII in the early steps of the photosynthetic solar energy conversion acting between P680 and plastoquinone (Klimov, 2003). There were no differences observed in total phenolics content due to temperature in either cultivar (Table 6.7). No change in total phenolics content due to temperature was also reported in scots pine seedlings (Holopainen and Kainulainen, 2004). SA application did not affect pheophytins nor total phenolics content in either cultivar (Table 6.7).

Pigment concentrations are often used as an indirect measure of photosynthetic biomass (Hurley and Watras, 1991). The results of pigments concentrations and their ratios indicate that there was a reduction in photosynthesis as indicated by a reduction in photosynthesis pigments and biomass in heat-stressed plants of ivy geraniums. The elevated air temperature induced whitening in ivy geranium may be thermal-inhibition caused by impaired photosynthetic pigment synthesis or their degradation or both.

Stem fresh weight, leaf fresh weight, total fresh weight, stem dry weight, leaf dry weight and total dry weight were less in 'Beach' and 'Butterfly plants grown at elevated temperature (Table 6.8). Elevated air temperature reduced growth in ivy geranium similar to the findings for pansy, strawberry and wheat plants (Hamlin *et al*, 1999; Kadir *et al*, 2006; Tahir *et al*, 2005). No differences were found in total fresh to dry weight ratio, SWR or LWR due to temperature in 'Beach' or 'Butterfly' (Table 6.8).

Salicylic acid applications had no affect on stem fresh weight, leaf fresh weight, total fresh weight, stem dry weight, leaf dry weight, total dry weight, total fresh to dry weight ratio, SWR or LWR in 'Beach' or 'Butterfly' (Table 6.9). Thus, SA applications had no affect on growth of ivy geranium, similar to that observed for wheat cv. M-97 (Gunes *et al*, 2007).

Leaf area was reduced in 'Beach' and 'Butterfly' plants grown at elevated temperature (Table 6.10), indicating a reduction of leaf surface area for transpiration. These results are similar findings to which suggested high temperature reduced leaf expansion of strawberry plants (Kadir *et al*, 2006). Leaf expansion varies with leaf temperature (Tardieu *et al*, 1999). Leaf area ratio (LAR) was less at elevated temperature in both cultivars (Table 6.10). Reduced LAR indicates less photosynthesizing material to support respiring material within the plants of 'Beach' and 'Butterfly'. LAR of flame azalea plants was highest at 18^{o}C and decreased at higher temperatures (Malek, 1992).

No differences were found in specific leaf area (SLA) in 'Beach' and 'Butterfly' due to temperature (Table 6.10). This could be due to proportional reduction of whole plant leaf area with respect to leaf dry mass. Elevated temperature had no effect on leaf area index (LAI) in 'Beach', which may be due to proportional reduction of whole leaf

area to land area of the plant canopy. Salicylic acid did not affect leaf area, LAR, SLA or LAI index in 'Beach' or 'Butterfly' (Table 6.11), indicating that SA applications did not affect the overall growth of ivy geranium.

There was an interactive effect of temperature and SA application on LAI of 'Butterfly' (Table 6.12). Maximum LAI was obtained at ambient temperature with no SA application, which was not different from that obtained at elevated temperature with SA applications. LAI obtained at ambient temperature with SA application was not different from LAI obtained at elevated temperature without SA application. These results indicate that SA application reduced LAI at ambient temperature in 'Butterfly' but helped increase LAI at elevated temperature.

There was no interactive effect of temperature and SA application on foliar total Fe, Mn, Zn, Mg, or S content in 'Beach' or 'Butterfly'. Elevated temperature did not affect foliar total Fe in 'Beach' or 'Butterfly' (Table 6.13). Mn and Mg contents increased with increase in temperature in 'Beach' whereas they were not affected by temperature in 'Butterfly' (Table 6.13). These results are consistent with the findings that Mg and Mn levels in the leaves and roots of tomato plants were higher during summer than in winter (Darawsheh, *et al* 2006). Foliar Zn content was the same at both temperatures in 'Beach', whereas it was greater at elevated temperature in 'Butterfly' (Table 6.13). Foliar S content increased in 'Beach' and 'Butterfly' when grown at elevated temperature (Table 6.13).

The similar foliar total Fe concentration in healthy, green plants of 'Beach' and 'Butterfly' grown at ambient temperature and in heat-stressed, white plants grown at elevated temperature may be supported by the 'Chlorosis paradox' where chlorotic plants

had higher total Fe than green plants (Romheld, 2000). There may be inactivation of Fe or inhibition of its mobility within plants at elevated temperatures. Similar levels of foliar total Fe at both temperatures indicates that Fe uptake is not inhibited at elevated temperature. Another possible explanation may be that tissue analysis was done on a whole plant foliar tissue basis, white and green leaves were not separated for analysis. Fe may not be distributed evenly between older and new leaves, it may be more concentrated in the older, green leaves. It would be of interest to examine total and active Fe in white and green leaves of plants to answer the inactivation or immobility issue within the heat-stressed plants of ivy geraniums.

Elevated temperature resulted in greater accumulation of Mn in 'Beach' (Table 6.13), whereas Zn content increased with elevated temperature in 'Butterfly' (Table 6.13). 'Beach' responded to elevated temperature by reducing its growth. By slowing its growth, 'Beach' would retain higher foliar Fe, Mn and Zn concentration. 'Butterfly' continued to put out new growth at elevated temperature, which was white. The additional foliar tissue may be lacking foliar Fe, Mn, and Zn resulting in a dilution effect. This suggests that elevated temperatures may cause degradation of heat-sensitive enzymes or formation of antioxidants and heat shock proteins due to a lack of Fe, Mn, or Zn. Increased content of Zn in foliar tissues of 'Butterfly' at elevated temperature indicates a possibility of interruption of its translocation to the younger leaves, which may have a greater requirement for enzymes synthesis to combat heat stress.

The higher uptake rates of divalent cations in 'Beach' and 'Butterfly' grown at elevated temperature (irrespective of Fe-chelate application) supports the view of

Kochian (1993) that all divalent cations are taken up through the same channels in the plasma membrane of the root cell. Romheld (1987) classified *Pelargonium* as a Strategy-I plant, having Fe-efficiency mechanisms. Fe-efficiency mechanisms may also enhance the availability of Mn (Welch *et al*, 1993).

Deficiency of one micronutrient can stimulate the accumulation of the other micronutrients in deficient plants (Yu and Rengel, 1999). The younger leaves may be deficient in Fe due to insufficient retranslocation of Fe from roots. The deficiency of Fe may signal (shoot-phloem-root loop) an enhanced Fe-uptake response (Schmidt, 2003), resulting in greater accumulation of other cations like Mn in 'Beach' and Zn in 'Butterfly'.

Chelate as fertilizer in continuous liquid feed (20N-4.4P-16.6K, Peters peat-lite fertilizer) may act as an extractant and thus elevate the water-soluble levels of Mn and Zn recovered from the substrate (Kriej, 1998). Synthetic chelates, such as EDTA have been shown to enhance phytoextraction of Zn from soil (Groman *et al,* 2001). Similar findings have been documented in maize, which indicated higher mobilization of Zn from soil with an application of EDTA (Chen *et al*, 2004). This extraction effect may be greater at elevated temperatures, and contribute to increased concentrations of foliar Mn and Zn, even though neither was applied beyond that present in the liquid feed.

Foliar Mg content was greater in 'Beach' at elevated temperature whereas Mg was not different due to temperature in 'Butterfly' (Table 6.13). Foliar Mg content increased at elevated temperature in the cultivar with lesser susceptibility to whitening, indicating there may be a connection between foliar Mg content and whitening as Mg^{2+} enhances the stability of photosystem II against heat damage (Schreiber and Armond,

1978). Foliar S content was greater in 'Beach' and 'Butterfly' at elevated temperature (Table 6.13). Gadallah (1996) documented that temperature had a dominant effect on growth, and shoot and root sulfur content. S is taken up as SO_4^{2-} by the roots and is transported via the xylem to the leaves. The uptake and subsequent distribution of SO_4^{2-} to the leaves is closely regulated in response to demand (Sunarpi and Anderson, 1996). All enzymatic and most non-enzymatic antioxidants contain S. Glutathione, as the major non-protein thiol, plays a central role in reactive oxygen species (ROS) defense. Antioxidant defense systems are often constitutive, multifactorial elements of plant cell metabolism that are up regulated under the impact of biotic and abiotic stressors (Kandlbinder *et al* 2004). 'Beach' and 'Butterfly' may need more S at elevated temperatures to synthesize heat-shock proteins or antioxidants in response to temperature-stress. This may be the reason for higher concentrations of S in leaves at elevated temperature. Salicylic acid did not affect foliar tissue contents of Fe, Mn, Zn, Mg and S in 'Beach' nor in 'Butterfly' (Table 6.14).

The accumulation of Mn, Mg and S in 'Beach' and of Zn and S in 'Butterfly' indicate that there may be a greater nutrients requirement at elevated temperature. However, the translocation of all these nutrients to upper leaves in heat-stressed plants of ivy geranium may be impaired. The whitening process may deprive the cells of ivy geranium from more than one nutrient and 'Beach' responded by increasing the concentration of nutrients to meet the demand of plant cells during development.

Conclusion

Elevated air temperature causes whitening of ivy geranium. Whitening is first expressed in the youngest, developing leaves of ivy geraniums. Elevated temperatures severely reduced the growth, leaf area, fresh (stem, leaf and total) weight and dry (stem, leaf and total) weight of ivy geraniums. 'Beach' responded to heat stress by increasing its size less while 'Butterfly' did not after 6 weeks. Leaf area and leaf area ratio (LAR) were reduced at elevated temperature, indicating the presence of reduced photosynthesizing material to support respiring material with ivy geraniums.

Elevated temperature reduced photosynthetic pigments and their ratios in ivy geranium. Reduction in carotenoids concentration in 'Butterfly' indicated the possible role of reduced carotenoids causing whitening of ivy geraniums. Reduced total pheophytins concentration in both cultivars at elevated temperature is an indication of heat-induced functional disconnection in electron transfer, which could result in thermal-inhibition.

Similar levels of total Fe in both cultivars indicate no inhibition of Fe-uptake at elevated temperature. 'Beach' accumulated greater foliar Mn, Mg, and S content at elevated temperature. 'Butterfly' the more susceptible cultivar to whitening, had similar concentration of Mn but increased foliar Zn and S at elevated temperature.

SA application did not affect growth, leaf area, fresh (stem, leaf and total) weight, dry (stem, leaf and total) weight, fresh: dry weight ratio, LAR, SLA or foliar nutrient (Fe, Mn, Zn, Mg and S) contents in foliar tissues. It did reduce chlorophyll content at elevated temperature in 'Butterfly' but not in 'Beach'

Whitening in ivy geranium is a heat stress response, initially exhibited by young, developing leaves and is caused by elevated air temperature. Whitening is the result of an impaired photosynthetic pigments synthesis or their degradation or both. SA application did not affect growth or whitening in ivy geraniums at elevated temperature.

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Table 6.1. Average daily growth chamber temperature from 2 Feb to 16 March 2007. Day= 7am -5pm, and night = 6pm- 6am

	Unheated Temperature (^{o}C)		Heated Temperature (^{o}C)	
	Day-time	Night-time	Day-time	Night-time
Low	18.6	13.7	25.3	18.4
High	32.2	19.8	40.5	26.5
Average	27.7	16.1	35.8	22.3

Table 6.2. Effect of elevated air temperature on plant height, width, and growth index (GI) in ivy geranium 'Beach' and 'Butterfly'

	Beach			Butterfly		
Temperature	Height (cm)	Width (cm)	GI (cm^3)	Height (cm)	With (cm)	GI (cm^3)
At the start of experiment						
28^{o}C	16.5 a[x]	31.1 a	12542 a	13.6 a	23.9 a	6161 a
36^{o}C	16.3 a	30.1 a	11801 a	12.2 a	25.7 a	6523 a
2 weeks						
28^{o}C	16.1 a	35.6 a	16023 a	15.0 a	30.9 a	11230 a
36^{o}C	16.0 a	34.3 a	14948 a	12.9 a	30.2 a	9351 a
4 weeks						
28^{o}C	18.3 a	41.3 a	24334 a	16.3 a	36.5 a	16703 a
36^{o}C	17.5 a	36.6 a	18737 a	13.8 a	33.1 a	12143 a
6weeks						
28^{o}C	19.3 a	46.6 a	33049 a	16.9 a	42.3 a	23049 a
36^{o}C	18.3 a	36.4 b	19647 b	14.8 a	34.7 a	14119 a

[x]Means followed by same letters within column and within 2 weeks period are not different according to Fisher's protected LSD test (P=0.05).

Table 6.3. Effect of salicylic acid application on plant height, width, and growth index (GI) in ivy geranium 'Beach' and 'Butterfly'

Treatment	Beach Height (cm)	Beach Width (cm)	Beach GI (cm^3)	Butterfly Height (cm)	Butterfly Width (cm)	Butterfly GI (cm^3)
At the start of experiment						
0 μmol l^{-1}	17.0 a[x]	30.7 a	12733 a	13.2 a	24.6 a	6275 a
100 μmol l^{-1}	15.8 a	30.5 a	11610 a	12.7 a	25.1 a	6408 a
2 weeks						
0 μmol l^{-1}	16.6 a	36.0 a	16978 a	13.5 a	30.4 a	9798 a
100 μmol l^{-1}	15.4 a	33.9 a	13993 a	14.4 a	30.6 a	10783 a
4 weeks						
0 μmol l^{-1}	19.1 a	40.2 a	24359 a	14.5 a	35.5 a	14385 a
100 μmol l^{-1}	16.6 a	37.7 a	18713 b	15.7 a	34.1 a	14462 a
6 weeks						
0 μmol l^{-1}	19.5 a	42.6 a	28912 a	15.9 a	38.9 a	18976 a
100 μmol l^{-1}	18.0 a	40.4 a	23784 a	15.8 a	38.1 a	18192 a

[x]Means followed by same letters within column and within 2 weeks period are not different according to Fisher's protected LSD test (*P*=0.05).

Table 6.4. Effect of elevated air temperature on extent of whitening (on scale of 1 to 7, 1= 0%, 2= 17%, 3= 34%, 4 =50%, 5= 67%, 6= 84% and 7= 100% whitening) of ivy geranium 'Beach' and 'Butterfly' at the end of experiment (16 March 2007)

	Beach	Butterfly
Temperature		
28°C	1.04 b[x]	1.04 b
36°C	2.23 a	3.71 a
SA treatment		
0 μmol l^{-1}	1.73 a	2.29 a
100 μmol l^{-1}	1.54 a	2.46 a

[x]Means followed by same letters within column and within treatment are not different according to Fisher's protected LSD test (*P*=0.05).

Table 6.5. Effect of elevated air temperature on pigment concentrations ($\mu g \cdot cm^{-2}$) and pigment ratios in ivy geranium 'Beach' and 'Butterfly'

Temperature	Chl a	Chl b	Caro	Total Chl	Chl a: b	Chl a:Caro	Chl b:Caro	Total Chl:Caro
Beach								
28°C	24.4 a[x]	14.9 a	8.2 a	39.3 a	1.64 a	2.96 a	1.81 a	4.77 a
36°C	14.5 b	9.3 b	6.3 a	23.8 b	1.56 a	2.31 b	1.48 b	3.80 b
Butterfly								
28°C	21.7 a	12.8 a	6.9 a	34.5 a	1.70 a	3.14 a	1.86 a	5.00 a
36°C	12.1 b	8.0 b	4.7 b	20.1 b	1.52 a	2.58 b	1.71 a	4.29 b

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).

Table 6.6. Effect of salicylic acid on pigment concentrations ($\mu g \cdot cm^{-2}$) and pigment ratios in ivy geranium 'Beach' and 'Butterfly'

SA-treatment	Chl a	Chl b	Caro	Total Chl	Chl a: b	Chl a:Caro	Chl b:Caro	Total Chl:Caro
Beach								
0 $\mu mol\ l^{-1}$	19.7 a[x]	12.3 a	7.6 a	32.0 a	1.58 a	2.56 a	1.61 a	4.16 a
100 $\mu mol\ l^{-1}$	19.2 a	11.9 a	7.0 a	31.1 a	1.61 a	2.71 a	1.68 a	4.40 a
Butterfly								
0 $\mu mol\ l^{-1}$	17.9 a	11.2 a	6.1 a	29.0 a	1.59 a	2.91 a	1.83 a	4.75 a
100 $\mu mol\ l^{-1}$	15.9 b	9.7 b	5.6 a	25.6 b	1.62 a	2.81 a	1.73 a	4.54 a

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).

Table 6.7. Effect of elevated air temperature and SA treatment on total pheophytins and phenolic contents ($\mu g \cdot cm^{-2}$) in ivy geranium 'Beach' and 'Butterfly'

	Beach		Butterfly	
	Pheophytins	Total phenolics	Pheophytins	Total phenolics
Temperature				
28°C	29.0 a[x]	123.0 a	19.5 a	106.7 a
36°C	11.7 b	103.2 a	4.9 b	91.4 a
SA treatment				
0 $\mu mol\ l^{-1}$	23.5 a	87.8 a	13.4 a	97.5 a
100 $\mu mol\ l^{-1}$	17.2 a	138.3 a	11.0 a	100.6 a

[x]Means followed by same letters within column and within treatments are not different according to Fisher's protected LSD test (P=0.05).

Table 6.8. Effect of elevated air temperature on fresh (shoot, leaf and total) weight, dry (shoot, leaf and total) weight, fresh to dry weight ratio, shoot weight ratio (SWR) and leaf weight ratio (LWR) in ivy geranium 'Beach' and 'Butterfly'

Temperature	Shoot fresh wt (g)	Leaf fresh wt (g)	Total fresh wt (g)	Shoot dry wt (g)	Leaf dry wt (g)	Total dry wt (g)	Fresh: dry wt ratio	SWR	LWR
Beach									
28°C	47.1 a[x]	213.1 a	272.2 a	8.8 a	26.6 a	28.4 a	9.62 a	149.4 a	0.31 a
36°C	30.0 b	143.1 b	171.5 b	5.2 b	20.8 b	17.8 b	9.74 a	132.2 a	0.27 a
Butterfly									
28°C	45.0 a	194.5 a	234.9 a	7.8 a	23.5 a	23.2 a	10.16 a	148.3 a	0.32 a
36°C	25.8 b	104.1 b	125.6 b	4.1 b	16.6 b	13.0 b	9.67 a	139.0 a	0.29 a

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).

Table 6.9. Effect of salicylic acid application on fresh (shoot, leaf and total) weight, dry (shoot, leaf and total) weight, fresh to dry weight ratio, shoot weight ratio (SWR) and leaf weight ratio (LWR) in ivy geranium 'Beach' and 'Butterfly'

Treatments	Shoot fresh wt (g)	Leaf fresh wt (g)	Total fresh wt (g)	Shoot dry wt (g)	Leaf dry wt (g)	Total dry wt (g)	Fresh: dry wt ratio	SWR	LWR
Beach									
0 μmol l^{-1}	40.5 a.	181.9 a	224.5 a	7.2 a	24.4 a	23.5 a	9.63 a	139.7 a	0.29 a
100 μmol l^{-1}	36.5 a	174.3 a	219.2 a	6.7 a	23.1 a	22.7 a	9.73 a	141.9 a	0.29 a
Butterfly									
0 μmol l^{-1}	35.4 a	157.5 a	184.0 a	5.9 a	20.4 a	18.2 a	9.99 a	146.0 a	0.30 a
100 μmol l^{-1}	35.4 a	141.1 a	176.4 a	6.0 a	19.8 a	17.9 a	9.84 a	141.2 a	0.31 a

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).

Table 6.10. Effect of elevated air temperature on leaf area, leaf area index (LAI), leaf area ratio (LAR) and specific leaf area (SLA) in ivy geranium 'Beach' and 'Butterfly'

Temperature	Leaf area (cm^2)	LAI	LAR	SLA
Beach				
28°C	2962 a[x]	1.83 a	19.8 a	103.7 a
36°C	1854 b	1.64 a	14.1 b	96.8 a
Butterfly				
28°C	2486 a	*z	16.7 a	101.1 a
36°C	1375 b	*z	9.8 b	98.3 a

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).
*z = interaction of temperature and SA (Table 6.12).

Table 6.11. Effect of Salicylic acid application on leaf area, leaf area index (LAI), leaf area ratio (LAR) and specific leaf area (SLA) in ivy geranium 'Beach' and 'Butterfly'

Temperature	Leaf area (cm^2)	LAI	LAR	SLA
Beach				
28°C	2482 a[x]	1.74 a	17.6 a	99.4 a
36°C	2334 a	1.70 a	16.3 a	101.1 a
Butterfly				
28°C	2021 a	*z	13.6 a	102.3 a
36°C	1839 a	*z	13.0 a	97.2 a

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).
*z = interaction of temperature and SA (Table 6.12).

Table 6.12. Interaction of temperature and salicylic acid application on leaf area index (LAI) of ivy geranium 'Butterfly'

Temperature	SA treatment	LAI
28°C	0 μmol l^{-1}	2.18 a[x]
28°C	100 μmol l^{-1}	1.56 b
36°C	0 μmol l^{-1}	1.35 b
36°C	100 μmol l^{-1}	1.62 a b

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).

Table 6.13. Effect of elevated air temperature on foliar nutrient (Fe, Mn, Zn, Mg and S) contents of ivy geranium 'Beach' and 'Butterfly'

Temperature	Fe (ppm)	Mn (ppm)	Zn (ppm)	Mg (%)	S (%)
Beach					
28°C	98.13 a[x]	31.40 b	20.60 a	0.19 b	0.09 b
36°C	45.73 a	67.35 a	28.07 a	0.25 a	0.15 a
Butterfly					
28°C	44.4 a	26.73 a	14.65 b	0.17 a	0.08 b
36°C	58.5 a	40.22 a	24.28 a	0.19 a	0.15 a

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).

Table 6.14. Effect of Salicylic acid applications on foliar nutrient (Fe, Mn, Zn, Mg and S) contents of ivy geranium 'Beach' and 'Butterfly'

Treatments	Fe (ppm)	Mn (ppm)	Zn (ppm)	Mg (%)	S (%)
Beach					
0 μmol l^{-1}	47.82 a[x]	49.97 a	22.85 a	0.23 a	0.12 a
100 μmol l^{-1}	96.05 a	48.78 a	25.82 a	0.22 a	0.12 a
Butterfly					
0 μmol l^{-1}	59.87 a	33.77 a	20.40 a	0.18 a	0.12 a
100 μmol l^{-1}	43.03 a	33.18 a	18.53 a	0.18 a	0.12 a

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).

CHAPTER VII

REVERSAL OF WHITENING IN IVY GERANIUM WITH LOW NIGHT TEMPERATURE AND FE-EDDHA

Abstract

The development of whitening of the youngest leaves of actively growing ivy geranium (*Pelargonium peltatum* L.) has been observed as the season changes from late spring to summer. High temperature and iron deficiency have been suspected to cause this disorder. High night temperatures are thought to be more important than high day temperatures. A study was conducted to determine if low night temperatures reverse whitening in ivy geraniums. Rooted cuttings of ivy geranium 'Beach' and 'Butterfly' were potted on 28 July 2006 into 15 cm containers filled with sphagnum peat and perlite (70:30, by volume). Plants were fertilized with 250mg $N \cdot L^{-1}$ from 20N-4.4P-16.6K Peters Peat-lite fertilizer as a continuous liquid feed. The potted cuttings were grown until they developed a substantial root system. Within this time, the plants developed whitening. On 8 Sept 2006, plants were moved to average night temperature of 16^{o} or 22^{o}C while day temperature averaged 29^{o}C. Iron (Sprint 138, Fe-EDDHA, 6% Fe) was applied at 0mg Fe (control) or 54mg Fe drench per pot on 8 Sept and 8 Oct 2006. The experiment was a split plot design, split by temperature. Cultivars varied in their response to low night temperature. 'Beach' responded to low night temperature by reducing its plant width,

growth rate, and fresh to dry weight ratio whereas 'Butterfly' did not. 'Beach' reduced its rhizosphere pH at 22°C, whereas 'Butterfly' did not. Night temperature treatments did not affect plant width, growth index, SPAD chlorophyll index, fresh weight, dry weight, fresh to dry weight ratio, leaf area, leaf area index, photosysnthetic pigments, total phenolic contents, foliar tissue nutrient concentrations or their ratios in 'Butterfly'. In addition, Fe-chelate application did not reduce growth, leaf area, leaf area index, fresh weight, dry weight, or fresh:dry weight ratio of either cultivar. Fe-chelate application increased SPAD chlorophyll index in 'Beach'. Fe-chelate application helped to increase chlorophyll accumulation, particularly chlorophyll b as indicated by Chl a:b ratio in 'Beach', which recovered 100% from whitening. Higher but similar levels of total Fe in foliar tissues indicated no inhibition of Fe-uptake in 'Beach' or 'Butterfly' at either temperature.

Introduction

The leaves of ivy geranium turn white, expand less and develop an upward cupped appearance due to heat stress. This physiological disorder is referred to as "whitening". Plants are reported to resume normal, green growth as the temperature moderates in late summer and fall. There has also been observational evidence that applying Fe-chelate before whitening helped to reduce whitening. The whitening of the young foliage is thought to be caused by a high temperature induced iron deficiency.

Temperatures about 33°C are quite common during summers in the southeastern U.S. (Weather Channel, 2008). Plants respire by using the metabolites made during photosynthesis. However, photosynthesis and respiration are inhibited at high

temperatures and as temperature increases, photosynthetic rates drop before respiratory rates drop. At temperatures above the temperature compensation point, photosynthesis cannot replace the carbon used as a substrate for respiration. This imbalance between photosynthesis and respiration is one of the main reasons for the deleterious effects of high temperatures (Taiz and Zeiger, 2002). Greater warming at night is often considered more likely to increase 24h respiratory CO_2 emission more than photosynthetic CO_2 uptake (Volder *et al*, 2004). High night temperatures reduced grain growth and development in rice more than high day temperatures (Morita *et al*, 2005). The decline of carbohydrates in shoots as well as roots during summer may be due to the imbalance between carbon production in photosynthesis and consumption in respiration (Carrow, 1996; Liu and Huang, 2001; Xu and Huang, 2000). Reynolds *et al* (1990) concluded that chlorophyll loss at 40/30^{o}C undoubtedly had a major impact on biomass accumulation in *Solanum chacoense*. The effects of heat stress on the function of the acceptor site of photosystem II (PS II) were reversible after the heated cyanobacterium cells were cooled down to the growth temperature (Wen *et al*, 2005).

Iron has an important role in the formation of thylakoid membranes in higher plants. It functions to accept and donate electrons and plays important roles in the electron-transport chains of photosynthesis and respiration (Connolly and Guerinot, 2002). Iron deficiency affected dark respiration less than photosynthesis in leaves of sugarbeet (Morales *et al*, 1998). Iron deficiency is accompanied by a decrease in light harvesting pigments, chlorophylls and carotenoids (Morales *et al*, 1990; Morales *et al*, 1994). High Fe (Fe-EDDHA) levels in the nutrient solution were associated with a higher concentration of leaf pigments of chile peppers (Anchondo *et al*, 2001).

The objectives of this study were to determine if low night temperatures reverse whitening and to determine the effect of low night temperature and Fe-EDDHA on growth, photosynthetic pigments, and foliar nutrient composition of ivy geranium with whitening.

Materials and Methods

Rooted cuttings of ivy geranium, 'Beach' and 'Butterfly' were potted on 28 July, 2006 into 15 cm containers filled with sphagnum peat and perlite (70:30 by volume) with 0.96 kg gypsum /m^3, 7.7kg lime /m^3, and 0.32kg wetting agent (SaturAid, Debco Pty. Ltd., Tyabb, Australia) /m^3 added. Plants were fertilized with 250mg $N{\cdot}L^{-1}$ from 20N-4.4P-16.6K (Peters Peat-lite fertilizer, Scotts Company, Marysville, OH) as a continuous liquid feed. The potted cuttings were grown for a month until they developed roots to the edge of the container. Within this time, the cuttings of ivy geranium developed whitening. 'Beach' is known to be more resistant to whitening than 'Butterfly' (Personal communication, Harvey Lang, Fischer, USA).

Plants were moved to average night temperatures of 16^o or 22^{o}C (Table 7.1) while day temperature was 29^{o}C on average. Night temperature treatments were started on 8 Sept 2006. Iron (Sprint 138, Fe-EDDHA, 6% Fe, Becker Underwood Inc, Ames, Iowa) was applied at 0mg Fe (control) or 54mg Fe (i.e. 0.9g Fe-EDDHA in 60 ml of distilled water) drench per pot on 8 Sept 2006 and 8 Oct 2006.

Data was collected for plant height (from the rim of the container) and width (an average of two widths measured, one at the widest point and another at 90^o) at bi-weekly intervals. Growth index was calculated as: GI (cm^3) = 3.14*(width/2)^2*height.

Leachate pH and EC (soluble salts) were measured using the Virginia Tech pour-through technique at week 0, 2, 4, and 8 (Wright, 1986). The pH was measured using an Orion Model 920 pH meter (Orion Reseach Inc., Boston, MA). EC was measured in millimhos using YSI Model 35 electrical conductivity meter (Yellow Spring Instrument Co., Yellow Springs, OH). Leachate samples were analyzed for Fe, Zn, Mn, Mg and S content using inductively coupled plasma (ICP) atomic emission spectrometry (Optima 4300DV, PerkinElmer Instruments, Norwalk, CT) at the Mississippi State University Soil Testing Laboratory.

Extent of whitening was determined as visual rating on a scale of 1 to 7, where 1 = 0%, 2= 17%, 3= 34%, 4 =50%, 5= 67%, 6= 84% and 7 = 100% whitening before starting the experiment and at the end of the experiment. Symptoms were also recorded photographically. Recovery of plants from whitening was calculated as the difference in whitening from initial rating to final rating of extent of whitening.

Five recently mature leaves were harvested from each plant. One leaf disc was taken from each of the five leaves for one pooled sample for pigment analysis. Pigment concentrations [i.e. chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids (Caro)] were estimated spectrophotometrically by placing five 38.5mm^2 leaf discs in a vial with 5 ml of DMSO (dimethyl sulfoxide) and extracting for 24 hours by incubating in the dark at 20^{o}C. The absorptions of the extracts were determined at 664, 648 and 470 nm (Chl a, Chl b, Caro respectively) using Bio-Rad UV/VIS spectrophotometer (Bio-Rad Laboratories, Hercules, CA, USA). DMSO was used as the blank. Pigment calculations were made according to the equations used by Lichtenthaler (1987) and Chappelle *et al*, (1992). Pigment concentrations were expressed on leaf area basis ($\mu g \cdot cm^{-2}$).

The total phenolic contents of leaves were also determined spectrophotometrically using five 38.5mm^2 leaf discs placed in 10 ml of extractant, a mixture of methanol, distilled water, and hydrochloric acid in 79:20:1 ratio. The vials were incubated at 20^{o}C for 24 hours in the dark to allow complete extraction of phenolic compounds. Peak absorbance was at 320 nm when scanned between 200 and 900 nm using the Bio-Rad spectrophotometer. The phenolics content was determined by the protocol of Kakani *et al*, (2004) and expressed as $\mu g \cdot cm^{-2}$.

Plants were harvested at the media line at the end of experiment (1 Nov, 2006) to determine plant biomass and leaf area. Fresh weight of each plant was measured. Three plants per treatment were used to determine total leaf area (LA) using a portable leaf area meter (LI-3000, LI-COR. Lincoln, Nebraska). Leaf area index (LAI) is the leaf area per unit area of land (Hunt, 1982) and it was calculated as LAI= LA/(3.14*r^2) where LA= leaf area and r = radius of plant canopy.

After measuring fresh weight, one plant from each replication was rinsed with 0.1N HCl followed by rinsing thrice with deionized water. Plants were dried in an oven at 60^{o}C until dry before determining dry weight. Dry leaf samples were separated from stems. The dry leaves were ground through a 0.5 mm screen (20 mesh) using a Cyclotec sample mill (UDY corporation, Fort Collins, CO). Ground tissue samples were used to determine total Fe, Zn, Mn, Mg and S (Crouse, 2001) using inductively-coupled plasma (ICP) atomic emission spectrometry (Optima 4300DV, PerkinElmer Instruments, Norwalk, CT).

Experiment was a split plot design, split by temperature. There were three replications with 2 subsamples for each treatment. Plant size indices, plant fresh weights,

plant dry weights, pigments (chlorophylls and carotenoids), phenolic contents and foliar nutrient concentrations were analyzed by analysis of variance, using Proc-Glimmix (SAS software, SAS Institute, Cary, N.C.). Fischer's protected least significant difference (LSD) at P=0.05 was used to indicate significant differences between treatment means.

Results and Discussion

No interactions between night temperature and Fe-chelate applications were observed for plant height, width or growth index in either cultivar. There were no differences in plant height, plant width or growth index in either cultivar before starting the experiment (Table 7.2). Night temperature did not affect plant height, width or growth index in either cultivar after 2 weeks. Plant width was less after 4 weeks at 16^{o}C in 'Beach'. After 6 and 8 weeks the growth index and plant width in 'Beach' was greater at the warmer 22^{o}C temperature. Plant height, width and growth index were unaffected by night temperature in 'Butterfly' through the experiment (Table 7.2). 'Beach' responded to low night temperature by increasing less in size whereas 'Butterfly' did not, indicating a cultivar specific response to night temperature.

There were no differences in plant height, plant width and growth index in either cultivar before starting Fe-chelate applications. Fe chelate application did not affect plant height, width or growth index in either cultivar throughout the experiment (Tables 7.3).

Total fresh weight, total dry weight, LA, and LAI were not affected by night temperature in 'Beach' or 'Butterfly' (Table 7.4). Low night temperatures reduced fresh to dry weight ratio in 'Beach', but not in 'Butterfly'. These results indicate low night temperatures reduced water content in 'Beach', but not in 'Butterfly'.

Fe-chelate applications did not affect total fresh weight, total dry weight, fresh to dry weight ratio, LA, and LAI in 'Beach' or 'Butterfly' (Table 7.4). These results indicate that Fe-chelate applications did not affect growth in ivy geranium.

Night temperature did not affect SPAD-chlorophyll index of mature leaves in 'Beach' or 'Butterfly' (Table 7.5) throughout the experiment. This lack of response may be due to the recently mature leaf samples being uniformly green.

Fe-chelate application increased SPAD-chlorophyll index in 'Beach' after 6 weeks, but it was not different at the end of the experiment (Table 7.6). The first application of Fe-chelate applied at the start of the experiment (8 Sept) did not affect SPAD-chlorophyll index until after 4 weeks of growth. A second application of Fe on 8 Oct. occurred before measuring SPAD-chlorophyll index at week 6 and may have temporarily increased SPAD-chlorophyll index at 6 weeks. Fe-chelate application did not alter SPAD-chlorophyll readings in 'Butterfly' throughout the experiment (Table 7.6). This indicates that there is no problem in Fe-uptake in ivy geraniums with whitening. Since SPAD measurements were taken on recently matured leaves, these leaves had developed without whitening.

Leachate pH, EC (soluble salts), Fe, Mn, Zn, Mg and S contents were not different at the start of the experiment for 'Beach' and 'Butterfly' (Table 7.7). Leachate pH, EC, Mn, Zn, Mg and S contents were not different due to temperature after 2 weeks in 'Beach' or 'Butterfly' (Table 7.8). However, at 2 weeks there was an interaction effect of temperature and Fe-chelate application on leachate Fe contents for both cutivars (Table 7.9). 'Beach' and 'Butterfly' responded similarly where 22^{o}C and Fe-chelate application resulted in the maximum leachate Fe content. Leachate Fe levels at both temperatures

with no Fe-chelate application were not different from each other. Leachate sampling indicated that about 50% applied Fe was present in the leachate 15 days after application, which is consistent with the findings that Fe-chelates have the capacity to maintain soluble iron in the soil solution over time (Cantera *et al*, 2002). Leachate pH, EC, Fe, Zn, Mg and S contents in 'Beach' and 'Butterfly' were not different due to temperature after 4 weeks (Table 7.10). However, there was an interaction effect of temperature and Fe-chelate application on leachate Mn content in 'Beach' (Table 7.11). 22^{o}C temperature and Fe-chelate application increased leachate Mn content. Leachate pH was reduced at 22^{o}C at the end of experiment in 'Beach', whereas leachate EC, Fe, Zn, and Mg contents were unaffected by temperature after 8 weeks (Table 7.12). *Pelargonium* has Strategy I Fe-efficiency mechanism (Romheld, 1987). The roots of Strategy I plants release more protons when they are iron-deficient, lowering the rhizosphere pH and thereby increasing the solubility of Fe^{3+} (Vizzotto *et al*, 1999). Reduced pH at 22^{o}C in 'Beach' may have increased availability of soluble Fe^{2+}. 'Beach' reduced its soil solution pH after 8 weeks at 22^{o}C whereas 'Butterfly', which is more susceptible to whitening, did not have a reduced pH. These results are consistent with findings that different genotypes have different capacities to release H^{+}. Fe-stressed plants of *Actinidia* genotype D1 showed a higher capability to lower the pH of the nutrient solution as compared to plants of sel.2084 (Vizzotto *et al*, 1999).

Leachate pH, EC (soluble salts), Fe, Mn, Zn, Mg and S contents were not different at the start of the experiment for 'Beach' and 'Butterfly' (Table 7.13). Leachate pH, EC, Mn, Zn, Mg and S contents in 'Beach' and 'Butterfly' were not affected by Fe-chelate applications after 2 weeks (Table 7.14). Leachate EC, Mn, Zn, Mg and S

contents in ‘Beach’ and ‘Butterfly’ were not affected by Fe-chelate applications after 4 weeks (Table 7.15). Leachate pH in ‘Butterfly’, a cultivar susceptible to whitening, increased with application of Fe-chelate after 4 weeks, whereas ‘Beach’ did not. In both ‘Beach’ and ‘Butterfly’, leachate Fe increased with Fe-chelate application at weeks 4 and 8. Leachate EC, Zn, and Mg contents in ‘Beach’ and ‘Butterfly’ were not affected by Fe-chelate applications after 8 weeks (Table 7.16). Leachate pH increased with Fe-chelate application in ‘Beach’ but not in ‘Butterfly’ at 8 weeks.

There were no differences due to temperature or Fe-chelate application on initial and final extent of whitening in ‘Beach’ and ‘Butterfly’ (Table 7.17). There were no interactive effects of temperature and Fe-chelate on initial or final extent of whitening in either cultivar. The recovery was calculated as the difference between initial and final extent of whitening. There were no differences between temperature or Fe-chelate treatments in recovery from whitening in either cultivar (Table 7.17). Temperature did not influence recovery of plants from whitening. The lack of difference may be that both temperatures were conducive to recovery or that there was a narrow range of control (22^{o}C) and low night temperature (16^{o}C). Plants recovered to the same extent at both temperatures, which could explain no statistical difference in recovery of whitening. At the end of the experiment, none of the ‘Beach’ plants had white leaves as indicated by final extent of whitening (scale 1=no whitening) whereas ‘Butterfly’ had more than 17% (scale 2= 17% whitening). These results suggest that ivy geranium can recover from whitening if placed under low night temperature or treated with Fe-chelate.

The extent of whitening at the end of the experiment was not different with or without Fe-chelate application in ‘Beach’ and Butterfly’ (Table 7.17), which implies that

ivy geranium already having whitening do not need Fe-chelate for recovery from whitening. Whitening is likely due to a reversible inactivation of photosystem II due to high temperature. The effects of heat stress on the function of the acceptor site of PSII were reversible after the heated cyanobacterium cells were cooled down to the growth temperature (Wen *et al*, 2005).

Night temperatures did not affect Chl a, Chl b, Chl a+b, carotenoids (Caro), Chl a: b, Chl a: Caro, Chl b: Caro, Chl(a+b): Caro ratio or total phenolics in 'Beach' or 'Butterfly' (Table 7.18). Fe-chelate applications did not affect Chl a, Chl b, Chl (a+b), Caro, Chl a:Caro, Chl b:Caro, Chl(a+b):Caro ratios and total phenolics in 'Beach' or 'Butterfly' (Table 7.19). Chl a:b ratio was reduced due to Fe-chelate application in 'Beach', but not in 'Butterfly'. Fe-chelate application increased Chl b content in 'Beach', as indicated by the Chl a:b ratio. Similar findings have been documented for chile peppers and zonal geraniums (Anchondo *et al*, 2001; Lee *et al*, 1996).

There were no differences observed in foliar levels of total Fe, Zn, Mn, Mg or S due to night temperature in 'Beach' or 'Butterfly' (Table 7.20). However, both had enough foliar Fe (Mills and Jones, 1996), no toxicity symptoms were observed. Night temperature did not affect foliar nutrients of ivy geraniums already having whitening. A sufficient total Fe and Mn contents had accumulated in ivy geraniums, however, it may be unavailable to the plants with whitening to perform various metabolic functions or these nutrients are in an inactive form in the plants of ivy geraniums.

There were no differences in Fe:Mn, Fe:Zn and Mn:Zn ratios due to temperature in 'Beach' or 'Butterfly' (Table 7.21). There were no differences in Fe:Mn, Fe:Zn or

Mn:Zn ratios due to Fe-chelate application in ‘Beach’. However, Fe-chelate application increased Fe:Mn ratio in ‘Butterfly’.

Conclusion

‘Beach’ responded to low night temperature by reducing its growth index and fresh to dry weight ratio whereas ‘Butterfly’ did not. ‘Beach’ reduced its soil solution pH at 22^{o}C, whereas ‘Butterfly’ did not. This could be the reason ‘Beach’ recovers more readily from whitening.

There were no significant differences in the amount of recovery of plants from whitening under low night temperature. Plants recovered at the same rate at both temperatures, which could be the reason for no difference in recovery of whitening due to low night temperature.

Fe-chelate application did not reduce growth, leaf area, fresh weight, dry weight or fresh:dry weight ratio of either cultivar. Fe-chelate application helped to increase SPAD chlorophyll index in ‘Beach’. Although Fe-application did not help to reduce whitening in ivy geraniums, it helped to preserve chlorophyll accumulation, particularly chlorophyll b as indicated by Chl a:b ratio in ‘Beach’. Similar levels of total Fe in foliar tissues indicated no inhibition of Fe-uptake in ‘Beach’ or ‘Butterfly’ at either temperature. Whitening may be an inactivation or immobility of Fe.

Fe-chelate applications were not beneficial in ‘Beach’ or ‘Butterfly’ recovery from whitening. Fe-chelate appears to be only effective in preventing whitening.

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Table 7.1. High, low, and average air night temperature (between 18:00 and 6:00hr) for treatments 16°C and 22°C between 8 Sept and 1 Nov 2006

Night Temperature	High	Low	Average
16°C	18.2 °C	14.4°C	16.3°C
22°C	26.4 °C	18.0°C	22.2°C

Table 7.2. Effect of night temperature on plant height, width and growth index (GI) in ivy geranium 'Beach' and 'Butterfly' at start of the experiment and after 2, 4, 6 and 8 weeks

	Beach			Butterfly		
Night Temperature	Height (cm)	Width (cm)	GI (cm^3)	Height (cm)	Width (cm)	GI (cm^3)
At the start of experiment						
16°C	10.3 a[x]	17.8 a	5223 a	6.9 a	10.5 a	1264 a
22°C	9.7 a	18.3 a	5232 a	7.3 a	10.6 a	1294 a
2 weeks						
16°C	9.5 a	26.7 a	11245 a	7.0 a	13.1 a	2020 a
22°C	11.0 a	24.0 a	10006 a	7.2 a	13.6 a	2191 a
4 weeks						
16°C	9.4 a	24.6 b	9293 a	6.9 a	15.0 a	2759 a
22°C	11.5 a	30.7 a	17219 a	7.3 a	15.9 a	3176 a
6 weeks						
16°C	10.0 a	27.4 b	12044 b	7.4 a	16.3 a	3499 a
22°C	13.3 a	37.1 a	29407 a	7.1 a	17.6 a	4316 a
8 weeks						
16°C	11.2 a	30.0 b	16404 b	7.0 a	17.0 a	3562 a
22°C	14.9 a	40.2 a	38994 a	8.3 a	18.5 a	5644 a

[x]Means followed by same letters within column and within week are not different according to Fisher's protected LSD test (P=0.05).

Table 7.3. Effect of salicylic acid applications on plant height, width and growth index in ivy geranium 'Beach' and 'Butterfly' at start of the experiment and after 2, 4, 6, and 8 weeks

	Beach			Butterfly		
Fe-application (per pot)	Height (cm)	Width (cm)	GI (cm^3)	Height (cm)	Width (cm)	GI (cm^3)
Start of the experiment						
0 mg	10.3 a[x]	19.0 a	5949 a	6.8 a	10.4 a	1186 a
54 mg	9.7 a	17.1 a	4505 a	7.4 a	10.7 a	1372 a
2 weeks						
0 mg	9.9 a	27.9 a	12487 a	7.3 a	13.1 a	2083 a
54 mg	10.7 a	22.8 a	8764 a	6.9 a	13.6 a	2128 a
4 weeks						
0 mg	10.8 a	27.5 a	13688 a	7.4 a	15.2 a	2948 a
54 mg	10.1 a	27.8 a	12825 a	6.9 a	15.7 a	2987 a
6 weeks						
0 mg	12.5 a	32.5 a	22839 a	7.3 a	17.0 a	4068 a
54 mg	10.9 a	32.0 a	18612 a	7.2 a	17.0 a	3746 a
8 weeks						
0 mg	13.9 a	35.8 a	31261 a	7.6 a	18.2 a	5149 a
54 mg	12.2 a	34.5 a	24137 a	7.7 a	17.3 a	4057 a

[x]Means followed by same letters within column and within week are not different according to Fisher's protected LSD test (P=0.05).

Table 7.4. Effect of night temperature and Fe-chelate application on fresh weight, dry weight, fresh to dry weight ratio, leaf area (LA) and leaf area index (LAI) in ivy geranium 'Beach' and 'Butterfly' at the end of experiment

	Beach					Butterfly				
	Fresh wt (g)	Dry wt (g)	Fresh:dry wt ratio	LA (cm^2)	LAI	Fresh wt (g)	Dry wt (g)	Fresh:dry wt ratio	LA (cm^2)	LAI
Temperature										
16°C	155.7 a[x]	17.2 a	9.1 b	1885 a	8.70 a	36.5 a	3.56 a	9.1 a	500 a	5.60 a
22°C	205.8 a	21.0 a	9.8 a	2650 a	11.01 a	39.3 a	3.71 a	10.3 a	621 a	6.73 a
Fe-application										
0 mg	204.3 a	21.4 a	9.5 a	2272 a	8.87 a	39.9 a	3.71 a	10.64 a	636 a	6.57 a
54 mg	157.1 a	16.7 a	9.4 a	2263 a	10.84 a	36.0 a	3.56 a	8.77 a	485 a	5.75 a

[x]Means followed by same letters within column and within treatment are not different according to Fisher's protected LSD test (P=0.05).

Table 7.5. Effect of night temperature on SPAD chlorophyll index of ivy geranium 'Beach' and 'Butterfly' throughout the experiment measured at biweekly intervals

Temperature	Day of start	2 weeks	4 weeks	6 weeks	8 weeks
Beach					
16°C	46.2 a[x]	54.6 a	61.3 a	58.2 a	54.8 a
22°C	42.4 a	52.9 a	55.2 a	56.6 a	54.6 a
Butterfly					
16°C	28.5 a	26.7 a	26.0 a	32.5 a	39.2 a
22°C	25.7 a	25.5 a	28.2 a	30.4 a	36.3 a

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).

Table 7.6. Effect of Fe-chelate on SPAD chlorophyll index of ivy geranium 'Beach' and 'Butterfly' throughout the experiment measured at biweekly intervals

Fe-treatment	Day of start	2 week	4 week	6 week	8 week
Beach					
0 mg	45.9 a[x]	51.9 a	59.0 a	54.5 b	53.9 a
54 mg	42.7 a	56.0 a	57.5 a	60.3 a	55.5 a
Butterfly					
0 mg	28.1 a	24.6 a	25.8 a	29.8 a	37.3 a
54 mg	26.1 a	27.6 a	28.4 a	33.1 a	38.2 a

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).

Table 7.7. Effect of night temperature on leachate pH, EC, Fe, Mn, Zn, Mg and S content in the media of ivy geranium 'Beach' and 'Butterfly' before starting the experiment

Temperature	pH	EC	Fe	Mn	Zn	Mg	S
Beach							
16°C	5.95 a[x]	5.91a	0.86 a	0.03 a	0.49 a	349.54 a	27.47 a
22°C	5.95 a	5.29 a	1.24 a	0.03 a	0.47 a	272.82 a	18.38 a
Butterfly							
16°C	6.14 a	4.35 a	0.43 a	0.01 a	0.28 a	270.05 a	23.15 a
22°C	6.18 a	4.95 a	0.45 a	0.03 a	0.26 a	291.59 a	25.99 a

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).

Table 7.8. Effect of night temperature on leachate pH, EC, Fe, Mn, Zn, Mg and S content in the media of ivy geranium 'Beach' and 'Butterfly' after 2 weeks

Temperature	pH	EC	Fe	Mn	Zn	Mg	S
Beach							
16°C	5.35 a[x]	5.07 a	*z	0.08 a	0.29	182.75 a	4.96 a
22°C	5.25 a	5.26 a	*z	0.13 a	0.34 a	249.76 a	7.14 a
Butterfly							
16°C	5.59 a	4.11 a	*z	0.01 a	0.24 a	151.90 a	4.26 a
22°C	5.68 a	4.46 a	*z	0.008 a	0.19 a	175.33 a	4.56 a

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).
*z indicates interaction effects of temperature and Fe-chelate application (Table 7.8).

Table 7.9. Interaction of temperature and iron chelate on leachate Fe concentration in ivy geranium 'Beach' and 'Butterfly' after 2 weeks

Interactions		Beach	Butterfly
16°C	0mg Fe	1.21 c[x]	2.38 c
16°C	27mg Fe	18.83 b	12.48 b
22°C	0mg Fe	1.16 c	4.08 c
22°C	27mg Fe	23.38 a	25.13 a

[x]Means followed by same letters within column are not different according to Fisher's protected LSD test (*P*=0.05).

Table 7.10. Effect of night temperature on leachate pH, EC, Fe, Mn, Zn, Mg and S content in the media of ivy geranium 'Beach' and 'Butterfly' after 4 weeks

Temperature	pH	EC	Fe	Mn	Zn	Mg	S
Beach							
16°C	5.16 a[x]	4.59 a	10.8 a	*z	0.22 a	158.95 a	1.78 a
22°C	5.07 a	5.47 a	13.1 a	*z	0.27 a	200.84 a	2.38 a
Butterfly							
16°C	5.33 a	3.88 a	8.34 a	0.05 a	0.15 a	141.04 a	2.53 a
22°C	5.42 a	3.86 a	7.84 a	0.02 a	0.19 a	141.79 a	1.78 a

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).
*z indicates interaction effects of temperature and Fe-chelate application.

Table 7.11. Interaction of temperature and iron chelate on leachate Mn concentration in ivy geranium 'Beach'

Interactions		Leachate Fe
16°C	0mg Fe	0.17 ab[x]
16°C	27mg Fe	0.08 b
22°C	0mg Fe	0.18 ab
22°C	27mg Fe	0.22 a

[x]Means followed by same letters within column are not different according to Fisher's protected LSD test (*P*=0.05).

Table 7.12. Effect of night temperature on leachate pH, EC, Fe, Mn, Zn, Mg and S content in the media of ivy geranium 'Beach' and 'Butterfly' after 8 weeks

Temperature	pH	EC	Fe	Mn	Zn	Mg	S
Beach							
16°C	5.58 a[x]	2.56 a	14.51 a	N/A	0.17 a	83.84 a	N/A
22°C	5.33 b	2.78 a	15.22 a	N/A	0.17 a	96.46 a	N/A
Butterfly							
16°C	5.57 a	2.62 a	8.75 a	N/A	0.19 a	80.66 a	N/A
22°C	5.53 a	2.68 a	7.29 a	N/A	0.21 a	82.55 a	N/A

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).
N/A= Data not available

Table 7.13. Effect of Fe-chelate application on leachate pH, EC, Fe, Mn, Zn, Mg and S content of ivy geranium 'Beach' and 'Butterfly' before starting the experiment

Fe-treatment	pH	EC	Fe	Mn	Zn	Mg	S
Beach							
0 mg	5.94 a[x]	5.64 a	1.21 a	0.04 a	0.53 a	319.19 a	22.76 a
54 mg	5.96 a	5.55 a	0.89 a	0.02 a	0.43 a	303.17 a	23.09 a
Butterfly							
0 mg	6.17 a	4.50 a	0.44 a	0.02 a	0.32 a	266.25 a	23.71 a
54 mg	6.16 a	4.81 a	0.43 a	0.01 a	0.22 a	295.39 a	25.43 a

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).

Table 7.14. Effect of Fe-chelate application on leachate pH, EC, Mn, Zn, Mg and S content in the media of ivy geranium 'Beach' and 'Butterfly' after 2 weeks

Fe-treatment	pH	EC	Fe	Mn	Zn	Mg	S
Beach							
0 mg	5.28 a[x]	5.17 a	*z	0.09 a	0.31 a	236.66 a	6.72 a
54 mg	5.32 a	5.15 a	*z	0.12 a	0.32 a	195.85 a	5.38 a
Butterfly							
0 mg	5.61 a	4.17 a	*z	0.00 b	0.19 a	164.86 a	4.43 a
54 mg	5.65 a	4.41 a	*z	0.02 a	0.25 a	162.37 a	4.38 a

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).
*z indicates interaction effects of temperature and Fe-chelate application (Table 7.8).

Table 7.15. Effect of Fe-chelate application on leachate pH, EC, Fe, Mn, Zn, Mg and S content in the media of ivy geranium 'Beach' and 'Butterfly' after 4 weeks

Fe-treatment	pH	EC	Fe	Mn	Zn	Mg	S
Beach							
0 mg	5.08 a[x]	5.27 a	5.73 b	*z	0.24 a	196.45 a	2.59 a
54 mg	5.14 a	4.38 a	18.20 a	*z	0.25 a	163.35 a	1.57 a
Butterfly							
0 mg	5.33 b	3.87 a	2.12 b	0.02 a	0.17 a	149.60 a	2.32 a
54 mg	5.42 a	3.87 a	14.06 a	0.04 a	0.17 a	133.23 a	1.99 a

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).
*z indicates interaction effects of temperature and Fe-chelate application (Table 7.10)

Table 7.16. Effect of Fe-chelate application on leachate pH, EC, Fe, Mn, Zn, Mg and S content in the media of ivy geranium 'Beach' and 'Butterfly' after 8 weeks

Fe-application	pH	EC	Fe	Mn	Zn	Mg	S
Beach							
0 mg	5.30 b[x]	2.67 a	4.56 b	N/A	0.19 a	101.91 a	N/A
54 mg	5.60 a	2.67 a	25.17 a	N/A	0.15 a	78.39 a	N/A
Butterfly							
0 mg	5.55 a	2.47 a	1.38 b	N/A	0.21 a	82.35 a	N/A
54 mg	5.54 a	2.83 a	14.67 a	N/A	0.19 a	80.86 a	N/A

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).
N/A= Data not available.

Table 7.17. Effect of night temperature and Fe-chelate application on extent of whitening (on scale of 1 to 7, 1= 0%, 2= 17%, 3= 34%, 4=50%, 5= 67%, 6= 84% and 7= 100% whitening) in ivy geranium 'Beach' and 'Butterfly' before starting and at the end of the experiment

	Beach			Butterfly		
	Initial	Final	Recovery	Initial	Final	Recovery
Temperature						
16°C	1.50 a[x]	1.00 a	0.50 a	3.92 a	2.21 a	1.77 a
22°C	1.95 a	1.00 a	0.96 a	3.54 a	2.46 a	1.08 a
Fe-application						
0 mg	1.63 a	1.00 a	0.63 a	3.75 a	2.38 a	1.44 a
54 mg	1.83 a	1.00 a	0.83 a	3.71 a	2.29 a	1.42 a

[x]Means followed by same letters within column and within treatment are not different according to Fisher's protected LSD test (P=0.05).

Table 7.18. Effect of night temperature on Chl a, Chl b, Caro, total Chl (μg·cm^{-2}), their ratios and total phenolics in ivy geranium 'Beach' and 'Butterfly' at the end of the experiment

Temperature	Chl a	Chl b	Caro	Total Chl	Chl a:b	Chl a:Caro	Chl b:Caro	Total Chl:Caro	Phenolics
Beach									
16°C	24.86 a[x]	15.83 a	2.63 a	40.69 a	1.60 a	9.47 a	5.99 a	15.46 a	85.13 a
22°C	24.66 a	15.36 a	2.72 a	40.02 a	1.58 a	9.06 a	5.64 a	14.71 a	79.60 a
Butterfly									
16°C	22.61 a	13.43 a	2.46 a	36.04 a	1.69 a	9.13 a	4.99 a	12.95 a	76.66 a
22°C	22.21 a	11.75 a	2.13 a	31.96 a	1.74 a	9.49 a	5.48 a	14.98 a	68.06 a

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05).

Table 7.19. Effect of Fe-chelate applications on Chl a, Chl b, Caro, total Chl (μg·cm^{-2}), their ratios and total phenolics in ivy geranium 'Beach' and 'Butterfly'

Fe-application	Chl a	Chl b	Caro	Total Chl	Chl a:b	Chl a:Caro	Chl b:Caro	Total Chl:Caro	Phenolics
Beach									
0 mg	23.87 a[x]	14.72 a	2.58 a	38.60 a	1.63 a	9.29 a	5.70 a	15.18 a	85.38 a
54 mg	25.64 a	16.47 a	2.77 a	42.11 a	1.56 b	9.25 a	5.93 a	14.99 a	79.35 a
Butterfly									
0 mg	21.51 a	12.48 a	2.29 a	34.00 a	1.74 a	9.43 a	5.40 a	14.83 a	72.50 a
54 mg	21.29 a	12.69 a	2.29 a	33.99 a	1.69 a	9.18 a	5.07 a	13.09 a	72.20 a

[x]Means followed by same letters within column and within cultivar are not different according to Fisher's protected LSD test (*P*=0.05)

Table 7.20. Effect of night temperature and Fe-chelate application on ivy geranium 'Beach' and 'Butterfly' foliar nutrient content at the end of the experiment

	Beach					Butterfly				
	Fe (ppm)	Mn (ppm)	Zn (ppm)	Mg (%)	S (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Mg (%)	S (%)
Temperature										
16°C	577.34 a[x]	187.30 a	44.75 a	0.56 a	0.12 a	181.29 a	68.86 a	35.42 a	0.43 a	0.19 a
22°C	408.05 a	163.36 a	35.58 a	0.47 a	0.11 a	227.07 a	67.09 a	31.55 a	0.42 a	0.16 a
Fe-application										
0 mg	481.04 a	168.18 a	39.98 a	0.53 a	0.11 a	160.20 a	69.88 a	33.22 a	0.44 a	0.18 a
54 mg	504.35 a	182.48 a	40.35 a	0.50 a	0.12 a	248.17 a	66.06 a	33.75 a	0.41 a	0.17 a

[x]Means followed by same letters within column and within treatment are not different according to Fisher's protected LSD test (P=0.05).

Table 7.21. Effect of night temperature and Fe-chelate on foliar tissue Fe:Mn, Fe:Zn and Mn:Zn ratios of ivy geranium 'Beach' and 'Butterfly'

	Beach			Butterfly		
	Fe:Mn	Fe:Zn	Mn:Zn	Fe:Mn	Fe:Zn	Mn:Zn
Temperature						
16°C	3.03 a[x]	12.89 a	4.16 a	2.60 a	4.98 a	1.94 a
22°C	2.33 a	11.00 a	4.57 a	3.41 a	7.32 a	2.16 a
Fe-application						
0 mg	2.68 a	11.69 a	4.21 a	2.24 b	4.78 a	2.12 a
54 mg	2.68 a	12.20 a	4.52 a	3.77 a	7.52 a	1.99 a

[x]Means followed by same letters within column and within treatment are not different according to Fisher's protected LSD test (P=0.05).

CHAPTER VIII

CONCLUSION

Elevated root-zone temperature is not the cause of whitening of ivy geraniums. Ivy geraniums grew well under elevated root-zone temperatures without developing whitening or a reduction in growth. High levels of Fe-chelate drenches caused toxicity symptoms and suppressed plant biomass and water content of ivy geraniums.

Elevated air temperature causes whitening of ivy geranium. Elevated air temperature severely reduced the growth, leaf area, fresh (shoot, leaf and total) weight and dry (shoot, leaf and total) weight of ivy geraniums. 'Beach' increased its size less due to heat stress whereas 'Butterfly' did not. Elevated air temperature reduced photosynthetic pigments and their ratios. Reduction in carotenoids concentration in 'Butterfly' but not in 'Beach' indicated the possible role of carotenoids in reducing susceptibility of ivy geraniusm to whitening. Elevated temperatures reduced total pheophytins concentration and may be an indication of a heat-induced functional disconnection in electron transfer resulting in thermal-inhibition.

Higher levels of total Fe indicated no inhibition of Fe-uptake in 'Beach' at elevated temperature, whereas similar levels of Fe at both temperatures in 'Butterfly' suggest a possibility of inactivation or immobility of Fe within plants at elevated air temperature. An accumulation of foliar Mn and Zn in 'Beach' and foliar S in 'Butterfly'

at elevated temperature suggested the greater demand of these nutrients in heat stressed plants. Fe-chelate application did not reduce growth in either cultivar. Even though Fe-application did not help to reduce whitening in ivy geraniums, it helped to preserve chlorophyll in 'Butterfly'.

None of the given deficiency (–Micro, –Fe, –Zn, –Mn, –S or –Mg) treatments caused whitening in ivy geraniums after 10 weeks when plants were grown at 23/21^{o}C. Whitening in ivy geraniums is not a micronutrient deficiency. Deficiency treatments of all micronutrients and S reduced plant growth the most. 'Beach' had greater capacity to tolerate micronutrient, S and Mg deficiencies than 'Butterfly'.

Salicylic acid applications did not affect plant growth, leaf area, fresh (stem, leaf and total) weight, dry (stem, leaf and total) weight, fresh: dry weight ratio, LAR, SLA or foliar nutrient (Fe, Mn, Zn, Mg and S) contents. It did not help to reduce the extent of whitening in either cultivar. It did reduce chlorophyll content at elevated temperature in 'Butterfly' but not in 'Beach'

There were no differences in recovery of plants from whitening under low night temperature. 'Butterfly' started with greater whitening than 'Beach' and the plants recovered to about the same extent at both temperatures.'Butterfly' remained with greater whitening at the end of the study than 'Beach'. 'Beach' responded to low night temperature by reducing its growth index and fresh to dry weight ratio whereas 'Butterfly' did not. 'Beach' reduced its soil solution pH at 20^{o}C, whereas 'Butterfly' did not. These could be reasons that 'Beach' is more resistant to whitening. Fe-chelate application did not reduce plant growth. Fe-chelate applications were not beneficial in

‘Beach’ or ‘Butterfly’ recovery from whitening and appears to be only effective in preventing whitening.

Whitening in ivy geranium is a symptom of heat stress first expressed in young developing leaves. It is caused by elevated air temperature, followed by impaired photosynthetic pigments synthesis and/or degradation leading to whitening. No breakdown or reduction in carotenoids and pheophytins concentration help to reduce susceptibility of ivy geranium to whitening. Whitening is not due to inhibition of Fe-uptake at elevated temperature.