ABSTRACT

TAYLOR, MATTHEW DARON. Elucidation and Control of Sudden pH Decline of Geranium. (Under the direction of Dr. Paul V. Nelson.)

Sudden pH decline (SPD) describes the situation where crops growing at an appropriate pH, rapidly (within 1-2 weeks) cause the substrate pH to shift downward one to two units. The cause(s) of SPD by geranium is unknown. Studies have indicated that P deficiency will suppress NO_3^- uptake and cause acidification. If NO_3^- uptake is suppressed, the cation-anion uptake ratio may shift and if favored towards cations, net H^+ efflux will increase.

Geraniums were grown with or without P in hydroponic solutions containing either $^{15}NH_4^+$ or $^{15}NO_3^-$ for 24 hour periods to determine the impact of low P on NH_4^+ and NO_3^- uptake. The $^{15}NH_4^+$:$^{15}NO_3^-$ and the cation:anion influx ratio increased significantly when plants did not receive P, suggesting an increased acidification rate. Phosphorus stressed plants produced nearly two times the acidity per gram dry weight of plant than plants that received P, confirming increased acidification due to P deficiency.

Geraniums were grown in three experiments to test the effects of temperature on SPD. Experiment one tested the effect of four day/night temperatures (14/10, 18/14, 22/18, 26/22 °C) on substrate acidification. End of crop substrate pH declined from 6.8 to 4.6 as temperature increased. Tissue P of plants grown at the highest three temperatures was deficient. It was not possible to determine if the drop in substrate pH was a temperature effect or a combination of temperature and P. To resolve this, a second experiment tested a factorial combination of the three highest temperatures from the first experiment and five

pre-plant P rates (0, 0.065, 0.13, 0.26, or 0.52 $g \cdot L^{-1}$ substrate). Regardless of tissue P concentrations, which ranged from deficient to above adequate, substrate pH decreased with increasing temperature. The results indicate that temperature acted independent of tissue P and caused geraniums to acidify the substrate. In the third experiment the amount of acid produced by roots of plants grown at two day/night temperatures (22/18 and 26/22 °C) was quantified. Plants grown at high temperature produce 28% more acid produced per gram root. It is clear from this study that high temperature can cause SPD by geranium.

Geraniums were grown in a second set of three experiments to test the effects of light on SPD and P uptake. The first experiment tested the effect of four light intensities (105, 210, 575, and 1020 ± 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$) on substrate acidification. End of crop substrate pH declined from 6.0 to 4.8 as light intensity increased. Tissue P of plants grown at the highest two light levels were deficient. Since plants in the two lowest light treatments had adequate P, it was not possible to determine if the drop in substrate pH was a light effect or a combination of light and P. The second experiment tested a factorial combination of the three highest light levels from the first experiment and five pre-plant P rates (0, 0.065, 0.13, 0.26, or 0.52 $g \cdot L^{-1}$ substrate). When tissue P concentrations were deficient, pH decreased by 0.6 to 1 pH units within two weeks and P deficiency occurred more often with the highest light intensity. These data indicate that P deficiency caused substrate acidification and P uptake was suppressed by high light intensity. In a third experiment, cumulative P uptake per gram root and the rate of P uptake per gram root per day both decreased 20% when light intensity increased from 500 to 1100 $\mu mol \cdot m^{-2} \cdot s^{-1}$. This third study confirmed that high light suppresses P uptake, which can consequently cause SPD.

Elucidation and Control of Sudden pH Decline of Geranium

by
Matthew Daron Taylor

A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

Horticultural Science

Raleigh, North Carolina

2007

APPROVED BY:

_____
Dr. Paul V. Nelson
Chair of Advisory Committee

_____
Dr. Dean L. Hesterberg
Soil Science Minor Representative

_____
Dr. Thomas W. Rufty Jr.

_____
Dr. Brian E. Whipker

DEDICATION

To my wife Elizabeth “Lizbe”

Your undying support and encouragement made this work possible.

May our lives be rich in happiness and wondrous memories.

I Love You

BIOGRAPHY

I was born just outside State College, PA in a small town called Bellefonte. Growing up, I spent a lot of time outdoors enjoying nature and plants and was very active in the Boy Scouts of America. I continued my enjoyment of plants by obtaining a Bachelor of Science degree in Horticulture with a minor in Plant Pathology from The Pennsylvania State University. I was the first person in the history of Penn State to attain this minor. My undergraduate research included two individual projects. The first was with Dr. Eva Pell, and involved the impact of ozone pollution on specific growth characteristics of *Spartina alterniflora*. This led to my first journal publication. The second project addressed the role of ethylene in root hair production during low phosphorus stress with Dr. Jonathan Lynch and Dr. Kathleen Brown. These projects kindled my interest in research and prepared me for graduate school.

After my baccalaureate program at Penn State, I attained a position as a grower at Dan Schantz Farm and Greenhouses in Zionsville, PA. I managed six bays at their 20-plus acre greenhouse range. This was followed by a position at a College Gardens, a retail nursery in State College, PA. I then moved to Florida and obtained a Master of Science degree in Horticultural Science with a minor in Soil and Water Science from The University of Florida. My advisor was Dr. Salvador Locascio and my focus was on vegetable nutrition and blossom-end rot of tomato. Following my M.S. degree, was a six month international internship at a ten acre tomato production greenhouse in Honselerdijk, The Netherlands. The

internship primarily involved working with cherry and cluster tomato production, but also involved experience with greenhouse production techniques for cucumbers, African violets, cut mums, and potted plants. Upon returning to the United States, I obtained a job as the head grower for Woodring's Greenhouses in Bellefonte, PA. This was wonderful position that I thoroughly enjoyed. Of all my experiences in horticulture, I view this as one of the most significant due to the degree of difficulty and the amount of knowledge gained.

During my time as head grower, I once again yearned to be involved in horticultural research and was accepted into a Doctor of Philosophy program with Dr. Paul.V. Nelson at North Carolina State University. This project centered on determining the cause of sudden pH decline of geranium. The works of this project are contained within this dissertation. As I write this, I will be getting married in two weeks to the beautiful, and soon to be, Elizabeth Taylor. My biography ends here, for now, but my soon to be wife and I are excited to see what the future holds…..

ACKNOWLEDGMENTS

I would like to thank my parents, Dennis and Helen Taylor, who I love very much. Their character, kindness, and endless support throughout my life, have made me the person I am today. I could have not gotten this far without them.

Dr. Paul Nelson, where do I start, I could not have asked for more out of an advisor than what I received from him. He is a premier researcher with a brilliant mind. In addition to being an exceptional scientist, he is a wonderful person with a splendid personality. I will cherish the hours spend in his office discussing topics ranging from beer drinking to religion with some horticultural research in the mix. His selfless devotion to my graduate and subsequent career is greatly appreciated. Because of him I am a better researcher, writer, speaker, and person. He is one of my role models and will be for the rest of my life.

I greatly appreciate my research partnership with Dr. Jonathan Frantz, USDA-ARS Research Horticulturist. I thank him for funding and assisting with designing, interpreting, and sample analysis for each experiment carried out during my graduate program. I am also thankful for the opportunity to visit the research facilities and his home in Toledo, OH. The efforts of his research assistants Douglas Sturtz and Elisa Ruszkiewicz also helped facilitate this project.

Thanks to Dr. Carole Saravitz, Dr. Janet Shurtleff, Dr. Judy Thomas, and the students and staff at the North Carolina State University Phytotron for all the time and assistance contributed to setting up and execution of experiments. Much appreciation is given to Nancy Mingus for assisting with and teaching me many valuable lab techniques and safety procedures. I am grateful to Dr. Tom Rufty, for his assistance designing and interpreting the experiment measuring ammonium and nitrate uptake. I would also like to thank Mike Jennette, Dr. Shuijin Hu, and Cong Tu for assistance with ^{15}N analysis and I am much obliged for assistance in the Marye Anne Fox greenhouses from Mark Hardy, Diane Mays, William Reece, and Wesley Turner.

I appreciate the efforts of Rachel McLaughlin for helping me whenever I had a question about anything and for informing me when important deadlines were approaching. Thanks to Christie Gordon and the rest of the bookkeeping staff for helping and teaching me how to retrieve funds from the often delinquent university.

I would like to thank all the past and present graduate students who have helped me with many different matters, reassured me after difficult exams, and were very understanding of issues associated with being a graduate student.

I would like to thank committee members Dr. Dean Hesterberg, Dr. Tom Rufty, and Dr. Brian Whipker for review of my dissertation and their guidance throughout my program.

Finally, I would like to thank my soon to be wife Elizabeth. She is my inspiration and also makes an exceptional lab assistant.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

Effect of Phosphorus Deficiency and High Temperature on Ammonium and Nitrate Uptake and Acidification by *Pelargonium*.

(In the format appropriate for submission to Plant and Soil)

Matthew D. Taylor

Department of Horticulture Science, North Carolina State University, Raleigh, NC, USA 27695

Paul V. Nelson

Department of Horticulture Science, North Carolina State University, Raleigh, NC, USA 27695 Phone: 919-515-1191 email: paul_nelson@ncsu.edu

Thomas W. Rufty

Department of Crop Science, North Carolina State University, Raleigh, NC, USA 27695

Jonathan M. Frantz

USDA-ARS-ATRV, 2801 W. Bancroft, MS 604, Toledo, OH, USA 43606

Effect of Phosphorus Deficiency and High Temperature on Ammonium and Nitrate Uptake and Acidification by *Pelargonium*.

Key words: acidification, cation-anion balance, pH

Abstract

A sudden pH decline (SPD) of the substrate is a growing problem in geranium growth systems, and the cause is unknown. In this study, we investigate whether a P deficiency can cause SPD, and whether the effect is related to a shift in cation-anion balance. Geraniums (*Pelargonium x hortorum* Bailey 'Designer Dark Red') were grown in hydroponic solutions with or without P, and the hydroponics systems were located in a growth chamber programmed for light/dark temperatures of 22/18 or 26/22 °C. Acidification potential was measured by the amount of base required to maintain nutrient solution pH at 5.8. The results indicated nearly two times the amount of base were required to maintain a stable pH with P-limited plants. Using periodic exposures to $^{15}NH_4^+$ or $^{15}NO_3^-$, it was found that NO_3^- uptake was strongly inhibited as plants became P stressed. Tissue nutrient profiles showed that NO_3^- uptake inhibition was accompanied by an increase in the cation to anion uptake ratio. These results indicate that changes in cation and anion uptake, and the associated increase in net H+ extrusion, which occur under P-stress conditions can contribute to SPD in geranium culture systems. Rhizosphere acidification was also greater at higher temperature even though the cation and anion responses were unchanged in control plants. These results suggest the involvement of CO_2 generated by root respiration and the possible contribution of high temperature to SPD of geranium.

Introduction

One of the largest cultural problems facing ornamental plant growers is control of root substrate pH. During crop production, pH rises and falls with irrigation water, use of acidic fertilizers, and/or from insufficient limestone in the substrate. The shifts in pH are usually gradual enough to be detected in time and successfully adjusted. However, there are exceptions.

Geraniums are the highest valued plant of the 2.5 billion dollar bedding plant industry (USDA 2005). During the 1980's, many geranium producers began reporting a sporadic and unexplained decline in substrate pH. During the same time period, they began reporting frequent occurrence of high concentrations of Fe and/or Mn in leaf tissue and the appearance of toxicity symptoms (Bachman and Miller 1995). Logically, one would think the two effects were linked to one another. In organic based soilless substrate such as that often used with geranium, pH has a large affect on nutrient availability (Nelson 2003); as pH decreases, some micronutrients become more available and can become toxic. In a study with 'Ringo Scarlet" geraniums, for example, substrate pH decreases from the 6.5-6.8 range to 5.2-5.5 was accompanied by ~ 10 fold increases in tissue Fe, Mn, and Zn concentrations (Lee et al. 1996). Also, in a study with seedling geraniums, decreases in pH to < 5.5 led to the appearance of micronutrient toxicity symptoms (Bierman et al. 1987). The observations indicating a 'sudden pH decline' (SPD) in geranium systems and the associated micronutrient

toxicities have led to substantial economic loses and, in some cases, implementation of tedious pH adjustment techniques (e.g. application of flowable limestone). Up to this time, the cause(s) of SPD in geranium culture has remained unknown.

It seems likely that geranium plants contribute to the observed rhizosphere acidification, because SPD is not limited to a particular cultural condition. The observations with micronutrients can be confusing, as some nutrient deficiencies can cause decreases in pH. Iron deficiency, for example, caused pea, sugar beet, and bean to lower the pH from 7.0 to 4.0 in 6 to 10 hours (Landsberg 1981). The drop occurred in a NO_3^- nitrogen solution that normally causes substrate pH to rise. Zinc deficiency also led to substrate acidification in dicotyledonous species even when NO_3^- was the sole source of N (Cakmak and Marschner 1990). Nonetheless, it would seem unlikely that geranium SPD is caused by Fe and Zn deficiencies. Both are applied in adequate quantities by growers as standard procedures and, as noted above, SPD seems related with micronutrient toxicity, not deficiency.

Another possible cause of geranium SPD is P deficiency (Hinsinger 2001). Growers often rely on alkaline fertilizers to anticipate and offset pH declines, and P is low or absent in the formulations (e.g. N-P_2O_5-K_2O of 13-2-13, 15-0-15, 14-0-14). Studies with chickpea (Le Bot et al. 1990), barley (Rufty et al. 1991) and soybean (Rufty et al. 1993) have shown that P deficiency results in suppression of NO_3^- uptake. Because NO_3^- is the dominant anion under most nutritional conditions (Rufty 1982; Mengel and Kirkby 2001), a decrease in nitrate

uptake might be expected to increase the cation to anion uptake ratio and cause acidification of the substrate as plants maintain electrochemical neutrality. Consistent with this notion, nutrient media became more acidic in the experiments with chickpea (Le Bot et al. 1990). It is less clear whether P-stress affects NH_4^+ uptake, but if NH_4^+ uptake were depressed less than uptake of NO_3^- (Schjorring 1986), a cation to anion uptake shift and acidification still could occur. Indeed, acidification was measured with P stressed rice when NO_3^- and NH_4^+ were both present as nitrogen sources (Kirk and Van Du 1997). This relationship is particularly relevant with geranium, as fertilizers commonly contain both forms of inorganic nitrogen.

In the present study, we investigate geranium response to P stress and its possible contribution to SPD. Several questions are addressed. 1) Does rhizosphere pH decline when geranium plants progress into P stress? 2) Is the pH decline associated with adjustments in NO_3^- and NH_4^+ uptake? And, 3) Is there an adjustment in the cation to anion uptake ratio that might contribute to the downward pH change? The experiments additionally included examination of acidification and nutrient uptake responses at a higher root temperature. Temperature fluctuations often occur in greenhouse operations, and increasing temperatures can elevate plant metabolism and have consequences for root function. Indeed, recent experiments indicated that high temperature stimulated acidification in the geranium system (Taylor et al. 2007).

Materials and Methods

Unrooted geranium (*Pelargonium x hortorum* Bailey 'Designer Dark Red') cuttings were received on 4 dates (9/13/05, 10/12/05, 11/3/05, and 1/5/06), dipped for 30 s in 10% ZeroTol, a hydrogen peroxide based disinfectant (BioSafe Systems LLC, Glastonbury, CT), and rooted for 15-16 d in 1 mM $CaSO_4$. In each run, 160 uniformly rooted plants were distributed equally among four 200 L continuous flow hydroponic units located in a growth chamber with 9 m^2 of growing area and 2.13 m vertical clearance at The North Carolina State University Phytotron. The light period was a 9/15 h day/night period and cool white fluorescent and incandescent lamps separated from the growing area by a polycarbonate barrier supplied a PPF of 575 ± 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$. A 3 h low-light night interruption during the middle of the dark period was provided by incandescent lamps which supplied a PPFD of 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$. CO_2 concentrations were maintained between 300-400 ppm by controlled injection of commercial grade gas. The growth chambers were programmed for one of two day/night temperature regimes; 22/18 or 26/22 °C.

The control solutions consisted of 1.5 mM NH_4NO_3, 5 mM $Ca(NO_3)_2$, 2 mM KNO_3, 1 mM KH_2PO_4, 1.5 mM KCl, 2 mM $MgSO_4$, 4 ppm Fe as Fe-DTPA (10% Fe), 9.1 µM $MnSO_4 \cdot H_2O$, 0.76 µM $ZnSO_4 \cdot 7H_2O$, 46.3 µM H_3BO_3, 1.57 µM $CuSO_4 \cdot 5H_2O$, and 0.10 µM $Na_2MoO_4 \cdot 2H_2O$. The P stress treatments were established by excluding KH_2PO_4 from the

nutrient solution and increasing KCl to 2.5 mM. Solution pH of the tanks was kept at 5.8 by electronic monitoring and automatic additions of 5 mM $Ca(OH)_2$.

At specific dates during the experiment (see results), six plants were removed from each hydroponic tank at about 12:00 pm and placed into aerated 500 ml beakers located within the same growth chamber. Each beaker contained solutions identical to those from which plants were removed, except that the beakers contained solutions with the stable isotope ^{15}N. Half of the beaker solutions contained 15 A% $^{15}NH_4^+$ and the other half 5 A% $^{15}NO_3^-$. After 24 hours of exposure, plants were separated into roots and shoots. Roots were dipped in 1mM $CaSO_4$ for 30 s to remove ions from the apoplast (Neagle et al. 2005), and shoots were dipped in 0.1 N HCl to remove any external nutrients, and rinsed with deionized water. The roots and shoots were then dried in a forced-air oven at 60 °C for 48 h. All leaves with petioles were removed from stems for shoot analysis. Ground tissues were analyzed for N and ^{15}N using elemental N analysis and ratio mass spectroscopy, and P, K, Ca, Mg, S, Fe, Cu, Mn, B, and Zn content was determined with inductively coupled plasma optical emission spectroscopy (IRIS-Intrepid II, Thermo Fischer Sci., Waltham, MA). All tissue concentrations are expressed on a dry weight basis.

Statistically, the experiment was a split-split-plot design with temperature treatments as the whole plot factor, plus and minus P treatments as the sub-plot factor, and 3, 11, and 19 days after transplanting (DAT) as the sub-sub-plot factor (SAS Institute 2002). Error bars in

figures were determined using the standard error function of Sigmaplot (SPSS Inc., Chicago, IL). A single growth chamber was used for a total of 4 runs, and temperature was randomly assigned. Each run consisted of 2 replications of plus and minus P treatments at control or high temperature, giving a total of 4 replications.

Results

The experimental approach involved keeping the pH stable, to minimize the potential for confounding interactions with micronutrient toxicity, and monitoring the amount of base used assessed the acidification potential of the geranium system. The amounts of base used to maintain a pH of 5.8 were different in the + and –P treatments at both temperatures (Table 1). At 22/18 °C, plants grown in solutions devoid of P required 2.4 times the amount of titrating base than plants with sufficient P and there was a similar, but less dramatic effect at 26/22 °C.

With all plant parameters measured, it was not possible to separate temperature effects statistically. Therefore, data from the temperature treatments were combined. After 3 days without an external P supply, plants had noticeably lower dry weights and root to shoot ratios were increased (Fig. 1). An increase in root to shoot ratio is the typical, well documented response to a nutritional stress. The growth response in the –P treatment was

accompanied by a lowered P concentration in the shoots and roots (Fig. 2), as well as lowered concentrations of N in shoots and, after the day 3 harvest, in roots as well (Fig. 3).

Ammonium and nitrate uptake

Uptake of $^{15}NH_4^+$ and $^{15}NO_3^-$ was calculated from addition of ^{15}N contents in shoots and roots after 24 h of exposure to the specific ^{15}N treatments. For the three sampling dates, the mean root uptake of $^{15}NH_4^+$ was lower in plants without P than in the controls, 1.19 mg compared to 1.64 mg ^{15}N per gram root dry weight (Table 2). Uptake of $^{15}NO_3^-$ also was decreased in P limited plants, to 3.31 from 5.15 mg ^{15}N per gram root dry weight. Thus, the suppression with –P was four times greater than $^{15}NH_4^+$, in absolute terms (0.45 and 1.84 mg ^{15}N), and the $^{15}NH_4^+$ to $^{15}NO_3^-$ uptake ratio increased from 0.32 to 0.40 (Table 2). In addition, there was some indication that distribution of ^{15}N label between the shoot and root over the 24 h uptake period was altered by P stress. With $^{15}NH_4^+$, ~ 24% of the absorbed label was in the shoot in the P-limited conditions compared to 31% with the +P controls over the three treatment dates. With $^{15}NO_3^-$, 31% of the ^{15}N was in the shoot compared to 33% with the control (data not shown).

Total cation and anion uptake

To estimate total cation to anion uptake ratio, nutrient contents were converted to meq. Of all the nutrients measured in plant tissues (N, P, K, Ca, Mg, S, Fe, Cu, Mn, B, and Zn), the macronutrients represented ~ 99.0 % of the total molar concentration; therefore only

the macronutrients were used to evaluate changes in ion balance and the cation to anion uptake ratio. As shown in Table 3, uptake of all the ions was lower with the P limited plants. The NH_4^+ to NO_3^- uptake ratios were used to estimate the proportion of total N entering in the cation or anion form. The tissue nutrient contents indicated that P-limited plants had a significantly greater cation to anion uptake ratio than the control plants that received P (Fig. 4).

Discussion

The results of these experiments clearly show that a P limitation, if it occurs, could contribute to the SPD observed with geraniums. As we had hypothesized from the results of earlier studies on P stress (Schjorring 1986; Le Bot et al. 1990; Rufty et al. 1991, 1993), plants growing under a P limitation generated much higher amounts of acidity than control plants with an adequate P supply. The results indicated that the increase in acidity generation was associated with a shift in the cation to anion uptake ratio, and the primary controlling factor was inhibition of NO_3^- uptake.

The conceptual basis for ion balance in plants and its relationship to transport processes in roots are solidly embedded in transport physiology literature (Hodges 1973; Smith 1973; Raven and Smith 1976; Kirkby 1981; Glass 1988; Glass 2003). Cation uptake is linked with H^+ efflux and anion uptake with H^+ influx (i.e. OH^- efflux). Thus, a decline in

anion uptake greater than that in cation uptake, like that occurring here with P-stressed geranium, results in increase net H^+ release from the nutrient absorbing root cells.

It should be noted that there were positive and negative aspects to the experimental design used in our experiments. On the positive side, the maintenance of a stable solution pH by continual monitoring and automatic additions of base allowed assessment of the acidification potential of geranium without the confounding interactions that would come from micronutrient toxicities. On the other hand, rhizosphere acidification itself can cause adjustments in ion uptake favoring anions (Rufty 1982). We cannot know the extent that this response would have offset the observed rates of rhizosphere acidification, if solution pH had been allowed to move downward.

One of the interesting observations in these experiments was the increased generation of rhizosphere acidity with higher temperatures. This acidification occurred even though plant growth and the cation and anion balance was not statistically different from that at the lower temperature, which implicates additional factors. The most logical explanation is that additional acidification was associated with higher root respiration and increased release of CO_2. The CO_2 would react with H_2O to form H_2CO_3 (carbonic acid), which would partially dissociate into H^+ and HCO_3^- (bicarbonate), and result in pH decline. This would also account for the increase in acidity generation at the high temperature with the +P control plants (Table 1). It should also be mentioned that phosphorus stress can lead to changes in

root metabolism (Rabe and Lovatt 1986). Increased secretion of organic acids from roots is a known occurrence with P stressed plants (Hinsinger 2001), evidently due to increased up-regulation of PEP carboxylase activity (Raghothama 1999). While secretion may serve to increase P availability near the root surface, the effect on root zone pH seems relatively small compared to the changes occurring in net H^+-efflux (Hinsinger 2001).

The inhibition of NO_3^- uptake appears to follow the response profile seen with P-stessed plants in other studies. Inhibition can be measured before changes in growth can be detected, and it coincides with excess root accumulation of NO_3^- and the amino acid products of its assimilation, as translocation into the xylem is restricted (Rufty et al. 1993). Thus, the NO_3^- uptake inhibition with low P appears to involve the same feedback system that links the NO_3^- uptake rate with plant demand for N (Clarkson 1986; Imsande and Touraine 1994; Glass 2003). In our study, the initial increase in root N concentration on day 3 (Fig. 3) and lower % translocation of absorbed ^{15}N, are both consistent with restricted translocation of soluble N molecules into the xylem. At later sample times, root accumulation of soluble N would have been masked by the inhibition of uptake and the general decline in total N. The xylem transport inhibition could only have been resolved with separation of labeled ^{15}N fractions, which was not the level of resolution being sought in these experiments.

The suppressive regulation of NO_3^- uptake would have coexisted with or been superimposed on inhibition of NO_3^- uptake by NH_4^+. Many studies have shown that the

presence of NH_4^+ in nutrient media leads to decreased NO_3^- uptake, again probably involving the feedback loops controlling the NO_3^- uptake system (Breteler and Siegerist 1984; Kronzucker et al., 1999; Aslam et al., 2001; Glass 2003). An NH_4^+ inhibition of NO_3^- uptake would explain the cation to anion ratio being just above 1.0 in +P control plants. Dicots most often have cation to anion ratios of just under 1.0 if NO_3^- is the sole inorganic N source in nutrient media (e.g. Kirkby and Knight 1977; Kirkby 1981; Rufty et al. 1982).

Some similarities exist in the feedback loops for NO_3^- and NH_4^+ uptake (Glass, 2003), which helps to explain why uptake of both inorganic N forms were restricted under a P limitation. Of course, the NH_4^+ concentration in solution and NH_4^+ uptake were much lower than those for NO_3^-, and the absolute decrease in NH_4^+ was less than for NO_3^-, which led to rhizosphere acidification. Although P stress had little impact on S uptake, other studies have suggested that a close regulatory inter-relationship or 'co-regulation' (Clarkson et al. 1989) may often exist among the nutrient anion transport systems (for nitrate and sulfate, see also Smith 1980; Karmoker et al., 1991; Koprivova et al. 2000).

Acknowledgements

The authors thank Carole Saravitz and the phytotron staff, Nancy Mingus, Elizabeth Kirksey, Mike Jennette, Douglas Sturtz, Elisa Ruszkiewicz, Will Healy, Kristan McGuigan and Ball Floraplant for facilitation of this experiment.

References

Aslam M, Travis R L and Rains D W 2001 Inhibition of net nitrate uptake by ammonium in Pima and Acala cotton roots. Crop science. 41, 1130-1136.

Bachman G R and W B Miller 1995 Iron chelate inducible iron/manganese toxicity in zonal geranium. J. Plant Nutr. 18, 1917-1929.

Bierman J A Shoemaker C A Carlson W H 1987 Iron and manganese toxicity of seedling geraniums. HortScience. 22, 118

Breteler H and Siegerist M 1984 Effect of ammonium on nitrate utilization of dwarf bean. Plant Physiol. 75, 1099-1103.

Cakmak I and Marschner H 1990 Decrease in nitrate uptake and increase in proton release in zinc deficient cotton, sunflower, and buckwheat plants. Plant and Soil. 129, 261-268.

Clarkson D T 1986 Regulation of the absorption and release of nitrate by plant cells: a review of current ideas and methodology. In Lambers H Neetson J J and Stulen I. eds. Fundamental, Ecological and Agricultural Aspects of Nitrogen Metabolism in Higher Plants. pp. 3-27. Martinus Nijhoff Publishers, Dordrecht.

Clarkson D T Saker L R and Purves J V 1989 Depression of nitrate and ammonium transport in barley plants with diminished sulphate status. Evidence of co-regulation of nitrogen and sulphate intake. Journal of Experimental Botany 40, 953-963.

Glass A D M 1988 Nitrogen uptake by plant roots. ISI Atlas of Sci. 1, 151-156.

Glass A D M 2003 Nitrogen use efficiency of crop plants: Physiological contraints upon nitrogen absorption. Critical Rev. in Plant Sci. 22, 453-470.

Hinsinger P 2001 Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. Plant and Soil. 237, 173-195.

Hodges T K 1973 Ion absorption by plant roots. Adv. Agron. 25, 163-207.

Imsande J and Touraine B 1994 N demand and the regulation of nitrate uptake. Plant Physiol. 105, 3-7.

Karmoker J L Clarkson D T Saker L R RooneyJ M Purves J V 1991 Sulphate deprivation depresses the transport of nitrogen to the xylem and the hydraulic conductivity of barley (Hordeum vulgare L.) roots. Planta. 185, 269-278.

Kirk G J D and Van Du L 1997 Changes in rice root architecture, porosity, and oxygen and proton release under phosphorus deficiency. New Phytologist. 135, 191-200.

Kirkby E A 1981 Plant growth in relation to nitrogen supply. In Clark, F.E. and Rosswall, T., eds., Terrestial Nitrogen Cycles. Ecol. Bull. 33, 249-267.

Kirkby E A and Knight A H 1977 Influence of the level of nitrate nutrition on ion uptake and assimilation, organic acid accumulation and cation-anion balance in whole tomato plants. Plant Physiol. 60, 249-253.

Koprivova A Suter M Camp R O Brunold C Kopriva S 2000 Regulation of sulfate assimilation by nitrogen in Arabidopsis. Plant Physiol. 122, 737-746.

Kronzucker H J Glass A D M and Siddiqi M Y 1999 Inhibition of nitrate uptake by ammonium in barley: Analysis of component fluxes. Plant Physiol. 120, 283–291.

Landsberg E 1981 Organic acid synthesis and release of hydrogen ions in response to Fe deficiency stress of mono- and dicotyledonous plant species. J. Plant Nutr. 3, 579-591.

Le Bot J Alloush G A Kirkby E A and Sanders F E 1990 Mineral nutrition of chichpea plants supplied with nitrate or ammonium. II. Ionic balance in relation to phosphorus stress. J. Plant Nutr. 13, 1591-1605.

Lee C W Choi J M and Pak C H 1996 Micronutrient toxicity in seed geranium (*Pelargonium x hortorum* Bailey). J. Amer. Soc. Hort. Sci. 121: 77-82.

Mengel K and Kirkby E A 2001 Principals of plant nutrition. Kluwer Academic Publishers. Dordrecht, The Netherlands.

Naegle E R Burton J W Carter T E and Rufty T W 2005 Influence of seed nitrogen content of seedling growth and recovery form nitrogen stress. Plant and Soil. 271, 329-340.

Nelson P V 2003 Greenhouse operation and management 6th edition. Prentice Hall, New Jersey.

Rabe E and Lovatt C J 1986 Increased arginine biosynthesis during phosphorus deficiency. Plant Physiol. 81, 774-781.

Raghothama K G 1999 Phosphate acquisition. Annu. Rev. plant. Physiol. Plant. Mol. Boil. 50, 665-693.

Raven J A and Smith F A 1976 Nitrogen assimilation and transport in vascular land plants in relation to intracellular pH regulation. New Phytol. 76, 415-431.

Rufty T W 1982 Nitrate uptake, root and shoot growth, and ion balance of soybean plants during acclimation to root-zone acidity. Bot. Gaz. 143, 5-14.

Rufty T W Jr MacKown C T Israel D W 1990 Phosphorus stress effects on the assimilation of Nitrate. Plant Physiol. 94, 328-333.

Rufty T W Jr Siddiqi M Y Glass A D M and Ruth T J 1991 Altered ^{13}Nitrogen Nitrate Influx In Phosphorus Limited Plants. Plant Sci. 76, 43-48.

Rufty T W Jr Israel D W Volk R J Qiu J and Sa T 1993 Phosphate regulation of nitrate assimilation in soybean. J. Exp. Bot. 44, 879-891.

Schjorring J K 1986 Nitrate and ammonium absorption by plants growing at a sufficient or insufficient level of phosphorus in nutrient solution. Plant and Soil. 91, 313-318.

Smith I K 1980 Regulation of sulphate assimilation in tobacco cells. Effect of nitrogen and sulphate nutrition on sulphate permease and o-acetylserine sulphydrylase. Plant Physiol.

66, 877-883.

Smith F A 1973 The internal control of nitrate uptake into barley roots with differing salt contents. New Phytol. 71, 769-782.

Taylor M D Nelson P V and Frantz J M 2007 Effect of temperature on extreme substrate acidification by geranium (*Pelargonium x hortorum* Bailey). HortScience. 42, 874.

United States Department of Agriculture (USDA) 2005 Floriculture Crop Summary. National Agriculture Statistics Service. Sp. Cir 6-1(05). Washington, D.C.

Table 1. Effect of phosphorus treatment at high (26/22° C day/night) and control (22/18) temperatures on meq of titrating base consumed per gram plant to maintain pH of 5.8 in 200 L hydroponic tanks for 20 d.

	Temperature Treatment (° C day/night)	
	Control (22/18)	High (26/22)
Phosphorus Treatment	meq titrating base · g^{-1} dry weight plant	
Plus P	0.77	1.38
Minus P	1.83	2.33
Significance	**	***

** and *** significant at P = 0.01 and 0.0001 respectively.

Table 2. Main effects of P treatment on whole plant ^{15}N content (divided by g root dry weight) that entered the roots as $^{15}NH_4^+$ and $^{15}NO_3^-$ over a 24 hour period, and NH_4^+ to NO_3^- uptake ratio.

	Whole Plant ^{15}N Uptake		NH_4^+ to NO_3^- Uptake Ratio
	$^{15}NH_4^+$	$^{15}NO_3^-$	
P Treatment	mg $^{15}N \cdot g^{-1}$ root dry weight		
Plus P	1.64	5.15	0.323
Minus P	1.19	3.31	0.394
Significance	*	**	*
Difference in ^{15}N uptake	0.45	1.84	

* and ** Significant at P = 0.05 and 0.01, respectively.

Table 3. Main effect of P treatment 3, 11, and 19 days after transplanting (DAT) on the meq of macronutrients cations and anions absorbed by roots. Data are estimated from nutrient accumulation in shoots. The amount of NH_4^+ or NO_3^- was determined by measurement of ^{15}N uptake on sample dates and applying that ratio to the total shoot N observed on each individual DAT.

	Element or total amount of ions								
	Ca	K	Mg	N-(NH_4^+)	Total cations	P	S	N-(NO_3^-)	Total anions
3 DAT	----------meq----------								
P treatment									
Plus P	51.6	63.1	24.1	51.4	190	15.4	15.1	167.3	198
Minus P	46.5	57.8	21.9	49.6	176	8.4	14.5	153.0	176
Significance	NS	*	NS	NS	NS	*	NS	*	*
11 DAT									
P treatment									
Plus P	71.3	87.7	28.8	69.9	258	22.2	19.8	190.2	232
Minus P	50.3	67.0	22.4	66.9	207	5.3	15.4	145.6	166
Significance	***	*	**	NS	**	**	*	*	**
19 DAT									
P treatment									
Plus P	78.3	95.3	26.6	60.3	261	21.7	18.0	205.5	245
Minus P	53.1	64.5	20.8	58.3	197	3.75	14.2	150.6	169
Significance	*	**	*	NS	**	***	**	*	**

NS, *, **, *** Nonsignificant or significant at P = 0.05, 0.01,and 0.001 respectively.

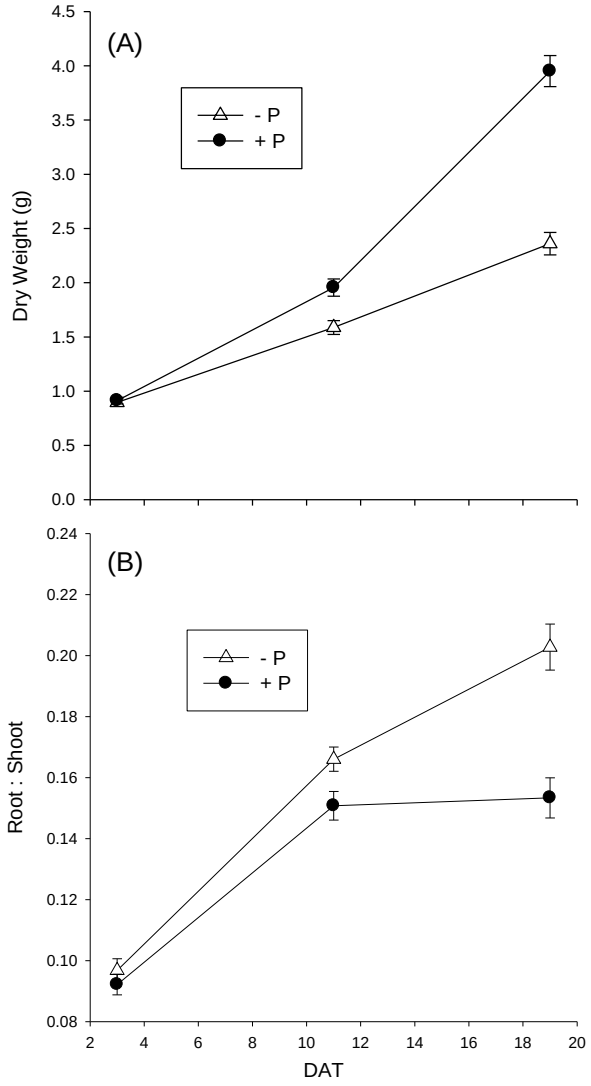


Figure 1. Total dry weight (A) and root to shoot dry weight ratio (B) of plants grown with or without P 3, 11, and 19 days after transplanting (DAT).

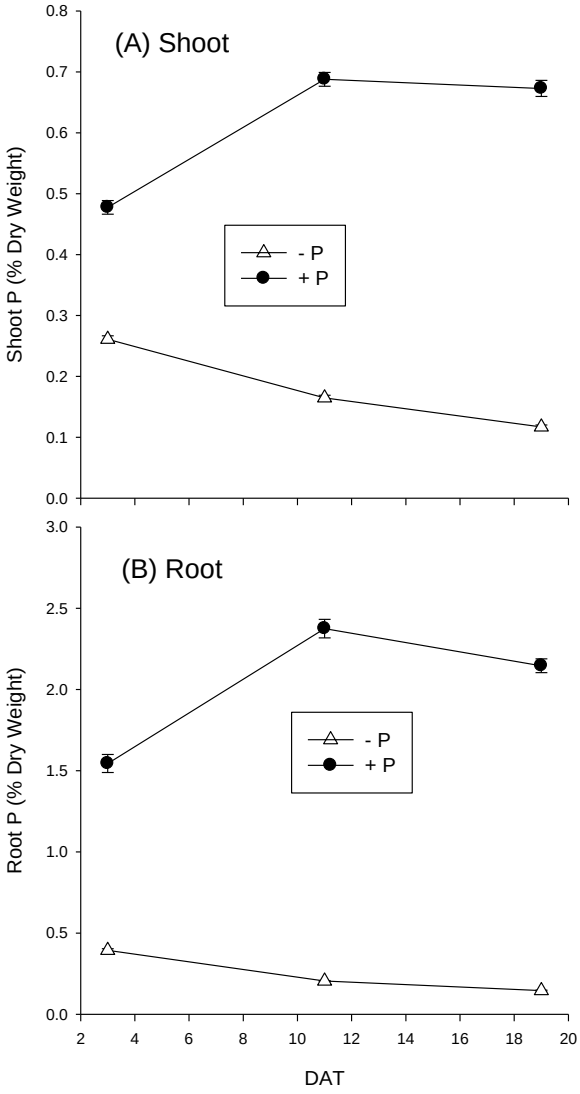


Figure 2. Shoot (A) and root (B) dry weight P percent of plants grown with or without P 3, 11, and 19 days after transplanting (DAT).

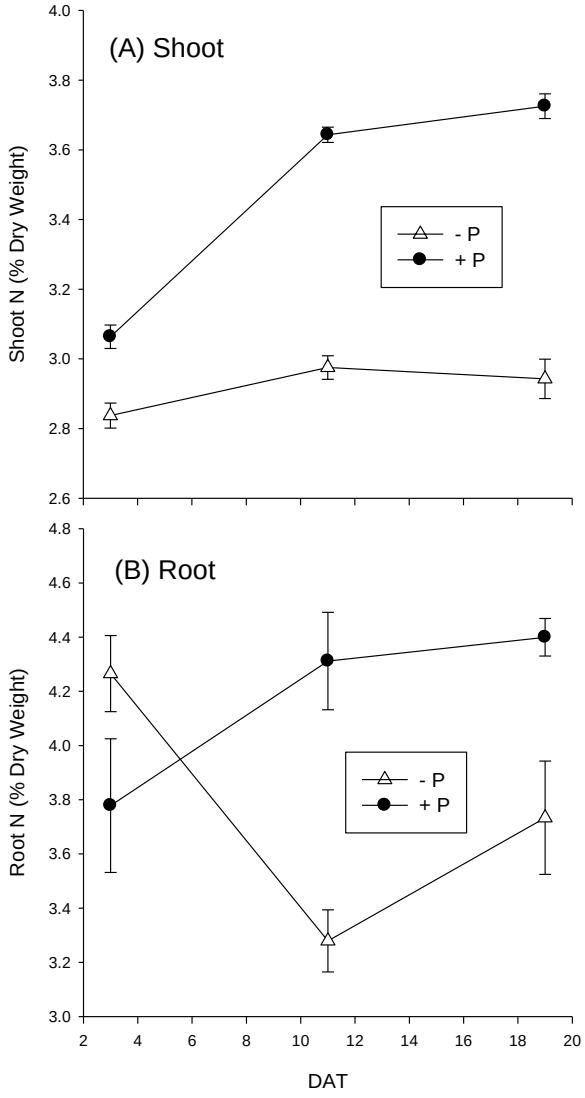


Figure 3. Shoot (A) and Root (B) dry weight N percent of plants grown with or without P 3, 11, and 19 days after transplanting (DAT).

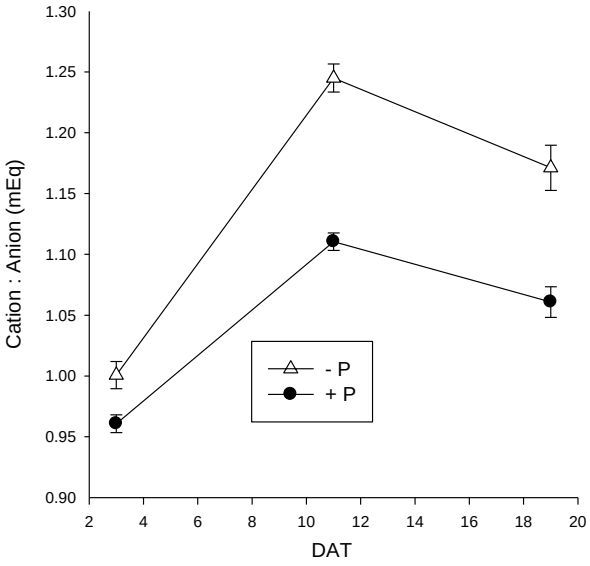


Figure 4. Uptake ratio of cations to anions in plants grown with or without P 3, 11, and 19 days after transplanting (DAT).

CHAPTER 2

High Temperature Induces Substrate Acidification by Geranium

(In the format appropriate for submission to the Journal of the American Society for Horticultural Science)

High Temperature Induces Substrate Acidification by Geranium

Matthew D. Taylor[1] and Paul V. Nelson[2]

Department of Horticultural Science, Campus Box 7609, North Carolina State University, Raleigh, North Carolina 27695-7609

Jonathan M. Frantz[3]

USDA-ARS Application Technology Research Unit, 2801 W. Bancroft, MS 604, Toledo, OH 43606

Received for publication_____ . Accepted for publication _____.

The authors gratefully acknowledge the scientists and staff of the North Carolina State University Phytotron; Ball Floraplant, Will Healy, and Kristan McGuigan for providing incite and plant material; and Nancy Mingus, Elizabeth Kirksey, Douglas Sturtz, and Elisa Ruszkiewicz for technical assistance.

[1]Graduate research assistant

[2]Professor and Corresponding Author. E-mail address: paul_nelson@ncsu.edu

[3]Research Horticulturist

Subject Category: Soil-plant-water relationships and Environmental stress physiology

High Temperature Induces Substrate Acidification by Geranium

*Additional index words***:** pH, phosphorus, *Pelargonium x hortorum* Bailey, acid quantification

Abstract. Sudden pH decline (SPD) describes the situation where crops growing at an appropriate pH, rapidly (within 1-2 weeks) cause the substrate pH to shift downward one to two units. 'Designer Dark Red' geraniums (*Pelargonium* x *hortorum* Bailey) were grown in three experiments to assess possible effects of temperature on SPD. The first experiment tested the effect of four day/night temperature regimes (14/10, 18/14, 22/18 and 26/22 ºC day/night) on substrate acidification. At 63 days after transplanting (DAT), substrate pH declined from 6.8 to 4.6 as temperature increased. Tissue P of plants grown at the highest 3 temperatures was extremely low (0.10-0.14% of dry weight), and P stress has been reported to cause acidification. It was not possible to determine if the drop in substrate pH was a singular temperature effect or a combination of high temperature and low P. To resolve this, a second experiment tested a factorial combination of the three highest temperatures from experiment one and five pre-plant P rates (0, 0.065, 0.13, 0.26, or 0.52 $g \cdot L^{-1}$ substrate). Regardless of tissue P concentrations, which ranged from deficient to above adequate, substrate pH decreased with increasing temperature. At 63 DAT, in the 0.065 and 0.13 P

treatments, tissue P was deficient and pH decreased with increasing temperature from 5.6 to 4.7 and 5.9 to 4.7, respectively. In the 0.26 P treatment, tissue P was adequate at the lowest temperature and there was no acidification. At the mid and highest temperatures, tissue P was deficient and statistically equivalent, yet pH decreased to 5.2 and 4.7, respectively. In the highest P treatment, tissue P levels were unaffected by temperature, above adequate, and pH declined with each increase in temperature from 6.5 to 5.0. The results at 63 DAT, once more showed that temperature acted independent of tissue P and caused geraniums to acidify the substrate. In the third experiment the amount of acidity produced by roots of plants grown at the two highest temperatures used in the first two experiments was quantified. Plants grown at the higher temperature produced 28% more acid per gram dry root. Results herein indicate that high temperature can induce SPD by geranium.

During the 1980s, many geranium producers observed a sporadic, unexplained decline in substrate pH. During the same time period, they also reported the occurrence of toxic concentrations of Fe and/or Mn in leaf tissue (Bachman and Miller, 1995). In organic-based soilless substrate, pH has a large affect on nutrient availability (Nelson, 2003). As pH decreases, most micronutrients become more available and can become toxic. In a study with 'Ringo Scarlet' geraniums, dry tissue Fe, Mn, and Zn concentration increased from 82 to 951, 118 to 9260, and 56 to 1340 $mg \cdot kg^{-1}$, respectively as final substrate pH decreased from ~6.5 to ~5.5 (Lee et al., 1996). The symptoms of micronutrient toxicity in geranium include: reduced leaf and plant size; black spots on young leaves; dark green color, large purplish or black spots, leaf cupping, and interveinal, marginal, or speckled chlorosis on the entire plant; cupping, and interveinal, marginal, or speckled chlorosis and/or necrosis on older leaves; and in severe cases complete necrosis of older leaves (Bachman and Miller, 1995; Brochat and Moore, 2004; Lee et al., 1996; Smith et al., 2004).

Sudden pH decline (SPD) describes the situation where crops growing at an appropriate pH, rapidly (within 1-2 weeks) cause the substrate pH to shift downward one to two units. Within a greenhouse firm or geographic area, one geranium crop may be affected while the subsequent crops are not, although all crops appear to be grown in a similar manner. Geraniums are the highest valued bedding plant of the $2.53 billion bedding plant industry (USDA, 2005) and SPD can cause severe economic loses and/or lead to tedious pH adjustments, such as application of flowable lime. The cause of SPD is unknown.

Apparently, an unknown signal(s) triggers an acidification process by roots. Three nutrient deficiencies known to cause plants to acidify substrate include: 1) Fe (Welkie and Miller, 1993), 2) Zn (Cakmak and Marschner, 1990), and 3) P (Schjorring, 1986 and Hinsinger, 2001). Pea (*Pisum sativum* L.), sugar beet (*Beta vulgaris* L.), and bean (*Phaseolus vulgaris* L.) lowered root substrate pH from around 7.0 to 4.0 in 6 to 10 hours when Fe was missing in the nutrient solution (Landsberg, 1981). The drop occurred in a NO_3^- nitrogen solution that normally causes the substrate pH to rise. Zinc deficiency has also been shown to cause acidification in dicotyledonous species even when NO_3^- was the sole source of N (Cakmak and Marschner, 1990). However, it is unlikely that Fe or Zn deficiency is the cause of SPD since both nutrients are applied sufficiently by most growers and the syndrome typically leads to Fe toxicity, not deficiency. On the other hand, P deficiency seems a likely contributor to the induction of SPD because growers rely heavily on alkaline fertilizers to offset pH decline, particularly in geranium, and P is low or absent in these alkaline fertilizers, i.e., 13-2-13, 15-0-15, 14-0-14 (N-P_2O_5-K_2O) (Nelson, 2003).

In addition to low P, there is evidence high temperature might also contribute to the occurrence of SPD. Micronutrient toxicity symptoms have been reported to be more common following high temperature stress (Smith et al., 2004). Taylor et al. (2007) showed that geraniums grown at 26/22 °C (day/night temperature) required more base to maintain hydroponic solution pH than plants grown at 22/18, indicating increased acidification. The

objectives of this research were to determine if high temperature causes geraniums to acidify the substrate (experiment 1), if the temperature effect is independent of tissue P concentration (experiment 2), and to quantify the amount of acidity produced due to high temperature and P stress in hydroponics (experiment 3).

Materials and Methods

Experiment 1. Rooted 'Designer Dark Red' (zonal) and 'Galleria Bright Violet' (ivy) geranium cuttings were fumigated for insects overnight with 0.5% Bifenthrin (Attain TR, Whitmire Micro-Gen Research Laboratories. St. Louis, MO 63122), planted the following day into 11 cm (606 cm^3) azalea pots, and grown for 63 d. The substrate consisted of 3:1 by volume peat:perlite amended with $MgSO_4$, Micromax micronutrient mix (The Scotts Company, Marysville, OH 43040), triple-superphosphate (TSP) (19.7% P) at 0.59, 0.89, 0.26 $g \cdot L^{-1}$ substrate, respectively, and flowable dolomitic limestone (Cleary Chemical Corporation, Dayton, NJ 08810) at 8.34 $mL \cdot L^{-1}$ substrate. Treatments were arranged in a factorial design with four temperature regimes (14/10, 18/14, 22/18 and 26/22 ºC day/night), and two cultivars. There were three replications and two plants per plot, giving a total of 48 plants. Plants were grown in growth chambers with 9 m^2 of growing area and 2.13 m vertical clearance in The Phytotron at North Carolina State University. The light regime consisted of a 9 h photosynthetic light period, 6 h of darkness, 3 h low light night interruption, and 6 h of darkness. During the photosynthetic period, cool white fluorescent and incandescent lamps separated from the growing area by a polycarbonate barrier supplied a PPF of 575 ± 25

$\mu mol \cdot m^{-2} \cdot s^{-1}$. The 3 h low light night interruption was provided by incandescent lamps and supplied a PPF of 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Carbon dioxide concentrations were maintained between 300-400 ppm by controlled injection of commercial grade gas. Plants were fertilized with each irrigation with a solution made from deionized water consisting of 1.4 mM NH_4NO_3, 3.6 mM $Ca(NO_3)_2$, and 4 mM KNO_3 (N, K, and Ca at 196, 156, 144 $mg \cdot L^{-1}$, respectively). Plants were fertigated when the substrate was dry.

Experiment 2. This experiment was similar to experiment one except it had one cultivar ('Designer Dark Red'), three temperature treatments (18/14, 22/18 and 26/22 ºC day/night), five P treatments (0, 0.065, 0.13, 0.26, and 0.52 $g \cdot L^{-1}$ substrate), and two harvest dates. Three replications, gave a total of 90 plants. Lime in the substrate was $CaCO_3$ powder (Fisher Scientific, Pittsburgh, PA 15275) supplied at 3.5 $g \cdot L^{-1}$ substrate, since the flowable dolomitic limestone caused substrate pH to rise for the first five weeks during experiment one (Fig. 1).

Experiments 1 and 2 measurements. Substrate pH was measured via the pour-through technique (Wright, 1986) every seven days for the length of both experiments and immediately after each pour-through, plants were fertilized. In experiment two, pour-through extracts taken 14, 28, 42, and 56 days after transplanting (DAT) were also analyzed for P. At 63 DAT in experiment one and at 28 and 63 DAT in experiment two, 4-6 recently mature leaves were removed from one plant, rinsed with 0.1 N HCl, and rinsed again with deionized

water. Plant samples from all three experiments were dried in a forced-air oven at 60 ºC for 48 h, weighed, ground in a Wiley Mill through a 25 mesh screen (1 mm particle size), and P content of both leaf samples and pour-through extracts were determined with inductively coupled plasma optical emission spectroscopy (IRIS-Intrepid II, Thermo Fischer Sci., Waltham, MA). Pour-though P concentrations could not be detected below the lower limit of 11 $\mu g \cdot L^{-1}$. All tissue concentrations are expressed on a dry weight basis. Experimental data was statistically analyzed as a complete block design with mean separation by Duncan's multiple range test at $p = 0.05$ (SAS Institute, 2002, Cary, NC 27513). Interactions were considered significant at $p \leq 0.05$. Error bars in figures from all three experiments were determined using the standard error function of SigmaPlot (SPSS Inc., Chicago, IL). In experiment two, the low temperature treatments were utilized for regression analysis of substrate pH over the time period 35-63 DAT using Microsoft Office Excel 2007 (Microsoft Cooperation, Redmond, WA 98052).

Experiment 3. 'Designer Dark Red' geranium cuttings, partially rooted in Oasis® Wedge® growing foam (Smithers-Oasis North America, OH 44240), were fumigated overnight as in experiments one and two. The following day they were placed in the same type of growth chambers used in experiments one and two. The temperature was 22/18 ºC day/night and plants were fertilized daily with a complete fertilizer containing N, P, K, Ca, Mg, Fe, S, Mn, B, Zn, Cu, Mo, and Na at 106, 10, 111, 54, 12, 5, 13, 0.1, 0.2, 0.01, 0.005, 0.005, and 11 $mg \cdot L^{-1}$, respectively. After 14 d, 96 rooted cuttings were transplanted into 12 plastic

containers measuring 27 x 32 cm and 22 cm tall. Containers were covered with plastic lids with eight evenly spaced 2.5 cm holes to hold plants. Half of the containers were placed in a second growth chamber set to a day/night temperature of 26/22 ºC. The hydroponic containers were aerated and filled with 19 L of solution consisting of 1.5 mM NH_4NO_3, 5 mM $Ca(NO_3)_2$, 2 mM KNO_3, 1 mM KH_2PO_4 (0 mM in minus P treatment), 1.5 mM KCl (2.5 mM in minus P treatment), 2 mM $MgSO_4$, 4 mg·L^{-1} Fe as Fe-DTPA (10% Fe), 9.1 µM $MnSO_4 \cdot H_2O$, 0.76 µM $ZnSO_4 \cdot 7H_2O$, 46.3 µM H_3BO_3, 1.57 µM $CuSO_4 \cdot 5H_2O$, and 0.10 µM $Na_2MoO_4 \cdot 2H_2O$. The volume of the containers was maintained at 19 L and the pH was set and maintained at 5.80 by additions of 5 mM $Ca(OH)_2$.

This experiment was a factorial design with two temperature treatments (22/18 and 26/22 °C day/night), two P treatments (0 and 1 mM P), and three sampling dates (7, 13, and 19 DAT) with three replicates, and two plants per plot. On each sampling date, at approximately 12:00 pm, two plants were removed from each hydroponic unit. Each plant was placed into a glass 500 mL beaker containing an aerated solution (pH of 5.80), identical to the solution in the 19 L units from which they were transplanted. The beakers were located within the same growth chamber and had polycarbonate lids, which held the plants. After 24 hours, plants were removed and roots were rinsed with deionized water so that all solution remaining on roots was washed back into the beaker. Plants were then separated into roots and shoots. Roots were dipped in 1 mM $CaSO_4$ for 30 s followed by deionized water to remove ions from the apoplast (Neagle et al., 2005), patted dry, and weighed. Shoots were weighed,

rinsed with 0.1 N HCl, rinsed again with deionized water, and then both roots and shoots were dried as previously described and weighed again. All leaves with petioles were removed from stems for shoot tissue analysis as described previously. Roots were also analyzed. Solutions in beakers were brought back to volume (500 mL) with deionized water and titrated with a standardized base to the initial pH of 5.80 to determine the amount of acid produced by roots.

Results

Experiment 1. For all temperature treatments and both cultivars, pH increased from less than 5.1 to greater than 6.2 during the first 35 d (Fig. 1). This is thought to be a consequence of the unexpected slow dissolution of the preplant flowable dolomitic limestone. All interactions were significant for substrate pH, tissue P percent, and shoot dry weight. Starting 49 DAT for both geranium cultivars, substrate pH decreased as temperature increased. By 63 DAT, substrate pH of plants grown at low (18/14), medium, (22/18), and high (26/22 ºC day/night) temperatures had decreased to 6.18, 5.49, and 4.53, respectively for 'Designer Dark Red' and 6.40, 5.77, and 4.76 for 'Galleria Bright Violet'. Leaf P concentration of both cultivars were significantly higher in plants that were grown at the extra-low temperature (14/10 ºC day/night) compared to all other temperature treatments, which were similar (Fig. 1). Krug et al. (2006), determined the lower end of the acceptable range for tissue dry weight P concentration in recently mature leaves for mature zonal geraniums is 0.17 to 0.19%. This indicates that all geraniums, other than those grown at

extra-low temperature, were approaching or experiencing P deficiency. Phosphorus deficiency was expressed as interveinal and marginal yellowing and reddening of lower leaves. Shoot dry weights of plants grown at high temperature were significantly higher than plants grown at all other temperature treatments, and the opposite occurred for plants grown at extra-low temperature, for both cultivars (Table 1). The number of fertigations increased with temperature for experiments 1 and 2 (Table 2).

Experiment 2. All interactions were significant for pH and tissue P concentration (Fig. 2). Substrate pH decreased as temperature increased with all P treatments, starting 28 DAT. Differences in substrate pH between temperature treatments generally increased over time and were greatest with the 3 highest P treatments, 63 DAT. Substrate pH of plants grown with zero P decreased with increasing temperature starting 7 DAT and decreased over time. At 63 DAT in the 0.065 and 0.13 P treatments, substrate pH decreased with increasing temperature from 5.6 to 4.7 and 5.9 to 4.7, respectively. At the two highest P rates, the temperature effects on substrate pH were statistically significant starting 35 DAT and by 63 DAT differences were greater than 1.5 pH units.

When comparing all low-temperature-grown plants, the slope of substrate pH was negative for plants that had end-of-crop tissue P concentration below 0.21 and was zero or positive when tissue P was greater than 0.20, starting 35 DAT (Table 3). The r^2 is well correlated when P was supplied but deficient. In the zero P treatment, the rate of pH decline began to

subside at 35 DAT. These data indicate that P deficiency of geraniums can cause substrate acidification.

Tissue P was adequate and ranged from 0.30 to 0.60 with all temperature and P treatments (except 0 P) at 28 DAT in experiment two (Fig. 2). At the end of the crop, tissue P was deficient and less than 0.15 percent in all plants grown with the three lowest P rates. Plants grown with 0.26 g P had end of crop tissue P concentrations that were at or just below adequate and plants grown at the highest P rate had tissue P above adequate (> 0.20). In most cases temperature treatments had little or no significant effect on tissue P concentration.

The only interaction that was significant for P concentration of substrate-pour-through-extracts was P rate x DAT and the temperature effect was nonsignificant (Fig. 3). As expected, substrate extract P concentrations increased significantly as P rate increased, at 14 DAT. The concentration of P decreased over time for all P treatments except the zero treatment, which was nondetectable on all sampling dates.

The main effects of temperature treatment and P rate on plant dry weights 28 and 63 DAT for experiment two are presented in Table 4. Plants grown at low temperature weighed significantly less than plants grown at medium and high temperature, at 28 DAT. High-temperature-grown plants weighed significantly less than all other plants at 63 DAT. These data indicate plants may have been under high temperature stress, which was not apparent in

experiment one. Plants that did not receive P weighed less than all other plants on both dates. At 63 DAT, plants from the 0.065 P treatment weighed less than all plants receiving higher P rates and plants from the 0.13 P treatment weighed significantly less than plants from the 0.52 P treatment.

Experiment 3. Shoot and root P concentrations were lower in plants that did not receive P on all sampling dates and all interactions were significant (Fig. 4). Shoot P was deficient 7 DAT and decreased over time in plants that did not receive P. Plants grown with P at high temperature had higher root tissue P on all DAT, higher shoot P 7 DAT, and lower shoot P 19 DAT. The amount of acid produced per gram dry weight of root was 23.6 meq from plants grown at high (26/22 º C day/night) temperature and 18.4 meq from plants grown at the control (22/18) temperature. This difference was significant at $p = 0.025$ and is a 28.3 % increase in acid production at high temperature. There was an interactive effect of P treatment and DAT on acid production. Acid production did not differ 7 DAT, but at 13 and 19 DAT acid production by roots of P deficient plants was less than plants that received P (Fig. 5). The dry weights of plants was unaffected by temperature, but was lower due to lack of P and this difference increased over time (Fig. 6)

Discussion

The results of all three experiments indicated that high temperature increases substrate acidification by geranium. Starting 35 DAT in experiment one, substrate pH decreased

significantly when tissue P was deficient (0.10-0.16%) and remained relatively constant or increased when tissue P was close to or above adequate (0.18-0.34%) (Fig. 1). Acidification due to P deficiency is a widespread phenomenon among dicotyledonous species (Marshner, 1995). It was not possible to determine if the pH effect was due to temperature alone or a combination of temperature and P deficiency. Increased growth was likely not responsible for increased acidification of 'Designer Dark Red' plants since dry weights of plants from the medium and low temperatures were not statistically different, while plants grown at medium temperature had a significantly lower substrate pH starting 56 DAT (Table 1).

In experiment two, geranium substrate pH decreased as temperature increased regardless of tissue P concentration (Fig. 2). At 28 DAT, tissue P was adequate in plants at all temperature and P treatments except 0 P. Yet, pH was lower in the high temperature treatment with all P rates except the highest. These data indicate temperature acted independent of tissue P concentration. At 63 DAT, in both the 0.065 and 0.13 P treatments, tissue P was deficient, unaffected by temperature treatment, and pH decreased with increasing temperature from 5.6 to 4.8 and 5.9 to 4.7, respectively. At the 0.26 P treatment, tissue P was adequate at low temperature and deficient at the two higher temperatures and again pH decreased as temperature increased. In the highest P treatment, all tissue P levels were adequate on both tissue sampling dates, yet at 63 DAT pH declined from 6.5 at the low temperature to 5.0 at the high temperature. Again, the results at 63 DAT indicate that temperature is acting independent of tissue P. In all cases when plants received P and temperature was high, pH

fell below 5.2 by 57 DAT. Dry weights of plants grown at medium and high temperatures were not significantly different 28 DAT and plants grown at high temperature weighed significantly less 63 DAT (Table 4). Since pH was lower with high temperature on both DAT, these data again indicate that increased growth was not responsible for increased acidification.

Acidification due to P deficiency is also indicated in experiment two. When comparing all of the low temperature treatments, substrate pH decreased when P was deficient. In Table 3, plants that received P and had tissue a P concentration less than the lower established value of 0.17 to 0.19 % had negative slopes and r^2 values that indicate a fit of the respective model. Plants grown without P were severely deficient by 35 DAT and may have lost the facility to lower substrate pH, which did occur prior to 35 DAT (Fig. 2). When plants were grown at low temperature and tissue P was above 0.19%, the slope was greater than or equal to zero and the r^2 showed that the model fit poorly. This indicates that P deficiency can cause geraniums to lower substrate pH.

Data in experiment three once again indicated that higher temperatures increase substrate acidification by geranium roots (Table 5). Acid production per gram root increased 28% when plants were grown at high temperature compared to the control temperature. The effect of P treatments on acid production by roots was unexpected. The effect of P treatment was not significant 7 DAT and plants that received P had increased acid production 13 and 19

DAT (Fig. 5). This was unexpected and may have been due to decreased acidification capacity, caused by severe P deficiency. Substrate acidification occurs when the net H^+ efflux from roots increases. The function of the rhizodermal H^+ ATPase pump is dependent on ATP, which decreases significantly in roots of plants under severe P stress (Chiera et al., 2004). Shoot P was less than or equal to 0.18% on the first sampling date (7 DAT) and was severely deficient (< 0.11%) by 19 DAT in plants not receiving P.

As the treatment temperature increased, the number of fertigations increased for experiment one and two. Depending on the pH of the fertilizer solution and the potential acidity/basicity of the fertilizer, increasing the frequency of fertigations could cause substrate pH to shift upward or downward. The pH of the fertilizer solution used in experiment one and two was 5.6, which is greater than the substrate pH from nearly all plants grown at medium and high temperature 63 DAT. Additionally, NO_3^- makes up 80% or more of the N in most basic fertilizers (Nelson, 2003). In experiment one and two, 90% of the N was NO_3^-.

Increased irrigations due to high temperature may also indirectly increase the rate at which lime is leached from the substrate. This may have been a factor in experiment one, when the lime source was flowable dolomitic lime that seemed to dissolve slowly over the first five weeks of the experiment. This effect was unlikely in experiment two. The lime source was readily dissolvable $CaCO_3$ powder that dissolved within the first week. In addition, the data from experiments one, two, and three, along with the magnitude of the effect on substrate pH

with a small increase in the number of irrigations, indicate that most of the observed effect in these studies was due to a direct effect of high temperature on substrate acidification by geranium. The indirect effect of temperature due to greater lime leaching from increased irrigation frequency is likely a contributing factor to SPD in a commercial setting.

It is clear from this study that high temperature can cause SPD of geraniums. In most cases, when the temperature was high (26/22 º C day/night), substrate pH dropped 0.5-1.3 pH units within a two week period. The recommended temperatures for the production of zonal geraniums ranges from 18 to 24º C for the day and 17 to 22º for the night period (Ball FloraPlant, 2007; Fischer USA, 2007; Hamrick, 2003). The temperatures used for the high temperature treatment in this study are just above these recommendations. It is highly likely, during normal geranium production, that temperatures will exceed these temperatures, especially in the southern regions of the US. To help prevent SPD, geranium growers should avoid P deficiency, which is known to cause substrate acidification, and try to maintain temperature within the recommended range. Although it has been determined that P deficiency and high temperature are associated with SPD, other factors are most likely involved. Growers need to constantly monitor substrate pH in order to head-off possible oncoming pH problems by using pH adjustment materials such as flowable limestone.

Literature Cited

Bachman, G.R. and W.B. Miller. 1995. Iron chelate inducible iron/manganese toxicity in zonal geranium. J. Plant Nutr. 18:1917-1929.

Ball FloraPlant. 2007 catalog. Ball Horticultural Company. USA.

Broschat, T. and K. Moore. 2004. Phytotoxicity of several iron fertilizers and their effects on Fe, Mn, Zn, Cu, and P content of African marigolds and zonal geraniums. HortScience. 39:595-598.

Cakmak, I. and H. Marschner. 1990. Decrease in nitrate uptake and increase in proton release in zinc deficient cotton, sunflower, and buckwheat plants. Plant and Soil. 129:261-268.

Chiera, J.M., J.F. Thomas, and T. W. Rufty. 2004. Growth and localized energy status in phosphorus-stressed soybean. J. Plant Nutr. 27:1875-1890.

Fischer USA. 2007. Producing high quality geraniums. Fischer Cultural Guide 2007-2008. 57-74. Colorado.

Hamrick, D. 2003. Ball Redbook 17th Edition. Volume 2: Crop Production. Ball Publishing. IL.

Hinsinger, P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. Plant and Soil. 237:173-195.

Krug, B.A., B.E. Whipker, B. Cleveland, and I. McCall. 2006. Taking the "art" out of interpreting tissue analysis for geranium. Proc. of the Southern Nursery Assn. Res. Conf. 15: 635-639.

Landsberg, E. 1981. Organic acid synthesis and release of hydrogen ions in response to Fe deficiency stress of mono- and dicotyledonous plant species. J. Plant Nutr. 3:579-591.

Lee, C.W., J.M. Choi, and C.H. Pak. 1996. Micronutrient toxicity in seed geranium (*Pelargonium x hortorum* Bailey). J. Amer. Soc. Hort. Sci. 121:77-82.

Marschner, H. 1995. Mineral nutrition of higher plants. Academic Press. NY.

Mills, H.A. and J.B. Jones Jr. 1996. Plant Analysis Handbook II. A Practical Sampling, Preparation, Analysis, and Interpretation Guide. MicroMacro Publishing, Inc., GA.

Naegle, E.R., J.W. Burton, T.E. Carter, and T.W. Rufty. 2005. Influence of seed nitrogen content of seedling growth and recovery form nitrogen stress. Plant and Soil. 271:329-340.

Nelson P.V. 2003. Greenhouse operation and management 6th edition. Prentice Hall, NJ.

Schjorring, J.K. 1986. Nitrate and ammonium absorption by plants growing at a sufficient or insufficient level of phosphorus in nutrient solution. Plant and Soil. 91:313-318.

Smith, B.R., P.R. Fisher, and W.R. Argo. 2004. Water-soluble fertilizer concentration and pH of a peat-based substrate affect growth, nutrient uptake, and chlorosis of container-grown seed geraniums. J. Plant Nutr. 27:497-524

Taylor, M.D., P.V. Nelson, J.M. Frantz, and T.W. Rufty. 2007. Effect of Phosphorus Deficiency and High Temperature on Ammonium and Nitrate Uptake and acidification by *Pelargonium*. HortScience. 42:890.

United States Department of Agriculture (USDA). 2005. Floriculture Crop Summary. National Agriculture Statistics Service. Sp. Cir 6-1(05). Washington, D.C.

Welkie, G.W. and G.W. Miller. 1993. Plant iron uptake physiology by nonsiderophore systems. pp. 345-369. In: Barton, L. L. and B. C. Hemming Eds. Iron chelation in plants and soil microorganisms. Academic Press, Inc., NY.

Wright, R.D. 1986. The pour-through nutrient extraction procedure. HortScience. 21:227-229.

Table 1. Effect of temperature treatment on shoot dry weight for ‘Designer Dark Red’ and ‘Galleria Bright Violet’ geraniums 63 days after transplanting in experiment 1.

	Cultivar	
	Designer Dark Red	Galleria Bright Violet
Temperature treatment	----------Dry weight (g)----------	
Extra-low (14/10 ºC Day/Night)	10.3 c[z]	5.8 d
Low (18/14)	22.4 b	13.2 c
Medium (22/18)	23.5 b	18.1 b
High (26/22)	25.4 a	20.8 a

[z] Mean separation by Duncan’s multiple range test within columns.

Table 2. Number of fertigations for each temperature treatment for experiments 1 and 2.

	Experiment 1	Experiment 2
Temperature treatment	-----Number of fertigations----	
Extra-low (14/10º C Day/Night)	16	-
Low (18/14)	25	21
Medium (22/18)	34	27
High (26/22)	37	33

Table 3. Effect of preplant P rate on tissue dry weight P percent 63 DAT for plants grown at low temperature and the corresponding r^2 value and slope for days after transplanting (DAT) (x-axis) versus pH (y-axis) trend line (not shown) starting 35 DAT in experiment 2.

P Rate ($g \cdot L^{-1}$ substrate)	---% P---	----r^2---	Slope
0	0.06 d[z]	0.32	-0.01
0.065	0.08 d	0.85	-0.02
0.13	0.14 c	0.70	-0.03
0.26	0.21 b	0.00	0.00
0.52	0.26 a	0.20	0.01

[z] Mean separation by Duncan's multiple range test.

Table 4. Main effects of temperature treatment and P rate on plant dry weights 28 and 63 DAT for experiment 2.

	DAT	
	28	63
Temperature treatment	----Dry weight (g)----	
Low (18/14 ºC Day/Night)	2.54 b[z]	13.05 a
Medium (22/18)	3.84 a	13.82 a
High (26/22)	3.67 a	11.73 b
P Rate ($g \cdot L^{-1}$ substrate)		
0	1.51 b	1.49 d
0.065	3.44 a	13.78 c
0.13	3.95 a	15.47 b
0.26	4.06 a	16.45 ab
0.52	3.78 a	17.15 a

[z]Mean separation by Duncan's multiple range test within columns.

Figure 1. Substrate pH, every seven days after transplanting (DAT) for 'Designer Dark Red' (A) and Galleria Bright Violet' (B) geraniums at extra-low (14/10 ºC day/night), low (18/14), medium (22/18), and high (26/22) temperatures in experiment one. Error bars represent standard errors (n = 3). On the right side of each temperature curve are the tissue dry weight P % 63 DAT. Mean separation by Duncan's multiple range test at $p = 0.05$.

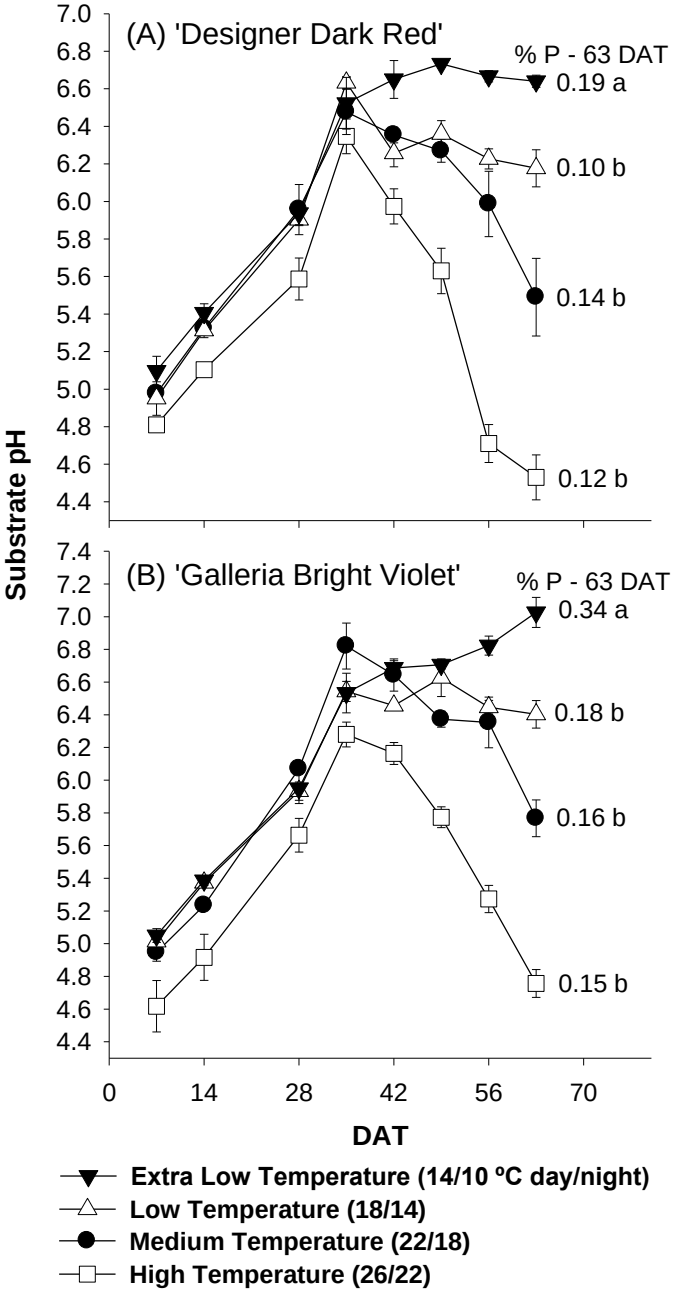
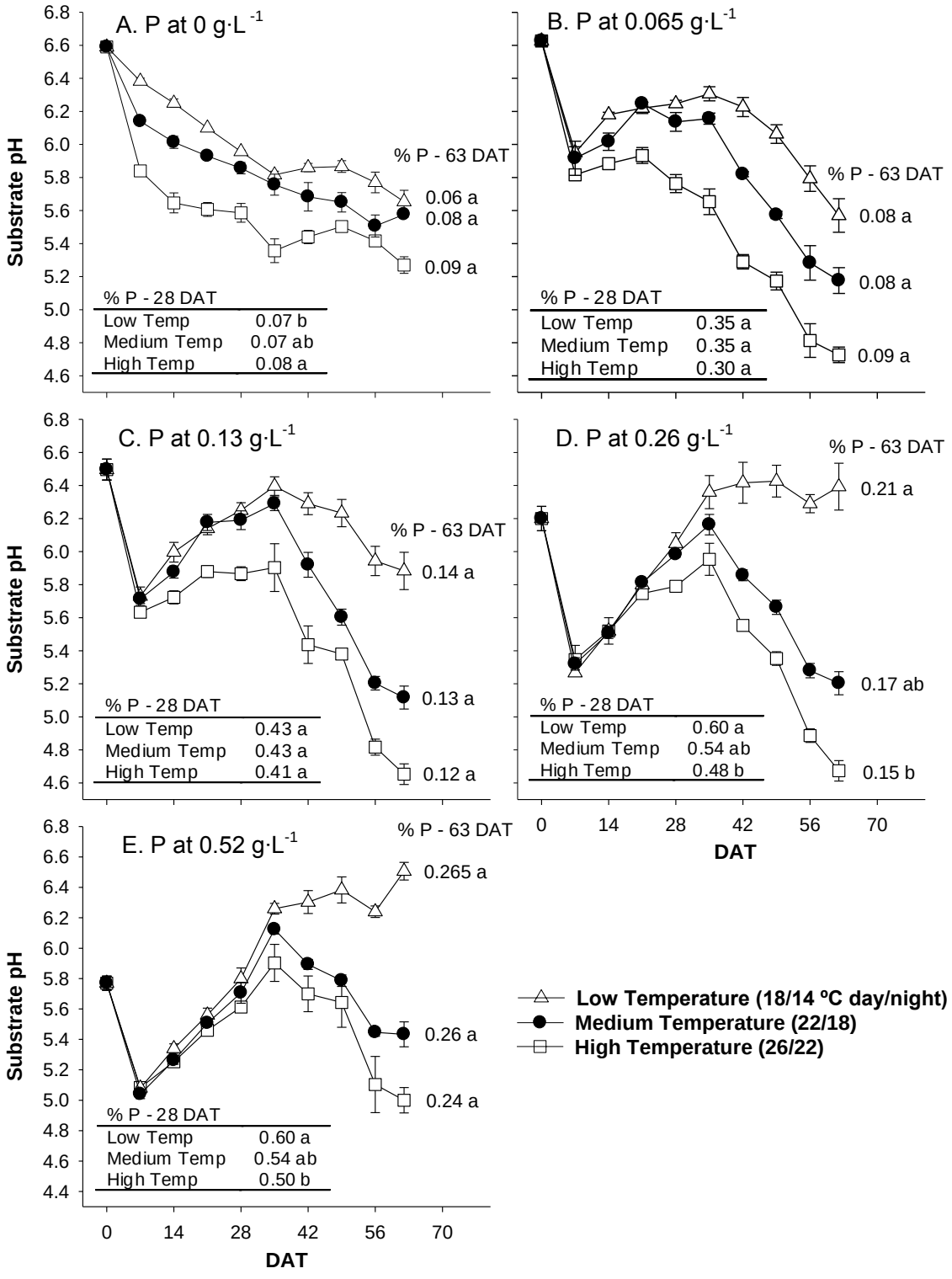

(A) 'Designer Dark Red'
% P - 63 DAT
0.19 a
0.10 b
0.14 b
0.12 b
(B) 'Galleria Bright Violet'
% P - 63 DAT
0.34 a
0.18 b
0.16 b
0.15 b
Substrate pH
DAT
Extra Low Temperature (14/10 ºC day/night)
Low Temperature (18/14)
Medium Temperature (22/18)
High Temperature (26/22)

Figure 2. Substrate pH, measured initially and every seven days after transplanting (DAT) for each P treatment (A. 0, B. 0.065, C. 0.13, D. 0.26, and E. 0.52 P at $g \cdot L^{-1}$ substrate) at low (18/14 ºC day/night), medium (22/18), and high (26/22) temperatures in experiment 2. Error bars represent standard errors (n = 3). In the lower left corner of each P treatment graph is a table with tissue dry weight P percent 28 DAT for that particular P treatment at each temperature. On the right side of each temperature curve is the tissue dry weight P percent 63 DAT. Mean separation by Duncan's multiple range test at $p = 0.05$.


A. P at 0 g·L-1
% P - 63 DAT
0.06 a
0.08 a
0.09 a
% P - 28 DAT
Low Temp 0.07 b
Medium Temp 0.07 ab
High Temp 0.08 a
B. P at 0.065 g·L-1
% P - 63 DAT
0.08 a
0.08 a
0.09 a
% P - 28 DAT
Low Temp 0.35 a
Medium Temp 0.35 a
High Temp 0.30 a
C. P at 0.13 g·L-1
% P - 63 DAT
0.14 a
0.13 a
0.12 a
% P - 28 DAT
Low Temp 0.43 a
Medium Temp 0.43 a
High Temp 0.41 a
D. P at 0.26 g·L-1
% P - 63 DAT
0.21 a
0.17 ab
0.15 b
% P - 28 DAT
Low Temp 0.60 a
Medium Temp 0.54 ab
High Temp 0.48 b
E. P at 0.52 g·L-1
% P - 63 DAT
0.265 a
0.26 a
0.24 a
% P - 28 DAT
Low Temp 0.60 a
Medium Temp 0.54 ab
High Temp 0.50 b
Substrate pH
DAT
Low Temperature (18/14 ºC day/night)
Medium Temperature (22/18)
High Temperature (26/22)

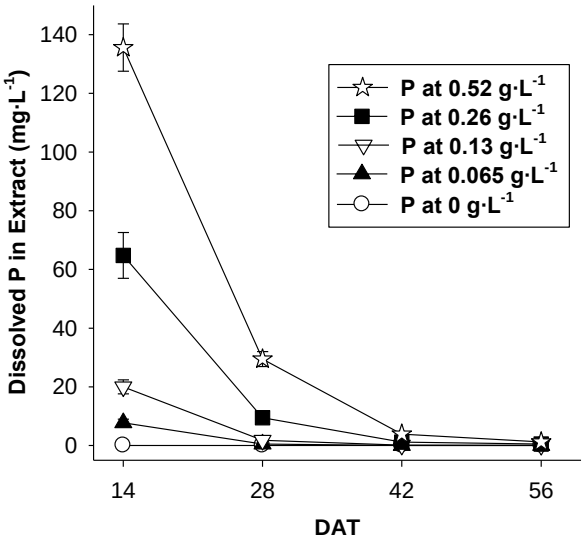


Figure 3. Pour-through extract P concentration at 14, 28, 42, and 56 days after transplanting (DAT) for treatments receiving 0, 0.065, 0.13, 0.26, and 0.52 P at $g \cdot L^{-1}$ substrate. Error bars represent standard errors (n = 9). Temperature treatments were combined.

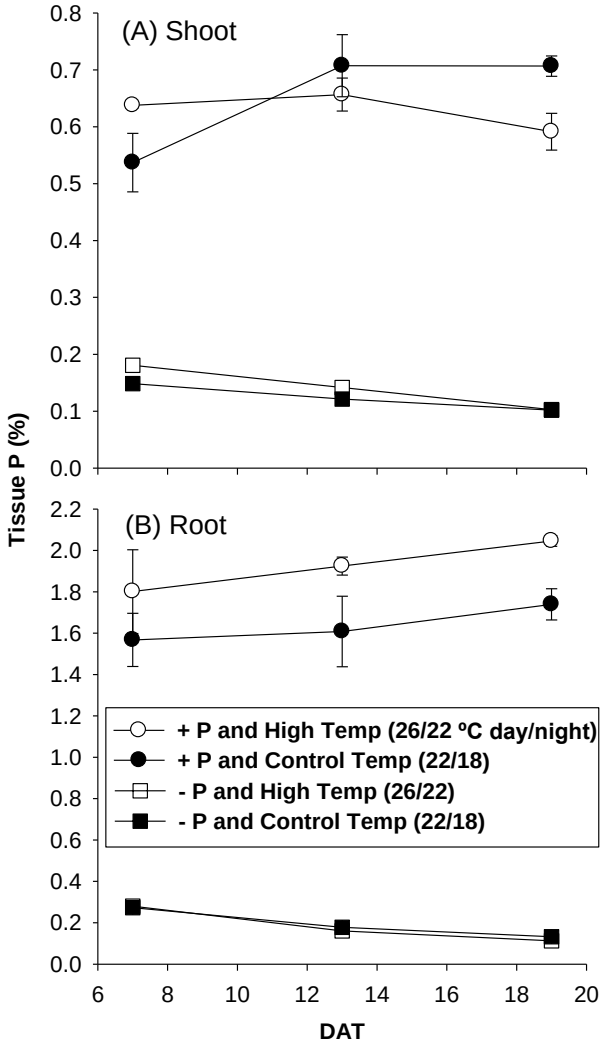


Figure 4. Shoot (A) and root (B) tissue dry weight P percent at 7, 13, and 19 days after transplanting (DAT) in plants grown at control (22/18 °C day/night) and high (26/22) temperatures with and without P. Error bars represent standard errors (n = 3).

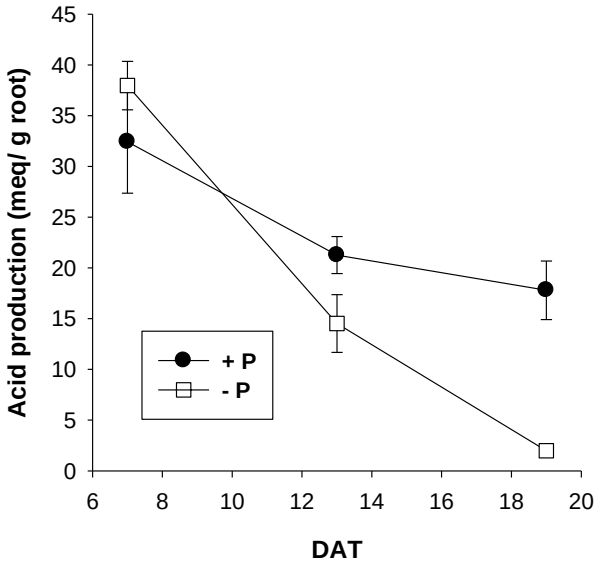


Figure 5. Acid produced per gram dry weight of roots for plants grown with and without P at 7, 13, and 19 days after transplanting (DAT). Temperature treatments were combined. Error bars represent standard errors (n = 12).

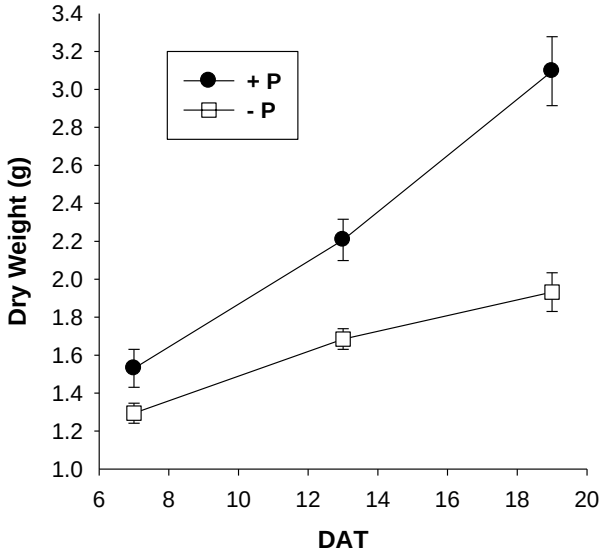


Figure 6. Whole plant dry weights at 7, 13, and 19 days after transplanting (DAT) of plants grown with or without P. Temperature treatments were combined. Error bars represent standard errors (n = 12).

CHAPTER 3

High Light Suppression of Phosphorus Uptake and Consequent Root Substrate Acidification by Geranium

(In the format appropriate for submission to the Journal of the American Society for Horticultural Science)

High Light Suppression of Phosphorus Uptake and Consequent Root Substrate Acidification by Geranium

Matthew D. Taylor[1] and Paul V. Nelson[2]

Department of Horticultural Science, Campus Box 7609, North Carolina State University, Raleigh, North Carolina 27695-7609

Jonathan M. Frantz[3]

USDA-ARS Application Technology Research Unit, 2801 W. Bancroft, MS 604, Toledo, OH 43606

Received for publication_____ . Accepted for publication _____.

The authors gratefully acknowledge the scientists and staff of the North Carolina State University Phytotron; Ball Floraplant, Will Healy, and Kristan McGuigan for providing incite and plant material; and Nancy Mingus, Elizabeth Kirksey, Douglas Sturtz, and Elisa Ruszkiewicz for technical assistance.

[1]Graduate research assistant

[2]Professor and Corresponding Author. E-mail address: paul_nelson@ncsu.edu

[3]Research Horticulturist

Subject Category: Soil-plant-water relationships and Environmental stress physiology

High Light Suppression of Phosphorus Uptake and Consequent Root Substrate Acidification by Geranium

Additional index words: *Pelargonium x hortorum* Bailey, pH, PPF

Abstract. Sudden pH decline (SPD) describes the situation where crops growing at an appropriate pH, rapidly (within 1-2 weeks) cause the substrate pH to shift downward one to two units. 'Designer Dark Red' geraniums (*Pelargonium* x *hortorum* Bailey) were grown in three experiments to assess possible effects of light on SPD and P uptake. The first experiment tested the effect of four light intensities (105, 210, 575, and 1020 ± 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$) on substrate acidification. At 63 d, substrate pH declined from 6.0 to 4.8 as light intensity increased. Tissue P of plants grown at the highest two light levels was extremely low (0.10-0.14 % of dry weight). P stress has been reported to cause acidification. Since plants in the two lowest light treatments had adequate P, it was not possible to determine if the drop in substrate pH was a direct light effect or a combination of light and P. The second experiment used a factorial combination of the three highest light levels from experiment one and five pre-plant P rates (0, 0.065, 0.13, 0.26, or 0.52 $g \cdot L^{-1}$ substrate) to assess this question. When tissue P concentrations were deficient, pH decreased by 0.6 to 1.0 pH units within two weeks and deficiency occurred more often with high light intensity. These data indicated

that P deficiency caused substrate acidification and indicated the possibility that P uptake was suppressed by high light intensity. The third experiment was conducted in hydroponics to determine the direct effect of high light intensity on P uptake. In this experiment, cumulative P uptake per gram root and the rate of P uptake per gram root per day both decreased 20 % when light intensity increased from 500 to 1100 $\mu mol \cdot m^{-2} \cdot s^{-1}$. It is clear from this study that P deficiency causes geraniums to acidify the substrate and that high light suppresses P uptake.

During the 1980s, many geranium producers observed a sporadic, unexplained decline in substrate pH. During the same time period, they also reported the occurrence of toxic concentrations of Fe and/or Mn in leaf tissue (Bachman and Miller, 1995). In organic-based soilless substrate, pH has a large affect on nutrient availability (Nelson, 2003). As pH decreases, most micronutrients become more available and can become toxic. In a study with 'Ringo Scarlet' geraniums, dry tissue Fe, Mn, and Zn concentration increased from 82 to 951, 118 to 9260, and 56 to 1340 $mg \cdot kg^{-1}$, respectively as final substrate pH decreased from ~6.5 to ~5.5 (Lee et al., 1996). The symptoms of micronutrient toxicity in geranium include: reduced leaf and plant size; black spots on young leaves; dark green color, large purplish or black spots, leaf cupping, and interveinal, marginal, or speckled chlorosis on the entire plant; cupping, and interveinal, marginal, or speckled chlorosis and/or necrosis on older leaves; and in severe cases complete necrosis of older leaves (Bachman and Miller, 1995; Brochat and Moore, 2004; Lee et al., 1996; Smith et al., 2004).

Sudden pH decline (SPD) describes the situation where crops growing at an appropriate pH, rapidly (within 1-2 weeks) cause the substrate pH to shift downward one to two units. Within a greenhouse firm or geographic area, one geranium crop may be affected while the subsequent crops are not, although all crops appear to be grown in a similar manner. Geraniums are the highest valued bedding plant of the $2.53 billion bedding plant industry (USDA, 2005) and SPD can cause severe economic loses and/or lead to tedious pH adjustments, such as application of flowable lime. The cause of SPD is unknown.

Apparently, an unknown signal(s) triggers an acidification process by roots. Three nutrient deficiencies known to cause plants to acidify substrate include: 1) Fe (Welkie and Miller, 1993), 2) Zn (Cakmak and Marschner, 1990), and 3) P (Schjorring, 1986 and Hinsinger, 2001). Pea (*Pisum sativum* L.), sugar beet (*Beta vulgaris* L.), and bean (*Phaseolus vulgaris* L.) lowered root substrate pH from around 7.0 to 4.0 in 6 to 10 hours when Fe was missing in the nutrient solution (Landsberg, 1981). The drop occurred in a NO_3^- nitrogen solution that normally causes the substrate pH to rise. Zinc deficiency has also been shown to cause acidification in dicotyledonous species even when NO_3^- was the sole source of N (Cakmak and Marschner, 1990). However, it is unlikely that Fe or Zn deficiency is the cause of SPD since both nutrients are applied sufficiently by most growers and the syndrome typically leads to Fe toxicity, not deficiency. On the other hand, P deficiency seems a likely contributor to the induction of SPD because growers rely heavily on alkaline fertilizers to offset pH decline, particularly in geranium, and P is low or absent in these alkaline fertilizers, i.e., 13-2-13, 15-0-15, 14-0-14 ($N\text{-}P_2O_5\text{-}K_2O$) (Nelson, 2003).

In addition to low P, high light may also be associated with SPD. Micronutrient toxicity symptoms have been reported to occur in geranium shortly after periods of high light (White, 1993). Tomato (*Lycopersicon esculentum* Mill.), Boston fern (*Nephrolepis exaltata* L.), and guinea grass (*Panicum maximum* Jacq.) have been shown to increase P uptake under higher light intensity (Anita et al., 2003., De Groot et al., 2001; Magalhaes and Wilcox, 1983;

Nowak et al., 2002). Most light treatments used in these studies were below what would normally occur during spring geranium production. It is possible that light intensities provided ranged from suboptimal to adequate. If higher light intensities were applied, a negative effect on P uptake could have occurred. The objective of this research was to assess the effect of high light intensity on substrate acidification and P uptake.

Materials and Methods

Experiment 1. Rooted 'Designer Dark Red' (zonal) and 'Galleria Bright Violet' (ivy) geranium cuttings were fumigated for insects overnight with 0.5 % Bifenthrin (Attain TR, Whitmire Micro-Gen Research Laboratories. St. Louis, MO 63122), planted the following day into 11 cm (606 cm^3) azalea pots, and grown for 63 d at The North Carolina State University Phytotron. The substrate consisted of 3:1 peat:perlite amended with $MgSO_4$, Micromax micronutrient mix (The Scotts Company, Marysville, OH 43040), triple-superphosphate (19.7 % P) at 0.59, 0.89, 0.26 $g \cdot L^{-1}$ substrate, respectively, and flowable dolomitic limestone (Cleary Chemical Corporation, Dayton, NJ 08810) at 8.3 $mL \cdot L^{-1}$ substrate. Treatments were arranged in a factorial design with four light treatments (105, 210, 575, and 1020 ± 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and two cultivars. There were three replications and two plants per plot, resulting in a total of 48 plants. Plants in the three lowest light treatments were grown in growth chambers with 9 m^2 of growing area and 2.13 m vertical clearance. Light was provided by cool white fluorescent and incandescent lamps separated from the growing area by a polycarbonate barrier. The 210 and 105 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light treatments were

achieved by using one and two layers of green shade cloth (army surplus mosquito netting), respectively. In the highest light treatment, plants were grown in chambers with 1.1 m^2 of growing area and 1.2 m vertical clearance. Light was provided by incandescent lamps and an equal ratio of metal halide to high pressure sodium lamps separated from the growing area by a polycarbonate barrier. The light regime for all light treatments consisted of a 9 h photosynthetic light period, 6 h of darkness, 3 h low light night interruption, and 6 h of darkness. The 3 h low light night interruption was provided by incandescent lamps and supplied a PPF of 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The day/night temperature in all chambers was 22/18 ºC. Carbon dioxide concentrations were maintained between 300 to 400 ppm by controlled injection of commercial grade gas. Plants were fertilized with each irrigation with a solution made from deionized water consisting of 1.4 mM NH_4NO_3, 3.6 mM $Ca(NO_3)_2$, and 4 mM KNO_3 (N, K, and Ca at 196, 156, 144 $mg \cdot L^{-1}$, respectively).

Experiment 2. This experiment was similar to experiment one except it had one cultivar ('Designer Dark Red'), three light treatments (210, 575, and 1020 ± 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$), five P treatments (0, 0.065, 0.13, 0.26, or 0.52 $g \cdot L^{-1}$ substrate), and two plants per plot. There were three replications, giving a total of 90 plants. Lime in the substrate was $CaCO_3$ powder (Fisher Scientific, Pittsburgh, PA 15275) supplied at 3.5 $g \cdot L^{-1}$, since the flowable dolomitic limestone caused substrate pH to rise for the first five weeks during experiment one (Fig. 1).

Experiments 1 and 2 measurements. Substrate pH was measured via the pour-through technique (Wright, 1986) every seven days for the duration of both experiments and immediately after each pour-through, plants were fertilized. In experiment two, pour-through extracts taken at 14, 28, 42, and 56 days after transplanting (DAT) were also analyzed for P. At 63 DAT, 4 to 6 recently mature leaves in both experiments and oldest leaves in experiment two were removed from plants, rinsed with 0.1 N HCl, and rinsed again with deionized water. Plant samples from all three experiments were dried in a forced-air oven at 60 ºC for 48 h, weighed, ground in a Wiley Mill through a 25 mesh screen (1 mm particle size), and P content of both leaf samples and pour-through extracts were determined with inductively coupled plasma optical emission spectroscopy (IRIS-Intrepid II, Thermo Fischer Sci., Waltham, MA). Pour-though P concentrations were not detectable (ND) below the lower limit of 11 $\mu g \cdot L^{-1}$. All tissue concentrations are expressed on a dry weight basis. In experiment two, total shoot P was determined by multiplying the dry weight of the shoots by the dry weight P % of recently mature leaves. Experimental data was statistically analyzed as a complete block design with mean separation by Duncan's multiple range test at $p = 0.05$ (SAS Institute, 2002, Cary, NC 27513). Error bars in both figures were determined using the standard error function of SigmaPlot (SPSS Inc., Chicago, IL).

Experiment 3. 'Designer Dark Red' geranium cuttings, partially rooted in Oasis® Wedge® growing foam (Smithers-Oasis North America, OH 44240), were fumigated overnight, as in experiments one and two. The following day, they were placed in the same type of growth

chambers used for the low and medium light treatments in experiments one and two. The temperature was 22/18 ºC day/night and plants were fertilized daily with a complete fertilizer containing N, P, K, Ca, Mg, Fe, S, Mn, B, Zn, Cu, Mo, and Na at 106, 10, 111, 54, 12, 5, 13, 0.1, 0.2, 0.01, 0.005, 0.005, and 11 $mg \cdot L^{-1}$, respectively. After 12 d, 48 rooted cuttings were transplanted into 12 opaque plastic buckets containing four L of nutrient solution. Buckets were covered with plastic lids with four evenly spaced 2.5 cm holes to hold plants and were placed into two chambers that were the same type as the high light chambers used in experiments one and two. The day/night temperature was 22/18 ºC and one of the chambers was shaded with standard window screen with a mesh size of 7.1 x 6.3 per cm^2, giving ~50 % light exclusion for the control light treatment. The hydroponic containers were aerated and filled with solutions consisting of 1.5 mM NH_4NO_3, 5 mM $Ca(NO_3)_2$, 2 mM KNO_3, 1 mM KH_2PO_4, 1.5 mM KCl, 2 mM $MgSO_4$, 4 $mg \cdot L^{-1}$ Fe as Fe-DTPA (10 % Fe), 9.1 µM $MnSO_4 \cdot H_2O$, 0.76 µM $ZnSO_4 \cdot 7H_2O$, 46.3 µM H_3BO_3, 1.57 µM $CuSO_4 \cdot 5H_2O$, and 0.10 µM $Na_2MoO_4 \cdot 2H_2O$. The volume of the containers was maintained by addition of deionized water every 2 to 3 days.

This experiment was a factorial design with two light treatments (500 and 1100 ± 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and two sampling dates (11 and 15 DAT), with three replication and four plants per plot. On each sampling date, plants were removed and roots were rinsed with deionized water so that all solution remaining on roots was washed back into the bucket. Plants were then separated into roots and shoots. Roots were dipped in 1 mM $CaSO_4$ for 30 s followed

by deionized water to remove ions from the apoplast (Neagle et al., 2005), patted dry, and weighed. Shoots were weighed, rinsed with 0.1 N HCl, rinsed again with deionized water, and then both roots and shoots were dried and weighed again. All leaves with petioles were removed from stems for tissue analysis as described previously. Roots were also analyzed. Solutions in buckets were brought back to volume with deionized water and analyzed for P content to determine P uptake by roots. Phosphorus concentration of hydroponic solution was determined by the colorimetric method of Murphy and Riley (1962).

Results and Discussion

Experiment 1. For all light treatments and both cultivars, pH increased from less than 5.3 to greater than 6.1 during the first 35 d (Fig.1). This unforeseen increase in substrate pH could be a consequence of an unexpectedly slow dissolution of the preplant flowable dolomitic limestone. All interactions were significant for substrate pH and tissue P percent. Starting 49 DAT, substrate pH of plants grown at high light (1020 $\mu mol \cdot m^{-2} \cdot s^{-1}$) were lower than plants from all other light treatments (Fig. 1). Substrate pH decreased to 4.78 for 'Designer Dark Red' and 4.80 for 'Galleria Bright Violet' by 63 DAT. At this point, substrate pH of 'Designer Dark Red' plants grown at very-low light (105 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was 5.96 and greater than plants at the low (210) and medium (575) light levels, which had statistically similar pH values of 5.61 and 5.49, respectively. 'Galleria Bright Violet' plants at the very-low, low, and medium light levels had statistically similar substrate pH levels of 6.12, 5.99, and 5.77.

Krug et al. (2006), determined the lower end of the acceptable range for tissue dry weight P concentration in recently mature leaves for mature zonal geraniums is 0.17 to 0.19 %. Although substrate pH decreased with increasing light, tissue P was deficient in all treatments except 'Designer Dark Red' plants grown at low light, which was marginal (Fig. 1). Acidification due to P deficiency is a widespread phenomenon among dicotyledonous species (Marshner, 1995). Phosphorus deficiency was also visually apparent in this experiment and expressed as interveinal and marginal yellowing and reddening of the lower leaves.

It is unclear why the increasing light levels caused the observed drop in substrate pH. In addition to high light intensity and P deficiency, larger root systems producing more acidity is possible, since dry weights of shoots increased with increasing light intensity for both cultivars (Table 1). A fourth possibility could be the increased number of fertigations (Table 2). As fertigation rate increases, lime may have been leached more quickly, causing a more rapid drop in substrate pH.

Experiment 2. All interactions were significant for substrate pH. At 7 DAT, substrate pH decreased with each increase in P rate for all light treatments (Fig. 2). Triple superphosphate was the P source and is composed primarily of monocalcium phosphate hydrate. A saturated solution of monocalcium-phosphate-hydrate has a pH of 1.0 to 1.5 indicating higher rates will cause lower initial substrate pH (Khasawneh et al., 1978). The effect of P rate on

substrate pH diminished after 7 DAT and was gone by 35 DAT. At all light intensities, substrate pH of plants grown without P began to decrease immediately and pH continued to decline for the length of the study. Plants that did not receive P had the lowest P % in all cases and would be expected to have the highest acidification rate due to the severity of the P deficiency. This effect did not occur and is most likely due to reduced plant growth (Table 3). Small, severely stunted plants would have less effect on rhizosphere acidification than larger plants with mild to moderate P deficiency. Plants grown with zero P were small in overall size, younger leaves were small and dark green, and older leaves were reddish, chlorotic, and necrotic, and eventually turned completely necrotic and senesced.

At 63 DAT under low light, substrate pH of plants grown with 0.065 P was 5.30 and lower than the substrate pH of all other plants (Fig. 2). Plants grown at low light and the 0.065 P rate had recently mature leaf P % of 0.14 at 63 DAT, which is below the accepted lower limit (0.17 – 0.19 %) (Table 3). All other P treatments at low light, except zero P, had recently mature leaf P % above 0.19 % and had the highest substrate pH, suggesting low pH was due to P deficiency. At 63 DAT under medium light intensity, substrate pH of plants that received the highest or lowest P rate were greater than the substrate pH of all intermediate P rates. All plants that received intermediate P rates had recently mature leaf P % that were below the lower limit, which again indicates that P deficiency caused substrate acidification (Table 3). The effect of P rate on substrate pH at high light intensity again showed the same effect. At 63 DAT, when the 0 P rate is excluded, substrate pH decreased from 5.74 to 4.87

as P rate decreased. Additionally, tissue P was deficient in all treatments except at the 0.52 P rate.

The light treatment x P rate interaction was significant for dry weight P % of recently mature leaves, shoot dry weight, and total shoot P (Table 3). Phosphorus concentration of recently mature leaves was significantly lower when plants were grown at high light compared to low light at all P rates and was lower than when plants were grown at medium light at the 0.065 and 0.52 P rate. This suggests that high light intensity suppressed P uptake. The dry weight P % of older leaves was not affected by the light x P rate interaction and also decreased significantly as light intensity increased (Table 4). Which again suggests that high light suppressed P uptake. Phosphorus content of older leaves also increased significantly with P rate.

Shoot dry weight was unaffected by light treatment when plants received no added P. At all other P rates, plants grown at low light weighed less than plants grown at medium and high light (Table 3). Plants grown at medium light weighed less than plants grown at high light at the 0.065 and 0.26 P rate. In contrast, the total amount of P in shoots was not significantly affected by light treatment at any P rate except 0.52 (Table 3). Thus, with the exception of low light at 0.52 P, the rate of P uptake per gram dry weight plant decreased with increasing light intensity. Since increased P uptake did not correspond with increased growth, P

concentration may have been lower due to growth dilution when plants increased in size due to higher light intensity.

An additional factor that could have caused lower tissue P % at higher light intensities was the higher number of fertigations required with increasing light intensity (Table 2). Higher number of fertigations could have caused increased leaching of preplant P, making less available for plant uptake. However, this does not appear to be true since the amount of P in pour-through substrate extracts averaged over all dates was unaffected by light treatment with the 0.065 and 0.52 P treatments (Table 5). Also, at the 0.13 P treatment, substrate extracts from plants grown at high and medium light intensity were statistically similar. At the 0.26 P rate, pour-through extract P concentrations did decrease significantly with increasing light intensity. This trend is apparent with other P treatments when statistics are not applied. The possibility that increased irrigations was responsible for lower tissue P seems likely; however, the effect of light on tissue P is more assertable than the effect on soil P. Phosphorus in pour-through soil extracts was greatest at 14 DAT for all P rates except zero, which did not change. Since higher light intensity indirectly increases irrigation frequency and potentially causes greater lime leaching, this could also be a contributing factor to SPD in a commercial setting.

Experiment 3. The data from experiments one and two indicate P deficiency causes substrate acidification by geranium and also indicates that high light intensity suppresses P uptake by

roots. The following experiment verifies that high light intensity suppresses P uptake. There was no significant effect of light treatment on pH of hydroponic solution, shoot and whole plant dry weight, or root and shoot P %. Mean values at high (1100 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and control (550 $\mu mol \cdot m^{-2} \cdot s^{-1}$) light levels were 5.68 and 5.34 for pH, 7.75 and 6.61 g for shoot dry weight, 8.80 and 7.38 g for total dry weight, 1.48 and 1.48 % for root P, and 0.60 and 0.62 % for shoot P, respectively. When plants were grown at the control light intensity, root dry weight was significantly lower than plants grown at high light (Table 6). Cumulative P uptake per gram root and rate of P uptake per gram root per day were significantly lower when plants were grown at the high light intensity compared to the control (Table 6). In two studies with tomato and one with Boston fern, increasing light from 250-750, 70-300, and 50-150 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively, caused P uptake to increase (Groot et., 2001; Magalhaes and Wilcox, 1983; Nowak et al., 2002). A study by Ming et al. (1999) showed that P uptake by *Dendrobium nobile* Lindl. increased 74 % when light intensity increased from 85 to 335 $\mu mol \cdot m^{-2} \cdot s^{-1}$ but decreased 29 % when light intensity increased from 335 to 840. These data indicate that increasing light intensity causes P uptake to increase, but there is a limit to this effect. Once this limit is reached, increasing light has a negative effect on P uptake. Experiment three verified this effect of supraoptimal light with geranium.

The main effects of DAT are also shown in Table 6 and as DAT increased, root dry weight increased and P remaining in the hydroponic tanks, cumulative P uptake per root weight, and P uptake per root weight per day decreased.

Conclusion

Clearly, high light intensity and P deficiency can be involved in SPD of geranium. It is not clear, however, if high light intensity contributes to substrate acidification if P is adequate. Further investigation of this question is warranted. In experiments one and two, when tissue P was below the range of 0.17 to 0.19 %, substrate pH dropped 0.6 to 1.0 pH units within a two week period. Phosphorus deficiency also occurred more often under the highest light intensity of 1020 $\mu mol \cdot m^{-2} \cdot s^{-1}$. In experiment three, increasing light from 500 to 1100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ decreased cumulative P and the rate of P uptake by 20 %, indicating excessive light has the potential to cause P deficiency in geranium. The daily light integral during the spring for most of the continental US ranges from 25 to 50 $mol \cdot m^{-2} \cdot d^{-1}$ (Fisher and Runkle, 2004). Eight hours per day of the high light treatments in this study provided a daily light integral of 29.4 (experiments one and two) and 31.7 (experiment three), which were below some of the levels experienced during spring geranium production. The recommended light intensity for the production of zonal geraniums ranges from 700 to 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fischer USA, 2007; Hamrick, 2003). These intensities are lower than the high light intensities used in this study and it is highly likely during normal geranium production that light will exceed these recommendations, especially in the late spring and in the southern regions of the US. To help prevent SPD, geranium growers should avoid P deficiency, which was shown in this study to cause substrate acidification. If possible, growers should also avoid extended periods of high intensity light, which can suppress P uptake and cause substrate acidification.

Additionally since other factors are likely involved with SPD, substrate pH must be regularly monitored to identify possible oncoming pH problems that can be corrected by application of flowable limestone.

Literature Cited

Bachman, G.R. and W.B. Miller. 1995. Iron chelate inducible iron/manganese toxicity in zonal geranium. J. Plant Nutr. 18:1917-1929.

Broschat, T. and K. Moore. 2004. Phytotoxicity of several iron fertilizers and their effects on Fe, Mn, Zn, Cu, and P content of African marigolds and zonal geraniums. HortScience. 39:595-598.

Cakmak, I. and H. Marschner. 1990. Decrease in nitrate uptake and increase in proton release in zinc deficient cotton, sunflower, and buckwheat plants. Plant and Soil. 129:261-268.

De Groot, C.C., L.F. M.Marcelis, R. Van Den Boogaard, H. Lambers. 2001. Growth and dry-mass partitioning in tomato as affected by phosphorus nutrition and light. Plant, Cell & Enviro. 24:1309-1317.

Fisher, P. and E. Runkle. 2004. Lighting up profits. Understanding greenhouse lighting. Meister Media Worldwide. OH.

Fischer USA. 2007. Producing high quality geraniums. Fischer Cultural Guide 2007-2008. 57-74. CO.

Hamrick, D. 2003. Ball Redbook 17th Edition. Volume 2: Crop Production. Ball Publishing. IL.

Hinsinger, P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. Plant and Soil. 237:173-195.

Khasawneh, F. E., E.C. Sample, and E.J. Kamprath. 1978. The role of phosphorus in agriculture. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, WI.

Krug, B.A., B.E. Whipker, B. Cleavland, and I. McCall. 2006. Taking the "art" out of interpreting tissue analysis for geranium. Proc. of the Southern Nursery Assn. Res. Conf. 15:635-639.

Landsberg, E. 1981. Organic acid synthesis and release of hydrogen ions in response to Fe deficiency stress of mono- and dicotyledonous plant species. J. Plant Nutr. 3: 579-591

Lee, C.W., J.M. Choi, and C.H. Pak. 1996. Micronutrient toxicity in seed geranium (*Pelargonium x hortorum* Bailey). J. Amer. Soc. Hort. Sci. 121:77-82.

Magalhaes, J.R., G.E. Wilcox. 1983. Tomato growth and mineral composition as influenced by nitrogen form and light intensity. J. Plant Nutr. 6:847-862.

Marschner, H. 1995. Mineral nutrition of higher plants. Academic Press. NY.

Ming, Z., B. ZhiLong, C. ShiJiang, W. ZhaoLiang, Z. YuJin, Z. LiQuan, L. QuanSen. 1999. Effects of temperature and light intensity on P uptake in dendrobe (*Dendrobium nobile* Lindl.). J. of Southwest Ag. University. 21:220-222.

Murphy, J. and Riley, J.P. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chem. Acta 27:31–36.

Naegle, E.R., J.W. Burton, T.E. Carter, and T.W. Rufty. 2005. Influence of seed nitrogen content of seedling growth and recovery form nitrogen stress. Plant and Soil. 271:329-340.

Nelson P.V. 2003. Greenhouse operation and management 6th edition. Prentice Hall, NJ.

Nowak, J. S. Sroka, B. Matysiak. 2002. Effects of light level, CO_2enrichment, and concentration of nutrient solution on growth, leaf nutrient content and chlorophyll fluorescence of Boston fern microcuttings. J. Plant Nutr. 25:2161-2172.

Schjorring, J.K. 1986. Nitrate and ammonium absorption by plants growing at a sufficient or insufficient level of phosphorus in nutrient solution. Plant and Soil. 91:313-318.

Smith, B.R., P.R. Fisher, and W.R. Argo. 2004. Water-soluble fertilizer concentration and pH of a peat-based substrate affect growth, nutrient uptake, and chlorosis of container-grown seed geraniums. J. Plant Nutr. 27:497-524

United States Department of Agriculture (USDA). 2005. Floriculture Crop Summary. National Agriculture Statistics Service. Sp. Cir 6-1(05). Washington, D.C.

Welkie, G.W. and G.W. Miller. 1993. Plant iron uptake physiology by nonsiderophore systems. pp. 345-369. In: Barton, L. L. and B. C. Hemming Eds. Iron chelation in plants and soil microorganisms. Academic Press, Inc., NY.

White, J.W. 1991. Geraniums IV. The grower's manual. Ball Publishing. IL.

Wright, R.D. 1986. The pour-through nutrient extraction procedure. HortScience. 21:227-229.

Table 1. Main effects of light and cultivar on dry weights of shoots of plants 63 DAT from experiment 1.

Light treatment	Dry weight (g)
Very-low light (105 µmol·m^{-2}·s^{-1})	4.3 d[z]
Low light (210)	10.6 c
Medium light (575)	20.8 b
High light (1020)	27.2 a
Cultivar	
Designer Dark Red	17.8 a
Galleria Bright Violet	13.6 b

[z] Mean separation by Duncan's multiple range test at $p = 0.05$.

Table 2. Number of fertigations for each light treatment for experiments 1 and 2.

	Experiment 1	Experiment 2
Light treatment	----Number of fertigations----	
Very-low light (105 $\mu mol \cdot m^{-2} \cdot s^{-1}$)	19	-
Low light (210)	20	17
Medium light (575)	34	27
High light (1020)	41	37

Table 3. Dry weight % P of recently mature leaves, shoot dry weight, and total shoot P determined from recently mature leaf P concentration from plants grown at low, medium, and high light 63 days after transplanting for experiment 2.

	----------P rate ($g \cdot L^{-1}$ substrate)----------				
	0	0.065	0.13	0.26	0.52
Light treatment	----------% P----------				
Low (210 $\mu mol \cdot m^{-2} \cdot s^{-1}$)	0.08 a	0.14 a	0.24 a	0.30 a	0.36 a
Medium (575)	0.07 ab	0.08 b	0.13 b	0.17 b	0.26 b
High (1020)	0.06 b	0.07 c	0.09 b	0.12 b	0.20 c
	----------Shoot weight (g)----------				
Low (210 $\mu mol \cdot m^{-2} \cdot s^{-1}$)	1.86 a	8.44 c	8.52 b	9.56 c	6.84 b
Medium (575)	1.53 a	14.96 b	17.05 a	17.16 b	18.40 a
High (1020)	1.42 a	18.38 a	20.13 a	21.22 a	22.19 a
	----------Total shoot P (g)----------				
Low (210 $\mu mol \cdot m^{-2} \cdot s^{-1}$)	0.12 a	1.21 a	2.04 a	2.87 a	2.48 b
Medium (575)	0.12 a	1.27 a	2.13 a	2.87 a	4.71 a
High (1020)	0.08 a	1.22 a	1.84 a	2.58 a	4.40 a

[z] Mean separation (in columns) by Duncan's multiple range test.

Table 4. Main effects of light and P rate on dry weight % P of oldest leaves 63 days after transplanting from experiment 2. Plants from the 0 P rate were insufficient for sampling.

Light treatment	% P
Low (210 µmol·m^{-2}·s^{-1})	0.24 a[z]
Medium (575)	0.18 b
High (1020)	0.13 c
P rate (g·L^{-1} substrate)	
0.065	0.098 d
0.13	0.16 c
0.26	0.21 b
0.52	0.25 a

[z] Mean separation by Duncan's multiple range test at $p = 0.05$

Table 5. Main effects of light and days after transplanting (DAT) on P concentration in pour-through substrate extracts in experiment 2.

	----------P rate ($g \cdot L^{-1}$ substrate)----------				
	0	0.065	0.13	0.26	0.52
Light treatment	----------soil P ($mg \cdot L^{-1}$)----------				
Low (210 $\mu mol \cdot m^{-2} \cdot s^{-1}$)	ND[z]	2.65 a[y]	9.37 a	25.7 a	41.8 a
Medium (575)	ND	2.26 a	4.13 b	16.8 b	40.0 a
High (1020)	ND	1.09 a	3.94 b	8.33 c	28.9 a
DAT					
14	ND	6.92 a	19.1 a	54.0 a	115.4 a
28	ND	0.93 b	3.21 b	10.9 b	25.2 b
42	ND	0.13 b	0.75 b	2.19 c	5.45 c
56	ND	0.02 b	0.18 b	0.58 c	1.54 c

[z] Not detectable (ND)

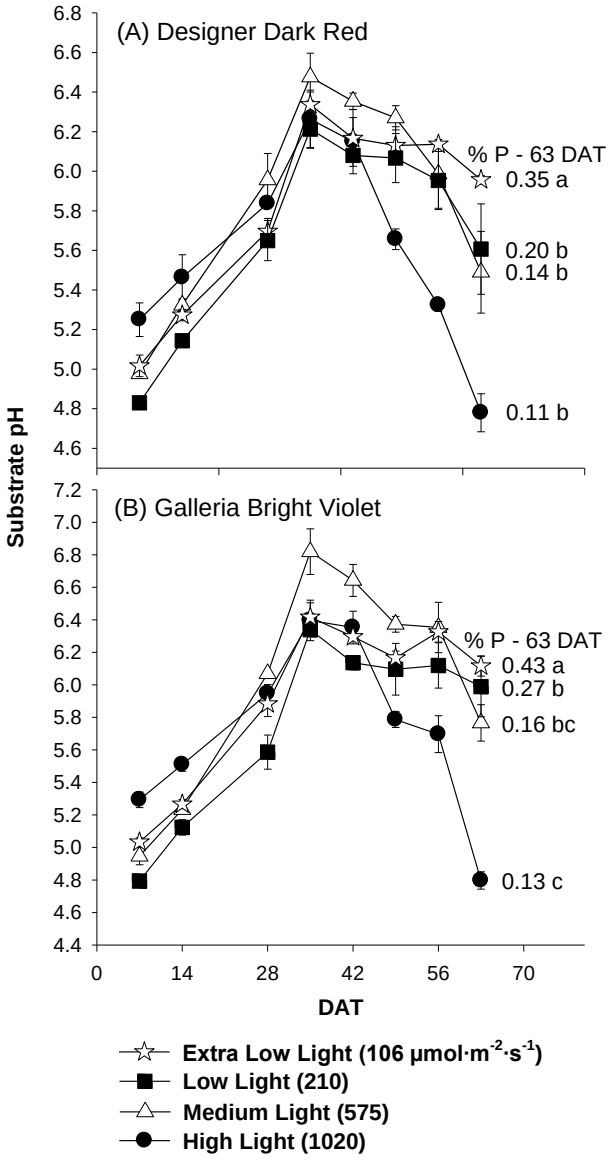
[y] Mean separation (in columns) by Duncan's multiple range test

Table 6. Main effect of light treatment and days after transplanting on root dry weight, the remaining P of 124 mg in 4 L of hydroponic solution, cumulative P uptake per gram dry weight of root, and P uptake per gram dry weight of root per day in experiment 3.

	Root dry weight	P remaining	P uptake per dry root weight	P uptake per root dry weight per day
Light treatment	--g--	--mg--	--mg·g^{-1}--	--mg·g^{-1}·d^{-1}--
Control (500 µmol·m^{-2}·s^{-1})	0.767	62.8	86.3	6.96
High (1100)	1.05	53.6	69.6	5.55
Significance	*	NS	**	**
Days After Transplanting				
11	0.538	78.7	87.3	7.94
15	1.28	37.7	68.5	4.57
Significance	***	**	**	***

NS, *, **, *** Nonsignificant or significant at P = 0.05, 0.01, and 0.0001 respectively.

Figure 1. Substrate pH measured every seven days after transplanting (DAT) for 'Designer Dark Red' (A) and Galleria Bright Violet' (B) geraniums at very-low (105 µmol·m^{-2}·s^{-1}), low (210), medium (575), and high (1020) light in experiment one. Error bars represent standard error (n = 3). On the right side of each light curve is the tissue dry weight P percent 63 DAT. Mean separation by Duncan's multiple range test.


(A) Designer Dark Red
% P - 63 DAT
0.35 a
0.20 b
0.14 b
0.11 b
(B) Galleria Bright Violet
% P - 63 DAT
0.43 a
0.27 b
0.16 bc
0.13 c
Substrate pH
DAT
Extra Low Light (106 µmol·m-2·s-1)
Low Light (210)
Medium Light (575)
High Light (1020)

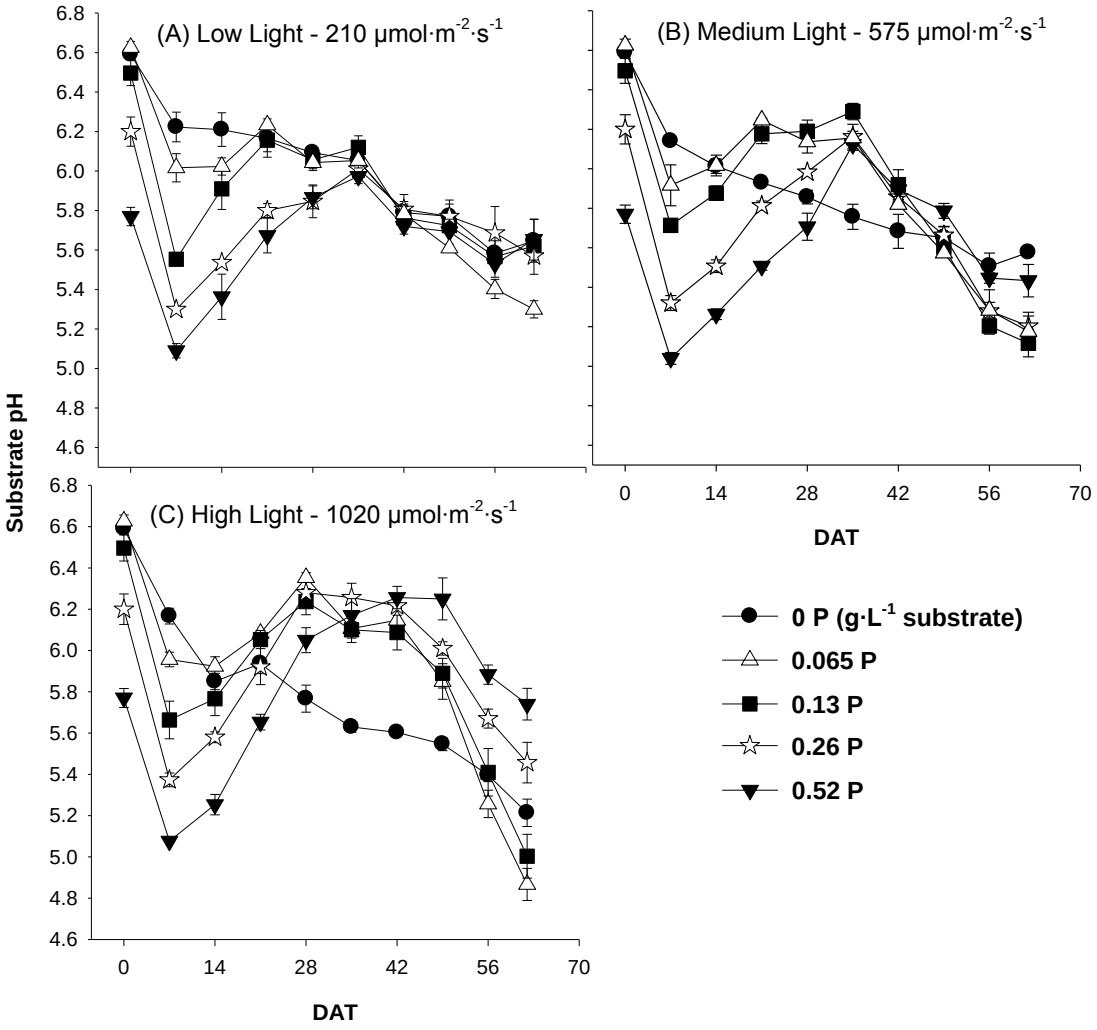


Figure 2. Substrate pH measured initially and every seven days after transplanting (DAT) for each P treatment (0, 0.065, 0.13, 0.26, and 0.52 P at $g \cdot L^{-1}$ substrate) at low (210 $\mu mol \cdot m^{-2} \cdot s^{-1}$), medium (575), and high (1020) light intensities in experiment 2. Error bars represent standard error (n = 3).

APPENDICES

Appendix A. Geranium Cultivar Survey.

Due to the lack of understanding of the cause of SPD of geranium we surveyed growers, producers, breeders, sales people, and professors during 2003 to determine tolerant and susceptible cultivars. Table 1 lists all cultivars that were mentioned to be tolerant or susceptible.

Table 1. All cultivars mentioned in 2003 survey inquiring about susceptibility to sudden pH decline. Susceptibility rating (SR) scale is 1-5 with 1 = highly susceptible, 2 = moderately susceptible, 3 = intermediate, 4 = mildly tolerant, and 5 = very tolerant. Table also includes the distribution company, series, cultivar, flower color, and type of geranium (ivy, regal, seed, or zonal).

Company	Series	Cultivar	Flower Color	Type	SR
Ball	Colorcade	purple	purple	ivy	2
Ball	Designer	dark red	dark red	zonal	1
Ball	Designer	grape improved	deep lavender	zonal	2
Ball	Designer	purple rose	purple rose	zonal	2
Ball	Designer	red	red	zonal	1
Ball	Designer	rose	rose	zonal	2
Ball	Designer	scarlet improved	scarlet	zonal	4
Ball	Designer	violet	violet	zonal	1
Ball	Designer	white	white	zonal	1
Ball	Fantasia	cardinal red improved	dark red	zonal	4
Ball	Fantasia	coral improved	coral	zonal	4
Ball	Fantasia	flame improved	bright red	zonal	4
Ball	Fantasia	flamingo rose improved	rose	zonal	4
Ball	Fantasia	neon rose	dark pink	zonal	4
Ball	Fantasia	pink shell	light pink	zonal	4
Ball	Fantasia	salmon	salmon	zonal	4
Ball	Fantasia	shocking pink	pink	zonal	4
Ball	Fantasia	strawberry sizzle	dark pink-white	zonal	2
Ball	Fantasia	violet	violet	zonal	4
Ball	Galleria	bright violet	bright violet	zonal	2
Ball	Galleria	ruby red	red	zonal	2
Ball	Showcase	dark salmon	dark salmon	zonal	2

Table 1 (continued)

Company	Series	Cultivar	Flower Color	Type	SR
Ball	Showcase	pink	pink	zonal	2
Ball	Showcase	scarlet	scarlet	zonal	2
Dumman	Atlantic	hot pink	hot pink	ivy	2
Dumman	Atlantic	salmon pink star	salmon pink	ivy	2
Dumman	Pinnacle	blue	blue	zonal	2
Dumman	Pinnacle	coral	coral	zonal	2
Dumman	Pinnacle	crimson red	crimson red	zonal	2
Dumman	Pinnacle	dark red	dark red	zonal	1
Dumman	Pinnacle	hot rose	hot rose	zonal	1
Dumman	Pinnacle	orange	orange	zonal	2
Dumman	Pinnacle	pink	pink	zonal	1
Dumman	Pinnacle	red	red	zonal	2
Dumman	Pinnacle	rose	rose	zonal	2
Dumman	Pinnacle	salmon impr.	salmon	zonal	2
Dumman	Pinnacle	soft lilac	soft lilac	zonal	2
Dumman	Pinnacle	teleman impr.	teleman	zonal	2
Dumman	Pinnacle	violet	violet	zonal	1
Dumman	Pinnacle	white	white	zonal	2
Dumman	Summit	dark red	dark red	zonal	4
Dumman	Summit	fire	fire	zonal	4
Dumman	Summit	lilac	lilac	zonal	4
Dumman	Summit	magenta	magenta	zonal	4
Dumman	Summit	pink	pink	zonal	4
Dumman	Summit	pink splash	pink splash	zonal	4
Dumman	Summit	salmon pink	salmon pink	zonal	4
Fischer	Butterfly	butterfly	light lavender	ivy	2
Fischer	Charmant	charmant	salmon	zonal	2
Fischer	Jazz	jazz	salmon blush	zonal	2
Fischer	Rocky Mountain	dark red	dark red	zonal	5
Fischer	Rocky Mountain	deep rose	deep rose	zonal	4
Fischer	Rocky Mountain	lavender	lavender	zonal	4
Fischer	Rocky Mountain	lavender pink 2001	lavender pink	zonal	4
Fischer	Rocky Mountain	light pink	light pink	zonal	4
Fischer	Rocky Mountain	light salmon	light salmon	zonal	3
Fischer	Rocky Mountain	magenta	magenta	zonal	4
Fischer	Rocky Mountain	orange	orange	zonal	4
Fischer	Rocky Mountain	red 2003	red	zonal	4
Fischer	Rocky Mountain	royal red	royal red	zonal	4

Table 1 (continued)

Company	Series	Cultivar	Flower Color	Type	SR
Fischer	Rocky Mountain	salmon 2004	salmon	zonal	4
Fischer	Rocky Mountain	salmon rose	salmon rose	zonal	5
Fischer	Rocky Mountain	scarlet	scarlet	zonal	4
Fischer	Rocky Mountain	violet	violet	zonal	4
Fischer	Rocky Mountain	white	white	zonal	4
Fischer	Schoena	helena	salmon	zonal	2
Fischer	Shiva	2003	red	ivy	4
Fischer	Tango	violet	bright violet	zonal	2
Goldsmith	Americana	dark red	dark red	zonal	2
Goldsmith	Elite	red	red	seed	2
Goldsmith	Maverick	apple blossom	white-pink	seed	2
Goldsmith	Maverick	coral	coral	seed	2
Goldsmith	Maverick	lavender	lavender	seed	2
Goldsmith	Maverick	light salmon	light salmon	seed	2
Goldsmith	Maverick	pink	pink	seed	2
Goldsmith	Maverick	quiksilver	pink	seed	2
Goldsmith	Maverick	red	red	seed	2
Goldsmith	Maverick	salmon	salmon	seed	2
Goldsmith	Maverick	scarlet	scarlet	seed	2
Goldsmith	Maverick	star	white-dark pink	seed	2
Goldsmith	Maverick	violet	violet	seed	2
Goldsmith	Maverick	white	white	seed	2
Goldsmith	Orbit	apple blossom	pink	seed	2
Goldsmith	Orbit	cardinal	red	seed	2
Goldsmith	Orbit	cherry	cherry	seed	2
Goldsmith	Orbit	coral	coral	seed	1
Goldsmith	Orbit	coral	coral	seed	2
Goldsmith	Orbit	deep salmon	deep salmon	seed	2
Goldsmith	Orbit	glow	dark pink	seed	2
Goldsmith	Orbit	hot pink	hot pink	seed	2
Goldsmith	Orbit	light salmon	light salmon	seed	2
Goldsmith	Orbit	orchid	orchid	seed	2
Goldsmith	Orbit	pink	pink	seed	2
Goldsmith	Orbit	red	red	seed	2
Goldsmith	Orbit	red	red	seed	2
Goldsmith	Orbit	rose	rose	seed	2
Goldsmith	Orbit	salmon	salmon	seed	2
Goldsmith	Orbit	scarlet	scarlet	seed	2

Table 1 (continued)

Company	Series	Cultivar	Flower Color	Type	SR
Goldsmith	Orbit	scarlet eye	red-white	seed	2
Goldsmith	Orbit	violet	violet	seed	2
Goldsmith	Orbit	white	white	seed	2
Oglevee	Classic Garden Collection	aurora	dark magenta	zonal	1
Oglevee	Classics	nicole	candy pink	ivy	2
Oglevee	Elegance	ballet	pink-hot pink	regal	4
Oglevee	Elegance	baroness	deep rosy red	regal	2
Oglevee	Elegance	bravo	cerise-white	regal	4
Oglevee	Elegance	burgundy	burgundy	regal	4
Oglevee	Elegance	camelot	rich purple-light purple	regal	4
Oglevee	Elegance	dandy	hot pink-purple	regal	4
Oglevee	Elegance	excalibur	white-rich rose	regal	4
Oglevee	Elegance	fascination	pink-rose-white	regal	4
Oglevee	Elegance	imperial	dark purple-white	regal	4
Oglevee	Elegance	jewel	magenta-white	regal	4
Oglevee	Elegance	lavender	lavender	regal	4
Oglevee	Elegance	light lavender splash	lilac-purple	regal	4
Oglevee	Elegance	pink chiffon	soft pink	regal	4
Oglevee	Elegance	purple bicolor	deep purple-white	regal	4
Oglevee	Elegance	raspberry swirl	rich raspberry-white	regal	4
Oglevee	Elegance	rose bicolor	rose-pink-cranberry	regal	4
Oglevee	Elegance	royalty white	white-rose	regal	4
Oglevee	Elegance	silver	white-violet	regal	4
Oglevee	Euro Collection	sassy dark red	dark red	zonal	2
Oglevee	Euro Collection	shocking violet	clear magenta	zonal	2
Oglevee	Fireball	fireball	deep scarlet	zonal	1
Oglevee	Global	deep lilac	lilac-orchid	ivy	2
Oglevee	Global	red	red	ivy	4
Oglevee	Global	ruby red	dark red	ivy	4
Oglevee	Global	salmon rose	rose-salmon	ivy	2
Oglevee	Patriot	bright pink	candy pink	zonal	2
Oglevee	Patriot	bright red	intense red	zonal	2
Oglevee	Patriot	bright violet	intense purple	zonal	2
Oglevee	Patriot	cherry rose	bright rose	zonal	2

Table 1 (continued)

Company	Series	Cultivar	Flower Color	Type	SR
Oglevee	Patriot	cranberry red	dark red	zonal	2
Oglevee	Patriot	evening glow	deep salmon	zonal	1
Oglevee	Patriot	kin	rich scarlet	zonal	3
Oglevee	Patriot	peach	mid salmon	zonal	1
Oglevee	Patriot	pink x2	mid salmon	zonal	2
Oglevee	Patriot	red	scarlet	zonal	2
Oglevee	Patriot	rose pink	vibrant lavender pink	zonal	2
Oglevee	Patriot	salmon	medium salmon	zonal	2
Oglevee	Patriot	sincerely yours	scarlet	zonal	2
Oglevee	Patriot	soft pink	pastel pink	zonal	2
Oglevee	Patriot	veronica	bright rose	zonal	2
Oglevee	Patriot	victoria	bright scarlet	zonal	2
Oglevee	Patriot	watermelon	warm coral	zonal	2
Oglevee	Patriot	white	white	zonal	2
S&G	Pinto	white	white	seed	2

Appendix B

Impact of Low Iron and Phosphorus Stresses on Acidification of Nutrient Solution by Geranium

Low Fe and low P can cause many plant species to acidify the substrate. Research was done to determine if low Fe or P stresses caused four geranium (*Pelargonium x hortorum* Bailey) cultivars to acidify nutrient solution. Two cultivars were susceptible (Patriot Salmon and Aurora) and two resistant (Rocky Mountain Dark Red and Rocky Mountain Salmon Rose) to substrate acidification based on a grower survey. Rooted geranium cuttings were transferred to 4 L containers containing modified Hoagland's solution with N supplied as 15% NH_4 and 85% NO_3. The plants were grown in a greenhouse for 44 days. Treatments consisted of a complete nutrient solution and two similar solutions devoid of either Fe or P. Solutions pH was set at 5.8, changed weekly, and tested 3 and 6 days after each change. Since all cultivars showed similar responses, results were combined. Twenty days after transplanting (DAT) plants in all treatments, including control, caused solution pH to fall below 5. At 37 DAT solution pH levels for control, minus Fe, and minus P treatments were 4.1, 3.7, and 3.6, respectively (Fig. 1). Results indicated that geranium is an acidifying plant when N is supplied as 15% NH_4 and 85% NO_3. Additionally, low Fe and low P stresses increase the acidification rate. Root and shoot dry weights of minus P plants were significantly lower that

weights of minus Fe plants (Tables 2 and 3). This indicated that plants under P stress had a higher specific rate of acidification than plants under Fe stress.

Table 2. Main effects of nutritional treatment on root dry weight.

Nutritional treatment	Dry weight (g)
Control	3.17 a[z]
Minus Phosphorus	2.34 b
Minus Iron	3.07 a
Cultivar	
Aurora	3.72 a
Patriot Salmon	2.59 b
Rocky Mountain Dark Red	2.64 b
Rocky Mountain Salmon Rose	2.48 b

[z]Mean separation by Duncans multiple range test.

Table 3. Main effects of nutritional treatment on shoot dry weight

Nutritional treatment	Dry weight (g)
Control	26.18 a[z]
Minus Phosphorus	12.18 b
Minus Iron	25.83 a
Cultivar	
Aurora	28.37 a
Patriot Salmon	19.16 b
Rocky Mountain Dark Red	19.16 b
Rocky Mountain Salmon Rose	18.91 b

[z] Mean separation by Duncans multiple range test.

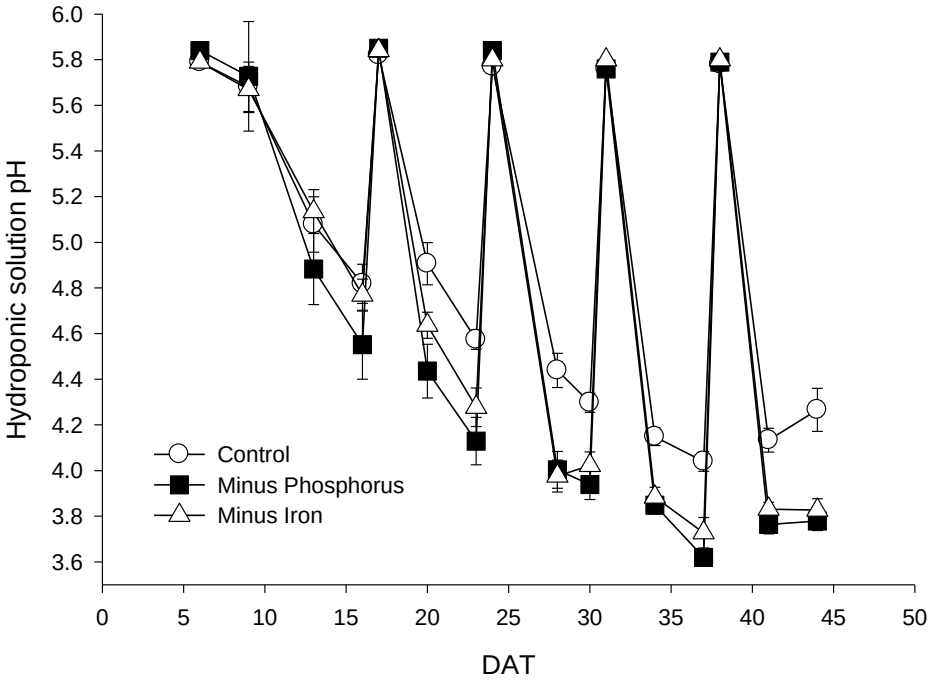


Figure 1. Hydroponic solution pH from plants grown with complete nutrient solution and two similar solutions devoid of either phosphorus or iron 6, 9, 13, and 16 days after transplanting (DAT) and 3 and 6 days after weekly hydroponic solution changes that started 17 DAT. When solutions were changed pH was set at 5.8. Error bars were determined using the standard error function (n = 3) of SigmaPlot (SPSS Inc., Chicago, IL).

Appendix C

Impact of Water Stress on Substrate Acidification by Geranium

It has been shown that plants with higher amounts of nitrate in the tissue are more susceptible to water stress. During periods of water stress plants may reduce nitrate uptake, which could increase the cation:anion uptake ratio and lead to substrate acidification. Geraniums (*Pelargonium x hortorum* Bailey 'Designer Dark Red') were grown in a 3:1 peat perlite substrate mix in a greenhouse for 60 days and fertilized once a week with N (10% NH_4^+ and 90 % NO_3^-) and K at 210 and 176 $\mu g \cdot L^{-1}$, respectively. All other nutrients were applied preplant. Irrigation treatments were established by irrigating the plants when soil moisture content reached 30 (wet), 22 (medium), or 14% (dry) by volume and determined with a WET sensor - WET 2 (Delta-T Devices Ltd., Cambridge, United Kingdom). Dry weight of shoots increased significantly from 19.0, 43.6, and 47.6 g per plant as irrigation treatment increased from 14, 22, or 30% by volume, respectively (Table 4). Irrigation treatment had no effect on substrate pH (Fig. 2), indicating that water stress is not the cause of sudden substrate pH decline of geranium.

Table 4. Main effects water treatment on shoot dry weight 60 days after transplanting.

Water treatment	Dry weight (g)
Wet	47.6 a[z]
Medium	43.6 b
Dry	19.0 c

[z] Mean separation by Duncans multiple range test.

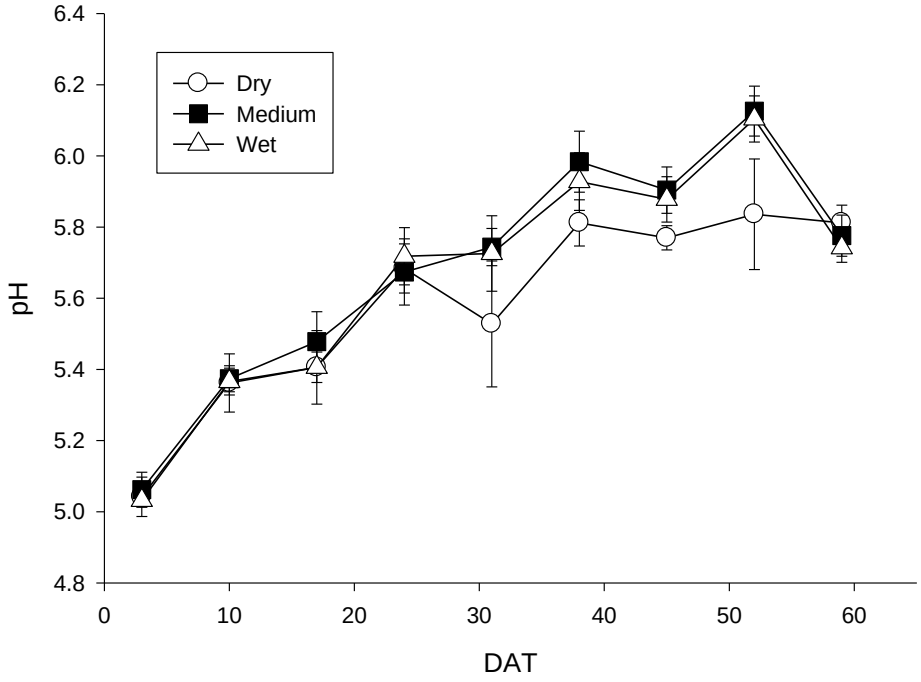


Figure 2. Substrate pH every 7 days starting 3 days after transplanting (DAT) determined by pour-through technique from plants grown under three irrigation treatments established by irrigating the plants when soil moisture content reached 30 (wet), 22 (medium), or 14% (dry) by volume. Error bars were determined using the standard error function (n = 3) of SigmaPlot (SPSS Inc., Chicago, IL).