

ABSTRACT

WILLIAMS, AMY LYNN. Foliar symptomology and tissue concentrations of five nutritionally deficient floriculture crops. (Under the direction of Paul V. Nelson.)

Tissue analysis standards and complete visual deficiency symptoms of N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, and B are crucial for monitoring plant nutrient status. Foliar analysis standards and visual symptoms of nutritional disorders for calibrachoa, angelonia, bracteantha, brachyscome 'Mini Yellow', and brachyscome 'Jumbo Mauve' have not been published and were the objectives of this study. These plants were grown hydroponically in a glass greenhouse at 35°N latitude. Nutrient treatments were based on the macronutrient composition of Hoagland's all-nitrate nutrient solution with altered micronutrient levels and 11 additional related solutions, each devoid of one essential nutrient. Visual symptoms were chronologically recorded and photographed.

Synoptic visual deficiency symptoms were as follows: N – Plants were stunted with smaller leaves. As symptoms progressed the plants developed a light green chlorosis and the lowest leaves developed a yellow chlorosis followed by a brown necrosis. P – Plants were smaller and all the foliage developed a dark green pigmentation, which progressed into a necrosis of the lower mature leaves. K – Plants develop chlorosis of the leaf tips and margins, which quickly progress into necrosis. Ca – Severe stunting and compactness would result, accompanied by chlorosis and necrosis of the shoot tips. Flowering would cease or be incomplete. Mg – Recently mature and mature leaves would develop a uniform or interveinal chlorosis, which would progress from a light green to yellow, and

then turn brown. S – Plants would be severely stunted and then develop a uniform lime-green chlorosis. Fe – A light green chlorosis would progress from the shoot tips to the mature leaves, which would progress into a light yellow followed by a white chlorosis and brown necrosis. Mn – A light green chlorosis of the entire plant, which would often be smaller in size. Necrosis would affect the recently mature leaves. Zn – Young leaves would develop a light green chlorosis and be slightly puckered. Cu – Plants were small and developed a blue-green pigmentation. Severe twisting and rolling of the young leaves was observed. B – Extreme rosetting and deformation of the shoot tips and young leaves resulted in short compact plants. Foliage was deep green and glossy with a thick, leathery texture.

The rate at which symptoms occurred is an indication of the species sensitivity to a particular nutrient deficiency. The chronological order in which nutrient deficiency symptoms occurred was as follows: that were first to occur by species were as follows: Calibrachoa – Fe, Ca, Mn, N, S, B, K, P, Cu, Zn and Mg. Angelonia – Ca, Fe, K, N, P, S, Cu, Mn, B, Zn and Mg. Bracteantha – Fe, Ca, B, K, N, P, Mg, S, Mn, Zn and Cu. Brachyscome ‘Mini Yellow’ – Fe, Ca, N, P, B, Mn, S, Mg, Cu, Zn and K. Brachyscome ‘Jumbo Mauve’ – Fe, N, B, Ca, P, K, S, Mg and Cu. Brachyscome ‘Jumbo Mauve’ plants were extremely resistant to Mn and Zn, because these symptoms did not appear during this trial and are not reported in the text.

**FOLIAR SYMPTOMOLOGY AND TISSUE CONCENTRATIONS OF FIVE
NUTRITIONALLY DEFICIENT FLORICULTURE CROPS**

By

AMY LYNN WILLIAMS

A thesis submitted to the Graduate Faculty of
North Carolina State University
In partial fulfillment of the
Requirements for the Degree of
Master of Science

HORTICULTURAL SCIENCE

Raleigh

2004

APPROVED BY:

Chair of Advisory Committee

BIOGRAPHY

Amy Lynn Williams was born and raised in Salisbury, Maryland to Martha H. Williams and Robert L. Williams, Jr. on May 6, 1978. She grew up with her younger sister Heather in Wicomico County, Maryland, near the Chesapeake Bay and Ocean City. She graduated from Parkside High School, Salisbury, Maryland in May 1996. Amy graduated with a B. S. degree in Biology at Salisbury State University, Salisbury, Maryland in May 2000. Following graduation she was employed by the USDA, Natural Resource Conservation Service, as a Biological Science Technician from June 2000 to October 2001. She also worked for CJZ Photography and Gee Wiz Design, as a photographer and graphic designer from January 1999 to October 2001. In January 2002, she began graduate studies in the Department of Horticultural Science at North Carolina State University, Raleigh, under the direction of Dr. Paul V. Nelson. Currently, Amy is completing the requirements for M.S. degree in the floriculture program under the direction of Drs. Paul V. Nelson, Brian E. Whipker, and John. M. Dole.

ACKNOWLEDGEMENTS

I would like to say a special thanks to Dr. Paul Nelson for offering such wonderful guidance, wisdom and dedication. It was a pleasure working with someone so devoted to his research and students. Thanks to Dr. Brian E. Whipker for reviewing and editing my never-ending flow of papers, I learned a lot from your precise direction. I truly appreciate all the opportunities Dr. Nelson and Dr. Whipker offered me during the time spent at NCSU. Thanks to Dr. John Dole for always having an open door and the key to all the proper plant names and Dr. Dan Bowman who served wonderfully on my committee. Thank you to Joseph Skipper and Suzi Gibson who helped with the manual labor, lab work and offered good conversation. I would like to thank Nancy Mingis for being a great friend, who was prepared to help and always had time to listen. A special thanks to James Gibson who helped me get my feet wet, I will always remember your friendship and supportive nature.

I would also like to give a special thanks to Martha & Robert Williams my mother and father. Without your visits and words of encouragement I would have been lost, I can see the pride in your eyes. Heather Williams my sister was always a phone call away and Hydie who kept me company and my feet warm. Thanks to my family and friends who have helped make this possible. Thanks goes out to Dr. Paul Kuk, a family friend who loaned me his computer on which all my work was done. I would have never come this far without his help. Thank you all for the many levels of support you have given, which blossomed into success. You all helped make this dream come true.

Thanks most of all to Andy my love, without his dedication, laughter, and arms to come home to, I would have been incomplete. I look forward to April 4th, 2004 our wedding day.

TABLE OF CONTENTS

	Page
LIST OF TABLES.....	vii
LIST OF FIGURES.....	viii
CHAPTER 1. FOLIAR SYMPTOMOLOGY AND TISSUE CONCENTRATIONS OF NUTRIENT DEFICIENT <i>CALIBRACHOA</i> PLANTS	1
ABSTRACT	1
OBJECTIVE	2
MATERIAL AND METHODS	3
RESULTS AND DISCUSSION.....	5
REFERENCES.....	15
CHAPTER 2. FOLIAR SYMPTOMOLOGY AND TISSUE CONCENTRATIONS OF NUTRIENT DEFICIENT <i>ANGELONIA</i> PLANTS	19
ABSTRACT	19
OBJECTIVE	20
MATERIAL AND METHODS	20
RESULTS AND DISCUSSION.....	21
REFERENCES.....	32
CHAPTER 3. FOLIAR SYMPTOMOLOGY AND TISSUE CONCENTRATIONS OF NUTRIENT DEFICIENT <i>BRACTEANTHA</i> PLANTS	36
ABSTRACT	36
OBJECTIVE	37
MATERIAL AND METHODS	37
RESULTS AND DISCUSSION.....	38
REFERENCES.....	50
CHAPTER 4. FOLIAR SYMPTOMOLOGY AND TISSUE CONCENTRATIONS OF NUTRIENT DEFICIENT <i>BRACHYSCOME</i> ‘MINI YELLOW’ PLANTS.....	54
ABSTRACT	54
OBJECTIVE	55
MATERIAL AND METHODS	55
RESULTS AND DISCUSSION.....	56
REFERENCES.....	67

CHAPTER 5. FOLIAR SYMPTOMOLOGY AND TISSUE CONCENTRATIONS OF NUTRIENT DEFICIENT <i>BRACHYSCOME</i> ‘JUMBO MAUVE’ PLANTS.....	71
ABSTRACT.....	71
OBJECTIVE.....	72
MATERIAL AND METHODS.....	72
RESULTS AND DISCUSSION.....	73
REFERENCES.....	82
SUMMARY	86
REFERENCES	92

LIST OF TABLES

CHAPTER 1. FOLIAR SYMPTOMOLOGY AND TISSUE CONCENTRATIONS OF NUTRIENT DEFICIENT *CALIBRACHOA* PLANTS

Table 1. Tissue nutrient concentrations at initial and advanced stages of deficiency in recently matured leaves of hydroponically grown calibrachoa16

CHAPTER 2. FOLIAR SYMPTOMOLOGY AND TISSUE CONCENTRATIONS OF NUTRIENT DEFICIENT *ANGELONIA* PLANTS

Table 1. Tissue nutrient concentrations at initial and advanced stages of deficiency in recently matured leaves of hydroponically grown angelonia33

CHAPTER 3. FOLIAR SYMPTOMOLOGY AND TISSUE CONCENTRATIONS OF NUTRIENT DEFICIENT *BRACTEANTHA* PLANTS

Table 1. Tissue nutrient concentrations at initial and advanced stages of deficiency in recently matured leaves of hydroponically grown bracteantha51

CHAPTER 4. FOLIAR SYMPTOMOLOGY AND TISSUE CONCENTRATIONS OF NUTRIENT DEFICIENT *BRACHYSCOME* ‘MINI YELLOW’ PLANTS

Table 1. Tissue nutrient concentrations at initial and advanced stages of deficiency in recently matured leaves of hydroponically grown brachyscome ‘Mini Yellow’68

CHAPTER 5. FOLIAR SYMPTOMOLOGY AND TISSUE CONCENTRATIONS OF NUTRIENT DEFICIENT *BRACHYSCOME* ‘JUMBO MAUVE’ PLANTS

Table 1. Tissue nutrient concentrations at initial and advanced stages of deficiency in recently matured leaves of hydroponically grown brachyscome ‘Jumbo Mauve’83

LIST OF FIGURES

CHAPTER 1. FOLIAR SYMPTOMOLOGY AND TISSUE CONCENTRATIONS OF NUTRIENT DEFICIENT *CALIBRACHOA* PLANTS

Figure 1. Days to develop initial deficiency symptoms for calibrachoa17

Figure 2. Dry weight of deficient nutrient plants expressed as a percentage of control plants at initial and advanced stages of deficiency for calibrachoa18

CHAPTER 2. FOLIAR SYMPTOMOLOGY AND TISSUE CONCENTRATIONS OF NUTRIENT DEFICIENT *ANGELONIA* PLANTS

Figure 1. Days to develop initial deficiency symptoms for angelonia34

Figure 2. Dry weight of deficient nutrient plants expressed as a percentage of control plants at initial and advanced stages of deficiency for angelonia.....35

CHAPTER 3. FOLIAR SYMPTOMOLOGY AND TISSUE CONCENTRATIONS OF NUTRIENT DEFICIENT *BRACTEANTHA* PLANTS

Figure 1. Days to develop initial deficiency symptoms for bracteantha52

Figure 2. Dry weight of deficient nutrient plants expressed as a percentage of control plants at initial and advanced stages of deficiency for bracteantha.....53

CHAPTER 4. FOLIAR SYMPTOMOLOGY AND TISSUE CONCENTRATIONS OF NUTRIENT DEFICIENT *BRACHYSCOME* ‘MINI YELLOW’ PLANTS

Figure 1. Days to develop initial deficiency symptoms for brachyscome ‘Mini Yellow’69

Figure 2. Dry weight of deficient nutrient plants expressed as a percentage of control plants at initial and advanced stages of deficiency for brachyscome ‘Mini Yellow’70

CHAPTER 5. FOLIAR SYMPTOMOLOGY AND TISSUE CONCENTRATIONS OF NUTRIENT DEFICIENT *BRACHYSCOME* ‘JUMBO MAUVE’ PLANTS

Figure 1. Days to develop initial deficiency symptoms for brachyscome ‘Jumbo Mauve’84

Figure 2. Dry weight of deficient nutrient plants expressed as a percentage of control plants at initial and advanced stages of deficiency for brachyscome ‘Jumbo Mauve’85

CHAPTER 1

FOLIAR SYMPTOMOLOGY AND TISSUE CONCENTRATIONS OF NUTRIENT DEFICIENT *CALIBRACHOA* PLANTS

Amy L. Williams¹, James L. Gibson¹, Paul V. Nelson¹, Brian E. Whipker¹, John M. Dole¹, Brenda Cleveland², and F.R. Walls²

¹Department of Horticultural Science, Campus Box 7609, North Carolina State University, Raleigh, North Carolina 27695-7609

²North Carolina Department of Agriculture and Consumer Services, Agronomic Division, Raleigh, NC 27607-6465

ABSTRACT

Foliar analysis standards and visual symptoms of nutritional disorders for calibrachoa have not been published and were the objectives of this study. *Calibrachoa* Cerv. Liricashowers 'Pure White' plants were grown hydroponically in a glass greenhouse at 35°N latitude. Nutrient treatments were based on the macronutrient composition of Hoagland's all-nitrate nutrient solution with altered micronutrient levels and 11 additional related solutions, each devoid of one essential nutrient. Visual symptoms were chronologically recorded and photographed. Iron deficiency occurred in four days

indicating a high level of susceptibility to this disorder. Calibrachoa was moderately susceptible to calcium, manganese and nitrogen deficiency, which occurred in 11- 13 days. P, K and B followed at 17 days, then Cu and Zn at 19 days.

Unique deficiency symptoms included the following: K resulted in pronounced chlorosis of the tips of older leaves prior to necrosis, there was considerable upward rolling of the margins on young and recently mature leaves on Cu and Zn deficient plants, and dark green pigmentation preceded symptoms of Mn, Zn and Cu deficiencies. Reduced plant biomass at incipient deficiency occurred for only Mg and Fe deficiencies. Minimal critical leaf concentrations for six nutrients were within the customary ranges for floricultural crops: 1.65% N, 1.7% K, 0.15% S, 77.5 mg·kg⁻¹ Fe, 34.9 mg·kg⁻¹ Mn, and 16 mg·kg⁻¹ Zn; while five nutrients were below the customary ranges: 0.16% P, 0.37% Ca, 0.11% Mg, 2.6 mg·kg⁻¹ Cu and 6.4 mg·kg⁻¹ B. The low P, Ca, Mg, Cu and B values may be due to the young growth stages of these plants compared to the older, more reproductive, stages associated with traditional standards in literature for floral crops. Detailed descriptions of the chronological development of deficiency symptoms are presented along with associated early and advanced stage leaf nutrient concentrations.

OBJECTIVES

Objectives of this study included (a.) generation of visual nutrient deficiency symptoms in the chronological order in which they appeared up to advanced stages for N, P, K, Ca,

Mg, S, Fe, Mn, Cu, Zn, and B and (b.) establishment of foliar analysis standards for these same nutrients at incipient and late stages of deficiency in calibrachoa.

MATERIALS AND METHODS

Unrooted stem cuttings of calibrachoa 'Pure White' were inserted in Oasis[®] LC1 foam cubes (Smithers Oasis, Kent, Ohio) containing only Ca and Mg from dolimitic limestone on December 21, 2001. The experiment was conducted in a glass greenhouse in Raleigh, N.C. at 35°N latitude that was set at night/cloudy day/clear day temperatures of 17/21/24 °C. During the establishment phase, cuttings were fertilized at each irrigation with mM concentrations of 0.35 NH₄, 5.15 NO₃, 0.35 PO₄, 1.0 K, 1.25 Ca, 1.0 Mg, and 36 µM Fe using the following reagent grade chemicals NH₄NO₃, KNO₃, K₂HPO₄, Ca(NO₃)₂·4H₂O, MgSO₄·7H₂O, and FeDTPA (1). Cuttings were grown with this nutrient regime until roots were visible at the edges of the rooting cube. Cuttings were pinched by removing 2 cm of growth from the terminal tip. After establishment, plants were transplanted on January 28, into 4.87-L aluminum painted plastic tubs with six circular holes in the lids. Six replications, each consisting of one tub with six rooted cuttings, were assigned to 12 treatments. To provide sufficient plant biomass for foliar analysis, plants were grown initially with a complete modified Hoagland's all nitrate solution: (macronutrients in mM) 15 NO₃, 1.0 PO₄, 6.0 K, 5.0 Ca, 2.0 Mg, and 2.0 SO₄ (2), plus µM concentrations of micronutrients: 72 Fe, 9.0 Mn, 1.5 Cu, 1.5 Zn, 45.0 B, and 0.1 Mo. On February 1, 2003 four treatments were induced that included a complete nutrient formula and complete minus one of the nutrients S, Cu or Zn. Because deficiency symptoms of N, P, K, Ca,

Mg, Fe, Mn and B typically develop more quickly, than S, Cu, and Zn (1), the introduction of these treatments were delayed until February 7, 2003 in order to have sufficient biomass for tissue sampling. Reagent grade chemicals and deionized water of 18-mega ohms purity was used to formulate treatment solutions. Tubs were inspected daily and deionized water was added as needed to maintain the nutrient solution volume for two weeks, and in subsequent weeks tubs were refilled with nutrient solution as needed. A complete replacement of nutrient solutions was done weekly.

Plants were monitored daily to document and photograph sequential series of symptoms on youngest, young, recently mature, and mature leaves as they developed. When the first visible symptoms occurred, the recently mature leaves were sampled from three replications of deficiency treatment and the control treatment to establish incipient deficiency tissue levels. When symptoms progressed to an advanced level, another set of recently mature leaves were sampled from the remaining three replications of the deficiency treatment and the control treatment to establish tissue concentrations associated with advanced deficiency. The tissue samples were first rinsed in deionized water, then washed in 0.2 N HCl for 30 s, and dried at 70 °C for 24 h. Dried tissue was ground in a stainless steel Wiley mill to pass 1 mm screen (20-mesh). Tissue was then analyzed for macro and micronutrients with the exceptions of N, using a Perkin Elmer 3300 Inductively Coupled Argon Plasma Emission Spectrophotometer (Perkin Elmer, Shelton, Conn.), while N was analyzed using Carlo Erba NA 1500 Series 1, O₂ combustion Nitrogen Analyzer (Carlo Erba, Lakewood, N.J.) at the N.C. Dept of Agriculture Laboratory, Raleigh.

The experiment was a randomized complete-block design with 6 blocks. Each block consisted of four plastic tubs of control treatment and one plastic tub of each deficient treatment (each tub was an experimental unit). All the data were subjected to ANOVA using PROC GLM SAS program (SAS Inst., Cary, N.C.). Where the F test indicated evidence of significant difference among the means, LSD ($P \leq 0.05$) was used to establish differences between means.

RESULTS AND DISCUSSION

Calibrachoa appears to be most sensitive to Fe deficiency due to the rapid appearance of symptoms four days after the initiation of treatment (Figure 1). Plants were moderately sensitive to Ca deficiency that followed at day 11, Mn and N at day 13, and S at day 15. Plants were fairly resistant to P, K, and B deficiency (day 17) as well as Cu and Zn (day 19). Mg was the slowest at day 34, probably due to the presence of dolomitic limestone in the Oasis rooting cubes. Only Fe and Mg deficiencies resulted in notable plant weight loss as an early symptom (Figure 2). Plants deficient of K weighed more than the control plants did, at this early deficiency stage. Plants deficient in all other nutrients had a similar dry weight as the control plants. During the advanced stage, all nutrient deficiencies resulted in lighter plants than the control. Following are the progressions of visual deficiency symptoms for each nutrient. Corresponding tissue concentrations at early and late stages are found in Table 1.

Three developmental stages of leaves are referred to in this paper and are described as follows. Young leaves are less developed and mature leaves are more developed than the recently mature leaves. Recently mature leaves are those that are approaching or have just reached mature size, color, shape, or any other distinguishing characteristic that would identify them as mature.

NITROGEN

Initial tissue N concentrations were 1.65% as compared to the control at 5.06% (Table 1) (day 13). Thirteen days after initiation of the N deficient treatment, all replications exhibited a smaller and shorter appearance than the control. The mature leaves were smaller and the primary lateral shoots were shorter than the control. On day 16, the lowest mature foliage of the primary stem developed either a uniform light yellow chlorosis or random patches of chlorosis. By day 20, axillary development was poor and the young to mature leaves were short and narrow, which gave the plants an overall stunted appearance. Faint chlorosis developed on the young leaves. Necrosis began with a tan to light brown papery burn on the tips and margins of the oldest mature leaves (day 22). By day 26, plants were extremely upright and compact in architecture compared to the cascading habit of the control. The lowest mature leaves exhibited random patches of yellow-green, yellow, and light yellow chlorosis. Some mature leaves were shriveled and showed a necrotic burn, which moved from the tip to the base of the leaf. Tissue N concentrations were 0.85% in deficient and 5.04% in control plants (Table 1) (day 26).

The N deficient plants weighed 5.1 g compared to the controls, which weighed 11.4 g (Figure 2).

PHOSPHORUS

Initial tissue P concentrations were 0.16% and 0.42% for the deficient and control leaves, respectively (Table 1) (day 17). The young leaves were medium green and the recently mature to mature leaves were unusually dark green. The mature leaves had a thin feather-like shape. Translucent, paper-thin light brown to tan necrotic spots appeared on the midveins and bordering middle regions of the mature leaves. By day 20, chlorosis quickly turned to a rusty brown necrosis of the lower mature leaves, which also had scorched leaf tips. These necrotic leaves became withered and turned dark brown by day 34 and even darker by day 39. This occurred not only on the mature leaves, but on the recently mature leaves as well. On day 46, plants had a dull dusty rust-brown cast. Necrotic spots appeared randomly on the young to mature leaves and began fusing together to form larger necrotic patches. As total necrosis of the mature basal leaves progressed, leaf tissue P concentrations were 0.05% in deficient and 0.36% in control plants (day 49) (Table 1). The P deficient plants weighed 14.5 g compared to the controls, which weighed 41.3 g (Figure 2).

POTASSIUM

Initial symptoms occurred at a tissue K concentration of 1.7% while the control was at 4.22% (Table 1) (day 17). The K deficient plants weighed 6.4 g compared to the controls, which weighed 4.6 g (Figure 2). The young and recently mature leaves developed a light green basipetal chlorosis, while the tips remained dark green. The mature leaves on the primary lateral shoots were a dusty, dull, deep, green color. Older foliage on the primary stem developed a dark green pigmentation. By day 29, the mature foliage was noticeably smaller and darker green with an obvious glossy sheen. Necrosis developed on day 34, as small brown spots on the margins of the oldest mature leaves. This necrotic region was bordered by a band of light green chlorosis on the inside of the leaf surface. Symptoms progressed toward the middle of the leaf. The brown necrotic spots began fusing to create a thin brown-black line on the margins of the tips of the mature leaves (day 43). Day 47, the young and recently mature leaves began to fold inward at the margins, which started at the base of the leaf and progressed toward the middle. A final leaf tissue sample was taken on day 50, resulting in a K concentration of 0.51% and 2.95% for the control (Table 1). The K deficient plants weighed 24.3 g compared to the controls, which weighed 41.3 g (Figure 2).

CALCIUM

Tissue concentrations for initial calcium deficiency were taken on day 9 when concentrations were 0.37% in deficient and 1.84% in control leaves and no symptoms

were apparent on the shoots (Table 1). The decision to harvest on this date was based on the condition of the roots, which were shorter in length and thicker in girth giving them a bristled stubby appearance. By day 11, the young leaves developed interveinal chlorosis and their midribs turned gray. These leaves exhibited a concave appearance, as the margins rolled inward. Interveinal chlorosis progressed down to the recently mature leaves while margins of the young leaves developed a thin green chlorosis. Moderate symptoms developed by day 15 when the tissue Ca concentration of deficient plants was 0.13%. At this point, the young leaves were stunted and had begun to curl and fold inward at the base. The tips were withered with a brownish black 'water soaked' appearance. By day 22, the necrotic shoot tips had begun to droop and many buds were smaller than the control or aborted completely. The buds and the youngest leaf tips developed a gray-brown necrosis. Young leaves began exhibiting a buckled or puckered appearance of the middle region of the leaf. Overall the plants had a tight compact canopy with no cascading habit. At this advanced deficiency stage tissue Ca concentration was 0.11% and 1.48% for the control plants (Table 1) (day 24). The Ca deficient plants weighed 7.2 g compared to the controls, which weighed 11.4 g (Figure 2).

MAGNESIUM

At the point of initial deficiency, tissue concentration of Mg in deficient plants was 0.11% while in the control it was 0.39% (Table 1) (day 34). The Mg deficient plants weighed 5.3 g compared to the controls, which weighed 14.5 g (Figure 2). Mature leaf

tips developed a greenish-yellow pigmentation with greenish-yellow semi-circular patches near the midrib. Interveinal chlorosis also developed on the young and recently mature leaves. By day 40, the interveinal chlorosis had become more distinct. The recently mature and mature leaves had a glossy appearance and the leaf architecture curved downward. Advanced symptoms occurred at tissue Mg concentrations of 0.10% and 0.28% for the control (Table 1) (day 40).

SULFUR

Initial symptoms occurred when deficient and control leaf tissue S concentrations were 0.15% and 0.44%, respectively (Table 1) (day 15). The young foliage appeared short and narrow with uniform greenish-yellow chlorosis. The recently mature foliage was also short, narrow and exhibited a light green chlorosis. By day 21, the recently mature leaves developed a greenish-yellow interveinal chlorosis. Four to five centimeters down from the shoot tip, the axillary shoots turned a yellow-green color (day 26). The mature leaves also developed a pale green pigmentation. On day 28, the young to recently mature leaves exhibited a light green to whitish-green interveinal chlorosis. By day 38, the axillary development was poor throughout the entire plant and the stunted size caused the plant to be stiff and inflexible to the touch. The tissue S concentration associated with these advanced symptoms was 0.21% while control plants were 0.61% (Table 1) (day 45).

IRON

Tissue Fe concentrations of $77.5 \text{ mg}\cdot\text{kg}^{-1}$ and 110.4 for control plants were recorded when symptoms first appeared (Table 1) (day 4). The Fe deficient plants weighed 2.1 g compared to the controls, which weighed 2.8 g (Figure 2). The young foliage tended to have a yellow-green leaf base and greenish-yellow tips. However, there were considerable variations of light green chlorosis in these leaves, including uniform and interveinal. By day 9, the young leaves were bright yellow and began to roll inward, which gave them a concave appearance. The recently mature leaves developed a faint interveinal chlorosis with light yellow margins and leaf base. The oldest mature leaves remained dark green, similar to the control. Young axillary shoots developed bright yellow chlorosis. By day 15 the recently mature leaves appeared concave and were yellow-green with an interveinal chlorosis. Shoot tips became whitish-yellow and developed brown necrosis on the basal petiole region of the young leaves. The necrosis caused these leaves to appear withered and shriveled. On day 17, the oldest mature leaves were still normal green but axillary shoots turned white followed by necrosis. Advanced symptoms occurred at a tissue Fe concentration of $40.6 \text{ mg}\cdot\text{kg}^{-1}$ and 68.0 for control plants (Table 1) (day 17).

MANGANESE

Initial tissue Mn concentrations were $61.6 \text{ mg}\cdot\text{kg}^{-1}$ and $107.7 \text{ mg}\cdot\text{kg}^{-1}$ for the control plants, respectively (Table 1) (day 13). The young and recently mature leaves developed

a uniform greenish-yellow chlorosis. Symptoms occurred when deficient and normal leaf tissue a faint, random, patchy interveinal chlorosis affected some recently mature leaves, which progressed, to a more visible interveinal chlorosis and the leaves took on a lobed appearance (day 20). By day 22, the young and recently mature leaves developed a distinct greenish-yellow interveinal chlorosis, with medium green veins. These leaves were wider than the control with a pronounced lobed appearance (day 24). The older mature leaves remained dark green. All axillary shoots had a light green chlorosis. On day 32, the mature leaves began showing greenish-yellow interveinal chlorosis and some leaves appeared folded or cupped and curled downward. The recently mature leaves developed papery brown marginal necrosis, which progressed to the entire leaf causing it to be completely withered. The young and recently mature leaf tips became curled. Tissue Mn concentrations were $6.3 \text{ mg}\cdot\text{kg}^{-1}$ and control plants were 70.4 at the time of these late symptoms (Table 1) (day 39).

COPPER

Initial symptoms occurred when deficient and control leaf tissue Cu concentrations were 2.6 and $9.4 \text{ mg}\cdot\text{kg}^{-1}$, respectively (Table 1) (day 19). Young leaves had an interveinal chlorosis and were spoon-like in appearance with tips and margins rolled upward. The architecture was extremely upright, with tightly compact shoot tips. The spoon-shape spread to the recently mature leaves and was still affecting the majority of young leaves (day 22). By day 26, the young leaves became completely rolled, forming a tube-like structure. The canopy became thicker with a defined whitish-green interveinal chlorosis

on the underside of the young leaves and a yellowish-green chlorosis on the upper surface of the leaves. The recently mature leaves developed random patches of chlorosis. The overall sizes of the plants were smaller than the control, with few to no flower buds present (day 28). Axillary growth was minute on the primary stem and not visible on the upper two thirds of the stem. On day 35, the young leaves developed brown necrosis at their tips and flower size was smaller than the control. The young and recently mature and mature leaves became narrow and started to roll inward at the margins (day 38). At day 40, the whitish-yellow interveinal chlorosis of the tubular young leaves intensified, while the smaller youngest leaves shriveled. Flowers were shriveled and some did not open, which led to abortion. By day 45, recently mature leaves developed random dark brown necrotic spots that spread to the majority of the leaf, which caused it to shrivel. Advanced tissue Cu concentrations were $2.3 \text{ mg}\cdot\text{kg}^{-1}$ and 9.9 for control plants (Table 1) (day 45). The Cu deficient plants weighed 8.1 g compared to the controls, which weighed 41.3 g (Figure 2).

ZINC

Initial Zn concentrations were $43.9 \text{ mg}\cdot\text{kg}^{-1}$ and 16.0 for the control and deficient plants at this initial stage (Table 1) (day 19). Margins of young and recently mature leaves began rolling inward. Random patchy interveinal or veinal chlorosis affected the recently mature leaves, which appeared to be more lobed than the controls. By day 22, the young and recently mature leaves had a defined concave appearance due to the margins rolling and folding inward. The mature leaves develop dusty, dull green pigmentation, while the

young to recently mature leaves had a spongy to rubbery texture. On day 26, the youngest leaves developed a faint brown necrosis, which appeared at the mid-regions of the leaf. A chlorosis developed on the base of the young leaves that was accompanied by random green and brown streaks and spots, which later became papery. The shoot apices of young leaves were very concave and the leaf tips began to bend downward and to one side. By day 28, a yellow-brown necrosis appeared on the mid-regions of the young and recently mature leaves, which caused these leaves to bend and turn left or right. By day 30, some recently mature leaves developed a mottled appearance that progressed to a greenish-brown marginal chlorosis. Young leaves developed brownish-black speckles, which fused to create larger necrotic patches. Leaves at the third node and below began to wither, with a necrosis that developed on the middle of the leaf. Plants were rigid to the touch with leaves that developed a thick texture. These late symptoms occurred at a tissue Zn concentration of $10.8 \text{ mg}\cdot\text{kg}^{-1}$ compared to 27.4 for control plants (Table 1) (day 30).

BORON

Deficient and control leaf tissue B concentrations were 6.4 and $32.1 \text{ mg}\cdot\text{kg}^{-1}$, respectively (Table 1) (day 17). A distinct interveinal chlorosis appeared on some young and recently mature leaves while others developed random patchy chlorosis. By day 20, shoot tips developed a rosette appearance, due to slower apical growth that led to shorter internodes. The recently mature leaves were slightly lobed with a glossy sheen, while the mature leaves were slightly darker green. On day 24, young leaves developed interveinal chlorosis and some also showed signs of deformity. Overall, plant texture became thick

and the stems were rigid. The chlorotic young leaves became more obvious as they turned to shades of yellow. The mature leaves were deep green with thick yellowish-white midveins (day 26). By day 32, short internodes and rosetted shoot tips, created a dense canopy. Flowers were not apparent because most buds aborted. On day 34, the few existing buds developed brown necrosis. This necrosis also developed on the margins and tips of the chlorotic young leaves. The young to mature leaves were noticeably smaller with a glossy waxy-like appearance (day 43). On day 46, the whole plant became obviously short because of poor axillary branching. There were no replications with visible flowers and all buds had aborted. The growing point had completely stopped, which resulted in extreme rosetting. These late symptoms occurred at a tissue B concentration of $2.4 \text{ mg}\cdot\text{kg}^{-1}$ and 37.4 for control plants (Table 1) (day 46). The B deficient plants weighed 16.6 g compared to the controls, which weighed 41.3 g (Figure 2).

REFERENCES

1. Pitchay, D. *Impact of 11 Elemental Nutrient Deficiencies on Shoot and Root Growth, and Foliar Analysis Standards of 13 Ornamental Taxa with Emphasis on Ca and B Control of Root Apical Meristem Development*; North Carolina State University: Raleigh, NC, **2002**; Ph.D. dissertation.
2. Hoagland, R.J.; Arnon D.I. *The Water-Culture Method for Growing Plants Without Soil*, Circ. 347 (Rev. Ed), California Agric. Exp. Sta.: Berkley, Calif., **1950**.

Table 1. Tissue nutrient concentrations at initial and advanced stages of deficiency in recently matured leaves of hydroponically grown calibrachoa.

Note: * denotes significance at $P \leq 0.05$, ** denotes significance at $P \leq 0.01$ for each control deficiency pairing.

Treatment	-N	-P	-K	-Ca	-Mg	-S	-Fe	-Mn	-Cu	-Zn	-B
Element	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn	B
Units	%	%	%	%	%	%	mg·kg ⁻¹	mg·kg ⁻¹	mg·kg ⁻¹	mg·kg ⁻¹	mg·kg ⁻¹
Control initial	5.06	0.42	4.22	1.84	0.39	0.44	110.4	107.7	9.4	43.9	32.1
Deficient initial	1.65**	0.16**	1.7**	0.37**	0.11**	0.15**	77.5**	34.9**	2.6**	16.0**	6.4**
Control advanced	5.04	0.36	2.95	1.48	0.28	0.61	68.0	70.4	9.9	27.4	37.4
Deficient advanced	0.85**	0.05**	0.51**	0.11**	0.10**	0.21**	40.6*	6.3**	2.3**	10.8**	2.4**

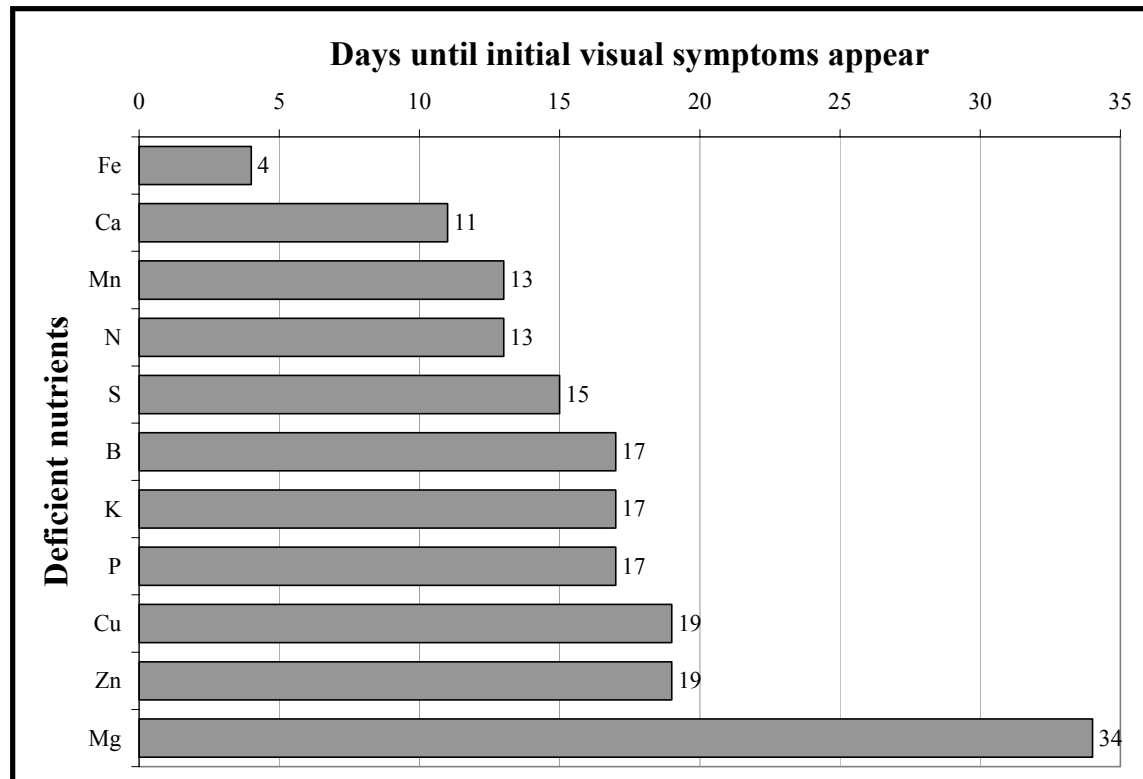


Figure 1. Days to develop initial deficiency symptoms for calibrachoa

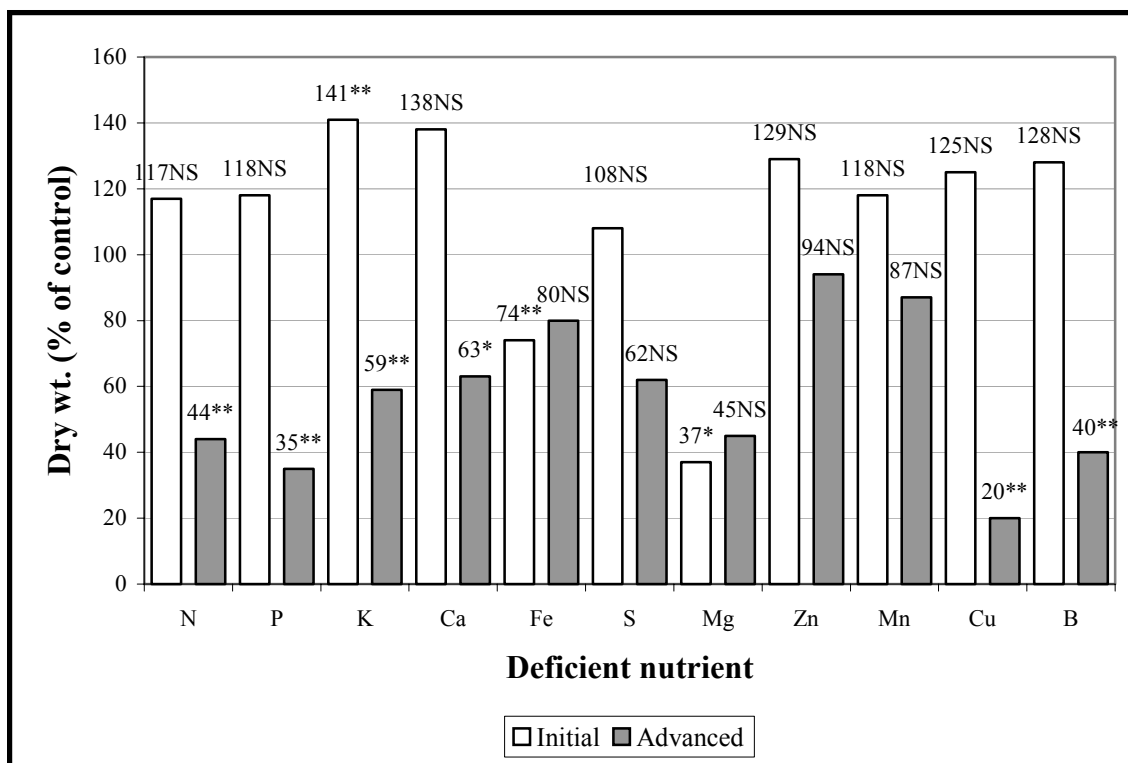


Figure 2. Dry weight of deficient nutrient plants expressed as a percentage of control plants at initial and advanced stages of deficiency for calibrachoa.

Note: NS denotes non-significance at $P \leq 0.05$, * denotes significance at $P \leq 0.05$, ** denotes significance at $P \leq 0.01$ for each control deficiency pairing.

CHAPTER 2

FOLIAR SYMPTOMOLOGY AND TISSUE CONCENTRATIONS OF NUTRIENT DEFICIENT *ANGELONIA* PLANTS

Amy L. Williams¹, Paul V. Nelson¹, Brian E. Whipker¹, John M. Dole¹, F.R. Walls² and Brenda Cleveland²

¹Department of Horticultural Science, Campus Box 7609, North Carolina State University, Raleigh, North Carolina 27695-7609

²North Carolina Department of Agriculture and Consumer Services, Agronomic Division, Raleigh, NC 27607-6465

ABSTRACT

Foliar analysis standards have not been published for *Angelonia angustifolia* ‘Carita Purple’. *Angelonia* plants were grown hydroponically in a glass greenhouse. Treatments consisted of a complete Hoagland’s all nitrate macronutrient solution with altered micronutrient content and 11 related solutions, each devoid of one essential nutrient. *Angelonia* appeared to be most sensitive to Ca deficiency due to the appearance of initial symptoms at day 12. N, P, K and Fe followed at day 16, S at day 20, Cu and Mn at day 24, B on day 26, Zn on day 31 and Mg on day 37.

Unique deficiency symptoms included considerable upward rolling of the margins and twisting of the young and recently mature leaves in Cu and Zn deficient plants and dark green pigmentation as the initial symptom of P and Cu deficiencies. Sulfur deficiency developed a dark reddish-purple pigmentation on the first to fifth internodes of the axillary branches as an advanced symptom. The minimum critical recently mature leaf standards were % 2.82 N, 0.29 P, 2.03 K, 0.24 Ca, 0.16 Mg, 0.11 S, and $\text{mg}\cdot\text{kg}^{-1}$ 80.3 Fe, 11.9 Mn, 2.5 Cu, 38.1 Zn, and 21.9 B. Detailed descriptions of the chronological development of deficiency symptoms are presented along with associated early and advanced stage leaf nutrient concentrations.

OBJECTIVES

Objectives of this study included (a.) generation of visual nutrient deficiency symptoms in the chronological order in which they appeared up to advanced stages for N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, and B and (b.) establishment of foliar analysis standards for these same nutrients at incipient and late stages of deficiency in angelonia.

MATERIALS AND METHODS

Materials and methodology were the same as reported by Williams et al. (1), with the following exceptions. Unrooted angelonia cuttings were inserted in Oasis[®] LC1 foam cubes on October 6, 2002 and were transplanted after establishment on November 7,

2002 into 4.87 L aluminum-painted plastic tubs. Angelonia leaves were opposite in orientation.

RESULTS AND DISCUSSION

Angelonia appeared to be most sensitive to Ca deficiency due to the rapid appearance of symptoms twelve days after the initiation of treatment (Figure 1). Plants were moderately sensitive to deficiencies of Fe that followed at day 16; K, N, and P at day 17; and S at day 20. Plants were fairly resistant to Mn and Cu (day 24), B (day 26), as well as Zn (day 31) deficiencies. Mg deficiency was the slowest at day 37, probably due to the presence of dolomitic limestone in the Oasis[®] rooting cubes. Only N deficient plants resulted in notable plant weight loss as an early symptom (Figure 2). During the advanced stage only Cu, N, P, K, and especially Mn deficiencies resulted in smaller plants than the control. Following are the progressions of visual deficiency symptoms for each nutrient. Corresponding tissue concentrations at early and late stages are found in Table 1. Low minimal critical nutrient concentrations may be due to the young growth stages of these plants compared to the older, more reproductive stages that are associated with traditional standards in the literature for ornamental crops.

Three developmental stages of leaves are referred to in this paper and are described as follows. Young leaves are less developed and mature leaves are more developed than the recently mature leaves. Recently mature leaves are those that are approaching or have just

reached mature size, color, shape, or any other distinguishing characteristic that would identify them as mature.

NITROGEN

Nitrogen deficiency appeared 16 days after inducement; these initial symptoms occurred on small, short plants with dry weights of 5.9 g and 8.4 g and tissue N concentrations of 2.82% and 5.06% for deficient and control treatments, respectively (Table 1) (Figure 2). The recently mature and upper mature leaves appeared short and narrow and the recently mature leaves exhibited a faint light green chlorosis. A few of the lowest mature leaves at the base of the primary stem had small light green patches (day 21). On day 23, the entire plant appeared thin and spindly with a noticeably less dense canopy. Young and recently mature leaves were short and narrow, while the mature leaves had similar, but more pronounced symptoms. At this point the lowest mature leaves developed a distinct yellow and green interveinal chlorosis, which began at the leaf base and progressed to the tip. Some of these lower mature leaves had a tan to brown necrosis of the leaf tip, while the middle regions were speckled with a random yellow-green chlorosis. The chlorosis of these lower mature leaves progressed to a light yellow color, which then turned bright yellow. Some of these leaves were tan and papery (day 25). Day 27, the entire plant size was smaller than the control and the density of the plants was thin. The older lower mature leaves continued to progress from a yellow-green to a bright yellow, followed by a tan to brown necrosis, which all began at the leaf base and moved toward the tip (day 29). Overall, the entire plant was uniformly chlorotic and exhibited a light green color

with a dull sheen (day 34). By day 38, the young, recently mature and upper mature leaves began curling upward and were slightly twisted. Plants were short and dramatically smaller with no axillary shoot elongation. Flowering was less, because there was an absence of flower buds present on the N deficient plants at these advanced symptoms. The lowest mature leaves developed a light green to yellow interveinal chlorosis, which eventually progressed to an orange-brown necrosis and then to a totally brown, withered necrosis. N tissue values at these advanced stages were 1.7% and 4.63% for deficient and control plants, respectively (Table 1) (day 38). The N deficient plants weighed 10.4 g compared to 19.5 g for the control (Figure 2).

PHOSPHORUS

Initial symptoms began on day 16 when P tissue concentrations were 0.29% compared to 0.63% for the control (Table 1). Phosphorus deficient plants developed a dark green pigmentation, which affected the entire plant. By day 23, a light green streaking developed on the second to fourth internodes of the stem, giving it a striped appearance. The plants were short with a dull dark green cast over the entire plant (day 25). By day 27, the first to third internodes had a purple striped coloration. This symptom progressed into a complete purple pigmentation at the third internode and a little more at the second. At this point, plants were slightly smaller and darker green in color (day 33). The margins of the young to recently mature leaves were smooth compared to the control, which had serrated margins (day 38). The dark green pigmentation persisted over the entire plant except for the first set of newly formed leaves at the tip of each shoot, which had a faint

chlorosis (day 41). By day 45, size difference resulted in large measure to a lack of shoot elongation. This caused the plants to have an upright architecture that was stiff to the touch. A noticeable symptom that remained with the plants was the darker green coloration of all the leaves. P tissue concentrations were 0.23% and 0.44% for the deficient and control plants, respectively (Table 1) (day 45). The most noticeable symptom was the size differences between the P deficient 17.6 g and control 28 g plants (Figure 2).

POTASSIUM

Initial tissue K concentrations were 2.03% for deficient plants compared to 3.47% for the control (Table 1) (day 16). Potassium deficiency initially appeared on the upper set of mature leaves. These mature leaves had light green chlorotic tips. By day 19, chlorotic leaf tips turned to a brownish-gray color, and then to a brown necrosis. Deficient plants resembled the controls, except for the necrotic tips of the mature leaves (day 23).

Approximately one centimeter of the leaf tips on the lower half of plants were tan to brown with a papery necrosis. These leaves also developed a dark green to brown necrosis along their margins (day 33). Symptoms of necrosis spread toward the middle of mature leaves by day 38. By day 41, mature leaves were gray-green to brown with withered tips, which curled upward. These advanced symptoms resembled the Zn deficiency treatments. At this point, K concentration in deficient leaves was 1.49% compared to 2.82% for the control (Table 1) (day 41). Dry weights for K deficient treatment were 19.1 g compared to 25.8 g for the control (Figure 2).

CALCIUM

Initial tissue Ca concentrations at this stage were 0.24% and 0.88% for the deficient and control leaves, respectively (Table 1) (day 12). Initial symptoms for Ca deficiency began with the roots. They were light brown and axillary roots were short and stubby. The first shoot symptoms developed on the young to recently mature leaves as a marginal interveinal chlorosis, which began at the base of the leaf and progressed to the middle. These young and recently mature leaves were glossy, small, slightly deformed, and bent downward at the middle of the leaf. By day 23, the young leaves had thin brown patches on the margins, from the middle to the base of the leaf. Advanced symptoms developed on the shoot tips with a dramatic necrosis (day 38). Few flower buds remained, because the majority had aborted or did not develop, due to the underdevelopment of the shoot tip, which also resulted in small compact plants. Those buds remaining were small, deformed and brown. The first pairs of young leaves were small and completely necrotic, while the remaining young leaves had either a uniform or interveinal chlorosis and were bent downward in the middle. Some young and recently mature leaves were so deformed the midveins developed a crater-like depression with a puckered appearance. The midveins of the recently mature leaves were also thin and brown. A speckled light green chlorosis appeared on the mid regions of the recently mature leaves and the margins developed a light green chlorosis. Recently mature leaves folded inward, beginning at the base, giving them a concave like appearance, while at the same time some curled at the leaf tip. The upper mature leaves had a light green to tan spotting or speckling on the

middle and base of the leaf, which also had light green chlorotic tips. Advanced tissue Ca concentrations of 0.15% and 1.18% for deficient and control plants, respectively (Table 1) (day 38).

MAGNESIUM

Magnesium tissue concentrations were 0.16% for deficient and 0.30% for control plants on day 37 when Mg symptoms originated (Table 1). A faint light green interveinal chlorosis or a random splotchy light green chlorosis appeared on leaf tips on the lower third of the shoots. The interveinal chlorosis was present on the mature leaves by day 41. As symptoms progressed mature leaves developed a tan necrosis of the margins, which moved inward toward the midveins on the middle of the leaf (day 43). A brown papery burn outlined the interior of this necrosis. The leaves most affected by the burn were positioned in the middle of the shoot. On day 46, the advanced symptoms remained on the mid to lower mature leaves as a distinct light green interveinal chlorosis, which originated at the middle of the leaf. These leaves developed a tan necrosis on the margins of the middle of the leaf, which progressed to a tan to brown mottled appearance. Necrosis moved toward the midveins and eventually affected the majority of the leaf. The affected leaves bent at the point of necrosis. Mg tissue concentrations were 0.13% for deficient and 0.24% for control plants, respectively (Table 1) (day 46).

SULFUR

Initial tissue S concentrations were 0.11% for deficient and 0.37% for the control plants, respectively (Table 1) (day 20). Deficient plants had limited axillary branch expansion and were short. The young and recently mature leaves were small and the recently mature leaves were light green and began curving and bending downward. By day 23, the young to recently mature leaves developed a lime-green coloration and were noticeably smaller, causing the entire plant to appear small. The internodes of the axillary shoots were half the length of the controls internodes and some developed a purple pigmentation near the shoot tip (day 27). The chlorotic plants appeared short and inflexible, while the leaves were thinner. By day 29, the first to fifth internodes of the axillary branches developed a dark reddish-purple pigmentation. Plants were approximately half the size of the controls. The shoots were short in length, which also had one and three fewer internodes than the controls. By day 35, entire plants had a dull lime-green cast and all leaves were short, narrow, and had unusually smooth margins. Advanced tissue S concentrations were 0.12% for deficient and 0.33% for control plants (Table 1) (day 35).

IRON

At the onset of the Fe deficiency, leaf concentrations were $80.3 \text{ mg}\cdot\text{kg}^{-1}$ for deficient and $110 \text{ mg}\cdot\text{kg}^{-1}$ for control plants (Table 1) (day 16). Symptoms started with a light green interveinal chlorosis of the recently mature and first to third set of mature leaves. The young and recently mature leaves developed a light green uniform chlorosis (day 18).

The young axillary shoots formed a light green uniform chlorosis (day 22). By day 27, the young to recently mature leaves and some mature leaves developed a light lime-green interveinal chlorosis. By day 30, interveinal chlorosis had affected the newly established axillary shoots and leaves. Interveinal chlorosis on the young, recently mature and mature leaves intensified. On day 35, some axillary shoots developed a light green to yellow chlorosis on the mid regions of the newly developed young leaves others exhibited a light green uniform chlorosis of all the leaves, and yet others developed a faint interveinal chlorosis on recently mature leaves. Flower quantity, size and distribution were similar to the control. Advanced tissue Fe concentrations were $81.8 \text{ mg}\cdot\text{kg}^{-1}$ in deficient and $99.6 \text{ mg}\cdot\text{kg}^{-1}$ in control plants (Table 1) (day 35).

MANGANESE

Initial tissue values for the Mn deficient plants were $11.9 \text{ mg}\cdot\text{kg}^{-1}$ and $108.7 \text{ mg}\cdot\text{kg}^{-1}$ for the control (Table 1) (day 24). Mn deficiency first developed as a faint light green uniform chlorosis over the upper half of the plant. The recently mature leaves appeared to have a faint light green marginal chlorosis, which had also developed on the mature leaves, but the width of the chlorosis was thinner and not as prominent as the recently mature leaves. Symptoms became more defined by day 29, with the light green uniform chlorosis appearing on the young, recently mature and mature leaves, except for the lowest mature leaves, which still resembled the control. Some of the recently mature to the first to third sets of mature leaves developed a light green marginal chlorosis. There was little to no elongation of the axillary shoots, which resulted in shorter plants (day 35).

Plants lost their glossy sheen and developed a dull lime green chlorosis, which enveloped the entire plant (day 37). A faint marginal interveinal chlorosis appeared on the young to first set of mature leaves by day 39, which gave these leaves a highlighted appearance. On day 46, the recently mature leaves began twisting upward at the middle and tips of the leaves. The marginal chlorosis on the young to mature leaves was more prominent by day 50. The plants also appeared small with a lime-green cast. On day 53, leaves had a soft and spongy texture, which resembled dehydration. The faint interveinal chlorosis remained only on the young leaves, while overall the plants were a lime-green color. Axillary branching appeared shorter and devoid of flower buds. Advanced Mn tissue concentrations were $11.4 \text{ mg}\cdot\text{kg}^{-1}$ and $83.6 \text{ mg}\cdot\text{kg}^{-1}$ for deficient and control plants (Table 1) (day 53). Mn deficient plants weighed 8.6 g, which was significantly less than the controls at 25.8 g (Figure 2).

COPPER

Initial Cu symptoms developed at tissue concentrations of $2.5 \text{ mg}\cdot\text{kg}^{-1}$ compared to $12.4 \text{ mg}\cdot\text{kg}^{-1}$ in control plants (Table 1) (day 24). Plants appeared to have a faint dull green cast accompanied by a basal chlorosis on the young and recently mature leaves. The young and recently mature leaves developed a curvature to the left or right within the middle of the leaf, giving it a boomerang appearance. These same leaves also developed a puckered or sunken midrib, which caused the leaves to curl or roll downward. Within two days of the initial symptoms, necrosis appeared. Small tan to brown patches developed on the margins at the midpoint where the leaf curved. The midrib of the mature leaves on the

fourth node began to pucker and these leaves curved to one side (day 27). Some of the mature leaf tips developed a small brown necrotic burn. By day 29, the entire plant appeared to have a dull, dark, blue-green cast. A light green marginal chlorosis of the first and second pair of mature leaves on the fourth to sixth nodes progressed to a tan to brown papery necrosis on the margins and tips of the leaves by day 37. Cu deficient plants were small and remained dull with a blue-green cast (day 39). Young leaves began to fold downward at the leaf base where the midveins was brown and necrotic. The necrosis on the first set of mature leaves developed light tan spots accompanied by a speckling of black dots. By day 50, the tan mature leaf tips progressed to a dark brown withered necrosis, some of which began to curl upward. Overall the blue-green plants were short with a dense canopy. On day 53, young and recently mature leaves developed a random splotchy chlorosis while the leaf tips twisted from the puckering of the midveins. Recently mature and mature leaf margins and midveins developed a brown spotted necrosis. Leaf tips of the mature leaves on the mid to lower region of the shoot curled upward. Flower buds aborted, but those flowers, which remained, were light pink in color compared to the dark purple flowers of the control. Advanced Cu tissue concentrations were $6.0 \text{ mg}\cdot\text{kg}^{-1}$ for deficient plants and $8.3 \text{ mg}\cdot\text{kg}^{-1}$ for controls plants (Table 1) (day 53). Cu deficient plants weighed 15.2 g, which was less than the controls at 25.8 g (Figure 2).

ZINC

Zn deficient plants showed symptoms on day 31, with tissue concentrations of 38.1 mg·kg⁻¹ and 86.2 mg·kg⁻¹ for the control plants (Table 1). A light green chlorosis appeared on the leaf tips of the recently mature and mature leaves of the fourth to sixth nodes. This chlorosis quickly developed into a brown necrosis, which progressed up the leaf, and ultimately turned into a brownish-gray necrosis. The necrotic leaf tips developed a tan to brown withered burn and began to roll upward (day 39). Some midveins of the young leaves collapsed and leaves folded downward in the middle. The upper half of the plants appeared to have a light green chlorosis (day 46). By day 50, the upper mature leaf tips were greenish gray to brown, which progressed to the middle of the leaf. These leaf tips curled upward and were papery in texture. On day 53, the lower half of the plant developed necrosis. These advanced symptoms appeared on the mature leaves as a brown papery necrosis, which twisted and curled upward. Deficient Zn tissue concentrations were 28.3 mg·kg⁻¹ compared to 28.3 mg·kg⁻¹ for the control plants (Table 1) (day 53).

BORON

Initial tissue B values were 21.9 mg·kg⁻¹ and 46.2 mg·kg⁻¹ for the deficient and control plants, respectively (Table 1) (day 26). Initial symptoms developed as short, stubby, thick primary and axillary roots with blackened root tips. Overall plants had a glossy sheen. By day 29, the young leaves developed a light green chlorosis, which appeared as either uniform or marginal. These young leaves were slightly deformed, twisted and small. On

day 34, shoots were compact and tips were rosetted. The recently mature leaves developed a light green marginal chlorosis. By day 38, buds were dramatically smaller and those, which were not aborted, were necrotic. The oldest leaves were darker green than the control. The mature leaves had a glossy sheen and appeared to have a wide leaf base. Axillary shoots had stopped elongating and shoot tips were severely rosetted. Overall, plants were rigid and the leaves were thick in texture. Advanced B deficient tissue concentrations were $22 \text{ mg}\cdot\text{kg}^{-1}$ and $29.6 \text{ mg}\cdot\text{kg}^{-1}$ for the deficient and control plants (Table 1).

REFERENCES

1. Williams, A. L.; Gibson, J. L.; Nelson, P. V.; Whipker, B. E.; Dole, J. M.; Cleveland, B.; Walls, F. R. *Foliar Symptomology and Tissue Concentrations of Nutrient Deficient Calibrachoa Plants*; North Carolina State University: Raleigh, NC, **2004**

Table 1. Tissue nutrient concentrations at initial and advanced stages of deficiency in recently matured leaves of hydroponically grown angelonia.

Note: * denotes significance at $P \leq 0.05$, ** denotes significance at $P \leq 0.01$ for each control deficiency pairing.

Treatment	-N	-P	-K	-Ca	-Mg	-S	-Fe	-Mn	-Cu	-Zn	-B
Element	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn	B
Units	%	%	%	%	%	%	mg·kg ⁻¹	mg·kg ⁻¹	mg·kg ⁻¹	mg·kg ⁻¹	mg·kg ⁻¹
Control initial	5.06	0.63	3.47	0.88	0.30	0.37	110.0	108.7	12.4	86.2	46.2
Deficient initial	2.82**	0.29**	2.03**	0.24**	0.16**	0.11**	80.3*	11.9**	2.5*	38.1**	21.9**
Control advanced	4.63	0.44	2.82	1.18	0.24	0.33	99.6	83.6	8.3	59.4	29.6
Deficient advanced	1.7**	0.23**	1.49**	0.15**	0.13**	0.12**	81.8**	11.4**	6.0**	28.3**	22.0**

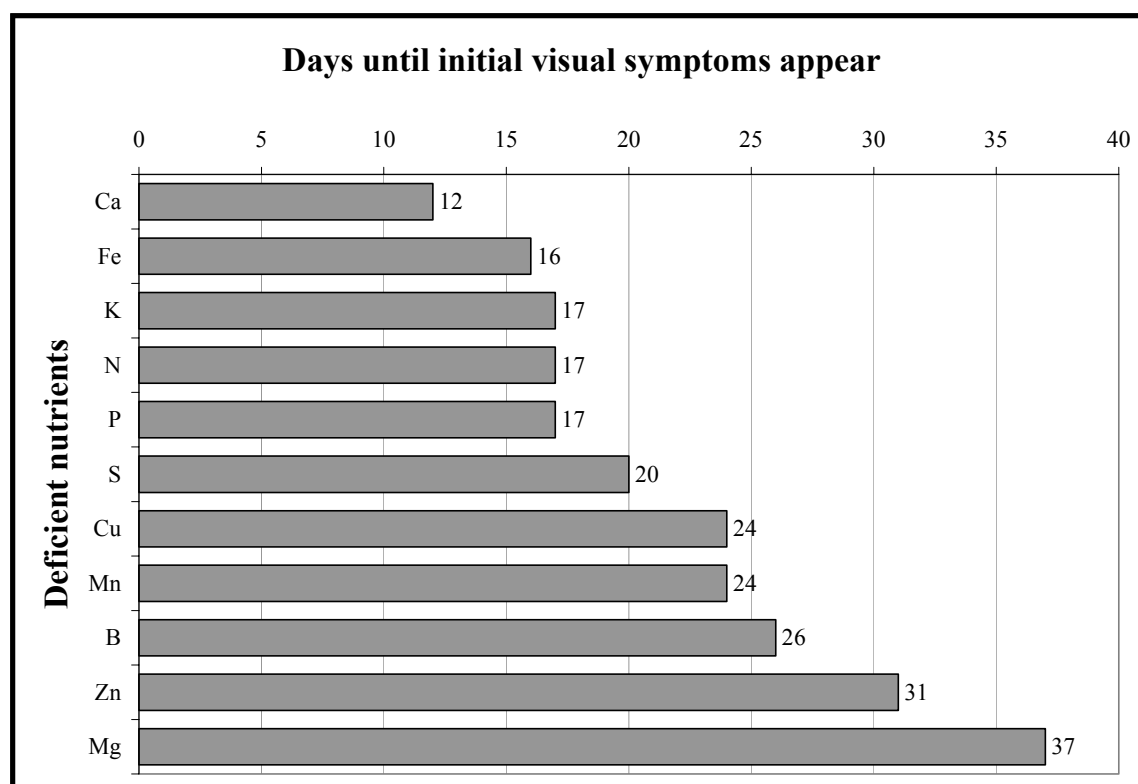


Figure 1. Days to develop initial deficiency symptoms for angelonia.

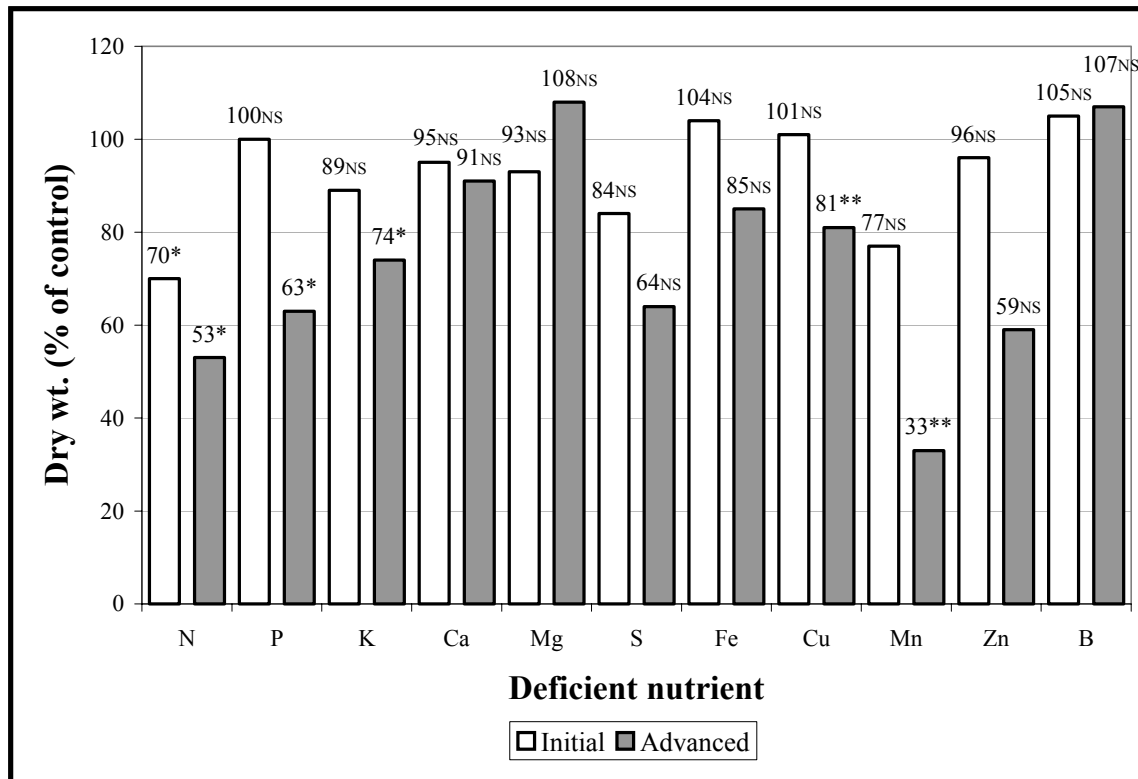


Figure 2. Dry weight of deficient nutrient plants expressed as a percentage of control plants at initial and advanced stages for angelonia.

Note: NS denotes non-significance at $P \leq 0.051$, * denotes significance at $P \leq 0.05$, ** denotes significance at $P \leq 0.01$ for each control deficiency pairing.

CHAPTER 3

FOLIAR SYMPTOMOLOGY AND TISSUE CONCENTRATIONS OF NUTRIENT DEFICIENT *BRACTEANTHA* PLANTS

Amy L. Williams¹, James L. Gibson¹, Paul V. Nelson¹, Brian E. Whipker¹, John M. Dole¹, Brenda Cleveland², and F.R. Walls²

¹Department of Horticultural Science, Campus Box 7609, North Carolina State University, Raleigh, North Carolina 27695-7609

²North Carolina Department of Agriculture and Consumer Services, Agronomic Division, Raleigh, NC 27607-6465

ABSTRACT

Foliar analysis standards have not been published for *Bracteantha bracteata* ‘Matilda Yellow’. *Bracteantha* plants were grown hydroponically in a glass greenhouse. Treatments consisted of a complete Hoagland’s all nitrate macronutrient solution with altered micronutrient content and 11 related solutions, each devoid of one essential nutrient. *Bracteantha* appeared to be most sensitive to Fe deficiency due to the appearance of symptoms at day 4. Ca and B followed at day 5, K at day 8, N at day 10, P and Mg on day 12, S and Mn at day 15, Zn on day 18 and Cu at day 20.

Unique deficiency symptoms included upward rolling of the margins and twisting of the recently mature leaves in Zn deficient plants and the young leaves of N and S deficient plants were thin and sword-like in appearance during initial symptoms of these deficiencies. The minimum critical recently mature leaf standards were % 2.70 N, 0.37 P, 1.90 K, 0.58 Ca, 0.08 Mg, 0.15 S, and $\text{mg}\cdot\text{kg}^{-1}$ 39.6 Fe, 13.6 Mn, 2.2 Cu, 32.4 Zn, and 21.1 B. Detailed descriptions of the chronological development of deficiency symptoms are presented along with associated early and advanced stage leaf nutrient concentrations.

OBJECTIVES

Objectives of this study included (a.) generation of visual nutrient deficiency symptoms in the chronological order in which they appeared up to advanced stages for N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, and B and (b.) establishment of foliar analysis standards in recently mature leaves for these same nutrients at incipient and late stages of deficiency in bracteantha.

MATERIALS AND METHODS

Materials and methodology were the same as reported by Williams et al. (1), with the following exceptions. Unrooted bracteantha cuttings were inserted in Oasis[®] LC1 foam cubes on March 6, 2002 and were transplanted after establishment on April 10, 2002 into 4.87 L aluminum-painted plastic tubs.

RESULTS AND DISCUSSION

Bracteantha was most sensitive to Fe deficiency as indicated by the rapid appearance of symptoms four days after the initiation of treatment. It was also very sensitive to Ca and B, which appeared five days after initiation (Figure 1). Plants were moderately sensitive to deficiencies of K with symptoms appearing day 8, N at day 10, and P and Mg at day 12. Plants were slower in developing S and Mn (day 15), Zn (day 18), and Cu (day 20) deficiencies. Only Zn deficient plants resulted in a notable plant weight difference when compared to the controls as an early symptom; those plants weighed less than control plants (Figure 2). With advanced symptoms Cu, B, P, K, Zn, Mn and especially N and S deficiencies resulted in smaller plants than the control. The following sections outline the progressions of visual deficiency symptoms for each nutrient. Corresponding tissue concentrations at early and late stages are listed in Table 1. Low minimal critical nutrient concentrations may be due to the young growth stages of these plants compared to the older, more reproductive stages, which are associated with traditional standards in the literature for ornamental crops.

Three developmental stages of leaves are referred to in this paper and are described as follows. Young leaves are less developed and mature leaves are more developed than the recently mature leaves. Recently mature leaves are those that are approaching or have just reached mature size, color, shape, or any other distinguishing characteristic that would identify them as mature.

NITROGEN

Initially, the plants appeared slightly smaller than the control with tissue N concentrations of 2.70% and 6.00% for the deficient and control plants (Table 1) (day 10). The roots were a dark, brownish-red color and longer than the control with fewer secondary and tertiary lateral roots. By day 13, a random light yellow chlorosis developed on the lowest mature leaves. Some of the oldest tips had a tan to brown burn that progressed from the tip of the leaf towards the base. Overall the plants were a dull, pale green color and all leaves appeared to be narrower than the control (day 15). Plants had an upright architecture with short axillary shoots, and were lighter green in color (day 19). The oldest leaves on the main stem developed a tip chlorosis. Leaf margins of the young leaves began to roll under, giving them a sword-like appearance. By day 23, plants were thin and spindly with no axillary shoot development and no elongation in the preexisting shoots. The young to mature leaves were light green compared to the control. The lowest mature leaves had a greenish-yellow to yellow chlorosis and some developed a necrosis. The lowest leaves progressed to yellow with some brown necrotic burning on the leaf tips with tissue concentrations at this advanced stage of 5.80% for the control and 1.70% for the N deficient plants (Table 1) (day 26). N deficient plants weighed 18.4 g, which was less than the controls at 56.8 g (Figure 2).

PHOSPHORUS

Phosphorus tissue concentrations were 0.37% for deficient and 0.80% for control plants on day 12 when P symptoms developed (Table 1). Overall the plants were a dull, dark green color and slightly more rigid than the control. The recently mature and mature leaves were slightly bowed and the roots appeared longer than the control. By day 15, the lower mature leaves developed a light green chlorosis and a papery brown necrotic tip burn on the margins, which were outlined by a thin yellow chlorosis. At this point, the plants were stunted and darker green in color, while the recently mature leaves appeared narrow compared to the control. The roots were darker, longer and denser than the control (day 17). The lowest mature leaves developed a yellow-green leaf tip chlorosis, which was followed by a brown papery necrosis. By day 24, the oldest mature leaves developed a faint purpling of the tips. Mature leaves developed a translucent gray-green coloration with light green to gray-brown necrotic spots, which fused and became brownish-black streaks on the leaves, which quickly shriveled. Overall plants were thin, spindly and stunted with less axillary growth (day 26). The leaves were inflexible and the mature leaves had a withered tan to brown necrosis, which started at the leaf tips and progressed back. These advanced symptoms occurred at tissue P concentrations of 0.07% and 0.50% for the deficient and control by day 30 (Table 1) (day 26). P deficient plants weighed 22 g, which was less than the controls at 41.8 g (Figure 2).

POTASSIUM

Initial tissue K concentrations were 1.90% and 7.60% for the control (Table 1) (day 8). A light green interveinal chlorosis appeared on the young leaves and the tips of the recently mature leaves. The young leaves were narrow and the margins rolled under slightly. Bud abortion of the youngest axillary buds developed. Overall the plants were darker green in color and slightly compact, due to the short axillary shoots (day 17). The youngest leaves developed a light green chlorosis, which progressed to a brown leaf tip necrosis. These leaves were shorter in length than the control. Young and recently mature leaves were slightly twisted and less upright (day 20). By day 22, the young leaves had obvious tip necrosis, which also appeared on the mature leaves. The downward bending leaves expressed a mottled-like appearance of dark green, light green and a brownish-black necrosis. Advanced symptoms developed by day 26, the entire plant was a dark blue-green color and slightly smaller than the control plants. The young leaves began turning slightly to one side. The mature leaves on the upper third of the plant had light green leaf tips. On those mature leaves brown necrotic spots then developed and fused into patches, which were sunken and crater-like. This necrosis began on the midvein and margins, with some random expression on the leaves. The leaf tips of the lowest oldest mature leaves developed a brownish-greenish-gray necrosis, which progressed to a brown necrosis. Advanced K tissue concentrations were 0.54% compared to 7.00% for the control (Table 1) (day 26). K deficient plants weighed 27.2 g, which was less than the controls at 35.6 g (Figure 2).

CALCIUM

Tissue concentrations during initial symptoms were 0.58% in deficient plants and 1.24% for the control (Table 1) (day 5). Initial Ca deficiency symptoms appeared in the roots 5 days after inducement. These roots appeared thick and stubby compared to the control. By day 10, the roots were shorter, denser, thicker and darker than the control. On day 13, the plants appeared short and compact (Figure 2). The young leaves were needle-like and deformed. Some developed a brownish-black withered leaf tip burn. These leaves also developed brown necrotic spots and patches giving them a water-soaked appearance. The recently mature leaves had a faint interveinal chlorosis on half of the leaf tips. The young leaves developed more necrotic black spots, while the recently mature leaves also developed dark black necrotic streaks and spots on the midvein (day 15). This necrosis developed rapidly and spread randomly on the leaf blade. By day 17, black spots appeared on the unopened flower petals. Necrosis affecting leaves turned grayish-brown and withered. Advanced symptoms developed by day 23, the young leaves were dark brown and withered starting at the leaf tips and progressed to the leaf base. The midvein on the recently mature leaves had black necrotic spots and some turned completely black. There was a complete necrosis of the leaves and the stems began collapsing, causing the flower heads to droop. Tissue Ca concentrations of advanced symptoms were 0.36% and 1.20% for the control (Table 1).

MAGNESIUM

Initial tissue Mg concentrations were 0.08% and 0.24% for the deficient and control by day 12 (Table 1) (day 12). Initial symptoms appeared as a light green, patchy interveinal chlorosis on the recently mature and mature leaves, which started at the tip and progressed toward the base. The margins had a light green chlorosis and some of the mature leaves had a small leaf tip necrosis. The recently mature and mature leaves were downward cupped. Faint light brown spots appeared on the chlorotic leaf tips and were fusing into patches (day 13). Overall the plant was light green color with yellow-green chlorotic patches and brown necrotic margins on the leaf tips of the recently mature and mature leaves (day 15). Recently mature and mature leaves increased in downward curling, which caused the plants to have a drooping architecture. The leaf tips of the recently mature leaves had yellow chlorotic patches, which progressed into a necrotic burn. Leaf tips of the mature leaves had faint, light green to dark green veins, which appeared web-like. The chlorosis started at the margins and moved upward (day 20). The leaf tips of the recently mature and mature leaves had a shriveled appearance. A dark brown necrosis began at either one side of the leaf or in the leaf middle, and was accompanied by a chlorotic papery green coloration, which bordered the necrotic area (day 22). A distinct interveinal chlorosis developed on the recently mature to mature leaves (day 27). The chlorosis of the young and recently mature leaves progressed to a dark brown necrosis on the leaf tips. At this advanced stage, tissue Mg concentrations were 0.10% and 0.22% for the deficient and control plants (Table 1) (day 27).

SULFUR

Initial tissue S concentrations were 0.15% and 0.27% for the control on day 15 (Table 1).

Limited axillary growth caused the plants to appear small, thin and spindly. The young and recently mature leaves were narrow, sword-like and light green in color.

By day 24, the plants were rigid, stiff and obviously stunted with no apparent axillary growth. The young and recently mature leaves developed a prominent narrow appearance due to the margins rolling under. The roots were longer than the control with less secondary and tertiary development. There were few to no flower buds present; however the remaining buds developed a light tan necrosis before aborting. By day 29, the necrotic buds turned brown and were much smaller than the controls. The shoots were uniformly greenish-yellow and the tips of all the leaves developed a whitish-yellow color 1mm in length, while some of the axillary shoots developed small brown spots on the leaves (day 31). Day 33, the leaf tips of the young leaves progressed to a yellow color while the tips of the leaves turned necrotic and brown. At this point, the young and recently mature leaves were a lime-green color while the lower mature leaves were dark green. The plants were severely stunted with no axillary shoot elongation and rigid. The young leaves developed small brown necrotic spots and the buds continued to abort (day 34). The recently mature and mature leaves developed a yellow chlorosis, which was followed by a brown, papery necrotic tip burn and a marginal burn. The roots were long with almost no secondary development and no tertiary or quaternary growth. Advanced tissue S concentrations were 0.05% and 0.18% for the control (Table 1) (day 34). S deficient plants weighed 10.4 g, which was less than the controls at 35.6 g (Figure 2).

IRON

Initial tissue Fe concentrations were 39.6 and 65.6 mg·kg⁻¹ for the control (Table 1) (day 4). Initial symptoms appeared on the young and recently mature leaves as a light green interveinal or uniform chlorosis. The chlorosis on the young and recently mature leaves progressed into a light green to yellow green interveinal chlorosis (day 5). Some of the young and recently mature leaf tips began twisting and turning, because the margins were rolling under. By day 10, the young leaves ranged from having a yellow-green interveinal chlorosis to a completely light yellow uniform chlorosis. The recently mature leaves had a greenish-yellow interveinal chlorosis and on some plants the chlorosis seemed to be on the base of the leaves. A faint whitish-yellow chlorosis appeared on the recently mature leaf margins, which quickly progressed to a necrosis of the leaf tips and resulted in marginal scorching (day 15). By day 19, unopened buds were a dark brownish-orange color compared to the control. The stems of the axillary shoots were light green at the base of the stem, which progressed to a light yellow-green at the stem apex. The lowest mature leaves remained a dark green, while the mature leaves directly above were light green. The recently mature leaves were light green to yellow with an interveinal chlorosis and the young leaves had a yellow uniform to interveinal chlorosis. Some of the recently mature leaves were a bleached yellowish-white color with white marginal tips and a brown necrosis. The young leaves had a light yellow to yellowish-white uniform chlorosis, with white to brown necrotic leaf tips. Tissue Fe concentrations at these advance stages were 28.7 and 59.6 mg·kg⁻¹ for the control (Table 1).

MANGANESE

Mn tissue concentrations for initial symptoms were 13.6 and 143.7 mg·kg⁻¹ for the deficient and control (Table 1) (day 15). Initial symptoms developed as a light green uniform chlorosis on the young and recently mature leaves, while the older mature leaves were dark green in color. The young and recently mature leaves were uniformly greenish-yellow and the leaf tips of the recently mature leaves were cupped downward (day 22). The young and recently mature leaves appeared swollen or wider and the midrib on some of the chlorotic recently mature leaves was a dark green color. By day 24, a lime-green chlorosis appeared on the young to recently mature leaves, while the lowest mature leaves were dark blue-green. The tips of the recently mature leaves developed a light yellow chlorosis with a faint brown necrosis (day 29). White to tan, shiny, sunken, undefined necrotic spots appeared randomly on the recently mature leaves followed by a brown papery necrosis on the tips (day 33). The lower one third of the mature leaves were dark green and developed a yellow marginal chlorosis on the leaf tips, which progressed to a brown leaf tip burn (day 37). By day 40, the marginal chlorosis on the lowest mature leaves widened and brown necrotic spots formed along the margins. The upper mature leaves were yellowish-green in color with dark green midveins and a brown tip necrosis. The mature and recently mature leaves had a yellowish-green interveinal chlorosis followed by silvery, tan, shiny random spots, which fused together to form patches. Tissue Mn concentrations were 4.4 and 127.7 mg·kg⁻¹ for the deficient and control at this advanced stage (Table 1) (day 40). Mn deficient plants weighed 27.7 g, which was less than the controls at 56.8 g (Figure 2).

COPPER

Tissue Cu concentrations for initial symptoms were 2.2 and 6.9 mg·kg⁻¹ for the control (Table 1). Plants developed a uniform light green color and fewer secondary branching on the roots (day 20). By day 23, the young and recently mature leaves of the shoot apex had a faint chlorosis. The recently mature and mature leaf tips cupped downward. The plants were shorter than the control and slightly more compact. The flower buds were light yellow compared to the control, which was a yellowish-orange. Some of the youngest shoots underneath the flower were dull green, puckered and deformed (day 29). By day 37, the young leaves were leathery, narrow and grayish-green with thin, needle shaped leaf tips, which began to twist. Some of the youngest leaves developed a brown leathery necrosis, which became withered, papery and twisted. The flowers were small and turned light yellow, while the buds developed brown necrotic spots. Overall the plants were a lime-green color and compact with a dense canopy. The lower mature leaves developed yellow chlorotic tips with brown necrotic spots, while the necrosis on the young leaves increased (day 40). The upper mature leaves curved and cupped upward. The leaf tips of the young leaves progressed into a brown necrosis. Tissue Cu concentrations at this advanced stage was 1.6 and 4.2 mg·kg⁻¹ for the control (Table 1).

ZINC

Tissue Zn concentrations were 32.4 compared to 52.2 mg·kg⁻¹ for the control at this initial stage (Table 1) (day 18). Zn deficient plants weighed 5 g, which was less than the controls at 6.8 g (Figure 2). Recently mature leaf margins were deformed causing the leaf tips to turn to one side, while some cupped downward at the leaf tips. The margins of the young and recently mature leaves rolled under, which also caused the leaves to twist and spiral. The young leaves were light green. By day 22, the young to the recently mature leaves developed random brown necrotic spots on the leaf tips, which progressed into large brown patches that then became withered. The young leaves turned completely necrotic, brown and brittle. The recently mature leaves developed dark brown necrotic spots, which fused to form brown necrotic patches. A faint to dark necrosis started at the leaf tips and progressed toward the leaf base. On day 24, the most severely affected plants had delayed flowering and some of the axillary buds aborted. Overall the plants were small, stunted and compact, due to the axillary shoots not elongating. Tissue Zn concentrations were 11.5 and 28.8 mg·kg⁻¹ for the deficient and control at this advanced stage (Table 1) (day 24). Zn deficient plants weighed 10.2 g, which was less than the controls at 15.3 g (Figure 2).

BORON

Tissue B concentrations were 21.1 and 59.3 mg·kg⁻¹ for the control (Table 1) (day 5). The young leaves were light green at the base and dark green at the tip. The first set of leaves

around the bud was deformed and light green in color. The mature leaf tips on the upper axillary shoot were twisted, due to the mid rib collapsing. By day 8, a veinal chlorosis appeared on the recently mature and mature leaves on the upper one third of the plant. The plants were rigid and brittle to the touch and the young and recently mature leaves twisted and spiraled. Flowers were incomplete with missing or deformed petals. By day 17, the plants were short and compact with a thick canopy; due to a reduction in shoot elongation. The mature leaves were wide and drooped downward. The basal margins were white and the center of the midvein developed a brown necrosis, which moved from the base to the tip. The roots were thin and less dense with fewer tertiary roots. The young axillary shoots were olive-green with a brown necrotic midvein. The young shoots were uniformly chlorotic and sparse on the main stem; the leaves of these shoots were deformed and twisted. The recently mature leaves were bent at the middle of the leaf and bowed downward or to one side. By day 23, the young leaves were obviously deformed and developed small brown necrotic spots. The plants had short internodes, which caused a rosetted appearance of the shoot apex. The growing point stopped developing, which resulted in small or aborted buds. The recently mature and mature leaves developed a light green marginal chlorosis. Plants were very compact, rosetted, thick, leathery, and rigid with glossy upper mature leaves. A brown necrosis developed on the margins of the young to mature leaves (day 26). Tissue concentrations at this advanced stage were 13.4 and 54.8 mg·kg⁻¹ for the deficient and control (Table 1). B deficient plants weighed 32.5 g, which was less than the controls at 56.8 g (Figure 2).

REFERENCES

1. Williams, A. L.; Gibson, J. L.; Nelson, P. V.; Whipker, B. E.; Dole, J. M.; Cleveland, B.; Walls, F. R. *Foliar Symptomology and Tissue Concentrations of Nutrient Deficient Calibrachoa Plants*; North Carolina State University: Raleigh, NC, **2004**

Table 1. Tissue nutrient concentrations at initial and advanced stages of deficiency in recently matured leaves of hydroponically grown bracteantha.

Note: * denotes significance at $P \leq 0.05$, ** denotes significance at $P \leq 0.01$ for each control deficiency pairing.

Treatment	-N	-P	-K	-Ca	-Mg	-S	-Fe	-Mn	-Cu	-Zn	-B
Element	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn	B
Units	%	%	%	%	%	%	mg·kg ⁻¹	mg·kg ⁻¹	mg·kg ⁻¹	mg·kg ⁻¹	mg·kg ⁻¹
Control initial	6.00	0.80	7.60	1.24	0.24	0.3	65.6	143.7	6.9	52.2	59.3
Deficient initial	2.70**	0.37**	1.90**	0.58**	0.08**	0.2**	39.6**	13.6**	2.2**	32.4**	21.1**
Control advanced	5.80	0.50	7.00	1.20	0.22	0.18	59.6	127.7	4.2	28.8	54.8
Deficient advanced	1.70**	0.07**	0.54**	0.36**	0.10**	0.05**	28.7**	4.4**	1.6**	11.5**	13.4**

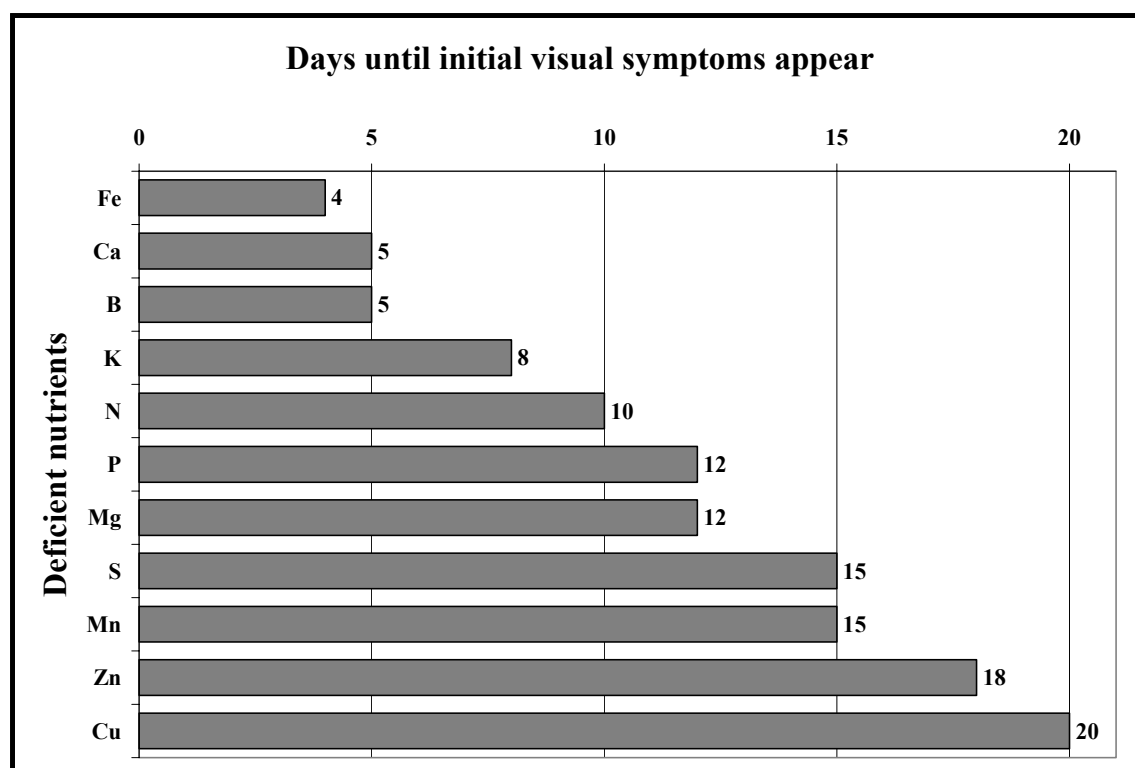


Figure 1. Days to develop initial deficiency symptoms for bracteantha.

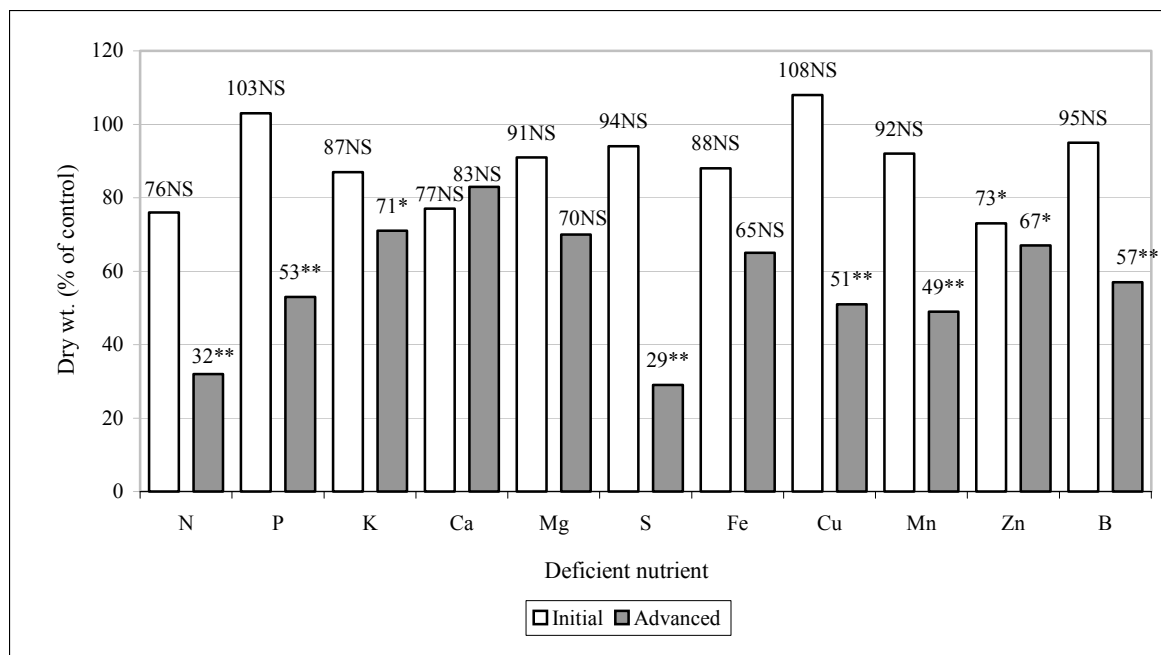


Figure 2. Dry weight of deficient nutrient plants expressed as a percentage of control plants at initial and advanced stages for bracteantha.

Note: NS denotes non-significance at $P \leq 0.05$, * denotes significance at $P \leq 0.05$, ** denotes significance at $P \leq 0.01$ for each control and deficiency pairing.

CHAPTER 4

FOLIAR SYMPTOMOLOGY AND TISSUE CONCENTRATIONS OF NUTRIENT DEFICIENT *BRACHYSCOME* ‘MINI YELLOW’ PLANTS

Amy Williams¹, Paul V. Nelson¹, Brian E. Whipker¹, John M. Dole¹, Brenda R. Cleveland² and F.R. Walls²

¹Department of Horticultural Science, Campus Box 7609, North Carolina State University, Raleigh, North Carolina 27695-7609

²North Carolina Department of Agriculture and Consumer Services, Agronomic Division, Raleigh, NC 27607-6465

ABSTRACT

Foliar analysis standards have not been published for *Brachyscome formosa* x *angustifolia* ‘Mini Yellow’. Deficiency symptoms do not exist for many nutrients in this species. *Brachyscome* ‘Mini Yellow’ plants were grown hydroponically in a glass greenhouse. Treatments consisted of a complete Hoagland’s all nitrate macronutrient solution with altered micronutrient content and 11 related solutions, each devoid of one essential nutrient. *Brachyscome* ‘Mini Yellow’ appeared to be most sensitive to Fe deficiency with the symptoms appearing at day 9. Ca and N followed at day 17, P at day

18, B at day 20, Mn at day 23, S on day 24, Mg on day 28, Cu and Zn on day 29 and K on day 33.

Unique deficiency symptoms included the following: Flowering ceased and older flowers turned necrotic with Cu and Zn, deficient mature leaves bent downward, due to a necrosis on the leaf bases. The minimum critical recently mature leaf standards were % 2.70 N, 0.37 P, 1.90 K, 0.58 Ca, 0.08 Mg, 0.15 S, and $\text{mg}\cdot\text{kg}^{-1}$ 39.6 Fe, 13.6 Mn, 2.2 Cu, 32.4 Zn, and 21.1 B. Detailed descriptions of the chronological development of deficiency symptoms are presented along with associated early and advanced stage leaf nutrient concentrations.

OBJECTIVES

Objectives of this study included (a.) generation of visual nutrient deficiency symptoms in the chronological order in which they appeared up to advanced stages for N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, and B and (b.) establishment of foliar analysis standards for these same nutrients at incipient and late stages of deficiency in brachyscome ‘Mini Yellow’.

MATERIALS AND METHODS

Materials and methodology were the same as reported by Williams et al. (1), with the following exceptions. Unrooted brachyscome ‘Mini Yellow’ cuttings were inserted in

Oasis ® LC1 foam cubes on October 6, 2002 and was transplanted after establishment on November 7, 2002 into 4.87 L aluminum-painted plastic tubs.

RESULTS AND DISCUSSION

Brachyscome ‘Mini Yellow’ appears to be most sensitive to Fe deficiency with appearance of symptoms appearing rapidly nine days after the initiation of treatment (Figure 1). Plants were moderately sensitive to Ca and N that followed at day 17, P at day 18, and B at day 20. Plants were fairly resistant to Mn (day 23) and S (day 24). Plants were most resistant to Cu and Zn deficiencies at day 29, followed by K, which was the slowest to develop (day 33). Cu, Mn, and Zn deficient plants resulted in notable plant weight reduction as an early symptom (Figure 2). With advanced symptoms B, Fe, Zn, and especially N nutrient deficiencies; plants weighed less than the controls. Plants deficient in all other nutrients did not significantly differ in weight to the control at these early and advanced deficiency stages. Following are the progressions of visual deficiency symptoms for each nutrient. Corresponding tissue concentrations at early and late stages of deficiency are listed in Table 1. Low minimal critical nutrient concentrations may be due to the young growth stages of these plants compared to the older, more reproductive stages that are associated with traditional standards in the literature for ornamental crops.

Three developmental stages of leaves are referred to in this paper and are described as follows. Young leaves are less developed and mature leaves are more developed than the recently mature leaves. Recently mature leaves are those that are approaching or have just

reached mature size, color, shape, or any other distinguishing characteristic that would identify them as mature.

NITROGEN

Initial N tissue concentrations were 3.83% and 6.23% for the deficient and control plants (Table 1) (day 17). Deficient plants had fewer axillary branches, with fewer leaves, which resulted in thin and spindly plants. These plants were short and small with thin canopies, and greenish-yellow basal mature leaves (day 19). A light green to yellow uniform chlorosis developed on the leaf tips of the lower mature leaves, which progressed to the entire leaf. The plants were upright in architecture with long flower stalks that protruded above the canopy (day 23). The young and mature leaves were light green with no axillary branching or shoot elongation, which caused the plants to appear severely thin and stunted (day 28). The entire plant developed a light yellowish-green uniform chlorosis by day 31, with an extremely thin canopy. By day 34, a tan to brown necrosis developed on the lowest mature leaves as a withered burn. Shoot elongation and axillary development stopped, which was accompanied by smaller and fewer leaves. Advanced tissue concentration were 1.39% for the deficient plants compared to 5.44% for the controls (Table 1) (day 34). N deficient plants weighed 7 g, which was less than the controls at 17.1 g (Figure 2).

PHOSPHORUS

Initial symptoms developed on day 18, with P tissue concentrations at 0.34% and 0.69% for deficient and control plants (Table 1). P deficient symptoms appeared as a dark green discoloration of the entire plant, especially the young foliage. By day 23, the entire plant was darker green in color than the control and had a slight dull sheen. The plants had thin canopies, which resulted from the small foliage (day 28). A few lower mature leaves developed a faint purple cast on the leaf tips accompanied by a deep, darker green coloration over the entire plant (day 34). With little to no shoot elongation these dark green plants were short and stunted (day 37). The mature leaves developed a yellowish-green chlorosis, which originated at the leaf tips and progressed toward the leaf base. These chlorotic leaves developed brown necrotic spots, which grew into larger patches (day 37). By day 38, the lower, withered mature leaves progressed to a tan to reddish-brown papery necrosis, which began at the leaf tips and progressed to the entire leaf. These plants were short, thin, and spindly and had a darker green pigmentation. P tissue concentrations at these advanced stages were 0.10% and 0.56% for deficient and control plants, respectively (Table 1).

POTASSIUM

Tissue values were 1.86% in deficient plants versus 5.20% in control plants (Table 1) (day 33). Initially, symptoms developed with a light green chlorosis on the young and recently mature leaves. The young and recently mature leaves had a light green chlorosis

with yellowish-green margins. Mature leaves had a faint interveinal chlorosis accompanied by a lighter green marginal chlorosis (day 38). By day 41, the plants were short and the light green interveinal and marginal chlorosis was evident on the young and recently mature leaves. This greenish-yellow to light green interveinal chlorosis became more obvious as it progressed on the young to mature leaves (day 41). On day 47, the plants were compact with a light green cast. The interveinal and marginal chlorosis was prominent, while the mature leaves developed a tan to brown necrotic burn on the leaflet tips. Young leaves had a light green uniform chlorosis and the recently mature leaves developed a light yellow to greenish-yellow interveinal chlorosis (day 55). Mature leaves progressed from a yellow to a tan chlorosis of the leaflet tips, which developed into a brown necrotic papery burn, causing the leaves to appear withered. Advanced symptoms appeared as short compact plants with tissue concentrations of 0.52% and 4.50% for deficient and control plants, respectively (Table 1) (day 55).

CALCIUM

Initially, Ca deficient young leaves were deformed and incomplete with tissue concentrations of 0.31% and 1.14% for the deficient and control plants (Table 1) (day 17). The young leaves were small and the deformation caused them to bend and twist within the middle of the leaf. The midveins collapsed causing the leaves to fold inward. These midveins and the leaflet tips had tan to brown necrotic spots, which were also on the mature leaf tips. Necrotic spots progressed to the recently mature and mature leaflet tips, and then developed toward the middle of these leaves (day 19). By day 23, the

young and recently mature leaves were small, severely deformed and shriveled. Flower buds were slightly smaller and turned brown to purple compared to the control. These leaves also began folding inward due to the collapse of the midvein. The mature leaves developed tan spots, which progressed into brown spots that formed necrotic patches on the leaf tips. These mature leaves developed a yellow chlorosis that progressed into a gray necrosis that began at the leaf tip and moved toward the base. At this point, some shoot tips were totally necrotic and the plants were compact. The shoot tips were shriveled and dry, which caused them to fold inward. The young leaves were small and deformed, this necrosis spread to the mature leaves. Flower stalks turned brown, which progressed to the buds and flowers that developed a necrosis and eventually aborted. Overall the plants were short, stunted and compact. The Ca tissue concentrations at this advanced stage were 0.27% and 0.99% for the deficient and control plants, by day 29 (Table 1) (day 23).

MAGNESIUM

Tissue concentrations for Mg deficient plants were 0.05% and 0.20% for deficient and control plants (Table 1) (day 28). Mg deficiency initially developed as an overall light green chlorosis. The mature leaves had a splotchy chlorosis, which began at the leaf tips and progressed towards the leaf base. By day 30, the mature leaves had a light green interveinal chlorosis. The plants continued to exhibit a light green uniform chlorosis, while the recently mature to upper mature leaves developed a light yellowish-green uniform chlorosis, which began at the leaf tips and progressed to the leaf base. The

mature leaves expressed a light yellow interveinal chlorosis. These leaves also contained a faint necrosis on the leaflet tips margins, which appeared as tan to brown spots (day 33). The chlorosis on the mature leaves advanced to a greenish-brown to tan necrosis on the margins of the leaflet tips. The recently mature leaves had a light green interveinal and uniform chlorosis, while some young leaves developed light green margins. The whole plant, except the lowest mature leaves had a light green pigmentation (day 36). By day 44, mature leaves had a distinct light yellow to yellow-green interveinal chlorosis, with deep green midveins. The recently mature leaves were light green with a faint interveinal chlorosis with light yellow-green leaf tips. The young to recently mature leaves developed a light green chlorosis along the margins that appeared as a fine thin line, which gave these leaves an outlined appearance. Flowering was less pronounced and the existing flowers were small with short petals compared to the control. Advanced Mg tissue concentrations were 0.05% and 0.25% in deficient and control plants (Table 1) (day 44).

SULFUR

The S tissue concentrations at this initial stage were 0.18% and 0.27% for the deficient and control leaves (Table 1) (day 24). Initial S deficiencies developed as short, thin plants with thin canopies. The entire plant exhibited a light green cast (day 29). By day 34, the shoot tips were lighter green than the rest of the plant and the plants were short and thin. The shoot tips advanced to a yellow-green color, which progressed down the rest of the shoot. The flower stalks from the flower down had a light greenish-yellow coloration.

The plants advanced to a lime-green chlorosis with little axillary branching, causing short shoots (day 37). Day 40, advanced symptoms progressed as short, thin-canopied plants with a lime-green chlorosis. The young to recently mature leaves were small and had a yellow-green chlorosis. Flower heads were small with thin short petals while the flower stalks and stems developed a red to purple cast. Tissue S concentrations for these advanced symptoms were 0.09% for the deficient compared to the 0.27% in the control plants (Table 1) (day 40).

IRON

The earliest of the deficiencies was Fe, tissue concentrations were 50.9 mg·kg⁻¹ and 62.5 mg·kg⁻¹ for deficient and control plants (Table 1) (day 9). Symptoms appeared as a light green chlorosis on the young and recently mature leaves. This chlorosis began at the leaf base and progressed to the tips. The young to recently mature leaves had a light lime-green chlorosis, while the youngest leaves were yellow-green (day 12). By day 14, the youngest leaves progressed to a light yellow, the young leaves were light yellow-green and the recently mature leaves were yellow-green to greenish-yellow. The chlorosis on all the leaves began at the leaf base and progressed to the tips. The lower mature leaves remained equal in color and size to the control. Tan to brown spots developed first on the margins of the young leaves and leaflets then progressed to the middle of the leaves and leaflets. These spots fused together causing a marginal burn. By day 16, the young leaves had a predominant light yellow uniform chlorosis while the recently mature leaves were light yellow-green with a random interveinal chlorosis. The necrotic spots on the young

leaves also appeared on the recently mature leaves, these spots fused to form patches on the margins of the leaflet tips, which progressed to the middle of the leaves. At this point, the young leaves began folding inward and developed a severe necrosis (day 18). The flower buds and young leaves were a whitish-yellow color, and the flower stalks had also turned a light yellowish-green to light green, which progressed from the base of the flower head downward. Shriveled and withered young leaves were covered with necrotic patches, which had originated at the leaf tips. The plants were more rigid than control plants, which gave them a more upright architecture. The most dramatic effect was that the lowest leaves, which remained a dark green comparable to the control leaf color, dramatic contrasting with the deficient chlorotic leaves. Advanced Fe tissue concentrations were $33.1 \text{ mg}\cdot\text{kg}^{-1}$ versus $59.7 \text{ mg}\cdot\text{kg}^{-1}$ for the control plants (Table 1) (day 18). Fe deficient plants weighed 4.5 g, which was less than the controls at 6.3 g (Figure 2).

MANGANESE

Mn tissue concentrations when initial symptoms appeared were $15.2 \text{ mg}\cdot\text{kg}^{-1}$ and $251 \text{ mg}\cdot\text{kg}^{-1}$ for deficient and the control plants (Table 1) (day 23). Mn deficient plants weighed 4.5 g, which was less than the controls at 4.8 g (Figure 2). The plants developed a light lime-green cast over its entirety; the young to upper mature leaves were most obvious. Mn deficient plants were slightly shorter and more compact than the control plant. Some upper mature leaves had a faint interveinal or a very thin marginal chlorosis; however the chlorosis was predominantly uniform. By day 28, a light lime-green uniform

chlorosis covered the deficient plants. The shoot tips were very chlorotic and the young to recently mature leaves appeared to have a faint yellow-green marginal chlorosis. The chlorotic plants were short with a dense canopy (day 33). The chlorosis on the young to recently mature leaves advanced to more apparent shades of yellow-green. By day 39, the recently mature leaf tips developed a greenish-tan blotchy chlorosis. At day 43, the plants were entirely light lime-green in color and short and compact, due to no shoot elongation. The chlorosis was most intense at the shoot tips, which progressed down the plant. A brownish-green necrosis formed on the leaflet tips of the mature leaves and the recently mature leaves developed random tan necrotic spots. Advanced Mn tissue concentrations were $8.4 \text{ mg}\cdot\text{kg}^{-1}$ and $94.5 \text{ mg}\cdot\text{kg}^{-1}$ for deficient and control plants (Table 1) (day 43).

COPPER

Initial Cu deficiency symptoms appeared on day 29, with tissue concentrations of $0.9 \text{ mg}\cdot\text{kg}^{-1}$ compared to $7.2 \text{ mg}\cdot\text{kg}^{-1}$ for control plants (Table 1). Cu deficient plants weighed 5.5 g, which was less than the controls at 10.1 g (Figure 2). Plants appeared slightly smaller and compact with a dull blue-green cast. Flowers were small, with petals half the length of control petals (day 40). Flowering was at half the amount visible on control plants and the petals were a pale yellow color. The dark blue-green cast increased over the entire plant (day 43). By day 51, other than the blue-green cast on the plants, the flowers were the most effected by the Cu deficiency. Flowering ceased and older flowers turned necrotic. These remaining flowers were small, with short and narrow petals. The flower stalks bent downward and from the base of the flower head moving down, it had a

light yellow to tan chlorosis, which progressed to a brown necrosis. Advanced tissue concentrations were $0.7 \text{ mg}\cdot\text{kg}^{-1}$ compared to $4.1 \text{ mg}\cdot\text{kg}^{-1}$ for control plants (Table 1).

ZINC

Zn tissue concentrations were $18.3 \text{ mg}\cdot\text{kg}^{-1}$ and $40.1 \text{ mg}\cdot\text{kg}^{-1}$ for control plants (Table 1) (day 29). Zn deficient plants weighed 6.2 g, which was less than the controls at 10.1 g (Figure 2). Small plants with thin canopies caused by short shoots and deformed and underdeveloped upper mature leaves were the first initial symptoms for Zn deficient plants. Plants remained short with a compact appearance by day 34, and developed a dark green cast by day 40. Necrosis developed on the basal region of the recently mature and upper mature leaves, which moved outward toward the leaf tips. The brownish-green necrosis caused the leaf base to become very thin and resulted in downward bending leaves (day 43). A faint brownish-green necrosis developed on the young light green chlorotic leaves along the midveins. The plants were generally a dark green color except for the young chlorotic leaves. The recently mature and mature leaves turned a pale yellow-green and the necrosis became paper thin, tan and brittle. These advanced symptoms developed by day 44, with Zn tissue concentrations at $18.3 \text{ mg}\cdot\text{kg}^{-1}$ compared to $40.4 \text{ mg}\cdot\text{kg}^{-1}$ for control plants (Table 1).

BORON

Initial symptoms developed with tissue concentrations of $17.7 \text{ mg}\cdot\text{kg}^{-1}$ and $59.5 \text{ mg}\cdot\text{kg}^{-1}$ for deficient and control plants (Table 1) (day 20). Primary and secondary roots appeared short, thick and stubby. By day 24, the roots developed a stubby appearance, with secondary roots having black tips. The shoot tips were slightly rosetted with a few young deformed leaves. Leaf deformation became more pronounced on the new growth and the leaves were smaller, with a faint light green chlorosis (day 29). Shoot tips were rigid to the touch and contained very small flower buds. Day 34, plants were short and compact, with few flowers. Those new and existing flowers were very deformed with petals that were short and narrow. Young leaves were extremely small and deformed. Shoot tips were rosetted, due to the termination of growth at the apical meristem. The short compact plants took on a dark green color and became rigid and slightly brittle to the touch. At this point, all flowering ceased because flowers aborted, died, or opened without petals (day 40). Young and recently mature leaves were deformed with a light green chlorosis. Extreme rosetting of the shoots gave the plants a short compact appearance. The dark green plants were stiff and rigid compared to the control plants. Mature leaves developed a light green to yellow chlorosis, which began at the leaf tip. Upper leaves were thin and deformed, with no leaflets forming. Tissue concentrations for advanced symptoms were $8.2 \text{ mg}\cdot\text{kg}^{-1}$ compared to $58.5 \text{ mg}\cdot\text{kg}^{-1}$ for control plants (Table 1) (day 40). B deficient plants weighed 12.5 g, which was less than the controls at 17.1 g (Figure 2).

REFERENCES

1. Williams, A. L.; Gibson, J. L.; Nelson, P. V.; Whipker, B. E.; Dole, J. M.; Cleveland, B.; Walls, F. R. *Foliar Symptomology and Tissue Concentrations of Nutrient Deficient Calibrachoa Plants*; North Carolina State University: Raleigh, NC, **2004**

Table 1. Tissue nutrient concentrations at initial and advanced stages of deficiency in recently matured leaves of hydroponically grown brachyscome ‘Mini Yellow’.

Note: * denotes significance at $P \leq 0.05$, ** denotes significance at $P \leq 0.01$ for each control deficiency pairing.

Treatment	-N	-P	-K	-Ca	-Mg	-S	-Fe	-Mn	-Cu	-Zn	-B
Element	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn	B
Units	%	%	%	%	%	%	mg·kg ⁻¹	mg·kg ⁻¹	mg·kg ⁻¹	mg·kg ⁻¹	mg·kg ⁻¹
Control initial	6.23	0.69	5.20	1.14	0.20	0.27	62.5	251.0	7.2	40.1	59.5
Deficient initial	3.83**	0.34**	1.86**	0.31**	0.05**	0.18**	50.9*	15.2**	0.9**	18.3**	17.7**
Control advanced	5.44	0.56	4.50	0.99	0.25	0.27	59.7	94.5	4.1	40.4	58.5
Deficient advanced	1.39**	0.10**	0.52**	0.27**	0.05**	0.09**	33.1*	8.4**	0.7**	18.3*	8.2**

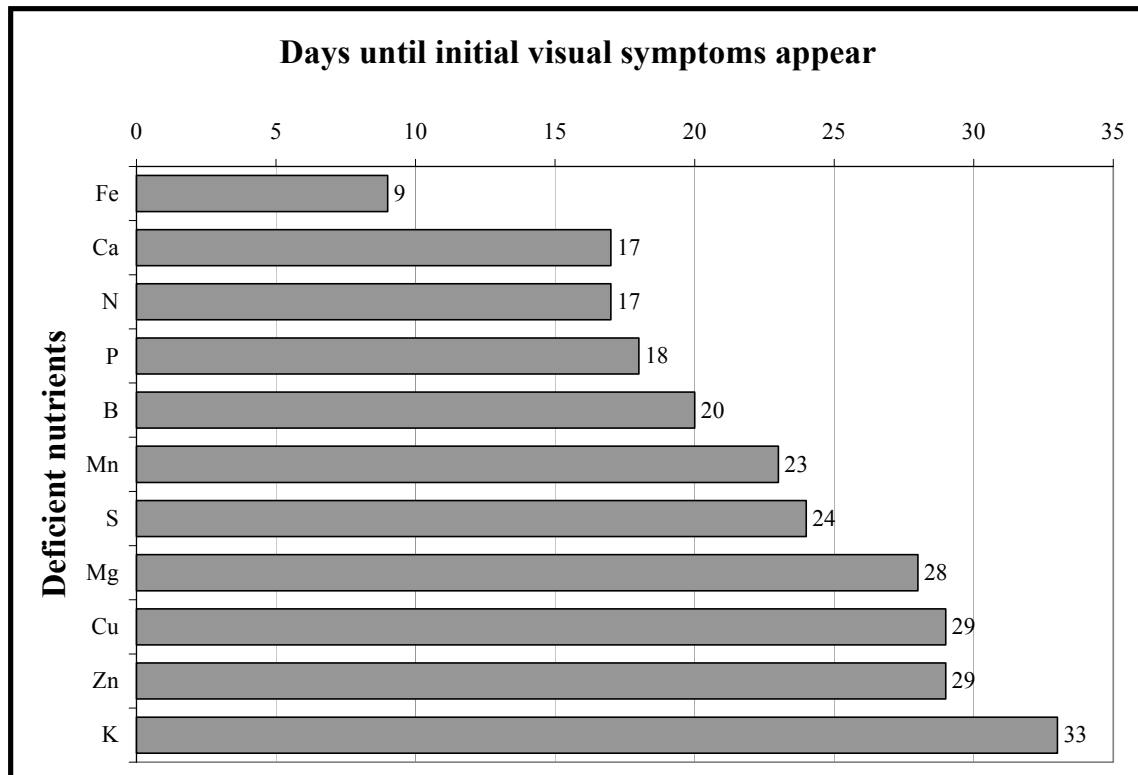


Figure 1. Days to develop initial deficiency symptoms for brachyscome ‘Mini Yellow’.

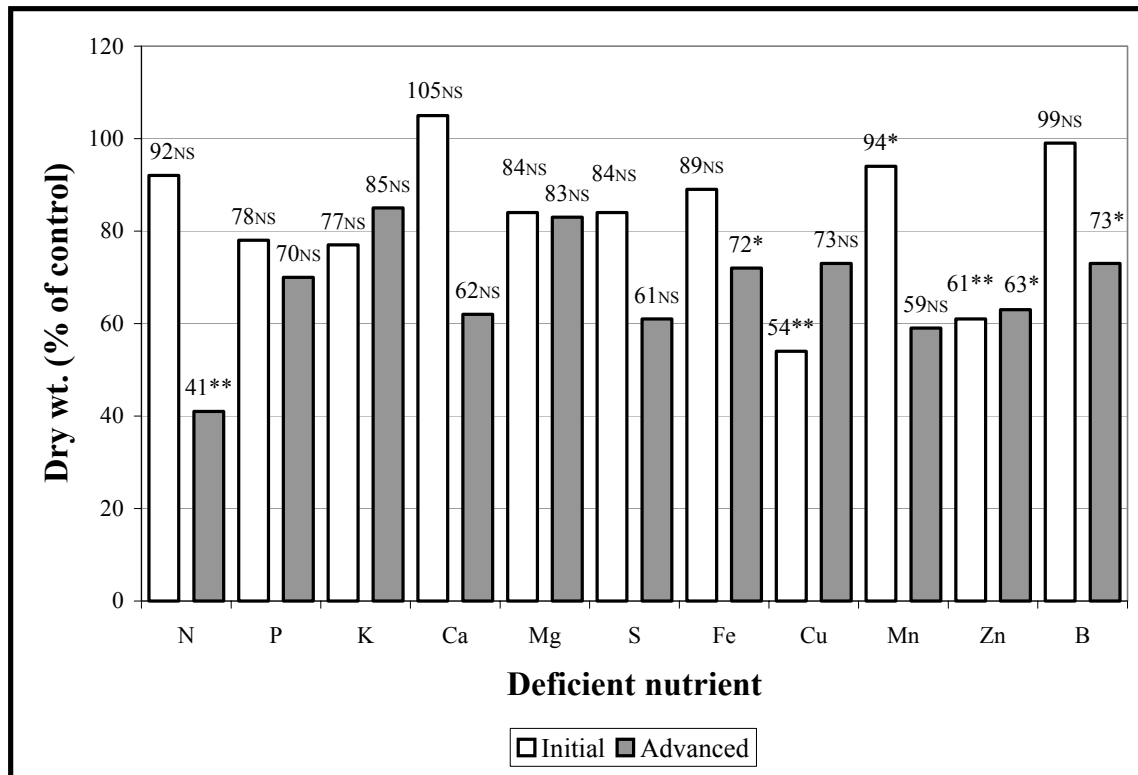


Figure 2. Dry weight of deficient nutrient plants expressed as a percentage of control plants at initial and advanced stages for brachyscome 'Mini Yellow'.

Note: NS denotes non-significance at $P \leq 0.05$, * denotes significance at $P \leq 0.05$, ** denotes significance at $P \leq 0.01$ for each control deficiency pairing.

CHAPTER 5

FOLIAR SYMPTOMOLOGY AND TISSUE CONCENTRATIONS OF NUTRIENT DEFICIENT *BRACHYSCOME* ‘JUMBO MAUVE’ PLANTS

Amy L. Williams¹, Paul V. Nelson¹, Brian E. Whipker¹, John M. Dole¹, Brenda Cleveland², and F.R. Walls²

¹Department of Horticultural Science, Campus Box 7609, North Carolina State University, Raleigh, North Carolina 27695-7609

²North Carolina Department of Agriculture and Consumer Services, Agronomic Division, Raleigh, NC 27607-6465

ABSTRACT

Foliar analysis standards have not been published for *Brachyscome* ‘Jumbo Mauve’. *Brachyscome* ‘Jumbo Mauve’ plants were grown hydroponically in a glass greenhouse. Treatments consisted of a complete Hoagland’s all nitrate macronutrient solution with altered micronutrient content and 11 related solutions, each devoid of one essential nutrient. *Brachyscome* ‘Jumbo Mauve’ appeared to be most sensitive to N, Fe, and B deficiencies due to the appearance of initial symptoms at day 5. Ca and P followed at day 12, K at day 17, S at day 31, Mg on day 40, and Cu on day 51.

Unique deficiency symptoms included Cu deficient plants having a random tan to brown necrotic spots on the underside of the recently mature and mature leaves and N deficient plants having flower bud stalks which were reddish-brown to purple, thin and drooped downward. The minimum critical recently mature leaf standards were % 4.3 N, 0.3 P, 2.3 K, 0.2 Ca, 0.1 Mg, 0.1 S, and $\text{mg}\cdot\text{kg}^{-1}$ 66.6 Fe, 1.3 Cu, and 47.8 B. Detailed descriptions of the chronological development of deficiency symptoms are presented along with associated early and advanced stage leaf nutrient concentrations.

OBJECTIVES

Objectives of this study included (a.) generation of visual nutrient deficiency symptoms in the chronological order in which they appeared up to advanced stages for N, P, K, Ca, Mg, S, Fe, Cu, Mn, Zn and B and (b.) establishment of foliar analysis standards in recently mature leaves for these same nutrients at incipient and late stages of deficiency in brachyscome ‘Jumbo Mauve’.

MATERIALS AND METHODS

Materials and methodology were the same as reported by Williams et al. (1), with the following exceptions. Unrooted brachyscome ‘Jumbo Mauve’ cuttings were inserted in Oasis[®] LC1 foam cubes on August 10, 2002 and were transplanted after establishment on September 17, 2002 into 4.87 L aluminum-painted plastic tubs.

RESULTS AND DISCUSSION

Brachyscome ‘Jumbo Mauve’ appeared to be most sensitive to N, Fe and B deficiency due to the rapid appearance of symptoms five days after the initiation of treatment (Figure 1). Plants were moderately sensitive to deficiencies of Ca and P that followed at day 12, and K at day 17. Plants were fairly resistant to S (day 31), Mg (day 40), as well as Cu (day 51) deficiencies. Plants were extremely resistant to Mn and Zn, because these symptoms did not appear during this trial and are not reported in the text. S and Cu deficient plants resulted in notable plant weight loss as an early symptom (Figure 2). During the advanced stage N, P, S, and B deficiencies resulted in smaller plants than the control. Following are the progressions of visual deficiency symptoms for each nutrient. Corresponding tissue concentrations at early and late stages are found in Table 1. Low minimal critical nutrient concentrations may be due to the young growth stages of these plants compared to the older, more reproductive stages that are associated with traditional standards in the literature for ornamental crops.

Three developmental stages of leaves are referred to in this paper and are described as follows. Young leaves are less developed and mature leaves are more developed than the recently mature leaves. Recently mature leaves are those that are approaching or have just reached mature size, color, shape, or any other distinguishing characteristic that would identify them as mature.

NITROGEN

Nitrogen deficient plants had tissue concentrations of 4.29% compared to 6.99% for the controls for these initial symptoms (Table 1) (day 5). Plants appeared small with less lateral shoot development, while some stems had a faint purple coloration. By day 12, all leaves were shorter and smaller than control leaves and early flowering was apparent. Plants were very prostrate, which caused the plants to appear thin and spindly (day 20). The leaves were small, rigid, and leathery. The nodes and stems developed a reddish pigmentation, while the lowest oldest leaves had a random light green to greenish-yellow chlorosis. By day 28, the chlorosis on the lowest leaves progressed into a greenish-yellow to yellow random chlorosis, which began in the middle of the leaves. The nodes and internodes developed a rusty-red coloration and the entire stem had a red tint, which was more prominent at the base of the plant (day 31). The lowest leaves had splotchy patches of yellow-orange and reddish-brown chlorosis. Some leaves progressed to a rusty brown color and then shriveled. The prostrate plants had firm stems and leaves. Day 35, the flower bud stalks were reddish-brown to purple, thin and drooped downward. By day 40, the plants were severely stunted, thin, and spindly with little to no axillary growth. The leaves were dramatically smaller in size and the lower leaves were yellow to brown and shriveled, while the entire plant was overall a light green color. Tissue N concentrations were 2.21% and 7.46% for deficient and control plants at this advanced stage (Table 1) (day 40). N deficient plants weighed 10.1 g, which was less than the controls at 21.5 g (Figure 2).

PHOSPHORUS

Initial P tissue concentrations for these initial symptoms were 0.27% compared to 0.58% for control plants (Table 1) (day 12). Plants appeared slightly smaller and had a medium green cast, while the lowest leaves developed a patchy chlorosis. By day 16, all leaves were dark green and had a canopy that appeared slightly thinner than control plants. The mature leaves developed dark green to faint blackish-brown margins of the leaflets (day 22). By day 33, the plants were smaller with less axillary branching and overall the plants were darker green. The leaves were thick giving the plants a rubbery texture. The lowest mature leaves had a yellow-green chlorosis, which progressed into a rusty, reddish-orange chlorosis on the leaflet tips and progressed toward the leaf base. This chlorosis quickly progressed into a tan to brown papery necrosis (day 37). Some recently mature to mature leaves developed a grayish-brown necrosis on the leaflets, which was papery and curled. The necrosis increased and progressed up the plant, resulting in brown, withered mature leaves (day 41). By day 45, the lower half of the plant was necrotic and leaves were withered and curled. Overall the plants developed an upright architecture (day 51). The lower necrotic half was withered, brittle and papery while, the remaining upper half of the plant was a dull dark green color and the young to recently mature leaves were small. Advanced P tissue concentrations were 0.23% and 0.61% for deficient and control plants by day 51 (Table 1). P deficient plants weighed 16.7 g, which was less than the controls at 23.7 g (Figure 2).

POTASSIUM

The K tissue concentrations were 2.32% and 4.77% for the deficient and control plants at this initial stage (Table 1) (day 17). Potassium deficient plants appeared slightly smaller and were a dark green color. The color difference was especially evident when the young light green control leaves were compared to the dark green K deficient leaves. The young to recently mature leaves were small in size. By day 26, the plants had an upright architecture, were thin and spindly with less leaf expansion, while some appeared short and compact. The recently mature leaves were small, dark green and developed a light green chlorosis on the margins. Plants were small with less axillary branching and the lowest mature leaves developed a light yellow to orange-yellow chlorosis (day 33). The dark green young leaves developed a lime-green chlorosis on the margins. The chlorosis on the lowest mature leaves progressed into a brown, shriveled necrosis, which curled at the leaf tips (day 37). By day 45, the plants drooped and appeared withered. The recently mature and upper mature leaves developed light yellow to tan chlorotic spots. The stems were thin and the plants were smaller. Overall the plants had a dark green cast, drooped and appeared water deprived (day 49). The shoot tips were compact with small leathery young leaves. The chlorotic spots on the recently mature and mature leaves progressed into tan to white translucent spots. Tissue K concentrations were 1.27% and 4.95% for deficient and control plants at this advanced stage (Table 1) (day 49).

CALCIUM

Tissue Ca concentrations were 0.18% and 0.67% for the deficient and control plants at this initial stage (Table 1) (day 12). Plants were short and compact with thick canopies and little to no flower buds apparent. Small pin sized tan spots quickly developed on the leaflet tips of the young to recently mature leaves (day 14). The chlorotic spots grew larger and more prominent on the young and recently mature to upper mature leaves (day 20). The spots progressed into depressions on the leaf surface, which appeared not only on the leaflet tips, but also in the mid regions of the leaflets. By day 24, the young to recently mature leaves were covered with a speckling of tan to brown spots. These spots fused together to make small necrotic patches. The flower stalks below the flower head developed a brown to black necrosis with slight indentations on the upper region of the stalk. This necrosis caused the stalks to bend and the flower head to droop downward. Flower petals turned brown and curled. The young dark green leaves were deformed due to the leaflet tips, which curled and folded inward. Advanced symptoms quickly developed by day 31, the tan speckling fused into large crater like patches of tan necrosis on the young to upper mature leaves. The leaflet tips of these leaves were brown, shriveled and some cupped upward. The midrib of the upper mature leaves turned brown and necrotic. The flower stalks collapsed completely and bent in half, while the flowers either failed to develop or open. Those flowers that opened had small whitish petals. Ca tissue concentrations were 0.14% and 0.83% for deficient and control plants (Table 1) (day 31).

MAGNESIUM

Magnesium deficient plants had tissue concentrations of 0.13% and 0.26% for deficient and control plants at this initial stage (Table 1) (day 40). Initially, the deficiency developed on the mid to lower mature leaves as a light green chlorosis that progressed into a yellow-green chlorosis. Recently mature leaves developed a faint chlorosis, while the lower chlorotic mature leaves developed a light yellow chlorosis on the leaflet tips (day 45). The recently mature and mature leaves progressed to random patches of chlorosis with a brown marginal necrosis. Overall plants were light green. The margins of the leaflet tips of the mature leaves turned yellow to brown, which gave it a bronzed appearance (day 50). These margins progressed to a thin brown necrosis, while the recently mature and mature leaves had a light green chlorosis. Advanced tissue Mg concentrations were 0.11% and 0.23% for the deficient and control plants (Table 1) (day 50).

SULFUR

Deficient S plants had tissue concentrations of 0.14% compared to 0.31% for control plants at this initial stage (Table 1) (day 31). S deficient plants weighed 10.4 g, which was less than the controls at 17.1 g (Figure 2). Plants were small, thin, had less axillary branching and the young to recently mature leaves were light green in color. The plants had an upright architecture and the petioles were thin and long compared to the control plants. The young leaves developed a light green chlorosis, which started in the middle of

the leaf near the midveins and progressed out (day 34). The internodes developed a faint red color, while the stalks of newly unopened buds turned a reddish-green to reddish-brown color, bent downward and drooped. Overall the plants were short, upright in architecture and developed a lime-green pigmentation (day 44). The stems had a faint reddish-brown to reddish-purple coloration mostly on the mid to basal region of the stem, which progressed to the top. By day 50, young to recently mature leaves were small and the lowest mature leaves developed a yellowish-green chlorosis. Plants were entirely lime-green in color, small, thin and spindly. Advanced S deficient tissue concentrations were 0.17% and 0.31% for deficient and control plants (Table 1) (day 50). S deficient plants weighed 18.5 g, which was less than the controls at 23.7 g (Figure 2).

IRON

Tissue Fe concentrations for this initial stage were $66.6 \text{ mg} \cdot \text{kg}^{-1}$ and 396 for the control plants (Table 1) (day 5). The young to recently mature leaves developed a faint, light green chlorosis on the basal and marginal regions. By day 10, the first sets of leaves at the shoot tips developed a uniform light green chlorosis. The chlorosis of the young and recently mature leaves progressed into an interveinal chlorosis, while the upper mature leaves developed a faint, light green chlorosis. The first sets of leaves progressed into a uniform light yellow chlorosis (day 16). The young leaves were extremely chlorotic, uniformly and interveinally, in shades of light yellow-green to light yellow. Recently mature leaves were uniformly and interveinally chlorotic in shades of light green to greenish-yellow. This chlorosis moved from the young to the upper mature leaves and

ranged from a light green to yellow, with either a interveinal or uniform chlorosis at the growing tips, while the lowest mature leaves remained a dark green color similar to the control plants. As symptoms progressed, the young leaves of the shoot tips turned uniformly and interveinally light yellow (day 22). Recently mature leaves were yellow-green and the veins turned to a light green color. At this point, the young shoot tips were light yellow to white and young to recently mature leaves progressed into a light yellow-green interveinal chlorosis with light green veins (day 31). Some upper mature leaves developed a light green marginal chlorosis, while the lower mature leaves remained a green color similar to the control plants. Advanced symptoms developed with tissue Fe concentrations of $69.5 \text{ mg}\cdot\text{kg}^{-1}$ and 116.5 for the control plants (Table 1) (day 31).

COPPER

Initial symptoms had tissue Cu concentrations of $1.3 \text{ mg}\cdot\text{kg}^{-1}$ compared to 5.3 for the control plants (Table 1) (day 51). Cu deficient plants weighed 24.5 g , which was less than the controls at 32.2 g (Figure 2). The young to recently mature leaves developed a uniform light green chlorosis. Recently mature to mature leaves were thick and the leaflets appeared slightly deformed. Random tan to brown necrotic spots developed on the recently mature and mature leaves. These spots were more prominent on the undersides of the leaves than on the surface. The petioles of the deficient leaves were light green. The spots progressed into crater-like depressions on the leaf surface (day 55). These spots fused into large brown patches, which eventually turned into a papery necrosis. Small gray to black spots appeared on the patchy brown necrosis. As symptoms

progressed, the leaves developed faint marginal chlorosis and a glossy sheen. Advanced tissue Cu concentrations were $2.2 \text{ mg}\cdot\text{kg}^{-1}$ and 4.5 for the deficient and control plants, for these late stage symptoms (Table 1) (day 63).

BORON

Tissue B concentrations were $47.8 \text{ mg}\cdot\text{kg}^{-1}$ and 64.9 for deficient and control plants (Table 1) (day 5). Boron deficient plants had short internodes, which caused them to be short and compact with fewer buds. As symptoms progressed, the shoots failed to elongate, the plants developed a dark green cast and the leaves were thick and leathery (day 10). By day 16, the plants were dark green, short and compact with an upright architecture, which caused the plants to be rigid. The young leaves of the shoot tips were slightly deformed and folded around the bud; at this point the growing point had ceased and rosetting was apparent. The petioles of the young leaves were faint brown and slightly withered. Flowers were smaller, with petals missing; those petals remaining were short and thin compared to the control flowers. Some young and recently mature leaves were prostrate and did not open or expand. The recently mature leaves developed small tan to light brown patches, which appeared in the middle of the leaves (day 22). Petioles of these leaves were slightly longer and some developed a tan to brown necrosis on the margin of the petiole. Flowers petals were short, incomplete and some began to fold inward, which gave it a cup-like appearance. Existing petals turned white at the base, while the rest of the petal remained purple. By day 31, young to recently mature leaves were light green, small and deformed. Shoot tips were compact due to heavy resetting.

Overall, plants were short, upright and compact with thick, leathery leaves. Petioles of the recently mature and mature leaves were long with thick light green to white midribs. Advanced symptoms had tissue B concentrations of 22.1 mg·kg⁻¹ and 67 for the deficient and control plants (Table 1) (day 31). B deficient plants weighed 11.8 g, which was less than the controls at 17.1 g (Figure 2).

MANGANESE AND ZINC

Plants were extremely resistant to Mn and Zn, because these symptoms did not appear during this trial and are not reported in the text. These elements did not appear after 71 days, which was 20 days after the last initial symptoms were expressed and 7 days after the last advanced symptoms was expressed.

REFERENCES

1. Williams, A. L.; Gibson, J. L.; Nelson, P. V.; Whipker, B. E.; Dole, J. M.; Cleveland, B.; Walls, F. R. *Foliar Symptomology and Tissue Concentrations of Nutrient Deficient Calibrachoa Plants*; North Carolina State University: Raleigh, NC, **2004**

Table 1. Tissue nutrient concentrations at initial and advanced stages of deficiency in recently matured leaves of hydroponically grown brachyscome ‘Jumbo Mauve’.

Note: * denotes significance at $P \leq 0.05$, ** denotes significance at $P \leq 0.01$ for each control deficiency pairing.

Treatment	-N	-P	-K	-Ca	-Mg	-S	-Fe	-Cu	-B
Element	N	P	K	Ca	Mg	S	Fe	Cu	B
Units	%	%	%	%	%	%	mg·kg ⁻¹	mg·kg ⁻¹	mg·kg ⁻¹
Control initial	6.99	0.58	4.77	0.67	0.26	0.31	298.3	5.3	64.9
Deficient initial	4.29**	0.27**	2.32**	0.18**	0.13**	0.14**	66.6*	1.3**	47.8**
Control advanced	7.46	0.61	4.95	0.83	0.23	0.31	116.5	4.5	67.0
Deficient advanced	2.21**	0.23**	1.27**	0.14**	0.11**	0.17**	69.5**	2.2**	22.1**

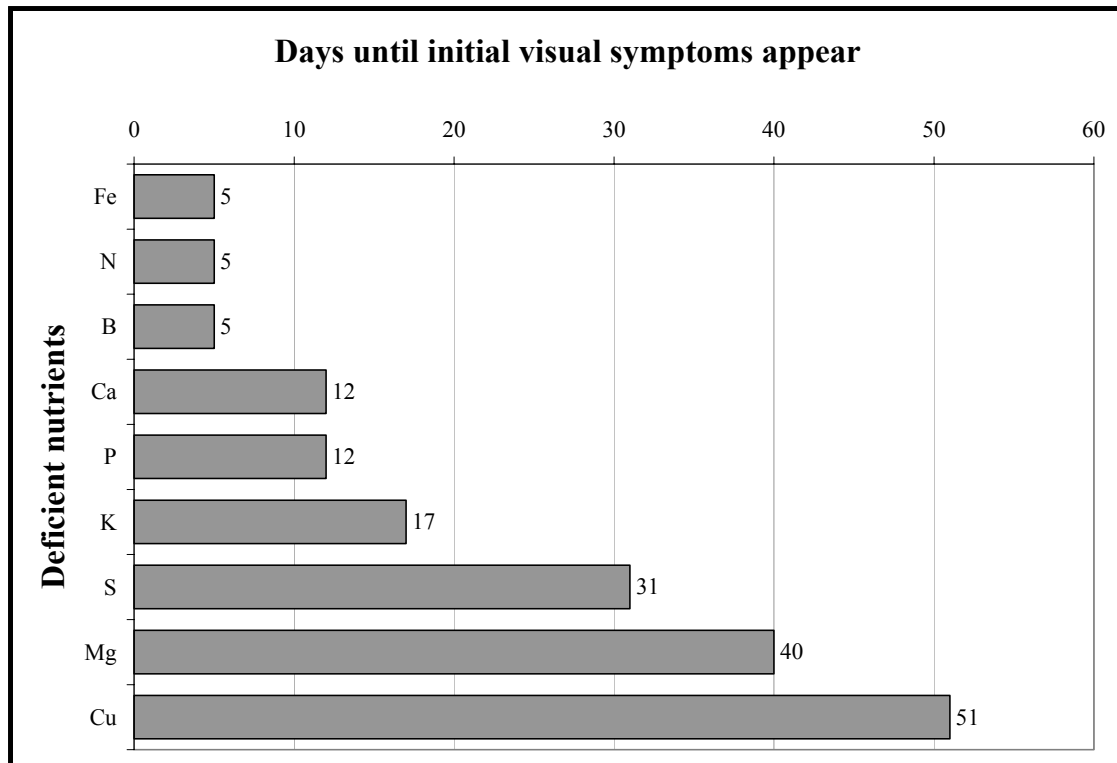


Figure 1. Days to develop initial deficiency symptoms for brachyscome ‘Jumbo Mauve’.

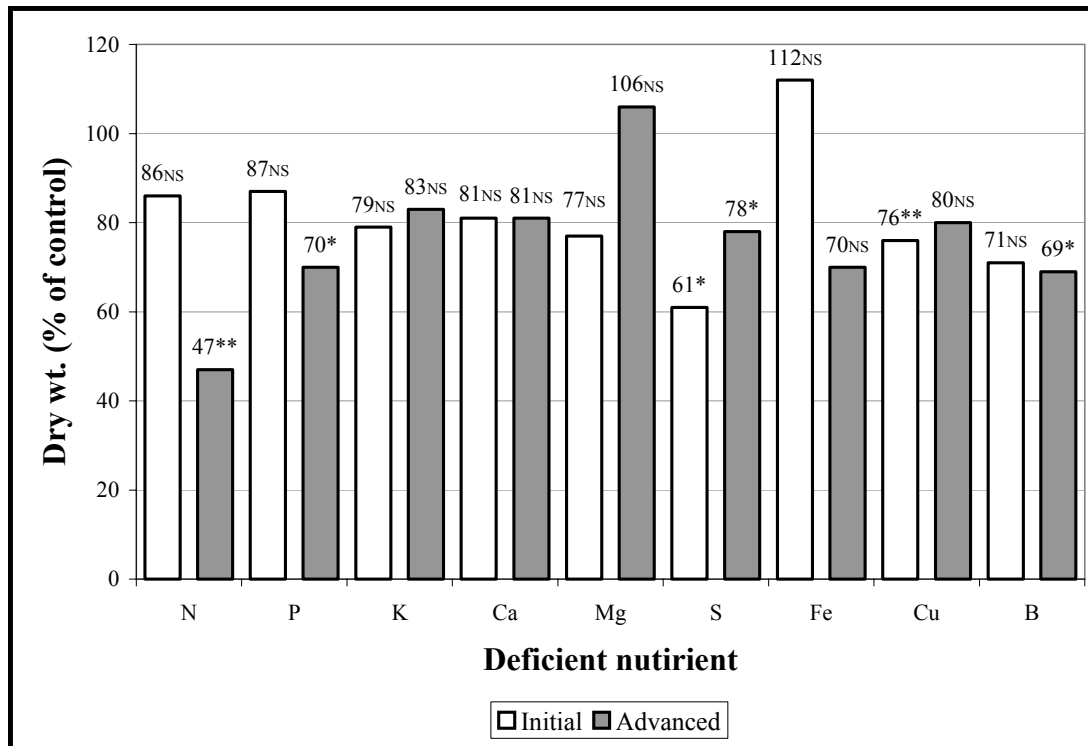


Figure 2. Dry weight of deficient nutrient plants expressed as a percentage of control plants at initial and advanced stages for brachyscome ‘Jumbo Mauve’.

Note: NS denotes non-significance at $P \leq 0.051$, * denotes significance at $P \leq 0.05$, ** denotes significance at $P \leq 0.01$ for each control deficiency pairing.

SUMMARY

Tissue analysis standards and complete visual deficiency symptoms of N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, and B are crucial for monitoring plant nutrient status. Foliar analysis standards and visual symptoms of nutritional disorders for calibrachoa, angelonia, bracteantha, brachyscome 'Mini Yellow', and brachyscome 'Jumbo Mauve' have not been previously published and were the objectives of this study. These plants were grown hydroponically in a glass greenhouse at 35°N latitude. Nutrient treatments were based on the macronutrient composition of Hoagland's all-nitrate nutrient solution with altered micronutrient levels and 11 additional related solutions, each devoid of one essential nutrient. Visual symptoms were chronologically recorded and photographed.

Symptomology

Synoptic visual deficiency symptoms were as follows: N – Plants were stunted with smaller leaves. As symptoms progressed the plants developed a light green chlorosis and the lowest leaves developed a yellow chlorosis followed by a brown necrosis. P – Plants were smaller and all the foliage developed a dark green pigmentation, which progressed into a necrosis of the lower mature leaves. K – Plants develop chlorosis of the leaf tips and margins, which quickly progress into necrosis. Ca – Severe stunting and compactness would result, accompanied by chlorosis and necrosis of the shoot tips. Flowering would cease or flowers would be incomplete. Mg – Recently mature and mature leaves would develop a uniform or interveinal chlorosis, which would progress from a light green to yellow, and then turn brown. S – Plants would be severely stunted and then develop a

uniform lime-green chlorosis. Fe – A light green chlorosis would progress from the shoot tips to the mature leaves, which would progress into a light yellow followed by a white chlorosis and brown necrosis. Mn – A light green chlorosis of the entire plant, which would often be smaller in size. Necrosis would affect the recently mature leaves. Zn – Young leaves would develop a light green chlorosis and be slightly puckered. Cu – Plants were small and developed a blue-green pigmentation. Severe twisting and rolling of the young leaves was observed. B – Extreme rosetting and deformation of the shoot tips and young leaves resulted in short compact plants. Foliage was deep green and glossy with a thick, leathery texture.

Unique Symptoms

Unique features among the deficiencies were: color changes within the stems, leaves and flowers; early flowering; leaf curling and flower toppling.

Color Change

Color change, specifically a reddening of foliage, occurred with S deficiency of angelonia and with N deficiency in brachyscome 'Jumbo Mauve'. Leaf reddening has been documented in many other plants as a physiological disorder, which can be induced by different abiotic stresses (1). Such stresses include high soil salinity, temperature, irradiation, UV-radiation and mineral deficiency (1). "The abnormal red coloring of leaves may be due to accumulation of red pigments from the flavonoid (C₆-C₃-C₆) group, namely anthocyanins, accompanied by chlorophyll degradation" (1). Under various stress conditions cotton (*Gossypium hirsutum* L.) anthocyanin levels increased up to

662% and chlorophyll content levels declined to 36% of the control, resulting in severe reddening of the cotton leaves (1).

Studies have also correlated N deficiency with increased anthocyanin levels and increased sugar content. For example, during N deficiency sucrose concentrations increased in the storage roots of sugar beets (scientific name) (2). Another study showed when the amounts of anthocyanins increased that sugar content in the Merlot and Cabernet Sauvignon grape (*Vitis vinifera* L.) juices also increased (3). Increased levels of anthocyanin were also found in cruciferous seedlings subjected to mineral nutrient deficiencies (4). When N and P deficiencies occurred, anthocyanin content increased and growth of the seedlings reduced (4). This reddening symptom varies with crop and the nutritional deficiency it accompanies. In strawberries (*Fragaria xananassa* Duchesne) a reddening occurred in N, P, K and Mg deficiencies (5). Seaside bentgrass (scientific name) turned red with N, Ca, and Mg deficiencies (6). N deficiency also caused reddening in grapes, chrysanthemums (*Dendranthema xgrandiflorum* Kitam.), and coleus (*Solenostemon scutellarioides* (L.) Codd.) (7, 8, 9), while Mg deficiency caused reddening in blueberries (*Vaccinium* L.) and cotton (10).

“Anthocyanins are members of a class of nearly universal, water-soluble, terrestrial plant pigments that can be classified chemically as both flavonoid and phenolic. They are found in most land plants, with the exception of the cacti and the group containing the beet. They contribute colors to flowers and other plant parts ranging from shades of red through crimson and blue to purple, including yellow and colorless” (11). Supporting

evidence suggests that the color change in flower petals from purple to pink of the copper deficient angelonia and the lightening of the yellow bracteantha petals were due to a decrease in anthocyanins. In both of cases the flower petal color seemed to lighten towards white. Many times flower color can also be determined by pH: for example, with hydrangeas (*Hydrangea macrophylla* (Thumb.) Ser.) lowering the pH can transition the flower color from pink to blue (12). Similarly, in calibrachoa the red-flowered cultivars had low pH and blue-flowered cultivars had high pH values in the vacuole (13). “The color and stability of an anthocyanin in solution is highly dependent on pH. They are most stable and most highly colored at low pH values and gradually lose colors as the pH is increased. As the pH rises, the anthocyanin can become almost colorless. This color loss is reversible, and the red hue will return upon acidification” (14). This same color loss can also be seen in copper deficiency. Copper availability is reduced as pH increases, allowing for speculation that the reduction in anthocyanin and the increase in pH is a result from copper deficiency.

Early Flowering

“Early flowering is thought to be triggered by forms of stress. Forms of stress may be drought, excessive soil moisture, nitrogen deficiency, root damage, fertilizer injury or chemical injury” (15). Early flowering can also be induced through the use of cultural techniques, such as photoperiod, accelerated growth and gibberellins (16). “In a study done with eastern cottonwood, combined treatments of water stress, root pruning and paclobutrazol was applied to 3-month-old rooted cuttings from mature trees. These cuttings had been subjected to root restriction and long days. All the treated plants

flowered, whereas no untreated plants formed flower buds” (17). Therefore, plants under stress sometimes flower early to assure reproduction. Grapes under nitrogen deficiency mature early to develop seed, however as a result they do not reach normal size (18). During our study, the stress of N deficiency caused early flowering in brachyscome ‘Jumbo Mauve’ and bracteantha ‘Matilda Yellow’.

Leaf Curling

Many nutrients are involved with cellular structure and stability. Deficiencies often cause necrosis, during this stage tissue dies and the formation of cells ceases within those areas. Leaf curling and bending can be a result of necrosis, for example: if the right margin of a leaf developed a necrosis, the cells on the left margin would continue to develop, which would then cause the leaf to bend toward the right. Cell division continuing in some regions and not in others often causes leaves to curl, twist or bend taking on many shapes. Cu deficiency caused the leaves of calibrachoa to develop a tubular structure, due to the margins on both sides rolling. B and Mn deficient calibrachoa leaves twisted and developed deformations. Cu deficient leaves of angelonia twisted to one side, resulting in the shoot tip to take on a pinwheel appearance. Ca and Zn deficient leaves of angelonia also developed a bending and curling appearance. Leaf tips bend and curl downward due to the breakdown of tissue during necrosis; this was seen in P, K, Mg, and Mn deficiencies of bracteantha leaves.

Flower Topple

Calcium is involved with cell elongation and division, which lends itself to plant structure and stiffness (18). Calcium deficiency often causes the tissue of leaves and stems to break down and become necrotic. Flower topple or topple disorder can occur with calcium deficiency; this occurs when the flower stalk weakens and the weight of the flower head causes the stalk to bend in half. This topple disorder was seen in this study with bracteantha and brachyscome 'Jumbo Mauve'. Topple disorder may be more frequently associated with tulips, daffodils, or iris.

Plant Sensitivity

The rate at which symptoms occurred is an indication of the species sensitivity to a particular nutrient deficiency. The chronological order in which nutrient deficiency symptoms occurred was as follows: that were first to occur by species were as follows: Calibrachoa – Fe, Ca, Mn, N, S, B, K, P, Cu, Zn and Mg. Angelonia – Ca, Fe, K, N, P, S, Cu, Mn, B, Zn and Mg. Bracteantha – Fe, Ca, B, K, N, P, Mg, S, Mn, Zn and Cu. Brachyscome 'Mini Yellow' – Fe, Ca, N, P, B, Mn, S, Mg, Cu, Zn and K. Brachyscome 'Jumbo Mauve' – Fe, N, B, Ca, P, K, S, Mg and Cu. Brachyscome 'Jumbo Mauve' plants were extremely resistant to Mn and Zn, because these symptoms did not appear during this trial and are not reported in the text. A combined average of days throughout the five crops the overall sensitivity was as follows: Very sensitive to Fe – 8 days, Ca – 11 and N – 12, Moderately resistant to B – 15, P – 15, K – 18, Mn – 19 and S – 21, Very resistant to Zn – 24, Cu – 29 and Mg – 30. Through this interpretation, Fe, Ca and N can be considered the most valuable nutrients to the five crops studied.

REFERENCES

1. Edreva, A.; Gurel, A.; Gesheva, E.; Hakerlerler, H. Reddeding of cotton (*Gossypium hirsutum* L.) leaves. *Biologia Plantarum* **2002**, *45* (2), 303-306.
2. Ulrich, A.; Hills, F. Sugar Beet Nutrient Deficiency Symptoms University of California: California, undated, 7.
3. Yokotsuka, K.; Nagao, A.; Nakazawa, K.; Sato, M. Changes in anthocyanins in berry skins of Merlot and Cabernet Sauvignon grapes grown in two soils modified with limestone or oyster shell versus a native soil over two years. *American Journal of Enology and Viticulture* **1999**, *50* (1), 1-12.
4. Hodges, DM.; Nozzolillo, C. Anthocyanin and anthocyanoplast content of cruciferous seedlings subjected to mineral nutreint deficiencies. *Journal of Plant Physiology* **1996**, *147* (6), 749-754.
5. Johanson, F. *Hunger in Strawberries*, Everett: Washington, 1980; 7,11,13,15.
6. Love, J. Mineral Deficiency Symptoms on Turfgrass. Wisconsin Academy of Sciences, Arts and Letters **1962**, *51*, 135-140.

7. Christensen, L.; Kasimatis, A.; Jensen, F. Macronutrients, *Grapevine Nutrition and Fertilization in the San Joaquin Valley*. The Regents of the University of California: California, 1978, 8.
8. Eysinga, J.; Smilde, K. *Nutritional disorders in chrysanthemums*, Centre for Agricultural Publishing and Documentation: Wageningen, 1980, 9, 17.
9. Cook, R.; Millar, C. Nitrogen deficiency. *Plant Nutrient Deficiencies*, Special Bulletin 353; Michigan State College: Michigan, 1953, 16.
10. Anonymous. *If They Could Speak*, Chilean Nitrate Educational Bureau, Inc.: New York, 1941, 12-13, 44.
11. Sullivan, J. Anthocyanin.
<http://www.charlies-web.com/specialtopics/anthocyanin.html> (accessed March 17, 2004)
12. Yoshida, K.; Toyama-Kato, Y.; Kameda, K.; Kondo, T. Sepal color variation of *Hydrangea macrophylla* and vacuolar pH measured with a proton-selective microelectrode. *Plant and Cell Physiology* **2003** 44 (3), 262-268.
13. Waterworth, R.; Griesbach, R. The biochemical basis for flower color in *Calibrachoa*. *Hortscience* **2001** 36 (1), 131-132.

14. Sims, C.; Morris, J. Effects of pH, Sulfur Dioxide, Storage Time and Temperature on the Color and Stability of Red Muscadine Grape Wine. *AJEV* **1984** 35 (1) 35-39.
15. Moore, M. Premature Flowering Management. The University of Georgia College of Agricultural & Environmental Sciences Cooperative Extension Service
<http://www.griffin.peachnet.edu/caes/tobacco/handbook/flowering98.html> (accessed March 17, 2004)
16. Chalupka, W.; Cecich, R. Control of the first flowering in forest trees. *Scandinavian Journal of Forest Research* **1997** 12 (1), 102-111.
18. Yuceer, C.; Kubiske, M.; Harkess, R.; Land, S. Effects of induction treatments on flowering in *Populus deltoids*. *Tree Physiology* **2003** 23 (7), 489-495.
19. Bennett, W. Rice. *Nutrient Deficiencies & Toxicities In Crop Plants*; APS Press: Minnesota, 1993, 177.