ABSTRACT

DHARMALINGAM S. PITCHAY. 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 (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. Limited information is available on visual disorder symptoms and foliar analysis standards for vinca, celosia, marigold, zinnia, salvia, pansy, impatiens, begonia, petunia, ornamental cabbage, snapdragon and New Guinea impatiens.

Synoptic visual deficiency symptoms were as follows: N - Symptoms began on lower leaves as uniform light green chlorosis to yellow and finally necrotic. Development of red to purple pigmentation was observed in marigold, snapdragon, begonia and ornamental cabbage. P - All foliage became deeper green, then lower leaves became purplish/reddish in some species, and finally necrotic. K - In the early stage of deficiency, plants became compact and deeper green. Later, necrosis developed on tips and margins of older leaves. Ca - Necrotic symptoms first developed on roots, then on young leaves and shoot apex. However, the shoot apices of New Guinea impatiens and impatiens did not develop necrosis. Mg - Interveinal chlorosis developed on older leaves followed by necrosis along the margins. S - The entire leaves became uniformly lighter green. Then the margins of all leaves became more chlorotic then the remainder of the leaves. Eventually, the margins developed necrosis. Fe - Young leaves developed interveinal chlorosis, generally from the base.

Then the entire lamina turned chlorotic. The chlorosis then gave way to bleached whitish-yellow. Mn - Young and recently matured leaves developed chlorosis, and mature leaves developed stippling of necrosis. Drastic reduction of shoot and root growth was common. Flowering was severely inhibited. Cu - Impaired flower development includes reduced size and premature abscission. Desiccation and sudden death of tissue occurred on recently mature leaves. Chlorosis was generally not a distinguishing feature. Zn - Young and recently matured leaves developed puckering, chlorosis, and necrosis. Some plants developed purple pigmentation. B - Foliage became darker green and glossy. Young and recently matured leaves became thick, leathery and brittle with severe distortions. The roots were short and stubby.

The rate at which symptoms occurred is an indication of the species sensitivity to a particular nutrient deficiency. The nutrient deficiency symptoms that were first to occur by species were as follows: begonia - N and Fe, celosia - N and K, impatiens - Ca, marigold - N and Fe, New Guinea impatiens 'Grenada' - Ca, New Guinea impatiens 'Timor' - Fe, ornamental cabbage - Ca and Fe, pansy - Fe, petunia - Fe, salvia - N, snapdragon - Ca, vinca - Fe, and zinnia - Ca.

Root and shoot apical meristems are specialized areas where cell proliferation and organogenesis take place. The absence of Ca, B and Ca\B greatly reduced primary root growth relative to control within 12 hours. The number of abnormal nuclei decreased in the order of Ca > Ca\B > B deficient treatments. Nuclei in both Ca and Ca\B deficiency treatments showed a significant decrease in volume. Boron deficiencies initially resulted in an increased mitotic index (MI),

concurrent with root apical meristem (RAM) distortion. Most of the increase in root diameter could be explained by an increase in the number of cell files in the cortex. Ca and B deficiencies together reduced both the MI and root tip distortion. Lack of B arrests cell death, and lack of Ca arrests cell division. The absence of B triggered cell division in the peripheral lateral root cap. This indicates that B may help to mitigate Ca deprivation effects in Ca deficient environment.

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

By

DHARMALINGAM S. PITCHAY

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APPROVED BY:

Brian E. Whipker, Ph.D.

Nancy G. Creamer, Ph.D.

Udo Blum, Ph.D.

Paul V. Nelson, Ph.D.
Chair of Advisory Committee

DEDICATION

This dissertation is dedicated to my loving wife

Endumathi

I would not have made this far without your support

BIOGRAPHY

Dharmalingam S. Pitchay was born and raised in Sungkai, Perak State, Malaysia. At an early age, he developed his passion for gardening inspired by his family. In 1981, Dharma graduated from the University of Agriculture Malaysia with a Bachelor of Agriculture Science degree majoring in Horticulture. Upon graduation, he worked for the Department of Agriculture in Malaysia. His responsibilities were to transfer research technologies to the end users in commercial horticultural farms and to provide advisory services pertaining to cultural techniques, plant propagation and nursery management. In 1996, Dharma received his Master of Science degree in Horticulture from West Virginia University. He then continued with his graduate education at North Carolina State University in 1998 majoring in physiology of plant nutrition under the direction of Dr. Paul V. Nelson, and completed his Doctor of Philosophy degree in 2002. During his journey, Dharma married Endumathi Muthu in December 1985.

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TABLE OF CONTENTS

Page

LIST OF TABLES xii

LIST OF FIGURES xviii

CHAPTER 1. VINCA FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS 1

ABSTRACT 2

INTRODUCTION 3

MATERIALS AND METHODS 4

RESULTS AND DISCUSSION 6

REFERENCES 17

CHAPTER 2. FRENCH MARIGOLD FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS 23

ABSTRACT 24

INTRODUCTION 25

MATERIALS AND METHODS 26

RESULTS 26

DISCUSSION 35

REFERENCES 38

CHAPTER 3. ZINNIA FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS 43

ABSTRACT 44

INTRODUCTION 45

MATERIALS AND METHODS 46

Page

RESULTS 46

DISCUSSION 54

REFERENCES 57

CHAPTER 4. SALVIA FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS 62

ABSTRACT 63

INTRODUCTION 64

MATERIALS AND METHODS 65

RESULTS 65

DISCUSSION 75

REFERENCES 78

CHAPTER 5. CELOSIA FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS 83

ABSTRACT 84

INTRODUCTION 85

MATERIALS AND METHODS 86

RESULTS 86

DISCUSSION 95

REFERENCES 97

CHAPTER 6. IMPATIENS FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS 102

ABSTRACT 103

INTRODUCTION 104

Page

MATERIALS AND METHODS 105

RESULTS 105

DISCUSSION 114

REFERENCES 118

CHAPTER 7. PANSY FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS 123

ABSTRACT 124

INTRODUCTION 125

MATERIALS AND METHODS 126

RESULTS 126

DISCUSSION 135

REFERENCES 138

CHAPTER 8. PETUNIA FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS 143

ABSTRACT 144

INTRODUCTION 145

MATERIALS AND METHODS 146

RESULTS 146

DISCUSSION 156

REFERENCES 159

Page

CHAPTER 9. BEGONIA FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS 164

ABSTRACT 165

INTRODUCTION 166

MATERIALS AND METHODS 167

RESULTS 167

DISCUSSION 176

REFERENCES 179

CHAPTER 10. ORNAMENTAL KALE FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS 184

ABSTRACT 185

INTRODUCTION 186

MATERIALS AND METHODS 186

RESULTS 187

DISCUSSION 196

REFERENCES 200

CHAPTER 11. SNAPDRAGON FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS 205

ABSTRACT 206

INTRODUCTION 207

MATERIALS AND METHODS 208

RESULTS 208

DISCUSSION 215

REFERENCES 217

Page

CHAPTER 12. NEW GUINEA IMPATIENS FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS 223

ABSTRACT 224

INTRODUCTION 225

MATERIALS AND METHODS 226

RESULTS 226

DISCUSSION 238

REFERENCES 241

CHAPTER 13. CELLULAR ANALYSIS OF Ca, B, AND Ca AND B DEPRIVATION 247

ABSTRACT 248

INTRODUCTION 249

MATERIALS AND METHOD 251

RESULTS 253

DISCUSSION 258

CONCLUSION 260

REFERENCES 261

CHAPTER 14. THE IMPACT OF Ca, B, AND Ca\B DEPRIVED NUTRIENT SOLUTION ± SUBSTRATES ON ROOT GROWTH AND DEVELOPMENT 269

INTRODUCTION 270

MATERIALS AND METHODS 270

RESULTS 273

Page

DISCUSSION 277

APPENDICES 291

APPENDIX I. POTENTIAL FACTORS CONFOUNDING NUTRIENT ACCUMULATION AND DEFICIENCY SYMPTOMS EXPRESSION IN THIS STUDY 292

APPENDIX II. pH OF 12 TREATMENT NUTRIENT SOLUTIONS BEFORE AND AFTER REPLACEMENT 294

APPENDIX III. ANALYSIS OF 11 NUTRIENT ELEMENTAL CONCENTRATIONS FOR EACH NUTRIENT DEFICIENT TREATMENT 295

LIST OF TABLES

Page

CHAPTER 1. VINCA FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Table 1. Tissue nutrient concentration at early and advanced stages of deficiency in recently matured leaves of vinca grown hydroponically 19

CHAPTER 2. FRECH MARIGOLD FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Table 1. Tissue nutrient concentration at early and advanced stages of deficiency in recently matured leaves of marigold grown hydroponically 40

CHAPTER 3. ZINNIA FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Table 1. Tissue nutrient concentration at early and advanced stages of deficiency in recently matured leaves of zinnia grown hydroponically 59

CHAPTER 4. SALVIA FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Table 1. Tissue nutrient concentration at early and advanced stages of deficiency in recently matured leaves of salvia grown hydroponically 80

CHAPTER 5. CELOSIA FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Table 1. Tissue nutrient concentration at early and advanced stages of deficiency in recently matured leaves of celosia grown hydroponically 99

CHAPTER 6. IMPATIENS FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Table 1. Tissue nutrient concentration at early and advanced stages of deficiency in recently matured leaves of impatiens grown hydroponically 120

Page

CHAPTER 7. PANSY FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Table 1. Tissue nutrient concentration at early and advanced stages of Deficiency in recently matured leaves of pansy grown hydroponically 140

CHAPTER 8. PETUNIA FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Table 1. Tissue nutrient concentration at early and advanced stages of deficiency in recently matured leaves of petunia grown hydroponically..... 161

CHAPTER 9. BEGONIA FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Table 1. Tissue nutrient concentration at early and advanced stages of deficiency in recently matured leaves of begonia grown hydroponically 181

CHAPTER 10. ORNAMENTAL CABBAGE FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Table 1. Tissue nutrient concentration at early and advanced stages of deficiency in recently matured leaves of ornamental cabbage grown hydroponically 202

CHAPTER 11. SNAPDRAGON FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Table 1. Tissue nutrient concentration at early and advanced stages of deficiency in recently matured leaves of snapdragon grown hydroponically 220

CHAPTER 12. NEW GUINEA IMPATIENS FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Table 1a. Tissue nutrient concentration at early and advanced stages of deficiency in recently matured leaves of 'Timor' grown hydroponically..... 242

Table 1b. Tissue nutrient concentration at early and advanced stages of deficiency in recently matured leaves of 'Grenada' grown hydroponically..... 242

Page

CHAPTER 14. THE IMPACT OF Ca, B, AND Ca\B DEPRIVED NUTRIENT SOLUTION ± SUBSTRATES ON ROOT GROWTH AND DEVELOPMENT

Table 1. Summary of a factorial combination of four nutrient formulations and four substrates tested in this study 273

Table 2. Effects of –Ca, -B, and –Ca\-B on shoot variables of tomato plants grown in four different substrates 279

Table 3. Effects of –Ca,-B, and –Ca\-B on root variables and shoot to root ratios of tomato plants grown in four different substrates..... 280

Table 4. Summary of analysis of variance of substrate types [a) solution, b) sand, c) solution + sand, d) peat] and nutrition [control, -Ca, -B and -Ca/-B], and their interactions on plant height..... 281

Table 5. Summary of analysis of variance of substrate types [a) solution, b) sand, c) solution + sand, d) peat] and nutrition [control, -Ca, -B and -Ca/-B], and their interactions on stem diameter 282

Table 6. Summary of analysis of variance of substrate types [a) solution, b) sand, c) solution + sand, d) peat] and nutrition [control, -Ca, -B and -Ca/-B], and their interactions on leaf area..... 283

Table 7. Summary of analysis of variance of substrate types [a) solution, b) sand, c) solution + sand, d) peat] and nutrition [control, -Ca, -B and -Ca/-B], and their interactions on nodes..... 284

Table 8. Summary of analysis of variance of substrate types [a) solution, b) sand, c) solution + sand, d) peat] and nutrition [control, -Ca, -B and -Ca/-B], and their interactions on shoot dry weight 285

Table 9. Summary of analysis of variance of substrate types [a) solution, b) sand, c) solution + sand, d) peat] and nutrition [control, -Ca, -B and -Ca/-B], and their interactions on root dry weight 286

Table 10. Summary of analysis of variance of substrate types [a) solution, b) sand, c) solution + sand, d) peat] and nutrition [control, -Ca, -B and -Ca/-B], and their interactions on root surface area 287

Page

Table 11. Summary of analysis of variance of substrate types [a) solution, b) and, c) solution + sand, d) peat] and nutrition [control, -Ca, -B and -Ca/-B], and their interactions on root length 288

Table 12. Summary of analysis of variance of substrate types [a) solution, b) sand, c) solution + sand, d) peat] and nutrition [control, -Ca, -B and -Ca/-B], and their interactions on shoot/root dry weight ratio 289

Table 13. Summary of analysis of variance of substrate types [a) solution, b) sand, c) solution + sand, d) peat] and nutrition [control, -Ca, -B and -Ca/-B], and their interactions on shoot/root surface area 290

APPENDIX III. ANALYSIS OF 11 NUTRIENT ELEMENTAL CONCENTRATIONS FOR EACH NUTRIENT DEFICIENT TREATMENT

Table 1. Vinca mean tissue nutrient concentration (n = 3) at early stages of deficiency in recently matured leaves of hydroponically grown plants 296

Table 2. Vinca mean tissue nutrient concentration (n = 3) at advanced stages of deficiency in recently matured leaves of hydroponically grown plants 297

Table 3. French marigold mean tissue nutrient concentration (n = 3) at early stages of deficiency in recently matured leaves of hydroponically grown plants 298

Table 4. French marigold mean tissue nutrient concentration (n = 3) at advanced stages of deficiency in recently matured leaves of hydroponically grown plants 299

Table 5. Zinnia mean tissue nutrient concentration (n = 3) at early stages of deficiency in recently matured leaves of hydroponically grown plants 300

Table 6. Zinnia mean tissue nutrient concentration (n = 3) at advanced stages of deficiency in recently matured leaves of hydroponically grown plants 301

Table 7. Salvia mean tissue nutrient concentration (n = 3) at early stages of deficiency in recently matured leaves of hydroponically grown plants 302

Table 8. Salvia mean tissue nutrient concentration (n = 3) at advanced stages of deficiency in recently matured leaves of hydroponically grown plants 303

Page

Table 9. Celosia mean tissue nutrient concentration (n = 3) at early stages of deficiency in recently matured leaves of hydroponically grown plants 304

Table 10. Celosia mean tissue nutrient concentration (n = 3) at advanced stages of deficiency in recently matured leaves of hydroponically grown plants 305

Table 11. Impatiens mean tissue nutrient concentration (n = 3) at early stages of deficiency in recently matured leaves of hydroponically grown plants 306

Table 12. Impatiens mean tissue nutrient concentration (n = 3) at advanced stages of deficiency in recently matured leaves of hydroponically grown plants 307

Table 13. Pansy mean tissue nutrient concentration (n = 3) at early stages of deficiency in recently matured leaves of hydroponically grown plants 308

Table 14. Pansy mean tissue nutrient concentration (n = 3) at advanced stages of deficiency in recently matured leaves of hydroponically grown plants 309

Table 15. Petunia mean tissue nutrient concentration (n = 3) at early stages of deficiency in recently matured leaves of hydroponically grown plants 310

Table 16. Petunia mean tissue nutrient concentration (n = 3) at advanced stages of deficiency in recently matured leaves of hydroponically grown plants 311

Table 17. Begonia mean tissue nutrient concentration (n = 3) at early stages of deficiency in recently matured leaves of hydroponically grown plants 312

Table 18. Begonia mean tissue nutrient concentration (n = 3) at advanced stages of deficiency in recently matured leaves of hydroponically grown plants 313

Table 19. Ornamental cabbage mean tissue nutrient concentration (n = 3) at early stages of deficiency in recently matured leaves of hydroponically grown plants 314

Table 20. Ornamental cabbage mean tissue nutrient concentration (n = 3) at advanced stages of deficiency in recently matured leaves of hydroponically grown plants 315

Table 21. Snapdragon mean tissue nutrient concentration (n = 3) at early stages of deficiency in recently matured leaves of hydroponically grown plants 316

Page

Table 22. Snapdragon mean tissue nutrient concentration (n = 3) at advanced stages of deficiency in recently matured leaves of hydroponically grown plants 317

Table 23. New Guinea impatiens 'Timor' mean tissue nutrient concentration (n = 3) at early stages of deficiency in recently matured leaves of hydroponically grown plants 318

Table 24. New Guinea impatiens 'Timor' mean tissue nutrient concentration (n = 3) at advanced stages of deficiency in recently matured leaves of hydroponically grown plants 319

Table 25. New Guinea impatiens 'Grenada' mean tissue nutrient concentration (n = 3) at early stages of deficiency in recently matured leaves of hydroponically grown plants 320

Table 25. New Guinea impatiens 'Grenada' mean tissue nutrient concentration (n = 3) at advanced stages of deficiency in recently matured leaves of hydroponically grown plants 321

LIST OF FIGURES

Page

CHAPTER 1. VINCA FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Figure 1. Plant height at various times after the start of deficiency treatments for vinca 20

Figure 2. Days from initiation of treatments to leaf sampling for nutrient analysis for vinca at early and advanced stages of deficiency..... 21

Figure 3. Dry weight of deficient nutrient plants expressed as a percentage of control plants at early and advanced stages for vinca 22

CHAPTER 2. FRENCH MARIGOLD FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Figure 1. Days from initiation of treatments to leaf sampling for nutrient analysis for marigold at early and advanced stages of deficiency 41

Figure 2. Dry weight of deficient plants expressed as a percentage of control plants at early and advanced stages of marigold..... 42

CHAPTER 3. ZINNIA FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Figure 1. Days from initiation of treatments to leaf sampling for nutrient analysis for zinnia at early and advanced stages of deficiency..... 60

Figure 2. Dry weight of deficient plants expressed as a percentage of control plants at early and advanced stages of zinnia 61

CHAPTER 4. SALVIA FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Figure 1. Days from initiation of treatments to leaf sampling for nutrient analysis for salvia at early and advanced stages of deficiency 81

Figure 2. Dry weight of deficient plants expressed as a percentage of control plants at early and advanced stages of salvia..... 82

Page

CHAPTER 5. CELOSIA FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Figure 1. Days from initiation of treatments to leaf sampling for nutrient analysis for celosia at early and advanced stages of deficiency 100

Figure 2. Dry weight of deficient plants expressed as a percentage of control plants at early and advanced stages of celosia 101

CHAPTER 6. IMPATIENS FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Figure 1. Days from initiation of treatments to leaf sampling for nutrient analysis for impatiens at early and advanced stages of deficiency 121

Figure 2. Dry weight of deficient plants expressed as a percentage of control plants at early and advanced stages of impatiens..... 122

CHAPTER 7. PANSY FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Figure 1. Days from initiation of treatments to leaf sampling for nutrient analysis for pansy at early and advanced stages of deficiency 141

Figure 2. Dry weight of deficient plants expressed as a percentage of control plants at early and advanced stages of pansy..... 142

CHAPTER 8. PETUNIA FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Figure 1. Days from initiation of treatments to leaf sampling for nutrient analysis for petunia at early and advanced stages of deficiency..... 162

Figure 2. Dry weight of deficient plants expressed as a percentage of control plants at early and advanced stages of petunia 163

Page

CHAPTER 9. BEGONIA FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Figure 1. Days from initiation of treatments to leaf sampling for nutrient analysis for begonia at early and advanced stages of deficiency 182

Figure 2. Dry weight of deficient plants expressed as a percentage of control plants at early and advanced stages of begonia 183

CHAPTER 10. ORNAMENTAL CABBAGE FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Figure 1. Days from initiation of treatments to leaf sampling for nutrient analysis for ornamental cabbage at early and advanced stages of deficiency 203

Figure 2. Dry weight of deficient plants expressed as a percentage of control plants at early and advanced stages of ornamental cabbage 204

CHAPTER 11. SNAPDRAGON FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF N, P, Ca, Fe, Mn, Cu, Zn and B

Figure 1. Days from initiation of treatments to leaf sampling for nutrient analysis for snapdragon at early and advanced stages of deficiency 221

Figure 2. Dry weight of deficient plants expressed as a percentage of control plants at early and advanced stages of snapdragon 222

CHAPTER 12. NEW GUINEA IMPATIENS FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Figure 1a. Days from initiation of treatments to leaf sampling for nutrient analysis for 'Timor' at early and advanced stages of deficiency 243

Figure 1b. Days from initiation of treatments to leaf sampling for nutrient analysis for 'Grenada' at early and advanced stages of deficiency 244

Figure 2a. Dry weight of deficient plants expressed as a percentage of control plants at early and advanced stages for 'Timor' 245

Figure 2b. Dry weight of deficient plants expressed as a percentage of control plants at early and advanced stages for 'Grenada' 246

Page

CHAPTER 13. CELLULAR ANALYSIS OF Ca, B, and Ca and B DEPRIVATION

Figure 1. Longitudinal section of tomato root apical meristem (RAM) grown in complete nutrients. The marked area 5 mm from quiescent center is where mitotic index (MI) calculations were done 264

Figure 2. Root growth of Ca, B, and Ca\B deprived treatments 265

Figure 3. Alterations in RAM morphology 24 hrs after Ca, B, and Ca and B deprivation. (a) Control treatment, (b) B deprived treatment, (c) Ca deprived treatment, and (d) Ca and B deprived treatment. Bar, 5 mm 266

Figure 4**.** RAM morphology at 48 hrs after Ca, B, or Ca and B deprivation. (a) Control treatment, (b) B deprived treatment (c) Ca deprived treatment and (d) Ca and B deprived treatment. Bar, 5 mm 266

Figure 5. Number of cellular files at 48 hr in control and Ca, B, Ca\B deprived RAM 267

Figure 6. Nuclear dimensions at 48 hr in control, and Ca, B and Ca\B deprived RAM. Mean separation by least significant difference (LSD) at $P<0.01$ is indicated by small cap letters 267

Figure 7. Mitotic index (MI) of Ca, B, and Ca\B deprived treatments 268

APPENDIX II. pH OF 12 TREATMENT NUTRIENT SOLUTIONS BEFORE AND AFTER REPLACEMENT

Figure 1. pH of nutrient solution at initial (day zero) and on day 6 for marigold 294

CHAPTER 1

VINCA FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Dharmalingam S. Pitchay[1], James L. Gibson[1], Brenda Cleveland[2], Paul V. Nelson[1], F.R. Walls[2], and Brian E.Whipker[1]

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

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

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VINCA FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Dharmalingam S. Pitchay[1], James L. Gibson[1], Brenda Cleveland[2], Paul V. Nelson[1], F.R. Walls[2], and Brian E.Whipker[1]

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

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

ABSTRACT

Current foliar analysis standards for vinca (*Vinca rosea* L.) are associated with acceptable growth, but do not indicate boundary values between deficient and acceptable growth. Deficiency symptoms have not been described for many nutrients in this crop. Vinca plants were grown hydroponically in a glass greenhouse. Treatments consisted of a complete nutrient solution and 11 additional solutions, each devoid of one essential nutrient. Visual deficiency symptoms and plant size were chronologically recorded for shoots and roots. In the absence of N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, and B dry weight basis concentration in the youngest fully expanded leaves at the time of early deficiency symptoms were 2.66, 0.07, 0.73, 0.32, 0.11, 0.09 (%), 29.0, 9.3, 1.4, 12.3, and 10.5 ($mg \cdot kg^{-1}$) respectively. Fe was the first deficiency symptom to occur at 4 days followed at

day 5 by K and B, at day 7 by Mg, at day 9 by N, Ca and Mn, and at day 16 by P. Deficiency symptoms for Zn, Cu, and S were the slowest to develop at days 39, 45, and 59, respectively. At the early deficiency stage, a significant reduction in shoot dry weight was observed in N, P, Ca, Mg, Mn, and B deficient plants. At the advanced deficiency stage, a reduction in the shoot dry weight was noted in all deficient plants except those deprived of Mg and S. Detailed descriptions of the chronological development of deficiency symptoms are presented along with associated early and advanced stage leaf nutrient concentrations.

INTRODUCTION

Vinca (*Vinca rosea* L.) is an important crop in the bedding plant industry, and is used in traditional and modern medicine for its alkaloids, which have anti-tumor properties (1). In spite of its importance, the only nutrient disorder symptoms reported are those of Fe deficiency (2,3). Foliar nutrient standards have also been inadequately described. Survey ranges for 11 essential nutrients have been reported (4,5). Values in these ranges are associated with acceptable growth, but do not establish the minimum and maximum critical sufficiency values. Present fertilizer formulation is solely based on a general recommendation of 2:1:2 (N: P_2O_5: K_2O) ratio (6). For cost-effective production of vinca, it is imperative for the industry to have complete descriptions of nutrient disorder symptoms for visual diagnosis and definitive foliar analysis standards.

The first objective of this study was to characterize visual symptoms of nutrient deficiencies in the chronological order in which they appear from early to advanced

stages for N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, and B in vinca. The second objective was to establish foliar analysis standards for these same nutrients that include an early stage of deficiency and a level associated with a late stage of deficiency.

MATERIALS AND METHODS

Vinca 'Blue Pearl' was sown on 8 August 2000 in Oasis LC1 foam cubes (Smithers Oasis, Kent, OH.) containing only Ca and Mg from dolomitic limestone. The experiment was conducted in a glass greenhouse in Raleigh, NC at 35°N latitude that was set at night/cloudy day/clear day temperatures of 17/21/24 °C. During the establishment phase, seedlings were fertilized at each irrigation with the following mM concentrations of 0.35 NH_4-N, 5.15 NO_3-N, 0.35 PO_4, 1.0 K, 1.25 Ca, 1.0 Mg, and 36 µM Fe using the following reagent grade chemicals NH_4NO_3, KNO_3, K_2HPO_4, $Ca(NO_3)_2 \cdot 4H_2O$, $MgSO_4 \cdot 7H_2O$, and FeDTPA. Seedlings were grown with this nutrient regime until the first set of true leaves developed.

After establishment, plants were transplanted on 10 September 2000, into 4.87 liters aluminum-painted plastic tubs with 6 or 9 circular holes in covers. Three replications with six seedlings and another three replications with nine seedlings (one plant/hole) were assigned to 12 treatments. The three replications with nine plants were sampled when an early stage of visible symptoms occurred and the remaining three replications with six plants were sampled when advanced visible symptoms occurred. Six treatments were induced on 10 September 2000 that included complete nutrient formula, and complete minus one of the nutrients K, Mg, S, Cu, and Zn.

Since deficiency symptoms of N, P, Ca, Fe, Mn, and B tend to develop earlier, these treatments were induced on 15 September 2000 to provide additional time to accumulate plant biomass for foliar analysis.

The treatment consisted of a complete modified Hoagland's all nitrate mM concentrations of macronutrients, 15 NO_3-N, 1.0 PO_4-P, 6.0 K, 5.0 Ca, 2.0 Mg, and 2.0 SO_4-S (7), plus μM concentrations of micronutrients, 72 Fe, 9.0 Mn, 1.5 Cu, 1.5 Zn, 45.0 B, and 0.1 Mo, and 11 additional solutions each devoid of one essential nutrient (N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, or B). The following reagent grade chemicals KNO_3, $Ca(NO_3)_2 \cdot 4H_2O$, KH_2PO_4, $MgSO_4 \cdot 7H_2O$, $CaCl_2$, KCl, $NaNO_3$, NaH_2PO_4, Na_2SO_4, $MgCl_2$, FeDTPA, $MnCl_2 \cdot 4H_2O$, $ZnCl_2 \cdot 7H_2O$, $CuCl_2 \cdot 5H_2O$, H_3BO_3, and $Na_2 2MoO_4 \cdot 2H_2O$ and deionized water of 18-mega ohms purity was used to formulate treatment solutions. During the first week, tubs were inspected daily and deionized water was added as needed to maintain the nutrient solution volume. In subsequent weeks, tubs were topped 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 as they developed. When the first visible symptoms occurred, the youngest fully expanded leaves were sampled from three replications of deficiency treatment and the control to establish early deficiency tissue levels. When symptoms progressed to an advanced level, another set of recently fully expanded leaves were sampled from the remaining three replicates of the deficiency treatment and the control 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 seconds, and again rinsed in

deionized water for 30 seconds. The samples were then oven dried at 70°C for 24 hours, and ground in a mortar and pestle. Tissue was then analyzed for macro and micronutrients with the exceptions of N, using the Perkin Elmer 3300 Inductively Coupled Argon Plasma Emission Spectrophotometer, Norwalk, CT while total N was analyzed using Carlo Erba NA 1500 Nitrogen Analyzer at the plant tissue laboratory of the North Carolina Department of Agriculture and Consumer Services, Agronomic Division, Raleigh.

The experiment was a randomized complete block design with six blocks. Each block consisted of four plastic tubs that served as control treatments 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, NC). 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

Following are the progressions of visual deficiency symptoms for each nutrient. The number of days to each symptom refers to time from the start of deficiency treatments. Early and late leaf sampling for foliar analysis was carried out on days as indicated in Figure 2 and the resulting recently matured leaf concentrations are presented in Table 1. This study was undoubtedly impacted by unavoidable covarying factors such as balancing ions sodium in the K, Ca and Mg treatment and chloride in the N, P, and S deficient treatment formulation. A list of potentially confounding factors in this study can be found in the appendix A.

Nitrogen

At initial stage, day nine, abnormalities included lighter green (dull olive-green) lower leaves, plant shorter, leaves fewer, smaller, and more lanceolate, axillary shoot smaller, and plant mass 56% of control. At this point, the tissue N concentration was 2.66% as compared to control 5.99%. As symptoms progressed, leaf orientation changed from horizontal to upright, more so in young leaves, with young leaves reaching an angle of 45° between adaxial leaf surface and stem above the leaf. As a consequence, shoot tips appeared to be buried in foliage (day 12). By day 17, the normally white pigmentation of the leaf veins turned yellow (veinal chlorosis) and spread out with time from each vein until it covered the entire leaf width. This syndrome began at the tip of the oldest leaves, progressed toward the leaf base and eventually moved up the plant to younger leaves. Flowering was delayed, although flower buds were present. The petiole and the main stem developed deeper pink pigmentation than normal (day 21). By day 25, the plants had six nodes with short internodes compared to the control, which had nine nodes with expanded internodes. This resulted in smaller plants (Figure 1). At this point, the tissue N concentration was 1.04%.

Phosphorus

On day 16, entire plants were darker green, shorter, had slightly smaller leaves and the primary roots appeared longer. By 21 days, only 3 to 4 lateral shoots had developed at the base of deficient plants, versus 6 to 7 on control plants, causing the

upper stems to be exposed. There were fewer nodes with smaller lateral shoots. Stems and petioles were deeper pink. These symptoms occurred at tissue P concentration of 0.07%. Leaves were upright by day 27. Symptoms continued with leaves changing to lighter olive green with pronounced blue tinges in the interveinal regions. Leaf orientation changed from upright to horizontal and slightly drooping. The tip of matured leaves curled downward. Leaves became flaccid. Axillary shoot growth was severely limited along the upper two-thirds of the shoot (day 38). A clear dark blue tinge along the mid-leaf lamina initially turned yellow and then developed brown necrotic patches on day 42. By day 50, necroses enlarged to encompass almost 50 percent of the mature leaves. The flowers were fewer, smaller, and lighter colored. Primary and secondary roots were longer with missing tertiary roots when compared to the normal. Primary roots extended further beyond the zone of secondary roots in deficient plants. At this point, the tissue P concentration was 0.04%.

Potassium

Deficiency began on day 5 with darker green foliage over the entire plant. Slower growth of leaves and internodes was associated with the darker pigment. By day 10, plants were noticeably compact with drooping lower leaves, while the tips of recently matured leaves curled downward. At this point, the tissue K concentration was 0.73%. By day 15, leaves had turned from darker green to lighter green than the control, and large, light green-gray desiccated spots developed at the acropetal end of lower leaves. Desiccated tissue rapidly changed to a light brown necrosis (day 18). This syndrome

repeated itself in interveinal areas at the acropetal end of leaves, spread toward the base of these leaves and then spread to leaves up the stem. Meanwhile, small black necrotic spots appeared randomly at the acropetal end of old leaves and then followed the same positional progression as desiccation (day 23). By day 27, puckered tissue was apparent along the mid-vein of recently matured and older leaves. Plants were considerably stunted with leaves reduced to about 30% of normal size, and lateral shoot length was only about 25% of control (day 31). Primary and secondary roots were fewer and shorter. Flowers were fewer and smaller than in the control. The tissue K concentration at this point was 0.28%.

Calcium

Symptoms began simultaneously on immature and recently matured leaves on day 9, as small black spots of 1 to 2 mm in size and upward puckering of leaf tissue between secondary veins. At this time, the tissue Ca concentration was 0.32%. The necrotic spots changed color to brown and eventually tan, and as they did, they enlarged. These symptoms appeared over the entire blade of immature leaves and at the basipetal end of recently matured leaves (day 10). These symptoms developed on young leaves on lateral shoots by day 11. Then a second occurrence of necrotic spots developed on these same leaves between the original spots (day 12). At this point, the lateral margins of young leaves curled downward. On immature leaves the basal 80% and on recently mature leaves the basal 30% of the margin of each leaf curled. By day 14, black necrosis occurred on the petiole causing it to shrivel and the leaf to hang downward and

eventually abscise. The tissue Ca concentration at this point was 0.28%. Deficient plants had shorter primary roots and longer secondary roots than the control plants. Light brown necrosis formed along the longitudinal axis of primary roots, but not at the tips. This necrosis did not cause these roots to die, nor did it inhibit the development of secondary roots that had already originated from these primary roots.

Magnesium

Symptoms began on day 7, with appearance of a faint light-green coloration along lower leaf margins. By day 15, symptoms developed on the mature leaves at the second node from the base of the plants, as chlorosis along the leaf margin, a short distance back from the tip. The symptoms described occurred at tissue Mg concentration of 0.11%. By day 16, chlorosis slowly spread inward leaving the tip and base of these leaves green. These symptoms spread to the other mature leaves (day 18). Although there was a faint interveinal chlorosis on the older leaves, it never became pronounced. The tip of older leaves then developed gray-green desiccation that gave way to tan and brown necrosis (day 23). By day 27, necrosis spread basipetally, particularly in the interveinal regions. This syndrome repeated itself on lateral shoots (day 31). Roots were proportionately smaller than in the control. The tissue Mg concentration at this point was 0.09%.

Sulfur

The S deficient plants were similar to the control during the first 7 weeks. Then slowly, whole plants turned lighter green and appeared larger than normal (day 59). By day 72, chlorosis developed along the acropetal margin of mature leaves along the mid-portion of the plants. This condition occurred at tissue S concentration of 0.09%. By day 76, interveinal chlorosis had slowly spread inward. Reddish pigmentation had developed on leaf petioles and stems. Flower size was smaller and petals overlapped less than in the control and eventually were separated. Roots were proportionately larger (day 80). The chlorotic symptoms progressed further until day 88, with no signs of necrosis, and the tissue S concentration at this point was 0.07%.

Iron

The first sign of deficiency appeared on day 4. Pigmentation of recently mature leaves became lighter green from the basal end, while on the immature leaves, light chlorosis developed along the acropetal margins just behind the tips. By day 7, chlorosis occurred on 100% of young leaves, 80% of the recently matured leaves, and 70% of the lower leaves. Meanwhile, light green pigmentation along the acropetal margins of young leaves spread inward and toward the leaf base in an interveinal pattern. Young leaf tips remained green. These symptoms developed when the tissue Fe concentration was 29.0 $mg \cdot kg^{-1}$. By day 9, interveinal chlorosis of young leaves progressed to a uniform chlorosis over the entire leaf except the tip and base. Petioles and leaf stems became pink.

Chlorosis continued to spread until entire young leaves were uniformly light green (day 12). The shade of green became continually lighter until it was yellow and eventually whitish-yellow (day 17). At this point, on day 21, entire margins of young leaves rolled upward. Lateral shoot leaves became whitish-yellow, but did not roll. Leaf orientation of young leaves was upright in deficient plants and horizontal in control plants. Primary root length was only 50% of the control. The lateral roots were also shorter with missing tertiary roots. Flowering was delayed and the flower buds were small and bleached compared to the control plants (day 25). At this point, the tissue Fe concentration was 16.4 $mg \cdot kg^{-1}$.

Manganese

On day 9, orientation of leaves above the recently mature leaf was more upright than the control. Light interveinal chlorosis appeared on young expanding leaves. The lateral shoots were small. This was followed by loss of leaf sheen on day 13. On day 16, light yellow-green pigmentation appeared on fully expanded leaves. The minimum critical concentration of Mn was 9.3 $mg \cdot kg^{-1}$. An overall light olive-green color developed on recently matured leaves (day 21). This chlorosis spread to upper and lower leaves except the oldest leaves (day 27). By day 37, the entire plants appeared olive-green with acropetal necrotic (brownish-black) spots on recently matured leaves. The necrosis progressed inwards and the syndrome extended to lower leaves with larger interveinal and marginal necrotic patches, on day 49. Flowers were smaller (2.3 vs. 3.8 cm diameter) and lighter than the control. Meanwhile, primary and secondary roots were fewer, shorter,

and thinner and tertiary roots were fewer. The tissue Mn concentration dropped to 5.2 $mg \cdot kg^{-1}$.

Copper

The plants appeared similar to the control during the first 5 weeks. By week 6, plants appeared smaller with deeper green leaves and greatly reduced root development. By day 45, young leaf lamina failed to expand in width, particularly along the basal three quarters of the leaves. At this time, the tissue Cu concentration was 1.4 $mg \cdot kg^{-1}$. By day 50, young leaves developed chlorosis in a mild veinal pattern. The chlorosis began in the basipetal region and progressed toward the acropetal. These symptoms spread down the plants and towards the growing shoots (day 55). Flowers were delayed and fewer than in control plants. Flower petals became angular and separated rather than rounded and overlapping. Petals had white bases (day 81). By day 88, primary and lateral roots were proportionately reduced to about 80% of the control size. Stems appeared light green (pinkish in control) with fewer and smaller lateral shoots. This occurred at tissue Cu concentration of 1.0 $mg \cdot kg^{-1}$.

Zinc

Symptoms began on day 39 with narrow, slightly distorted immature leaves that were lightly chlorotic at their base. By day 42, leaf orientation from the third node upward was upright. Axillary shoot development was poor. The tissue Zn concentration

at this time was 12.3 $mg \cdot kg^{-1}$. The symptoms then moved downward to the recently matured leaves resulting in narrow, pointed appearance (day 51). By day 64, older leaves developed interveinal chlorosis that progressed to yellowish-gray color. Yellow stripes developed over some secondary veins. The reduction in axillary shoot development continued which led to the lower $2/3^{rd}$ of the plants having a visible main stem. Necrosis developed on older leaves beginning at the acropetal region. As the symptoms advanced (day 72), there were fewer nodes (four) as compared to the control (six). Red pigmentation appeared on the leaf petioles and the short and thin internodes. The flower size was reduced by 70% with gaps in between the petals. The petals were diamond shaped with pointed tips curved upwards. The flower petal pigmentation faded from purple-pink to white. The tissue Zn concentration dropped further to 6.4 $mg \cdot kg^{-1}$.

Boron

The initial symptoms first appeared on the root system rather than the shoot with swollen brown root tips (day 5). By day 12, the growing points including the young and newly matured leaves were darker green than the lower leaves when compared to the control. The shiny light green pigmentation present in the young and newly maturing leaves of control plants were lacking in the B deficient plants. The leaf form was altered with a rounded leaf tip. As the deficiency advanced (day 21), the thickened leaves progressed to leathery and brittle texture. Flowering was delayed. Tissue B concentration at this point was 10.5 $mg \cdot kg^{-1}$. By day 38, mid-veins were twisted with a zigzag pattern causing wavy and uneven lamina with dimpled surface on maturing leaves. The shoot

apical meristem growth was inhibited, deformed (twisted) and clustered together with the immediate axillary shoots. The loss of apical dominance was noted with lateral shoots growing taller than the main shoot apical meristem. Partially developed flowers and buds were aborted. Overall, the internodes were similar in length to the control, except the sixth and the seventh internodes from the base, which were shorter and stacked together forming a rosette appearance. The stems were thick and greenish white as compared to the control. The symptoms progressed further, with mid and lower leaves slightly drooping downward (day 42). The growing points with asymmetric young leaves were severely twisted. The matured and newly matured leaves developed random yellowish chlorosis as the B stress persisted (day 50). The primary roots were shorter by 20%. The secondary roots were also short with fewer or missing tertiary roots. The tips of the primary, secondary and tertiary roots were swollen with brown coloration. The tissue B concentration dropped further by this time to 7.9 $mg \cdot kg^{-1}$.

Time to deficiency

Fe was the first deficiency to occur at 4 days followed at day 5 by K and B, at day 7 by Mg, at day 9 by N, Ca and Mn, and at day 16 by P. Zn, Cu, and S were the slowest to develop at days 39, 45, and 59. S deficiency may have been delayed due to the acquisition of S from SO_2 in the atmosphere (8).

Shoot dry weight

Nutrient deficiencies which resulted in a smaller dry weight (percent reduction) as compared to the control at the early deficiency stage were N (13%), B (30%), P (34%), Mn (40%), Mg (47%), and Ca (49%) (Figure 3). By the time of advanced deficiency, there were reductions in dry weight of all nutrients except Mg and S. Mg deficient plants were equal in dry weight to control plants. Sulfur deficient plants were significantly heavier than control plants at the early stage and did not differ at advanced stage. This suggests accumulation of photosynthate not used in certain protein synthesis (9). Vinca appears to be most sensitive to Fe deficiency (2,3) due to the rapid appearance of symptoms at 4 days. The delayed development of advanced symptoms of Fe deficiency may provide an opportunity for recovery.

The rapid and extensive suppression of height and weight (Figures 1 and 3) of N deficient plants indicates the importance of N in early growth and development of plants (10). The decline in shoot and increase in root dry weight of P deficient plants compared to the control indicates a shift in carbon partitioning to the root system (10).

Unique Visual Deficiency Symptoms

Unique visual deficiency symptoms of vinca included: N – veinal chlorosis of lower leaves, pinkish pigmentation on petiole and main stem, and upright leaf orientation; P – dark blue tinge at mid-leaf lamina that turned into brown necrotic patches on mature leaves, fewer nodes, and pink pigmentation on stems and petioles; K – compact dark

green plants at first followed by puckering of mature leaves and droopy appearance; S – increased plant size, chlorosis along the acropetal margin and interveinal chlorosis of mature leaves with reddish pigmentation on leaf petioles and stems; Cu – mild veinal pattern of basipetal chlorosis on young leaves, fewer flowers with angular petals, and exceptionally severe reduction in root size; Zn – chlorotic immature leaves, necrosis on old leaves, red pigmentation on internodes and petioles, and malformed and faded flowers; B – swollen brown root tips. Based on the age of affected plant tissue and the rate of deficiency progression between tissues, it can be concluded that the rate of translocation is very high for N and K, high for P and Mg, low for S, Zn, Cu, Mn, and B, and nil for Ca and Fe.

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The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of the products named, nor criticism of similar ones not mentioned. Appreciation is expressed to Tom Abramowski of Rockwell Farms, N.C., the North Carolina Commercial Flower Growers' Association, and Fred C. Gloeckner Foundation for financial support, Fred C. Gloeckner Inc. for providing the seeds and Smithers-Oasis for providing the propagation medium.

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Table 1. Vinca tissue nutrient concentration at early and advanced stages of deficiency in recently matured leaves of hydroponically grown plants in this study and published standards.

Treatment	**-N**	**-P**	**-K**	**-Ca**	**-Mg**	**-S**	**-Fe**	**-Mn**	**-Cu**	**-Zn**	**-B**
	Percent dry wt.						$mg \cdot kg^{-1}$ of dry wt.				
Element	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn	B
Control early	5.99	0.70	3.64	1.74	0.40	0.20	55.0	122.0	7.5	17.4	55.8
Deficient early	2.66	0.07	0.73	0.32	0.11	0.09	29.0	9.3	1.4	12.3	10.5
Control advanced	5.10	0.99	3.56	1.59	0.45	0.22	54.5	108.0	6.8	12.2	134.3
Deficient advanced	1.04	0.04	0.28	0.28	0.09	0.07	16.4	5.2	1.0	6.4	7.9
P value	0.00	0.02	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
Published*	2.72 - 6.28	0.28 - 0.64	1.88 - 3.48	0.93 - 1.13	0.32 - 0.78	0.22 - 0.50	72.0 - 277.0	135.0 - 302.0	6.0 - 16.0	30.0 - 51.0	21.0- 49.0

*Mills and Jones, 1996. Survey average of concentrations associated with acceptable crop performance, minimum critical concentrations not identified.

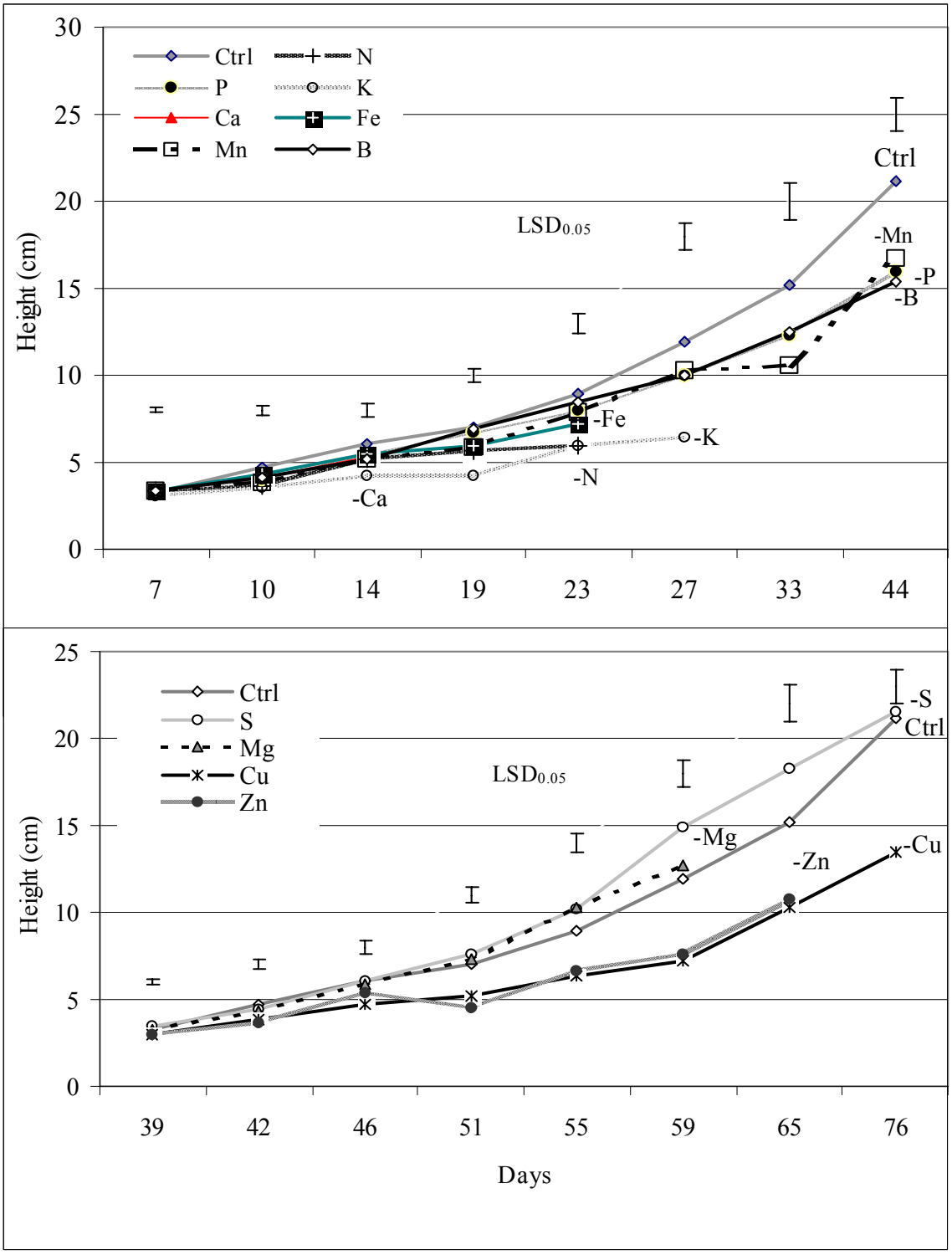

Figure 1. Vinca plant height at various times after the start of deficiency treatments.

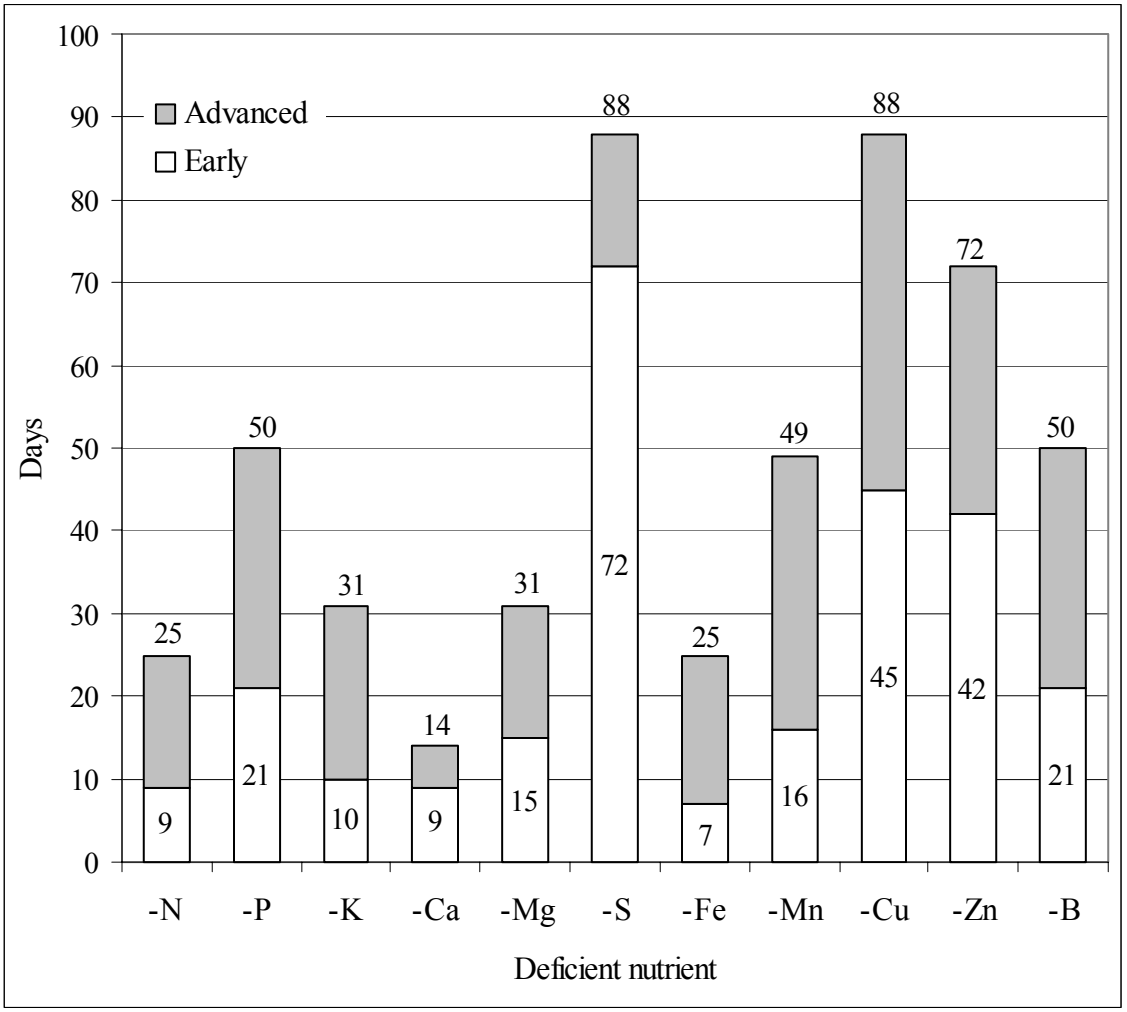

Figure 2. Days from initiation of treatments to leaf sampling for nutrient analysis for vinca at early and advanced stages of deficiency.

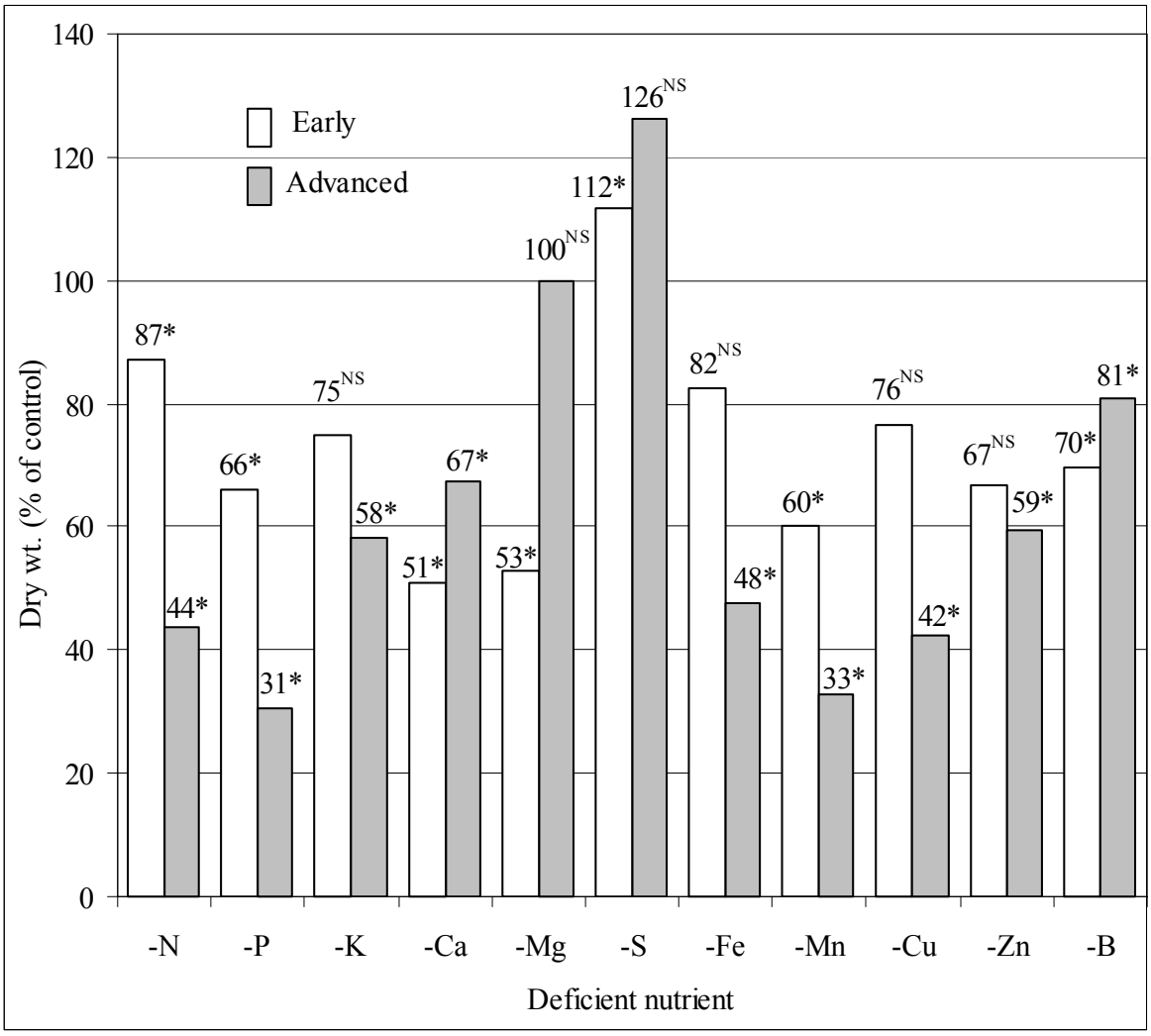

Figure 3. Vinca dry weight of deficient nutrient plants expressed as a percentage of control plants at early and advanced stages.

Note: ^{NS}, * Nonsignificant or significant at $P< 0.05$ for each deficiency versus control comparison.

CHAPTER 2

FRENCH MARIGOLD FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Dharmalingam S. Pitchay[1], James L. Gibson[1], Paul V. Nelson[1], Brian E.Whipker[1] and C. Ray Campbell[2]

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

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

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Dharmalingam S. Pitchay[1], James L. Gibson[1], Paul V. Nelson[1], Brian E.Whipker[1], and C. Ray Campbell[2]

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

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

ABSTRACT

Current foliar analysis standards for marigold (*Tagetes patula* L.) consist of values associated with acceptable growth, but do not indicate boundary values between deficient and acceptable growth. Deficiency symptoms have not been identified for many nutrients in this crop. Marigold plants were grown hydroponically in a glass greenhouse. Treatments consisted of a complete nutrient solution and 11 additional solutions, each devoid of one essential nutrient. Deficiency symptoms and plant size were chronologically recorded for shoots and roots. In the absence of N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn and B dry weight basis in the youngest fully expanded leaf nutrient concentrations at the early deficiency stage were 1.52, 0.07, 0.55, 0.30, 0.11, 0.09 (%), 40.9, 11.3, 2.1, 9.7 and 10.9 ($mg \cdot kg^{-1}$), respectively. When visual symptoms of N, P, K,

Ca, Mg, Fe, Cu and B appeared, plants were similar to normal plants in dry weight while Mn, and Zn deficient plants weighed less than normal plants and S free plants weighed more than normal plants. At the advanced deficiency stage, all nutrient deficient plants weighed less than normal with a severe reduction in the dry weight of N, K, Fe, Mn, Cu and B deficient plants. The early visual symptoms developed earlier (within 14 days) in N, P, K, Ca, and Fe plants with delayed development (≥ 18 days) in Mg, S, Mn, Cu, Zn and B deficient plants. Progressions of deficiency symptoms are presented in the text.

INTRODUCTION

Marigold (*Tagetes patula* L.), besides being an important bedding plant, is also widely grown for nematicidal activity to control nematodes (1), and the flower petals are incorporated into chicken feed to improve egg quality (2). In spite of its significant role in the agricultural sector, limited information is available on foliar nutritional standards and critical levels. These consist of a survey range values associated with acceptable growth and not necessarily critical boundaries, reported by Mills and Jones (3). To produce a premium crop on commercial scale, precise nutritional standards would be indispensable. Thus, this study was formulated, first, to characterize visual symptoms of nutrient deficiencies in the chronological order in which they appear from incipient to advanced stages for N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, and B in marigold. The second objective was to establish the minimum critical level foliar analysis standards, and a level associated with late stages of deficiency for the above listed nutrient elements.

MATERIALS AND METHODS

The protocol for this experiment was described in Pitchay et al., (4). Exceptions included: the test crop marigold 'Safari Series Gold'; the sowing date on 14 August 1999; and the initiation of all treatments on 26 August 1999. Shoot dry weight and height values are based on measurements while all other size comparisons with control plants are visual assessments.

RESULTS

Following are the progressions of visual deficiency symptoms for each nutrient element. Early and late leaf sampling was carried out on days as indicated in Figure 1 and the resulting recently matured leaf dry weight concentrations are presented in Table 1.

Nitrogen

On day 6, (after the start of the minus N treatment), whole plants appeared lighter green, more so on the lower leaves. Plants were shorter and smaller. They had thin stems, fewer and smaller leaves (leaflets closely arranged on short rachis and petiole), and poorly developed axillary shoots. The vegetative stage was brief with accelerated development of the reproductive stage. Flower buds were visible by day 10, and as they developed, they were greenish yellow with reddish pedicels. Flowering commenced earlier than control plants. By day 12, more than 50% of the plants flowered, whereas in

the control, 50% flowering occurred on day 27. At this point, the tissue N concentration was 1.52%. By day 17, lower oldest leaves appeared yellowish. As the symptoms progressed, 90% of the plants were flowering (day 20) compared to 33% in control plants. The older leaves turned from light green to yellow. Patches of reddish pigmentation developed at the tips of the outermost leaflets of lower leaves, spread over to the adjacent leaflets, and progressed towards the base of the entire compound leaves. This reddish pigmentation slowly intensified and finally turned to acropetal necrosis on the outermost leaflet margins of lower leaves. Reddish pigmentation also spread to maturing leaves, stems, and pedicels. The young compound leaf orientation was upright at a 45-degree angle. By day 27, all the plants were flowering, with only one flower per plant. However, the flowers were smaller with fewer deep yellow petals. Primary and secondary roots were considerably fewer and shorter than normal. Overall, a severe reduction of plant dry weight was observed compared to the normal as well as the other deficient treatments at advanced stage (Figure 2). At this point, the tissue N concentration was 1.04%.

Phosphorus

Symptoms began on day 7, when entire plants, including the growing points, appeared darker green. On day 14, flowering and light purple pigmentation on the upper portion of the main stems were observed. The plants appeared slender because of thin stems with fewer and shorter lateral shoots. The tissue concentration at this point was 0.07%. On day 16, gray overcast followed by dark necrosis occurred on the outer leaflet

margins of matured compound leaves. As the symptoms progressed from day 17, petioles curled downward beginning at the base of lower leaves. The initial dark necrosis progressed into tan brown shriveled necrosis starting from the outer most leaflet margins spreading across the entire compound leaves (day 19). By day 20, these symptoms progressed from lower to upper areas of plants. Pronounced dark red pigmentation also appeared from mid to upper portions of the stems. Meanwhile, fifty percent of the plants were flowering compared to 33% in control plants. The flower had smaller ray petals. Almost 50% of the six to eight leaflets of the compound leaves were necrotic by day 22, when tissue P concentration was 0.06%. Primary roots were fewer, but considerably longer by 30% than roots of control plants. Secondary roots were fewer and were only found on the basal portion of the primary roots.

Potassium

Plants appeared darker green, including the young leaves and the growing points, as compared to the normal plants (day 14). The leaflets curled downward beginning at the tips. By day 20, the internodes remained shorter and the leaves were smaller with short rachis and petiole, giving rise to compact plants. Plant height was 75% of that of normal plants. At this point, 10% of the plants were flowering compared to 33% in control plants, and the tissue K concentration was 0.55%. By day 22, irregular black necrotic spots appeared randomly across the lower leaves' leaflet lamina more so on the basipetal area for a brief period. The necrotic spots turned brown with pronounced chlorosis on the mid-vein area of young and maturing leaves (day 24). As the symptoms progressed (day

35), the downward curling syndrome became severe and gave way to a drooping appearance. At this point, only 5% of the plants were flowering due to necrosis of buds prior to reaching swollen bud stage. These symptoms developed when the tissue concentration further dropped to 0.40%. In addition, fewer and shorter primary and secondary roots were observed.

Calcium

Root symptoms of brown discoloration with significantly fewer and shorter primary and secondary roots were the first indication of Ca deficiency (day 9). Black necrotic spots appeared in shoot tips and the basipetal end of new and young unfolding leaflets and progressed towards the outermost leaflet tips (day 12). At this point, the tissue Ca concentration was 0.30%. This was followed by scattered black necrotic spots developing on young and maturing leaves, petioles, and lateral shoots by day 14. The flower buds were also affected by the blackened necrotic symptoms. Spots of black necrosis turned brown starting in young leaves near the leaf base, and spread towards the leaf tips (day 16). Leaflets were distorted, necrotic and smaller. On day 20, these symptoms progressed to maturing and matured leaves. Severe necrosis developed on the growing points causing the shoot tips to die. Necrotic symptoms also developed on buds and pedicels causing the buds to collapse. Eventually (day 23), even the portion below the growing points died back, and wilting of lower leaves occurred due to petiole necrosis. The base of the main stems appeared to have a black thorny surface. A significant reduction in height by 64% of control was noted. At this point, the tissue Ca

concentration remained at 0.30%. Primary roots were fewer and shorter with necrosis along all of the axis except the terminal 2 to 3 cm, which appeared normal. Secondary roots were fewer, shorter and thicker and appeared normal.

Magnesium

Initially on day 24, older leaves developed dark discoloration on the leaflet margins. By day 25, interveinal chlorosis (dark tan) developed on these leaves when the tissue Mg concentration was 0.11%. Irregular necrotic patches followed this on the leaflet margins of matured leaves. Plant size and weight was not reduced (Figure 2). On day 27, distinct interveinal chlorosis with darker green lamina close to the mid-veins was observed on mature leaves. Light tan to brown irregular necrotic spots developed in the chlorotic region and progressed inward towards leaflet bases. The interveinal chlorosis progressed to tan necrosis (day 34). By day 42, the young and mature leaves became misshapen due to downward curling of the leaflets, resulting in droopy appearance of the plants. Overall, the plants looked smaller (Figure 2). The tissue Mg concentration dropped further to 0.06%.

Sulfur

The S deficient plants appeared slightly bigger than normal plants after three weeks of planting (Figure 2). Flowering was earlier than in normal plants with 70% of plants flowered by day 24, compared to 25% in control plants. Subsequently, plants

began to appear lighter green. By day 27, the deficient plants had 50% more flowers than the normal plants. The flowers had longer pedicels and deeper yellow petals. This resulted in more showy flowers as compared to the normal. Meanwhile, by day 34, the entire plants, including the petioles and stems, became uniformly lighter green and eventually greenish yellow (day 37). At this point, the tissue S concentration was 0.09%. This was followed by narrower leaflets, which gave way to less dense smaller sized plants (day 41). By day 49, the plants appeared light yellow with tissue S concentration of 0.10%. The roots were pale yellow though similar in size to those on normal plants.

Iron

Development of lighter green coloration in the shoot apex region marked the beginning of Fe deficiency symptoms (day 6). The light green coloration then spread to the young leaves beginning in the basal leaflets and progressed towards the terminal leaflets. Within the leaflets, the symptoms first appeared at the basipetal end and progressed towards the acropetal end (day 10). This was followed by the development of yellowish green interveinal chlorosis on maturing leaves. Chlorosis was also observed on the rachis, petioles, and stems (day 16). At this point, the tissue Fe concentration was 40.9 $mg \cdot kg^{-1}$. As the symptoms advanced, chlorosis became more intense and the interveinal pattern of green pigmentation became less conspicuous as the entire lamina turned yellowish (day 20). This pattern of yellowing progressed to lower mature leaves (day 22). The shoots and the young leaves of the plants appeared as clusters of yellow. Eventually, on day 27, yellowish coloration gave way to bleached white and then pale

necrosis on the leaflet margins of young leaves and shoot tips. The flowers were smaller and light yellow with bleached petal margins. The primary roots were thicker with brownish coloration. A significant reduction in plant size was also observed (Figure 2). By day 29, the tissue Fe concentration dropped further to 34.1 $mg \cdot kg^{-1}$.

Manganese

Dull light green appearance of plants comprised initial symptoms on day 22. This was followed by light tan stippling, similar to ozone damage (5), in an interveinal pattern on young and recently matured leaves (day 24). At this point, the tissue Mn concentration was 11.3 $mg \cdot kg^{-1}$. The lateral shoots were fewer and smaller. By day 32, only 10% as many plants as in the control were flowering. Flowers were smaller with missing petals. As the symptoms progressed, light tan stippling turned to large chlorotic spots. Acropetal tan necrosis was also observed across the leaves including the lower leaves. In some instances, there was a total loss of green pigmentation on lower leaves (day 36). Plants were smaller with smaller leaves. The leaves became dull and olive green, spreading downward to older leaves (day 39). By day 49, the plants appeared less dense with smaller young leaves. Flowering was reduced to less than 10% of the plants as most of the buds aborted. The flowers were lighter yellow as compared to normal. The tissue Mn concentration at this point dropped to 6.7 $mg \cdot kg^{-1}$. Primary and secondary roots were fewer and shorter by 30% of normal plant roots.

Copper

Initially, Cu deficient plants were smaller and developed narrow young leaves. Leaf color was normal (day 32). When the first flower bud on each stem developed to about 0.5 cm diameter most turned necrotic along with the adjacent leaf (day 35). At this point, the tissue Cu concentration was 2.1$mg \cdot kg^{-1}$. Younger leaves further up the stem remained green. As subsequent flower buds developed in this younger tissue, many died along with the adjacent leaf while the remaindure survived. This lead to some stems with alternating dead and live buds plus leaves. It appeared that young tissue was benefiting from mobilized Cu in lower dying tissues. The primary roots were fewer and shorter with tiny secondary roots. As symptoms advanced, plants became more stunted. The young leaves were deformed with needle like leaflets (day 36). Light tan stippling also appeared on some of the lower mature leaves (day 46). Eventually by day 54, all flower buds died. The primary and secondary roots remained fewer and shorter with secondary roots less than 0.5 cm long. The tissue Cu concentration remained at 2.1 $mg \cdot kg^{-1}$.

Zinc

Chlorosis developed at growing points at day 24. This was followed by a uniform yellow pigmentation along the mid-veins of narrow unfolding leaflets of the first three young leaves. Flowering was severely affected due to bud abortion. Only 5% percent of the plants flowered and had light yellow pigmentation. The ray petals were missing on some flowers (day 39). These symptoms occurred at tissue Zn concentration of 9.7

$mg \cdot kg^{-1}$. By day 46, chlorosis spread from the upper part of the plants to lower leaves beginning along the leaflet margins of lower leaves. By day 49, chlorosis on older leaves became intense, taking on a veinal pattern, and necrosis began at the leaflet tips. Flowers developed with fewer ray petals. Buds shriveled with pointed tips and finally aborted. By day 54, the entire plants appeared lighter green than normal plants. At this point, the tissue Zn concentration was 8.1 $mg \cdot kg^{-1}$. The primary roots were fewer and less dense than normal.

Boron

In B deficient plants, the visual symptoms first appeared on roots (day 18) rather than the shoots. The primary roots were short with swollen tips. The secondary roots also had swollen tips and were very short at the basipetal end of the primary roots and abnormally longer closer to the tips of the primary roots, resembling witches' broom-like growth. Shoot visual symptoms first appeared on day 22, with glossy darker green foliage when the tissue B concentration was 10.9 $mg \cdot kg^{-1}$. By day 26, the basal leaflets of young leaves appeared narrow and needle-like due to partial lamina development. By day 32, symptoms progressed further to lower leaves, which caused the upper portion of the plants to appear skeletal. Leaves were thick, stiff and leathery, and easily snapped. The plants appeared stunted due to short internodes close to the growing point (day 35). The shoot meristems stopped developing. Flowering was absent due to bud abortion. The flower buds appeared shriveled with longitudinal ridges and the cross section revealed hollow buds with missing floral parts. The witches' broom-like root symptoms became

severe with stubby and stiff primary and secondary roots and a greater degree of swollen tips (day 47). At this point, the B tissue concentration was 9.3 $mg \cdot kg^{-1}$. Significant reduction in size was also noted (Figure 2).

DISCUSSION

Ca deficiency began in the roots and quickly suppressed shoot growth. Foliar Ca concentration declined rapidly to a low point at early visual symptoms and remained at this concentration through the development of advanced symptoms. This indicates that growth declined sufficiently to keep pace with diminishing Ca supply. Sulfur deficiency took longer to develop specific deficiency symptoms. The S tissue concentrations at early and advanced stages were similar (Table 1). At early stage, these plants appeared larger than normal, as observed in several other species (5). More flowers formed in S deficient plants than in control plants. Plant size and the stability of leaf S concentration throughout deficiency stages could be due to the acquisition of S from the atmosphere (6). In Cu deficient plants, the advanced stage can only be identified based on the severity of bud die back syndrome, reduction in dry weight, and visual root symptoms and not the critical tissue values (Table 1).

Zn was the only deficiency that resulted in a reduction in shoot dry weight in the early stage of deficiency. However, at advanced stage, a reduction in shoot dry weight was observed in all except P, Ca and Mg deficient plants. Shoot dry weight of S and Cu deficient plants weighed less than 50%; B, K, Fe, and Mn deficient plants weighed less than 24%; and N deficient plants weighed less than 16% of control (Figure 2). This

confirms the paramount importance of N for driving growth and development (7). The vegetative stage was brief with accelerated development of the reproductive stage earlier by 15 days compared to control. However, the flowers were smaller and fewer that could be due to phytohormones and lack of photosynthate (5, 7).

Foliar analysis standards generated in this study for the French marigold were generally lower than those reported for African marigold by Johnson, (8). This could be due in part to cultivar differences (9, 10). The standard tissue micronutrient concentrations of Fe, Cu, Zn and B were far below the toxicity level (11). This means, for these nutrients, deficiency stress is more likely to occur than toxicity.

Root deficiency symptoms for P, Ca, Cu, and B were sufficiently significant to use for identifying these deficiencies. P deficient plants had fewer and longer primary roots, with fewer secondary roots found only on the basal portion of the primary roots. The primary and secondary roots of B deficient plants were shorter, with swollen tips. The primary roots of Cu deficient plants were fewer and shorter with tiny secondary roots. Ca deficient plants developed necrosis along all of the axis except the terminal. In Ca and B deficient plants, the visual deficiency symptoms first appeared on roots rather than shoots, which can be useful in diagnosing these deficiency symptoms, even before the shoots are affected.

Synoptic visual symptoms were as follows: N - yellowing and acropetal reddish pigmentation of lower leaves; P - darker green followed by black to brown necrosis of mature leaves and stems with purple pigmentation; K - darker green leaves, short internodes, and stocky and droopy appearance; Ca - blackened necrosis on growing points, shoots, and along the root axis; Mg - interveinal chlorosis of lower leaves; S -

light green foliage with numerous quality flowers; Fe - basipetal interveinal chlorosis of young leaves; Mn - overall dull light green appearance followed by light tan stippling on young and mature leaves; Cu - narrow young leaves with die back syndrome of buds and the adjacent leaves; Zn - chlorosis of young leaves and primary veins with shriveled and pointed buds; B - thick and needle like, darker green young leaves with arrest of growing point and swollen root tips.

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Table 1. Marigold tissue nutrient concentration at early and advanced stages of deficeincy in recently matured leaves of hydroponically grown plants in this study and published standards.

Treatment	**-N**	**-P**	**-K**	**-Ca**	**-Mg**	**-S**	**-Fe**	**-Mn**	**-Cu**	**-Zn**	**-B**
	Percent dry wt.						$mg \cdot kg^{-1}$ of dry wt.				
Element	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn	B
Control Early	5.53	0.37	3.60	2.12	0.34	0.21	104.3	208.7	9.3	25.7	43.9
Deficient Early	1.52	0.07	0.55	0.30	0.11	0.09	40.9	11.3	2.1	9.7	10.9
Control Advanced	5.10	0.19	3.34	2.10	0.60	0.29	151.0	212.0	18.5	36.6	72.7
Deficient Advanced	1.04	0.06	0.40	0.30	0.06	0.10	34.1	6.7	2.1	8.1	9.3
P value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Published*	3.32 - 3.62	0.49 - 0.54	2.79 - 2.88	2.36 - 2.72	1.33 - 1.44	1.34 - 1.44	92.0 - 115.0	275.0 - 558.0	19.0 - 25.0	76.0 - 97.0	34.0 - 40.0

*Mills and Jones, 1996. Survey average of concentrations associated with acceptable crop performance, minimum critical concentrations not identified.

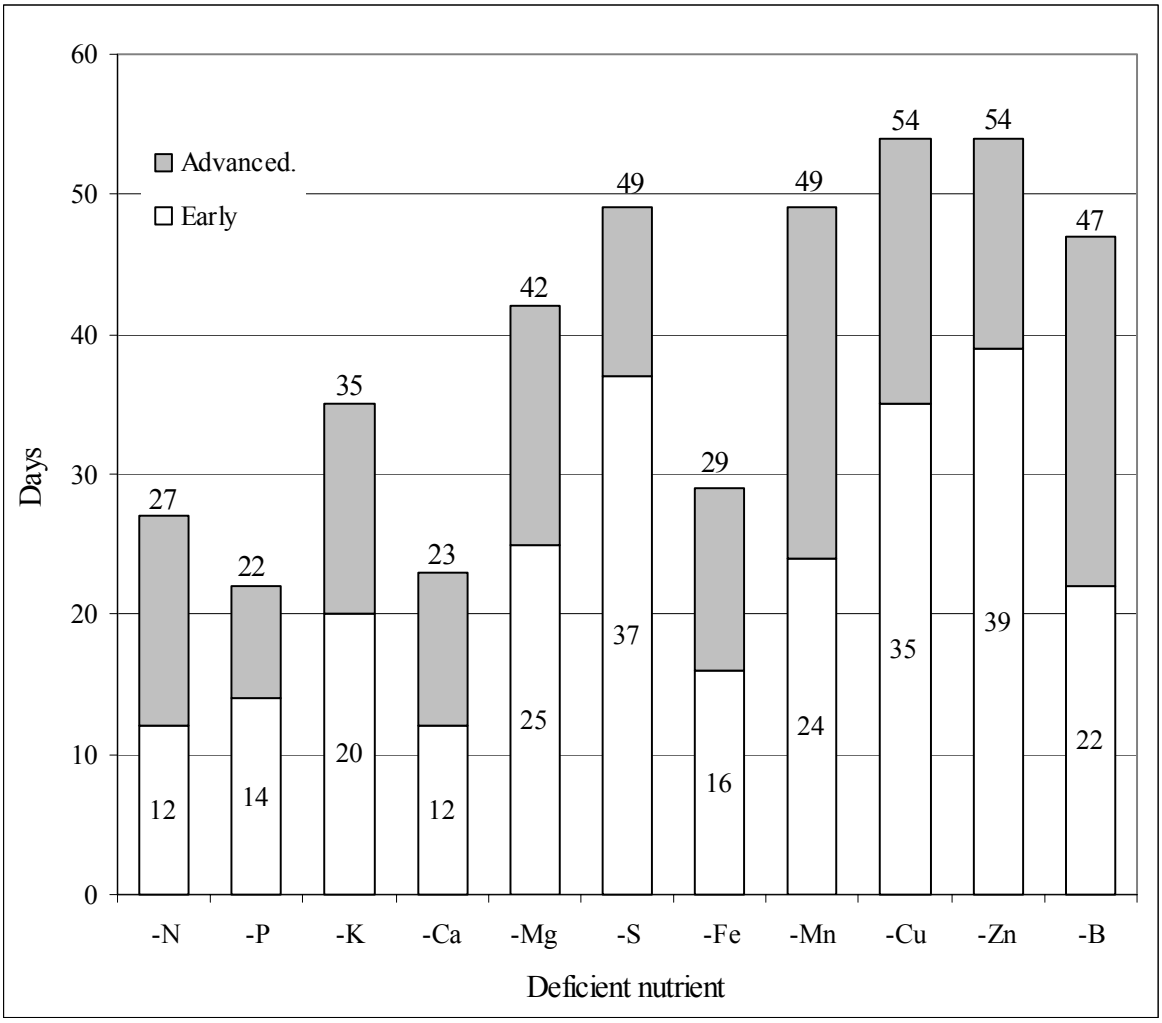

Figure 1. Days from initiation of treatments to leaf sampling for nutrient analysis for marigold at early and advanced stages of deficiency.

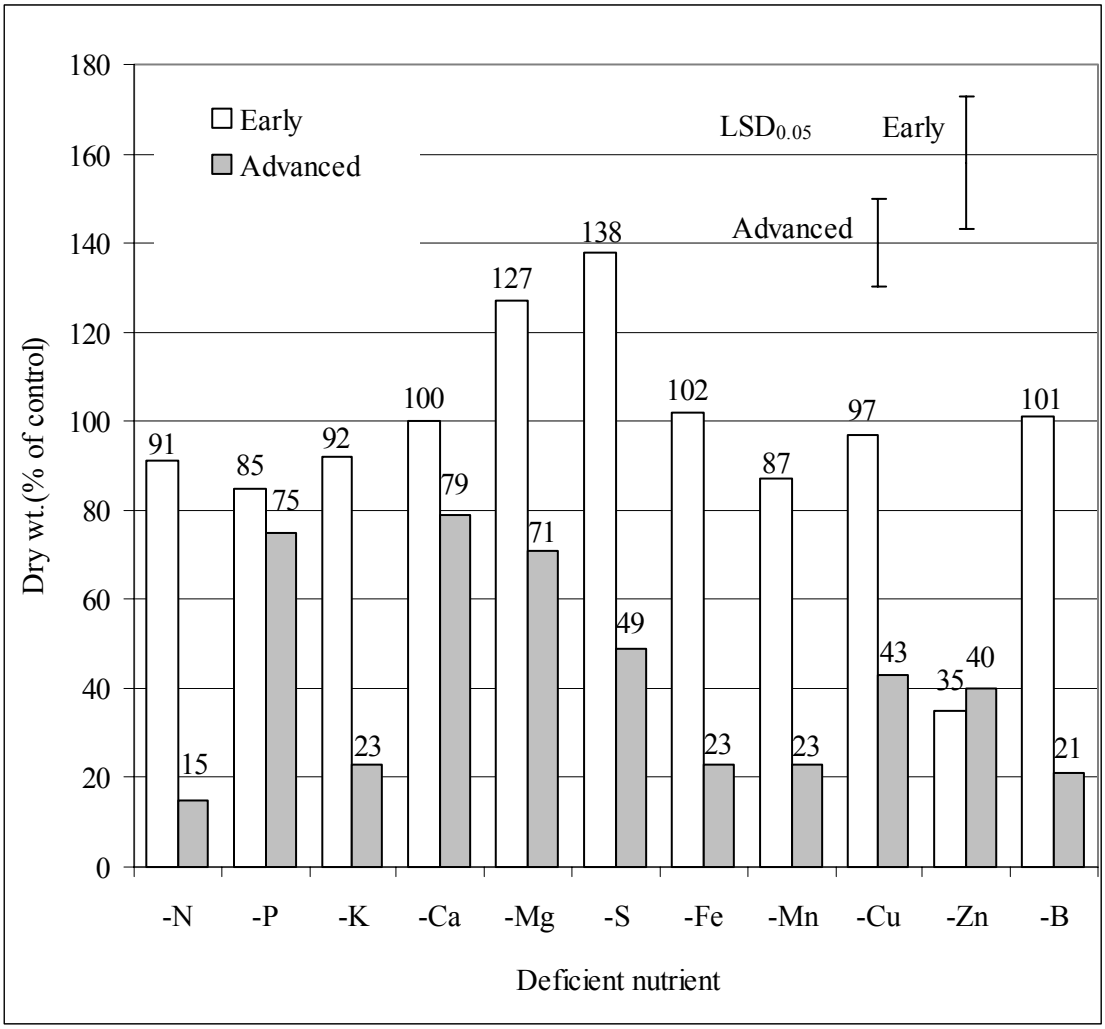

Figure 2. Marigold dry weight of nutrient deficient plants expressed as a percentage of control plants at early and advanced stages.

CHAPTER 3

ZINNIA FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Dharmalingam S. Pitchay[1], James L. Gibson[1], C. Ray Campbell[2], Paul V. Nelson[1] and Brian E.Whipker[1]

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

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

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Dharmalingam S. Pitchay[1], James L. Gibson[1], C. Ray Campbell[2], Paul V. Nelson[1] and Brian E.Whipker[1]

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

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

ABSTRACT

The present tissue analysis standards for zinnia (*Zinnia elegans* Jacq.) only indicate values based on survey range and not boundary values between deficient and acceptable growth. Moreover, complete visual deficiency symptoms have not been established for this crop. Zinnia plants were grown hydroponically in a glass greenhouse. Treatments consisted of a complete nutrient solution, and 11 additional solutions, each devoid of one essential nutrient. Deficiency symptoms and plant size were chronologically recorded for shoots and roots. Fully expanded youngest leaves were analyzed for nutrient concentration. Leaf dry weight concentrations associated with early deficiency of N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn and B were 1.66, 0.10, 0.89, 0.53, 0.10, 0.11 (%), 47.0, 10.8, 0.9, 9.2, and 23.4 ($mg \cdot kg^{-1}$), respectively. All nutrient deficient

plants weighed less than 90% of control at early stage. Shoot dry weight reduction was most pronounced in deficiencies of N, Ca, S, Mn, Cu, and Zn and least pronounced in Fe. On the other hand, at advanced stage a severe reduction of shoot dry weight was observed in P, K, B, N, and Mn deficient plants. The order of occurrence of early stage symptoms was Ca (4 days), N (7 days), K (7 days), P (10 days), Fe (12 days), Mn (28 days), Mg (29 days), B (30 days), Cu (42 days), Zn (42 days), and S (52 days).

INTRODUCTION

Zinnia (*Zinnia elegans* Jacq.) that comes in a variety of vibrant colors and forms with the ability of heat and drought tolerance has created a new wave of demand for the specialty cut flower and bedding plant industries. Owing to this fact, there is an anticipated need for foliar analysis standards and visual diagnosis of macro and microelement deficiencies that is vital for cost-effective zinnia production, as well as for minimal nutrient runoff. Current foliar analysis standards are limited. General guideline ranges for seven essential elements (1) and survey ranges for 11 essential elements (2) have been reported. Values in these ranges are associated with acceptable growth, but do not establish the minimum and maximum critical values. Present fertilizer formulation is solely based on a general recommendation of 2:1:2 (N: P_2O_5: K_2O) ratio (3) and/or on 100 $mg \cdot L^{-1}$ of N concentration (4). Besides soil testing, foliar analysis and visual diagnosis of nutrient deficiencies are valuable aspects of nutritional management program (3).

The objectives of this study were to characterize visual symptoms of nutrient deficiencies in chronological order as they appear from incipient to advance stages, and to establish foliar analysis standards that include early and advance deficiency stages for N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn and B.

MATERIALS AND METHODS

The protocol for this experiment was reported in Pitchay et al., (5). Exceptions included: the test crop zinnia 'Benary's Giant Orange'; the sowing date 28 August 1999; and the initiation of all treatments on 20 September 1999. Shoot dry weight and height values are based on measurements while all other size comparisons with control plants are visual assessments.

RESULTS

Following are the progressions of visual deficiency symptoms for each element that includes early and advanced stages. Early and late leaf sampling was carried out on days as indicated in Figure 1 and the resulting recently matured leaf concentrations are presented in Table 1.

Nitrogen

Initially, plants appeared lighter green. They had thin stems, shorter internodes and smaller leaves. Leaf orientation was similar to normal (day 5). Leaf blades appeared narrower from the basipetal to mid-leaf region. The lower leaves were lighter green with distinct veinal chlorosis. A development of light reddish pigmentation appeared on the first stem node from plant base. There was no sign of axillary shoots as compared to control (day 7). At this point, the tissue N concentration was 1.66%. Chlorosis progressed further from light green to yellowish green, more so on lower leaves. The young and the recently matured leaves were upright. The stems were thin and chlorotic (yellow) with the absence of axillary shoots. Meanwhile, the reddish pigmentation turned deeper red on the first node and lighter red on the second. The first internode was reddish and the rest of the internodes were chlorotic (day 13). By day 19, the cotyledons turned chlorotic (yellow) and blackened necrosis developed on the acropetal region starting from the lowermost leaves and progressed upwards. The flower buds were pointed with chlorotic sepals. By this time, the tissue N concentration dropped further to 1.23%. The primary root mass was lower by 50% and shorter by 60% than normal. Secondary roots were shorter and fewer, and were only found on the basal portion of the primary roots.

Phosphorus

In the beginning, some plants had slightly darker green leaves than normal (day 5). Thin stems, arching leaves and absence of axillary shoots made the plants look

smaller, by day 7. However, the roots were longer than normal by 30%. As the symptoms advanced (day 10), the plants appeared slender than normal due to longer and a greater number of internodes. Light purplish coloration developed on stem nodes. Leaf lamina was smaller and narrower with dark green older leaves and light olive green young and maturing leaves. At this point, the tissue P concentration was 0.10%. Grayish black necrosis developed at the acropetal region of mature leaves and progressed inwards and along the margins of the leaves. These symptoms started at the lower-most leaves and progressed upwards (day 13). By day 19, the entire lamina of the oldest leaves became necrotic. Flower buds were smaller with very short pedicels. The young leaves, especially the leaves below the buds were smaller as the symptoms progressed. At this point, the entire plants appeared lighter green, more so the younger leaves. Generally, the basipetal leaf regions were not as wide as normal. The tissue P concentration was 0.07%. On the other hand, the primary roots were numerous, and longer than normal by 35%. The secondary roots which were arising from the basal to mid-portion of the primary roots, were also numerous and longer.

Potassium

On day 5, the plants appeared darker green and shorter, and by day 7, dwarf, due to fewer nodes, shorter internodes and a height 50% of normal. Leaves were shorter lengthwise, with light white gray necrosis on the acropetal regions of oldest leaves when the tissue K concentration was 0.89%. By day 13, black necrosis developed at the acropetal ends, particularly at the margins, of oldest leaves covering 65% of leaf area.

These symptoms progressed up the plants. Meanwhile, light whitish gray necrosis appeared on the acropetal area of recently matured leaves. The internodes including the new ones remained short. The lateral and axillary shoots failed to develop. Finally, by day 19, black necrosis of the oldest leaves progressed to brown necrosis, covering almost 75% of the lamina, and the leaves appeared to be drooping. At this point, the tissue K concentration was 0.37%. Flowering was delayed with very short pedicels. The roots were thinner, although the length and the number of primary and secondary roots were similar to normal.

Calcium

Misshapen newly developing leaves with black necrotic spots was the first symptom of Ca deficiency (day 4). The tissue Ca concentration at this point was 0.53%. The terminal end of young leaves expanded laterally more than the basal two thirds of the leaves, yielding spoon shaped leaves with black necrosis at leaf mid-portion (day 5). On day 7, margins of newly developed young leaves curled and folded downwards causing severe distortion. Meanwhile, the black necrotic spots of young leaves turned brown and spread to about 75% of the mid-lamina. Necrosis also appeared on growing points, and mature leaves of some plants, and inhibition of axillary shoots resulting in smaller plants. The tissue Ca concentration dropped further to 0.44%. The primary roots were also shorter (25% of normal) with fewer and shorter secondary roots than normal.

Magnesium

The first symptoms appeared on day 23 with reduction of plant height and lateral shoot development. Faint interveinal chlorosis (light green) appeared on the acropetal region of lower leaves (day 25), which then advanced to greenish yellow coloration and spread inwards (day 29). At this point, the tissue Mg concentration was 0.1%. These symptoms then progressed to upper leaves (day 30). Eventually, a distinct interveinal chlorosis appeared on the lower leaves as well as the young leaves (day 35). Finally, by day 41, the interveinal chlorosis gave way to tan brown necrosis on the margins of lower leaves. The lateral shoots were fewer than normal; the flowers were smaller, and fewer (60% of normal). The tissue Mg concentration remained at 0.1%.

Sulfur

The first visual symptoms began to appear on day 46 with light green coloration on lower leaves, which then slowly progressed to the upper part of the plants (day 52). At this point, the tissue S concentration was 0.11%. The plants appeared smaller with less lateral branching. Flowering was earlier than in normal plants. All plants flowered. By day 58, the lower-most leaves turned greenish yellow and the young leaves of the primary and lateral shoots turned chlorotic from the base outward. Stems were thinner and lighter green. The leaf veins were also lighter green (day 63). At this point, lateral shoots also flowered and the tissue S concentration was 0.12%.

Iron

Visual symptoms first appeared on the basipetal region of immature leaves and stems as light green coloration on day 7. The basipetal chlorosis including the veins of the young and maturing leaves turned yellowish green, while interveinal chlorosis appeared on the acropetal area of the lamina, by day 12. The tissue concentration at this point was 47.0 $mg \cdot kg^{-1}$. By day 19, the entire young leaves turned greenish yellow. The internodes were shorter resulting in shorter plants. A faint interveinal chlorosis progressed to older leaves and young leaves of the lateral shoots. The symptoms on the older leaves further progressed to intense interveinal chlorosis. The axillary shoot development was 50% of normal by day 23. The interveinal chlorosis of the lateral shoots also intensified by day 29. The interveinal chlorosis pattern was distinct on lower leaves, and plant height was equal to normal on day 35. By day 41, almost 95% of the young leaves were yellow and interveinal chlorosis gave way to uniform chlorosis on mature leaves. The plants appeared taller than the normal. At this point, the tissue Fe concentration increased to 63.3 $mg \cdot kg^{-1}$.

Manganese

Development of interveinal chlorosis (light green) on young and recently matured leaves on day 23 was the first indication of visual symptoms. Leaves were smaller and narrower at the basipetal region. By day 28, the primary, secondary, and tertiary veins were green and readily apparent leaving the chlorotic tissue in small circular spots. At

this point, the tissue Mn concentration was 10.8 $mg \cdot kg^{-1}$. The small circular spots progressed to light white spots, randomly across the leaves (day 36). The white spots gave way to tan necrotic spots on mature leaves. These spots appeared larger on lower leaves (day 42). Necrosis of leaf margins began to appear on lower leaves. The main stems were thin with no axillary shoots (day 42). By day 52, necrosis progressed to about 40% of the leaf margins and lateral branching was only 10% of that of normal plants. Flowering was limited and delayed. Flowers were smaller (50% of control) due to missing ray petals, and the second whorl ray petals were reduced in number and were misshapen, narrow and light colored. The primary and secondary roots were fewer, thinner and shorter. The overall volume of the roots was 10% of normal plants. The tissue Mn concentration dropped to 9.2 $mg \cdot kg^{-1}$ at this time.

Copper

The reductions in plant height and size due to fewer nodes and shorter internodes by day 36 were the only first visual symptoms. This was followed by the appearance of upward cupping of young leaves, and minimal development of axillary shoots. Overall, the root system appeared smaller by 50% than normal (day 42). At this point, the tissue Cu concentration was 0.9 $mg \cdot kg^{-1}$. As the symptoms advanced, the plants were shorter. Flowering was delayed, although flower buds were apparent. By day 46, 70% as many plants as in the control were flowering with poorly developed ray florets. Tan to white necrosis developed along the margins of mid leaves (day 50). Plants remained short with lateral branching occurring only at the upper nodes (day 56). Meanwhile, the necrosis enlarged and developed as tan papery appearance on mid leaves and a blackened

appearance on lower leaves. The young leaves were not as pointed, and were shorter lengthwise by day 63. At this point, tissue Cu concentration was 1.2 $mg \cdot kg^{-1}$.

Zinc

Reduction of plant height was the only early visual symptom observed (day 30). Chlorosis (light green) then occurred on the immature tissue of shoots, basipetal area of very young leaves, and veins and mid-leaf portion of young leaves. The young leaves were upright, and there were no lateral shoots on the upper portion of the plants (day 36). By day 42, light tan necrotic patches developed at the mid-leaf region of young leaves and on immature leaves at the axillary shoot tips. Meanwhile, necrotic spots developed on recently matured leaves. At this point, the tissue Zn concentration was 9.2 $mg \cdot kg^{-1}$. As the symptoms advanced, the mid-leaf area necrotic patches turned gray, which then progressed to large brown necrotic patches. Necrosis also developed on some of the leaf veins. At this point (day 46), only the primary shoots were flowering while the lateral shoots had only initiated buds as compared to the control plants where the primary and lateral shoots were flowering. By day 52, necrosis became more severe with puckering of recently matured leaves. The tissue Zn concentration at this point was 8.1$mg \cdot kg^{-1}$.

Boron

Initially, plants appeared darker green and short due to shorter internodes at the upper portion of the plants, followed by tan brown necrosis of young leaf tips (day 28). Then leaves turned stiff and puckered in a horizontal orientation. By day 30, symptoms

progressed to necrosis of primary veins and light gray necrosis of mid-lamina area of maturing leaves with downward arching of lower leaves. At this point, the tissue B concentration was 23.4 $mg \cdot kg^{-1}$. The acropetal regions of very young leaves were chlorotic while the tips were necrotic. Primary and secondary roots appeared shorter with necrotic tips. As the symptoms advanced, young and maturing leaves were deformed with pointed and wavy tips while the recently matured leaf lamina appeared narrower with dented morphology (day 36). This was followed by chlorosis of primary, secondary and tertiary veins. Lower leaves began to droop at their bases. Lateral shoots were absent. Internodes were shorter at the upper portion of plants. Meanwhile, shoot apices stopped growing and flower buds aborted. Overall, the leaf texture was thick and brittle. Primary and secondary roots were short (only 10% of normal length), thick, and stiff with stubby tips. Secondary roots emerged in close proximity to the tips of the primary roots (day 42). By this time, the tissue B concentration dropped to 15.1 $mg \cdot kg^{-1}$.

DISCUSSION

The speed with which deficiencies occurred (Figure 1) is a good indication of susceptibility. Ca deficiency occurred first at 4 days followed by N and K at 7 days, P at 10 days, and Fe at 12 days. Deficiencies requiring a long time to occur were Mn at 28 days, Mg at 29 days, B at 30 days, Cu and Zn at 42 days, and S at 52 days.

Shoot dry weight reduction (Figure 2) was an early symptom of all deficiencies in zinnia. Shoot dry weight reduction was most pronounced in deficiencies of N, Ca, S, Mn, Cu, and Zn and least pronounced in Fe. Based on the 12 bedding plants addressed in this

study (data not shown in this paper), zinnia biomass reduction in S deficient plants was unusually large.

The initial dark green pigmentation in P and K deficient plants can be mistaken for healthy plants. However, these symptoms can be diagnosed by observing the roots (for P), and shoots (for K). P deficient plants tend to have large root system in response to acquire the limiting element (6-9). K deficient plants appeared dwarf, since K is the core cellular cation (10) that is essential for cell extension and turgidity (11). Apart from N, K is the second highest tissue concentration needed for optimal plant growth (Table 1). Delayed flowering is also an indication that the demand for element K is high in zinnias, as has also been previously reported (12).

Mn is an oxidizing agent that prevents super oxide from scavenging leaf tissue (13), thus, the absence of Mn may have caused the degradation of leaf tissue from chlorotic to white spots. Extensive damage in Zn deficient plants may have been due to the insufficiency of Zn to prevent reactive oxygen species from damaging the membranes (14), when the tissue concentration dropped below 9.2 $mg \cdot kg^{-1}$ (Table 1).

Synoptic visual deficiency symptoms were as follows: N - inhibited plant growth, light green lower leaves turned yellow and finally necrotic, and pinkish red first nodes turned red; P - dark green lower leaves developed grayish black necrosis, light purplish nodes, smaller buds with short pedicels, and increased root length; K- fewer nodes, and shorter internodes, darker green lower leaves turned whitish gray then developed black, and finally brown necrosis and drooped; Ca - young leaves and growing points developed black necrotic spots and appeared distorted, the roots were shorter; Mg - light green interveinal chlorosis of lower leaves turned greenish yellow and finally yellow with tan

brown necrotic margins; S - entire plants became greenish yellow, flowering was early and all plants flowered; Fe - light green shoots and young leaves turned yellow, maturing leaves turned from interveinal to total chlorosis; Mn - interveinal chlorosis with light tan necrotic spots on young and mature leaves; Cu - tan to white necrosis on margins of mid leaves of the plants turned papery; Zn - chlorotic/bleached mid-lamina of young and recently mature leaves; B - deformed, stiff, and leathery young leaf lamina, and stubby root tips.

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Table 1 . Zinnia tissue nutrient concentration at early and advanced stages of deficiency in recently matured leaves of hydroponically grown plants in this study and published standards.

Treatment	**-N**	**-P**	**-K**	**-Ca**	**-Mg**	**-S**	**-Fe**	**-Mn**	**-Cu**	**-Zn**	**-B**
	Percent dry wt.						$mg \cdot kg^{-1}$ of dry wt.				
Element	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn	B
Early control	5.00	0.60	4.77	1.43	0.96	0.26	55.1	128.0	4.1	20.0	56.6
Early deficiency	1.66	0.10	0.89	0.53	0.10	0.11	47.0	10.8	0.9	9.2	23.4
Advanced control	4.70	0.82	4.39	1.14	0.76	0.22	87.1	202.0	3.9	27.1	72.2
Advanced deficiency	1.23	0.07	0.37	0.44	0.10	0.12	63.3	9.2	1.2	8.1	15.1
P value	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.02	0.00	0.01	0.00
Published*	5.78	0.74	3.25	2.37	1.48	0.37	81.0	300.0	23.0	115.0	67.0

*Mills and Jones, 1996. Survey average of concentrations associated with acceptable crop performance, minimum critical concentrations not identified.

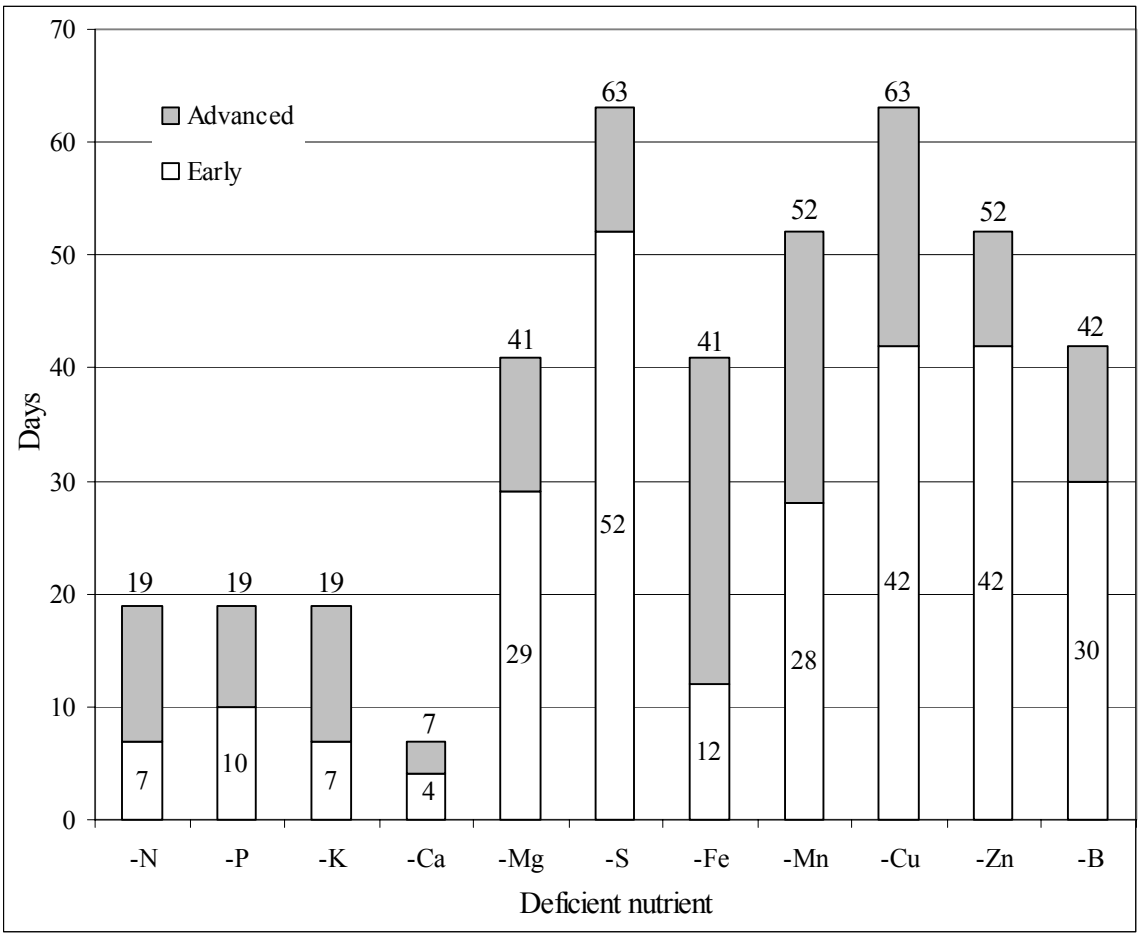

Figure 1. Days from initiation of treatments to leaf sampling for nutrient analysis for zinnia at early and advanced stages of deficiency.

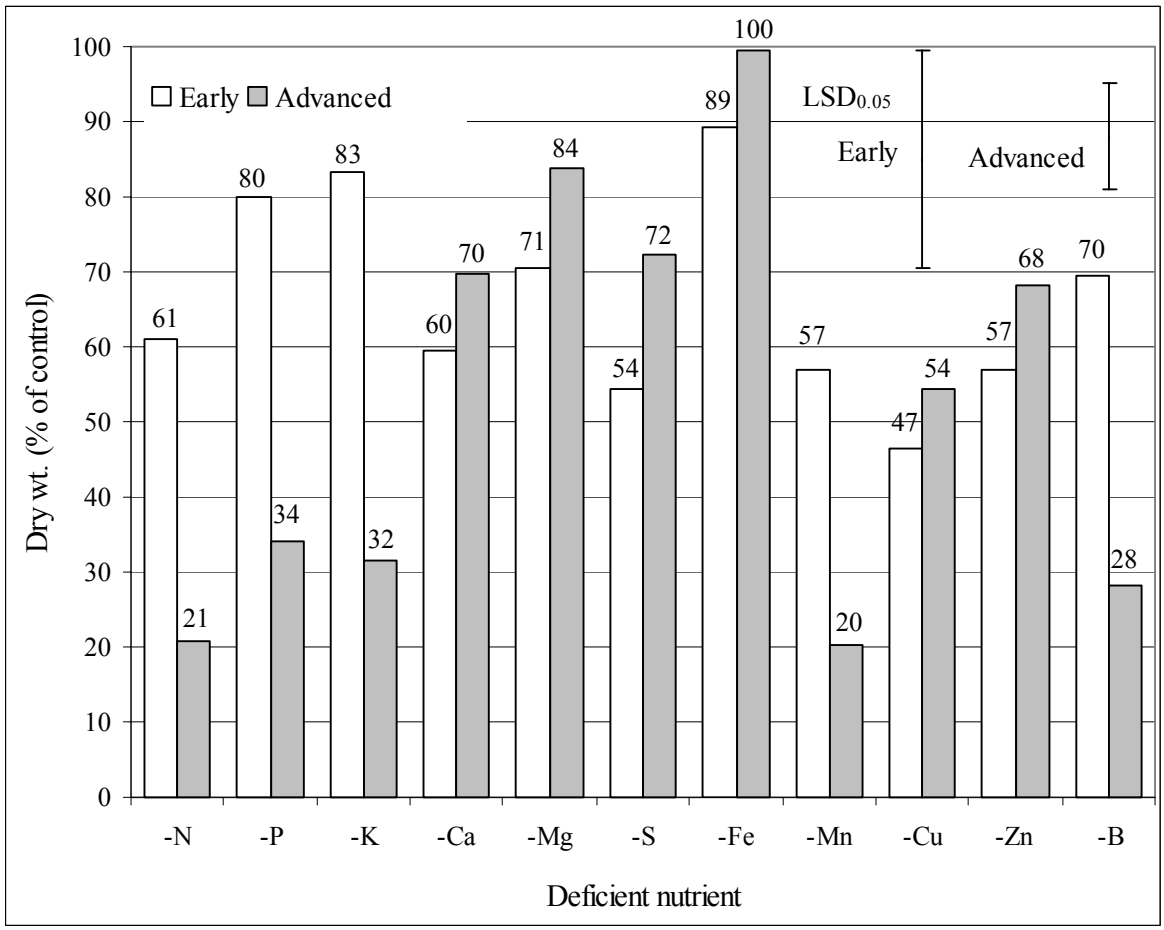

Figure 2. Zinnia dry weight of deficient plants expressed as a percentage of control plants at early and advanced stages.

CHAPTER 4

SALVIA FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Dharmalingam S. Pitchay[1], James L. Gibson[1], Paul V. Nelson[1], F.R. Walls[2], Brian E.Whipker[1], and Brenda Cleveland[2]

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

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

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Dharmalingam S. Pitchay[1], James L. Gibson[1], Paul V. Nelson[1], F.R. Walls[2], Brian E.Whipker[1], and Brenda Cleveland[2]

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

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

ABSTRACT

Tissue analysis standards are crucial for monitoring plant nutrient status for salvia. Complete visual deficiency symptoms have not been established for this crop. Salvia *(salvia splendens* Sell ex Roem. & Schult*)* plants were grown hydroponically in a glass greenhouse. Treatments consisted of a complete nutrient solution, and 11 additional solutions, each devoid of one essential nutrient. Deficiency symptoms and plant size were chronologically recorded for shoots and roots. Fully expanded youngest leaves were analyzed for nutrient concentration. In the absence of N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn and B, tissue dry weight concentrations at early deficiency stage were 2.22. 0.07, 0.22, 0.66, 0.09, 0.10 (%), 52.0, 5.1, 1.8, 12.0, and 9.3 ($mg \cdot kg^{-1}$), respectively. At early stage, shoot dry weight reduction was most pronounced in deficiencies of Mn (84%), Ca (79%),

Cu (77%), N (71%), and Zn (68%), and least pronounced in S (29%), Mg (27%), and Fe (12%). On the other hand, at advanced stage a severe reduction of shoot dry weight was observed in Ca and Mn (91%), N (87%), Cu (75%), Zn (70%), P (69%), and K (62%) deficient plants. The order of visual symptoms at early stage from first to last was N (7 days), Ca (9 days), Fe (10 days), K (12 days), B (14 days), P (18 days), Mn (62 days), Cu (80 days), Mg (81 days), Zn (84 days), and S (90 days).

INTRODUCTION

Salvia (*Salvia splendens* Sell ex Roem. & Schult**)** is one of the popular bedding plants used in landscapes. Limited information is available on visual disorder symptoms and foliar nutritional standard for salvia. Current foliar analysis standards guidelines are based on survey ranges for 11 essential elements (1). Values in these ranges are associated with acceptable growth, but do not establish the minimum and maximum critical values. Present fertilizer formulation is solely based on a general recommendation of 2:1:2 (N: P_2O_5: K_2O) ratio (2). Each element plays a unique role in plant growth and development. Thus, each elemental disorder has its own characteristic symptoms, which also vary according to species and within cultivars (3). Therefore, growers could use visual symptoms as a diagnostic tool for monitoring nutrient status, which is cost-effective and time saving.

The objectives of this study were to characterize visual symptoms of nutrient deficiencies in chronological order as they appear from incipient to advance stages, and

to establish foliar analysis standards that include early and advanced deficiency stages for N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn and B.

MATERIALS AND METHODS

The protocol for this experiment was reported in Pitchay et al., (4). Exceptions included: the test crop salvia 'Cover Girl'; the sowing date 21 December 1999; and the initiation of Mg, S, Mn, Cu, and Zn treatments on 22 February 2000 and N, P, K, Ca, Fe, and B treatments on 2 March 2000. Shoot dry weight and height values are based on measurements while all other size comparisons with control plants are visual assessments.

RESULTS

Following are the progressions of visual deficiency symptoms for each element including early and advanced stages. Early and late leaf sampling was carried out on days as indicated in Figure 1 and the resulting recently mature leaf concentrations are presented in Table 1.

Nitrogen

Plants appeared lighter green, more so on the lower older leaves (day 7). Plants were shorter (70% of control) and smaller (day 9). By day 14, plants had thinner stems,

shorter internodes, fewer and smaller leaves with light reddish petioles, and poorly developed axillary shoots. Flowers were also smaller and fewer, proportionate with plant size. First internodes from plant bases appeared shorter and reddish green when the tissue N concentration was 2.22%. The plants appeared skeletal and unlike control plants, flowers were absent on lateral shoots. By day 19, lower leaf veins turned lightly chlorotic. Plant size was 50% of control. Leaves were misshapen with the mid-leaf region broader and the length shorter by 50% of the control. As the symptoms progressed, there was no development of new leaves (day 22). Abscission of some flowers and cessation of growing tips was observed. Overall, inflorescence size and number of flowers were reduced by 40% and 70% of control respectively. At this point, whole plants appeared yellowish green. The older leaves turned yellow followed by a tinge of red pigmentation at the acropetal tips of the leaves, and eventually, the petioles turned reddish (day 25). The number of nodes was similar to that of the control; however, internode length and caliper size was reduced. Since growth halted, the number of leaves was only 20% of control with almost 90% reduction in total leaf area. Eventually by day 29, a uniform bleached yellow chlorosis developed across the entire lamina of lower most leaves when the tissue concentration was 2.01% N.

Phosphorus

Initially (day 18), the foliage appeared darker green. Irregular black necrotic spots developed randomly on margins and across the lamina of older leaves by day 20. At this point, the tissue P concentration was 0.07%. The plants had fewer leaves (40% of

control), fewer flowers, and fewer and poorly developed lateral shoots. The necrotic spots spread to maturing leaves (day 23). By day 26, necrotic spots progressed to necrotic patches. These patches enlarged, more so in the acropetal region. By this time, the necrotic patches turned from black to gray (day 28). Finally (day 31), these necrotic patches also appeared on recently matured leaves of lateral shoots while the tissue P concentration remained at 0.07%. At this point, the plant height was 83% of control, and the leaf size was 75% of control. The lateral shoots remained shorter and smaller compared to control. The inflorescences were narrower, and less compact due to fewer flowers. The primary and secondary roots were fewer compared to normal. However, the primary roots were longer while the secondary roots were shorter than control.

Potassium

Appearance of darker green plants was the beginning of visual symptoms. Petioles were also green, unlike control, which were brown (day 12). Plant growth was substantially reduced before specific symptoms developed. By day 15, the plants were shorter by 50% of control, and appeared dwarf due to poorly developed lateral shoots, and shorter internodes, petioles, and pedicels. At this point, the tissue K concentration was 0.22%. Mature leaves developed black necrosis along the margins including the tips. An extensive interveinal upward puckering also developed away from the margins of mature leaves (day 20). Subsequently, the black necrosis progressed inward leaving behind gray, dry, scorch-like margins. At this point, plants appeared compact with a height of 66% and leaf size 74% of control (day 25). By day 27, necrosis along the

margins of mature leaves progressed inward covering about 30 to 40% of the lamina. Eventually (day 30), chlorosis (greenish yellow) developed adjacent to the necrotic areas on some of the mature leaves when the tissue K concentration was 0.20%. Inflorescences were shorter with fewer flowers, and appeared compact due to shorter pedicel internodes. Inflorescence length was severely reduced at the first and second nodes from the base. Overall, the root volume was similar to that of control plants with lighter brown coloration (control dark brown).

Calcium

Initially, plants appeared smaller, and shorter by 47% of control. They had proportionately smaller and fewer leaves, and poor lateral branching. The root growth was limited (10% of control) and necrosis developed along the primary root axis with black root tips (day 9). By day 11, upper portion of the plants including the inflorescences appeared weak and bent when the tissue Ca concentration was 0.66%. From then on there was absolutely no plant growth. Black necrotic spots developed on recently matured leaves, and the young leaves of the lateral shoots. The necrosis began at the basipetal region (day 13) and extended to mid-leaf area including the primary and secondary veins (day 15). Top-most internodes shriveled and resulted in withering of the inflorescences (day 17). The older leaves appeared normal and the plant height was 30% of control. At this point, the roots appeared dark brown with shorter and thicker secondary roots, and breakdown of tissue occurred in between the terminal and mid-portion of the primary roots. This was followed by extensive brown necrosis in shoot tips

and inflorescences. As symptoms progressed, the older leaves began drooping (day 19). By day 21, black necrotic spots at the basipetal region of the recently matured leaves turned brown and enlarged surrounded by chlorotic tissue. Some of these mature leaves abscised and the plants had only about four leaves per plant (16% of control). Meanwhile, the roots were totally necrotic and began to rot. Young leaves of the lateral shoots and the upper portion of the plants including the inflorescences and growing tips developed extensive necrotic lesions and died. At this point, the tissue Ca concentration was 0.46%.

Magnesium

Initially, plants appeared lighter green (day 81). By day 84, chlorosis (yellowish green) developed on older leaves as a silhouette on leaf margins particularly toward the acropetal region. By day 88, the chlorosis progressed inwards as interveinal chlorosis, and the tissue Mg concentration at this point was 0.09%. Meanwhile, interveinal upward puckering developed on mature leaves. Plants were shorter than the control by 15%. They had fewer leaves, shorter petioles and poorly developed lateral shoots. Roots were lighter colored than control roots. Interveinal upward puckering of mature leaves intensified and became chlorotic. Chlorosis of leaf margins turned to light brown necrosis by day 94, and at this point, the plant height was 73% of control. The interveinal chlorosis progressed extensively and light brown necrotic spots formed in the interveinal area, more so in puckered areas by day 97. By day 100, necrosis also developed along the leaf margins of recently matured leaves and acropetal tips of young leaves. At this point, the tissue concentration was 0.07%. Flower inflorescences were shorter due to shorter internodes than control. Primary and secondary roots were fewer and shorter compared to normal.

Sulfur

Overall, plants appeared lighter green more so on recently matured leaves (day 90). Lateral branching was delayed, although axillary shoots were present. Mature leaves developed silhouette-like yellowish green chlorosis on the leaf margins (day 92), and at this point, the tissue S concentration was 0.10%. The chlorosis on leaf margins uniformly spread inward, while the axillary shoot leaves developed uniform chlorosis across the entire lamina (day 95). Stems were also slightly chlorotic with plant height 90% of control. By day 97, as the symptoms progressed, the chlorosis of the interior part of the leaf lamina intensified to greenish yellow, while the margins turned necrotic (day 102). At this point, the tissue S concentration was 0.13%. The young leaves of lateral shoots including the growing tips turned uniformly chlorotic with greenish yellow pigmentation. Petioles had slight yellowish pigmentation than control. Overall, the number of inflorescences was fewer due to less lateral branching. The root system was similar to control plants. Plant height was 90% of control.

Iron

Initial symptoms appeared on young leaves of terminal and axillary shoots on day 10, as acropetal light green chlorosis. By day 13, chlorosis turned yellowish green and progressed inward in an interveinal pattern. At this point, the Fe tissue concentration was 52.0 $mg \cdot kg^{-1}$. Acropetal yellowish green chlorosis also developed on young leaves of lateral shoots. Yellowish green interveinal chlorosis of terminal shoot leaves intensified to more yellowish green (day 15). Meanwhile, acropetal chlorosis of lateral shoots

progressed inward as interveinal chlorosis. Overall, the plants had fewer leaves and less lateral branching. Primary roots were fewer and similar in color to the control. In addition, tusk-like thick, new primary roots developed from the crowns. Older leaves developed interveinal chlorosis (day 17). Meanwhile, recently matured leaves gave way to total chlorosis except in the basipetal region. At this point, stems including the lateral stems appeared lighter green, and lower-most flower sepals including bracts turned bleached yellow. By day 22, the young leaves of lateral shoots, including the tips, became totally chlorotic. As the symptoms advanced (day 25), the acropetal region of young leaves became bleached yellow and progressed inward in an interveinal pattern. By day 29, light tan necrosis developed along the margins and tips of young and recently matured leaves and progressed inwards. At this point, the lateral shoot tips appeared bleached. Flowers began fading while the lateral flower buds turned bleached with red tips. Petioles were also chlorotic. The tissue Fe concentration increased to 69.6 $mg \cdot kg^{-1}$. Roots were thicker with light brown pigmentation. Primary roots were fewer and longer with numerous short comb-like secondary roots of equal length (0.5 cm) and spacing on the primary roots.

Manganese

Severe reduction of plant growth was the early sign of Mn deficiency. The plants had thinner stems, shorter internodes, poorly developed lateral shoots, smaller and fewer leaves, and few primary roots (5% of control) with an insignificant number of secondary roots (day 62). Meanwhile, the foliage turned lighter green, more so on older leaves,

which then became chlorotic (yellowish green) along the margins and tips, while the rest of the lamina remained lighter green (day 70). The tissue Mn concentration at this point was 5.1 $mg \cdot kg^{-1}$. Interveinal chlorosis was barely perceptible. These symptoms spread to young and recently matured leaves (day 74). Subsequently, yellow stippling appeared on the acropetal margins and tips of lower most leaves, which then progressed inward across the lamina (day 82). By day 85, yellow stippling also appeared on the young leaves. The yellow stippling advanced to brown necrotic stippling along the margins of older leaves, which then enlarged affecting about 30% of the lamina. Stippling across the lamina of mature leaves enlarged to white to bleached yellow color with brown necrotic spots in the center (day 90). Plants remained smaller with no flowering. Root growth was severely suppressed with only a few strands of lighter colored primary roots (5% of control) and very few secondary roots (day 95). At this point, the tissue Mn concentration was 4.2 $mg \cdot kg^{-1}$.

Copper

Plants appeared smaller with a height 66% of control (day 80). They had smaller lanceolated narrow basal leaves (30% of control), and thinner stems. Leaf color was normal. By day 86, young and recently matured leaves developed puckering and upward cupping starting from the basipetal areas and progressed outward. At this point, the tissue Cu concentration was 1.8 $mg \cdot kg^{-1}$. By day 92, chlorosis formed along the margins (exclusive of the tips) of recently matured leaves. Meanwhile, the basipetal region of mature leaves developed chlorosis along the margins and the interveinal areas. However,

some of the mature leaves on the third node from the top of lateral shoots developed desiccation over the terminal 30 to 40% starting from the tips. As the flower buds opened, many abscised. Buds that survived were lighter and eventually dried. The tips of the inflorescences also dried. By day 96, puckering also developed in the mid-lamina region of older leaves while the symptoms became more severe on recently matured leaves. Margins and tips of young and recently matured leaves were deformed due to partial formation of tissues in those areas. Eventually, the chlorosis on margins and interveinal areas of mature leaves turned necrotic (day 100). All the flowers and buds on the primary shoot inflorescences abscised. On lateral shoots, the poorly developed inflorescences retained only a few immature buds. The plant height was 60% of control. The plants had fewer (50% of control) and shorter light colored primary and secondary roots. The tissue Cu concentration remained at 1.8 $mg \cdot kg^{-1}$.

Zinc

Overall, plants had fewer and smaller leaves and poorly developed lateral branching. The plant height was 70% of control (day 84). Initially, recently matured leaves developed chlorosis along the margins and tips followed by upward puckering across the lamina. At this point, the tissue Zn concentration was 12.0 $mg \cdot kg^{-1}$ (day 86). These leaves then curled downward at the acropetal region, while mature leaves developed upward puckering across the lamina (day 87). By day 91, the chlorosis along the margins and tips of the recently matured leaves intensified to purplish pigmentation, which then turned to bronze colored necrosis by day 96. The interveinal zone of these

leaves developed bronze colored necrotic spots. Meanwhile, mature leaves developed a faint interveinal chlorosis across the lamina, and yellowish green chlorosis along the margins and tips. By day 99, these symptoms progressed to bronze colored necrosis along the margins and tips with latent light gray colored necrotic spots in the interveinal zone. By day 101, necrosis of recently matured leaves became severe and enlarged, while the remaining lamina were totally chlorotic (yellowish). Necrotic and chlorotic symptoms of mature leaves also intensified. The abaxial surface of mature and recently matured leaves turned chlorotic with development of a distinct veinal pattern. The young leaves developed chlorosis along the margins and interveinal areas. Overall, flowers were smaller, fewer, and lighter colored with poorly developed pinkish colored corollas that abscised prematurely leaving only the calyx. The inflorescence stalks were thinner and shorter. At this point, the tissue Zn concentration was 11.3 $mg \cdot kg^{-1}$. The root system was lighter colored and consisted of fewer and longer primary roots with numerous and longer secondary roots.

Boron

Initially day 14, the foliage appeared slightly darker green. This was followed by development of light chlorosis along the margins of young leaves (day 16). At this point, the tissue B concentration was 9.3 $mg \cdot kg^{-1}$. The young and recently matured leaves developed upward puckering across the lamina and began to curl downward at the leaf bases. By day 20, these symptoms began to spread to the acropetal region of mature leaves, while axillary and lateral shoots turned necrotic. Flowers were poorly developed,

and appeared faded in color with raised veination. Most of the flower buds were aborted on the primary inflorescences, while the lateral shoot inflorescences died retaining only the necrotic bracts. As symptoms progressed, young leaves either became deformed or rolled severely (day 23). The chlorosis along the margins turned necrotic (day 28). Leaves had thick and leathery texture. Undersurface of young leaves developed raised veins and random, sporadic tissue corking. There were numerous axillary shoots with tiny leaves which had black necrotic tips. At this point, the primary shoot inflorescences were barren with no flowers and buds. Lateral shoot inflorescences appeared shriveled and dried-up. The tissue B concentration was 8.8 $mg \cdot kg^{-1}$. Primary, secondary and tertiary roots were short and thick with stubby root tips. Lateral roots with brown tips that appeared close to the tips of primary, secondary and tertiary roots were longer.

DISCUSSION

N was the first deficiency to occur at 7 days followed by Ca (9 days), Fe (10 days), K (12 days), B (14 days), and P (18 days). Mn, Cu, Mg, Zn and S were the slowest to develop at days 62, 80, 81, 84 and 90, respectively. The delayed symptom of Mn deficient plants may be due to the supply of Mn in the seeds (5). The progression from early to advanced stage was quicker in Ca, P and S deficient plants. The interval between early and advanced stage (necrotic symptoms) ranges from 12 to 22 days for all the elements except for Cu and Mn deficiency, which took about 30 and 33 days respectively. An extended delay in S deficiency symptoms was consistently observed in marigold, vinca and zinnia (data not shown). This could have been due to the acquisition

of S from SO_2 in the atmosphere (6). Ca deficiency symptoms first appeared on roots rather than shoots and rapid disintegration of root tissue occurred. Thus, it is least possible to recover from Ca deficiency.

Plants can acquire various adaptations to overcome Fe deficiency (5). Although Fe deficient plants developed symptoms much earlier than other deficient elements, there was little effect initially on plant size (Figure 2). Moreover, the roots were abnormally thick. These could be some of the means of salvia to adapt to the Fe stress condition. On the other hand, increased Fe concentrations in recently matured leaves at the advanced stage of symptoms (Table 2) might indicate the presence of physiologically inactive iron in this tissue (5).

Absence of Mn paralyzed photosystem II (7). This could account for suppressed photosynthate in plants that resulted in severe cessation of root and shoot growth. It is also interesting to note that flowering was inhibited only in Mn deficient plants.

The appearance of necrosis along the margins of older leaves in K deficient plants may be mistaken for P deficiency. This misdiagnosis can be solved by closely observing the specific area in the plant tissue. In K deficient plants, necrosis appeared along the margins and tips of the lamina, and in P deficient plants, necrosis appeared not only along the margins, but also randomly across the lamina. Purple pigmentation is generally associated with P deficiency (8). However, in the case of salvia, purple pigmentation of older leaves only appeared in Zn deficient plants. Both N and S deficient plants developed uniform chlorosis on older leaves. These two deficiencies can be differentiated by pink pigmentation of leaf tips and petioles that forms in N deficient, but not S deficient plants.

Synoptic visual deficiency symptoms were as follows: N - small sized plant, older leaves turned lighter green to yellow with reddish pigmentation on acropetal region, reddish petioles, and reddish green internodes; P - dark green older leaves with irregular black necrotic spots most pronounced on margins and lamina, but also appearing across the lamina, enlarged to gray patches and less compact inflorescences; K - dark green older leaves with necrotic margins and interveinal puckering, and compact inflorescences; Ca - basipetal black necrotic spots on young and recently matured leaves, and internal necrosis of top node and roots; Mg - older leaves developed silhouette of chlorosis on margins with interveinal chlorosis that turned to tan brown necrosis; S - mature leaves including the petioles and stems developed uniform lighter green to greenish yellow chlorosis and necrotic margins ; Fe - young leaves developed acropetal then interveinal chlorosis, finally entire lamina became bleached with acropetal necrosis; Mn – leaves developed light green to yellowish green chlorosis with yellow stippling across the lamina that turned to brown necrosis, and root growth was suppressed; Cu - margin of young and mature leaves were chlorotic and deformed with upward cupping and puckering and finally desiccation from tips; Zn - downward cupping, puckering and chlorosis on margins of recently matured leaves, which turned purplish then bronze necrosis; B - puckering, downward rolling, and deformed margins of thick and leathery textured young and recently matured leaves, and roots were thick and short with stubby roots tips.

ACKNOWLEDGEMENTS

The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of the products named, nor criticism of similar ones not mentioned. Appreciation is expressed to Tom Abramowski of Rockwell Farms, N.C., the North Carolina Commercial Flower Growers' Association, and Fred C. Gloeckner Foundation for financial support, Ernst Benary of America Inc. for providing the seeds and Smithers-Oasis for providing the propagation medium.

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Table 1 . Salvia tissue nutrient concentration at early and advanced stages of deficiency in recently matured leaves of hydroponically grown plants in this study and published standards.

Treatment	**-N**	**-P**	**-K**	**-Ca**	**-Mg**	**-S**	**-Fe**	**-Mn**	**-Cu**	**-Zn**	**-B**
	Percent dry wt.						$mg \cdot kg^{-1}$ of dry wt.				
Element	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn	B
Control early	5.06	0.38	5.27	2.66	0.54	0.24	75.0	242.1	6.4	30.3	68.66
Deficiency early	2.22	0.07	0.22	0.66	0.09	0.10	52.0	5.1	1.8	12.0	9.3
Control advanced	4.81	0.38	5.30	2.98	0.59	0.22	114.0	268.3	6.0	32.7	79.7
Deficiency advanced	2.01	0.07	0.20	0.46	0.07	0.13	69.6	4.2	1.8	11.3	8.8
P value	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.02	0.00
Published*	2.38 - 5.61	0.30 - 1.24	2.90 - 5.86	1.00 - 2.5	0.25 - 0.86	0.73	60.0 - 300.0	30.0 - 284.0	7.0 - 35.0	25.0 - 115.0	25.0 - 75.0

*Mills and Jones, 1996. Survey average of concentrations associated with acceptable crop performance, minimum critical concentrations not identified.

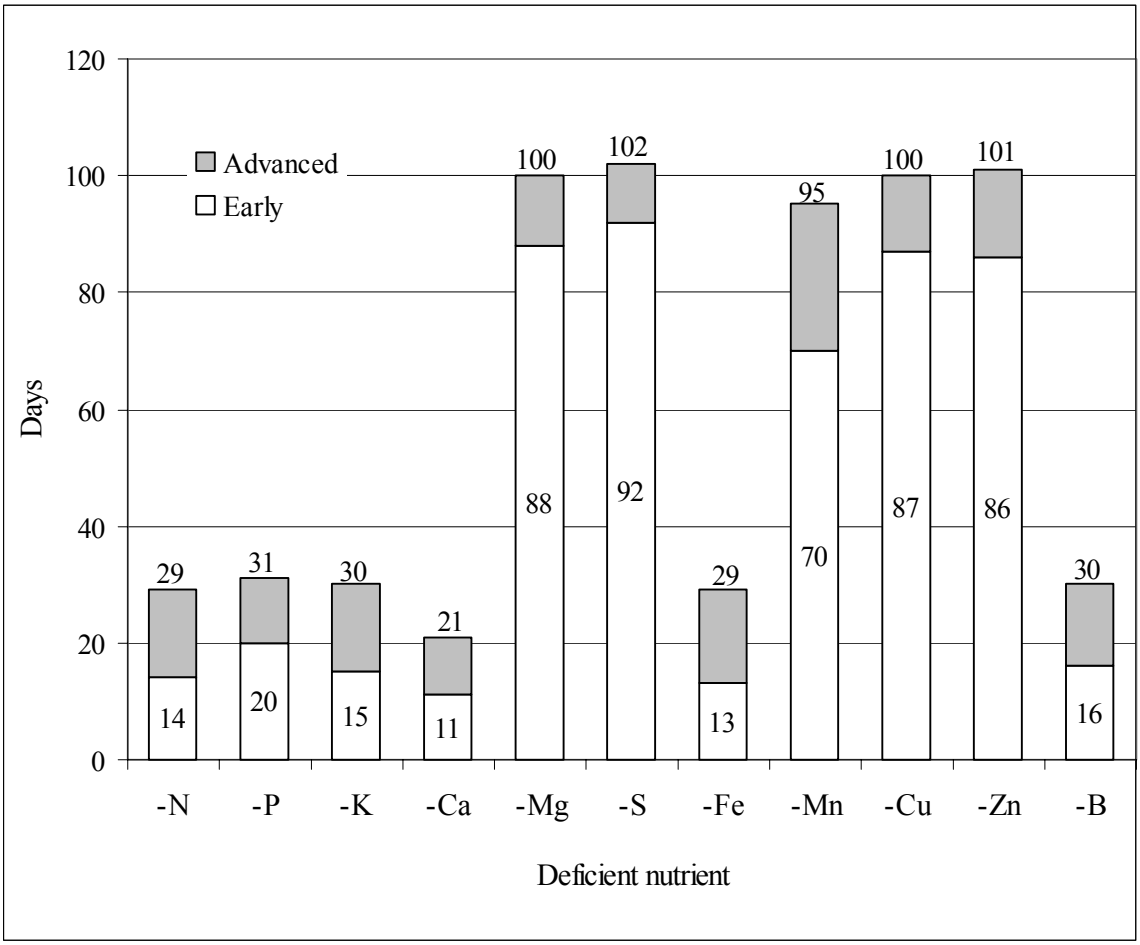

Figure 1. Days from initiation of treatments to leaf sampling for nutrient analysis for salvia at early and advanced stages of deficiency.

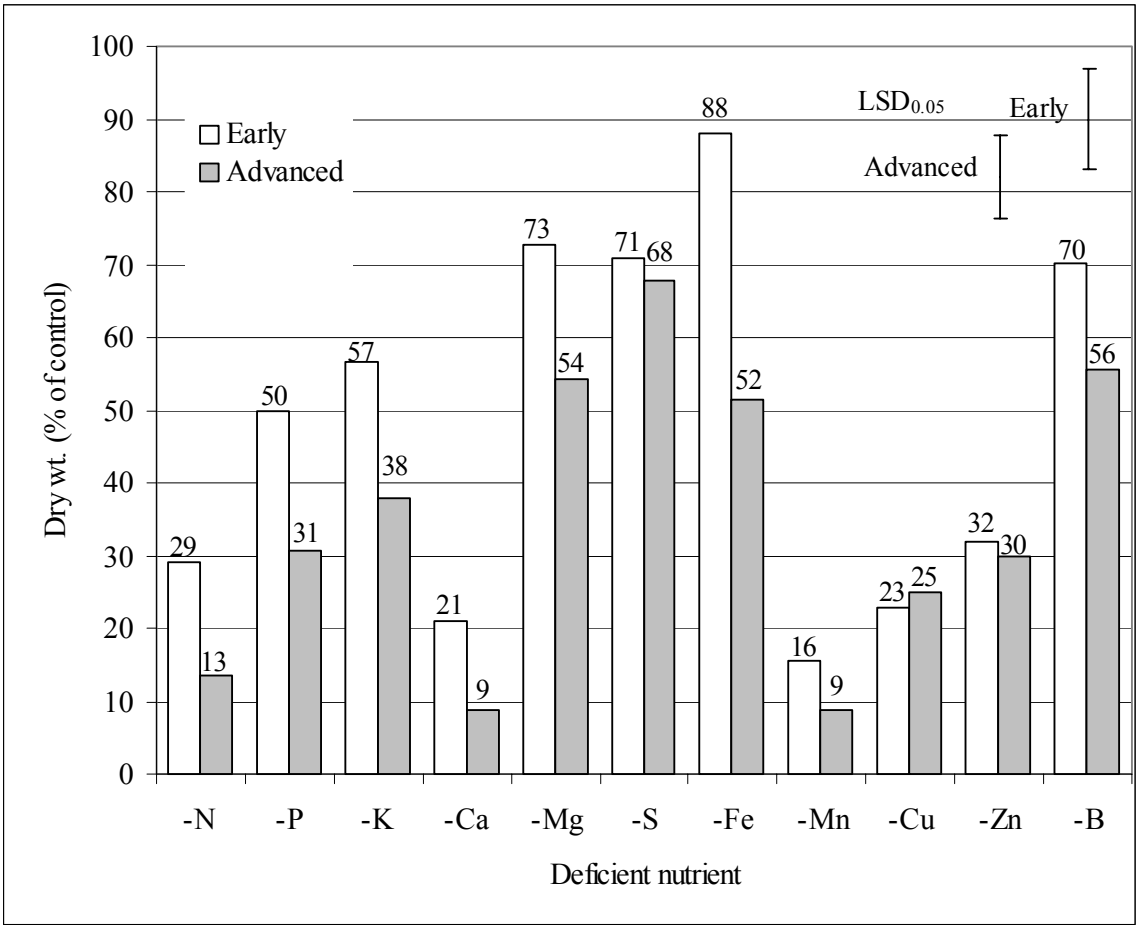

Figure 2. Salvia dry weight of deficient plants expressed as a percentage of control plants at early and advanced stages.

CHAPTER 5

CELOSIA FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Dharmalingam S. Pitchay[1], James L. Gibson[1], F.R. Walls[2], Brian E.Whipker[1], Brenda Cleveland[2], and Paul V. Nelson[1]

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

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

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Dharmalingam S. Pitchay[1], James L. Gibson[1], F.R. Walls[2], Brian E.Whipker[1], Brenda Cleveland[2], and Paul V. Nelson[1]

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

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

ABSTRACT

Tissue analysis standards and complete visual deficiency symptoms have not been established for celosia (*Celosia argentea* Plumosa group), which are crucial for monitoring plant nutrient status. Celosia plants were grown hydroponically in a glass greenhouse. Treatments consisted of a complete nutrient solution, and 11 additional solutions, each devoid of one essential nutrient. Deficiency symptoms and plant size were chronologically recorded for shoots and roots. Fully expanded youngest leaves were analyzed for nutrient concentration. Nutrient dry weight basis concentrations in recently matured leaves at early stages of deficiencies of N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn and B, were 3.71, 0.09, 4.81, 0.64, 0.11, 0.17 (%), 54.9, 13.7, 1.8, 7.6 and 8.9 ($mg \cdot kg^{-1}$) respectively. At the early deficiency stage, shoot dry weight deficit compared to the

control plants was most pronounced in deficiencies of B (45%), Cu (39%), and Mn (31%), and least pronounced in Mg (2%), K (2%), N (0%), and S (2% increase). On the other hand, at the advanced deficiency stage a severe deficit of shoot dry weight was observed in plants deficient in Mn (55%), B (50%), P (47%), Cu (44%), Ca (37%), and K (33%). The order of visual symptoms at early stage from first to last was N and K (8 days), Zn (14 days), S (15 days), P and Fe (16 days), B (17 days), Mn (22 days), Mg (23 days), Ca (27 days), and Cu (32 days).

INTRODUCTION

Celosia (*Celosia argentea* Plumose group) with vibrant colored flowers is a popular bedding plant widely used in landscapes. In some parts of the world, celosia is also grown as a food crop (1). Therefore, it is vital to produce quality celosia crops to meet the consumer demand. Limited information is available on visual disorder symptoms and foliar nutritional standards. Current foliar analysis standards guidelines are based on survey ranges for 11 essential elements (2). Values in these ranges are associated with acceptable growth, but do not establish the minimum and maximum critical values. Present fertilizer formulation is solely based on a general recommendation of a 2:1:2 (N: P_2O_5: K_2O) ratio (3). Each elemental disorder has its own characteristic symptoms, which also vary according to species and within cultivars (4). Therefore, for monitoring nutrient status, growers could use visual symptoms as a diagnostic tool, which is cost-effective and time saving.

The objectives of this study were to characterize visual symptoms of nutrient deficiencies in chronological order as they appear from incipient to advance stages, and to establish foliar analysis standards at early and advanced deficiency stages for N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn and B.

MATERIALS AND METHODS

The protocol for this experiment was described in Pitchay et al., (5). Exceptions included: the test crop celosia 'Century Red', the sowing date 16 August 2000, and the initiation of Mg, S, Mn, Cu, Zn, and B treatments on 6 October, and N, P, K, Ca, and Fe treatments on 13 October. Shoot dry weight and height values are based on measurements while all other size comparisons with control plants are visual assessments.

RESULTS

Following are the progressions of visual deficiency symptoms for each element. Early and late leaf sampling was carried out on days as indicated in Figure 1 and the resulting recently matured leaf dry weight concentrations are presented in Table 1.

Nitrogen

Symptoms began on day 8, as a silhouette of purplish-pink pigmentation along the leaf margins of young and older leaves. These leaves appeared green with a light shade of purplish-red pigmentation over the entire lamina, more so on young leaves. The tissue N

concentration was 3.71%. By day 12, plants appeared smaller. They had thinner stems, lanceolated smaller leaves (85% of control), and less lateral branching. Leaf orientation was upright, more so in young leaves. The silhouette of purplish-pink pigmentation along the margins of the oldest leaves gradually spread inwards covering the entire lamina by day 16, while the silhouette on mid-plant leaves progressed inwards as purplish spots, randomly across the lamina. The purplish pigmentation was more intense on acropetal region of the leaves. This syndrome moved up the plants to younger leaves. Flowering was earlier than in control plants. As the symptoms progressed, older leaves developed red pigmentation on the margins, which then progressed inward and totally covering the entire lamina, by day 22. This syndrome progressed up the plants to younger leaves. By day 25, oldest leaves developed necrosis that started at the tips and moved inward toward the leaf bases. Meanwhile, the red-pigmented older leaves developed patches of necrosis on mid-lamina region. The plant height and leaf size was reduced by 10% and 30% of control, respectively. The tissue N concentration was 2.82% at this stage.

Phosphorus

Initially, on day 16, plants appeared smaller with slightly darker green foliage. The plants also appeared skeletal due to thinner stems, lanceolated smaller leaves (40% of the width of control leaves), and poorly developed lateral shoots. Meanwhile, oldest leaves developed a silhouette of purplish brown pigmentation along the margins when the tissue P concentration was 0.09%. By day 21, purplish-brown pigmentation spread across the entire lamina of oldest leaves. This syndrome sequentially progressed up the plants to other leaves (day 24). Meanwhile, the young leaves developed light shades of purplish

pigmentation along the margins. By day 29, primary and secondary veins developed pinkish pigmentation on the adaxial surface, and purple pigmentation on the abaxial surface. The petioles were more pinkish than in control plants. Plants had smaller inflorescences (40% of control) with thin pedicels and less compact flowers, giving an overall stretched appearance. Slowly, by day 42, the purplish-brown pigmentation of oldest leaves acropetally (away from the tips) advanced to dull brownish-purple pigmentation covering the entire lamina. This syndrome sequentially progressed up the plants. By day 53, overall foliage appeared lanceolated and reduced in size by 50% of control. The inflorescences were smaller (40% of control), thinner and spiky (not as plume-like as that of control) giving an overall stretched appearance. The lateral shoots remained smaller. Plant height was about 90% that of control plants. The tissue P concentration was 0.05% at this point.

Potassium

Smaller plants with slightly darker green leaves constituted the early symptoms of K deficiency. Leaves were lanceolate, and darker green pigmentation was more intense along the margins (day 8). The tissue K concentration was 4.81%. By day 10, a silhouette of reddish-purple pigmentation developed along the margins of leaves, more so on recently matured leaves. Lower mature leaves developed reddish pigmentation along the margin area (day 22), which then interveinaly progressed inwards (day 26). Because of uneven expansion of leaf tissues, recently matured and younger leaves developed curvature at the acropetal region. Recently matured and older leaves developed internal

water soaked-like lesions randomly across the lamina (day 43). By day 45, dark gray necrosis developed on primary and secondary veins of recently matured and older leaves. Eventually, necrosis enlarged covering about 30% of the lamina on some of the recently matured leaves. By day 50, necrosis became bleached while the green-pigmented area intensified to darker green pigmentation. Plant height and leaf size was reduced by 20% and 15% of control. The tissue K concentration dropped to 1.81% at this point.

Calcium

In the beginning, plants appeared slightly shorter (10% of control) with darker green older leaves, and slightly pinkish younger leaves. The stems, petioles and the veins appeared more reddish than the control (day 27). At this point, the tissue Ca concentration was 0.64%. A faint reddish pigmentation appeared in the mid-lamina region of young leaves (day 30). This was followed by development of reddish spots on the mid-leaf region of young leaves (day 32). By day 34, white necrotic spots developed in the center of the red spots. These white spots enlarged. Eventually, by day 37, black necrosis developed on growing points of axillary and lateral shoots including the inflorescence tips and young leaves, causing the shoot tips to die. The plant height and leaf size was reduced by 20% and 15% of control respectively. At this time, the tissue Ca concentration was 0.47%.

Magnesium

Grayish interveinal pigmentation on recently matured leaves covering 5% of the lamina was the first sign of Mg deficiency (day 23). At this point, the tissue Mg concentration was 0.11%. The plants were shorter (90% of control). The grayish pigmentation then enlarged as maroon pigmentation covering about 40% of the lamina by day 25, and 80% of the lamina by day 29. As the symptoms progressed, patches of reddish pigmentation appeared on the maroon pigmented area, and the leaves appeared more reddish. Eventually, the pigmented area turned necrotic with surrounding dark brown tissue (day 34). As the symptoms advanced, plants appeared slightly shorter and smaller. The inflorescences were also smaller by 50% of control. The stems and the inflorescences were not as bright red as that of control. The lower leaves remained normal (day 39). The plant height and leaf size was reduced by 30% and 25% of control, respectively. The tissue Mg concentration was 0.08% at this point.

Sulfur

Plants appeared slightly shorter (90% of control) and lighter green, more so on recently matured and young leaves. Young and the recently matured leaves also had a light reddish silhouette along the margins (day 15). At this point, the tissue S concentration was 0.17%. Overall, the veins, petioles and stems were more prominent with bright red pigmentation (day 29). As the symptoms progressed (day 34), recently matured and older leaves developed reddish pigmentation in the acropetal region and

yellowish-green pigmentation in the basipetal area. Oldest leaves and young leaves remained lighter green while almost 80% of recently matured and older leaves appeared reddish (day 40). Yellow pigmentation developed on the distal portion of individual spikes within the inflorescence (day 53). Overall, the plants appeared slightly shorter and smaller (90% of control). At this point, the tissue S concentration was 0.14%.

Iron

Initially plants appeared slightly shorter (86% of control). Appearance of indistinct basipetal chlorosis (greenish yellow) on very young and young leaves was the first indication of Fe deficiency (day 16). At this point, the tissue Fe concentration was 54.9 $mg \cdot kg^{-1}$. The symptoms slowly progressed to mid-leaf region covering about 80% of the lamina (day 32). By day 37, recently matured leaves developed basipetal interveinal chlorosis (light yellowish green). Meanwhile, the chlorosis on young leaves turned yellowish green, more so on very young leaves. As the symptoms progressed, by day 42, the yellowish green chlorosis of young leaves vanished, and the entire leaves appeared chlorotic (yellow). However, some young leaves developed shades of light reddish pigmentation. By day 46, the basipetal interveinal chlorosis on recently matured leaves progressed to about 40% of the lamina. Interveinal chlorosis also appeared on mature leaves. The plant height was 76% of control while the leaf size was 85% of control. Flowering was similar to that of control. The tissue Fe concentration further dropped to 46.8 $mg \cdot kg^{-1}$.

Manganese

Initially, on day 22, plants appeared slightly lighter green due to the development of basipetal chlorosis (light green) of young and recently matured leaves. Plants were slightly smaller with smaller leaves, and thinner stems and pedicels. At this point, tissue Mn concentration was 13.7 $mg \cdot kg^{-1}$. Plant height was 80% of control with 50% reduction in inflorescence size. By day 41, recently matured and mature leaves developed interveinal chlorosis (greenish yellow). Tissue in the interveinal area of these leaves began to break down developing white necrotic patches, which enlarged to more than 50% of the lamina along the margins and mid-lamina areas, by day 48. Meanwhile, the acropetal area of young leaves randomly developed reddish stipplings with white necrotic spots in the center. Some young and mature leaf lamina, had shades of light red pigmentation across the lamina. The plant height was 69% of control. At this point, the tissue Mn concentration was 14.2 $mg \cdot kg^{-1}$.

Copper

Plants appeared smaller, and lighter green due to the development of light green pigmentation in the basipetal region of leaves, more so on recently matured leaves. Plants had thin stems and small leaves with basipetal upward cupping of young leaves (day 32). The young leaf lamina also appeared wavy. At this point, the plant height was 89% of control with tissue Cu concentration of 1.8 $mg \cdot kg^{-1}$. The light green pigmentation turned greenish white adjacent to primary and secondary veins, and spread outwards (day 39).

This tissue then turned pinkish, more so over the mid 80% of the lamina (day 42). The pinkish chlorosis faded and gave way to bleached light red coloration by day 46. This syndrome progressed down the plants to older leaves. Finally, by day 50, margins and the basipetal area of the recently matured leaves developed white necrosis covering about 70% of the lamina. The plant height was 85% of control. The tissue Cu concentration remained at 1.8 $mg \cdot kg^{-1}$.

Zinc

Initially, plants appeared smaller (day 14). Young and recently matured leaves developed interveinal orange pigmentation with numerous red spots in the acropetal area. The veins and margins of these leaves appeared red (day 17). The same effect was also observed on the abaxial surface of the leaves. The plant height was 68% of control with tissue Zn concentration of 7.6 $mg \cdot kg^{-1}$. By day 21, the red spots progressed inward towards the basipetal region covering 50 to 75% of the lamina. As the symptoms progressed, the spots enlarged into blotches of reddish orange pigmentation in the interveinal area. The young and recently matured leaves' orientation was upright compared to that of control (day 24). By day 28, the blotches of reddish orange pigmentation spread to about 80% of the lamina. As the symptoms advanced, breakdown of tissues occurred on the abaxial surface, and appeared translucent. On the adaxial surface of these leaves, the affected tissue developed interveinal orange yellow patches. On some leaves, a number of black necrotic spots developed randomly across the lamina. Inflorescences were smaller (40% of control) and orange-red rather than red as in the

control (day 32). The plants were shorter (71% of control) with smaller leaves (75% of control). The tissue Zn concentration was 7.7 $mg \cdot kg^{-1}$ at this point.

Boron

Overall, by day 17, the plants appeared shorter (62% of control) with shades of purplish pigmentation on the foliage, more so on the young and recently matured leaves. The purplish pigmentation was more prominent on the acropetal region and the margins of the leaves. At this point, the tissue B concentration was 8.9 $mg \cdot kg^{-1}$. By day 19, the veins of the abaxial surface of the young and recently matured leaves were reddish colored compared to that of control, which was green. The inflorescence size was less than 20% of control. The stems were thin with poorly developed axillary shoots. The nodes were stacked together with short internodes close to the upper portion of the primary shoots, and the plants appeared dwarf. As the symptoms progressed, by day 23, the matured leaves turned thick and leathery, and drooped at their bases. More than 80% of the lamina was purplish. By day 25, the dwarfing of internodes became severe causing the plants to appear compact with a height 40% of control. Older leaves developed chlorosis. Finally, black necrosis set in on the lateral shoots and inflorescences, and ultimately the growing points died (day 29). The tissue B concentration dropped to 7.9 $mg \cdot kg^{-1}$.

DISCUSSION

The rate at which symptoms occurred is an indication of the plant's sensitivity to a particular element (Figure 1). N and K deficiencies first occurred at 8 days followed by Zn at 14 days, S at 15 days, P and Fe at 16 days, B at 17 days, Mn at 22 days, and Mg at 23 days. Deficiencies requiring a long time to occur were Ca at 27 days and Cu at 32 days. Unlike other bedding plants (marigold, salvia, vinca, and zinnia –data not shown) celosia seems to be more sensitive to K, S, and less sensitive to Ca deficiencies. The delayed symptoms of Ca deficiency may have been due to the accumulation of Ca in plant tissues during the longer period of establishment in the control solution. Adedeji and Fanimokun (6) also found it took 35 days to establish Cu deficiency in celosia in sand culture.

At early deficiency stage, the most pronounced shoot dry weight deficit compared to the control was observed in B (45%), Cu (39%), Mn (31%), and least pronounced in P (27%), Ca (24%), Zn (14%), Fe (12%), and K and Mg (2%). However, shoot dry weight of N and S deficient plants were equal to control. At advanced stage, shoot dry weight reduction was severe in Mn (55%), B (50%), P (47%), Cu (44%), Ca (37%), and K (33%); and moderate in Zn (28%), S (26%), N (19%), Fe (18%), and Mg (17%) deficient plants (Figure 2).

As a rule of thumb, chlorosis is generally considered a nutritional deficiency symptom in plants. However, this standard rule is not applicable in celosia with red flowers. The pigmentation of stems, petioles, veins and pedicels were similar to that of inflorescences in celosia. Thus, nutrient deficiency visual symptoms are expressed as

somewhat reddish pigmentation and not as the usual chlorotic (yellowish) symptoms. Therefore, it is possible to misdiagnose a specific deficiency of an element solely based on visual symptoms. Tissue elemental concentration is an important tool in monitoring the nutritional status in celosia.

N is generally considered to be the nutrient required in greatest quantity for plant growth and development based on its high tissue concentration (7). However, in celosia, the tissue concentration of K was higher than N. This could mean that celosia requires a larger quantity of K than any other elements. It was also confirmed that K has a significant impact on growth and development of celosia plants (8, 9).

Synoptic visual symptoms were as follows: N - shades of purplish pigmentation on all leaves and acropetal purplish to red pigmentation of lower leaves turned necrotic; P - stunted growth, darker green to brownish purple pigmentation of oldest leaves with pinkish petioles, small and stretched inflorescences; K - darker green to reddish margins of matured leaves turned dark gray necrosis and curvature of leaves in the acropetal region; Ca - darker green older leaves, reddish spots of young leaves and growing points turned necrotic; Mg - gray interveinal pigmentation of matured leaves turned maroon then reddish and finally dark brown necrosis; S - lighter green recently matured leaves turned reddish in the acropetal and yellowish green in the basipetal areas while veins, petioles and stems had bright red pigmentation; Fe - yellowish green to greenish yellow pigmentation of young leaves turned totally yellow with shades of light reddish pigmentation; Mn - small plants, basipetal chlorosis of mature leaves turned to white necrosis, reddish stippling with white necrotic center on young leaves; Cu - wavy and upward cupping of young leaves, whitish green pigmentation on recently matured leaves

turned pinkish to bleached light red then to white necrosis; Zn - numerous brownish red spots enlarged to blotches of reddish orange pigmentation and finally a break down of tissues, and black necrotic spots on young and recently matured leaves; B - dwarf growth with droopy leaves, shades of purplish pigmentation on young and recently matured leaves, black necrosis on lateral shoot inflorescences.

ACKNOWLEDGEMENTS

The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of the products named, nor criticism of similar ones not mentioned. Appreciation is expressed to Tom Abramowski of Rockwell Farms, N.C., the North Carolina Commercial Flower Growers' Association, and Fred C. Gloeckner Foundation for financial support, Fred C. Gloeckner Inc. for providing the seeds and Smithers-Oasis for providing the propagation medium.

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Table 1. Celosia tissue nutrient concentration at early and advanced stages of deficiency in recently matured leaves of hydroponically grown plants in this study and published standards.

Treatment	**-N**	**-P**	**-K**	**-Ca**	**-Mg**	**-S**	**-Fe**	**-Mn**	**-Cu**	**-Zn**	**-B**
	Percent dry wt.						$mg \cdot kg^{-1}$				
Element	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn	B
Control early	5.72	0.44	7.96	2.45	0.77	0.27	72.7	258.3	9.7	27.9	28.4
Deficiency early	3.71	0.09	4.81	0.64	0.11	0.17	54.9	13.7	1.8	7.6	8.9
Control advanced	5.19	0.31	9.54	2.57	0.84	0.19	96.4	545.7	8.1	26.5	38.6
Deficiency advanced	2.82	0.05	1.81	0.47	0.08	0.14	46.8	14.2	1.8	7.7	7.9
P value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Published*	5.27	0.69	4.53	2.79	2.54	0.75	130.0	372.0	18.0	175.0	53.0

*Mills and Jones, 1996. Survey average of concentrations associated with acceptable crop performance, minimum critical concentrations not identified.

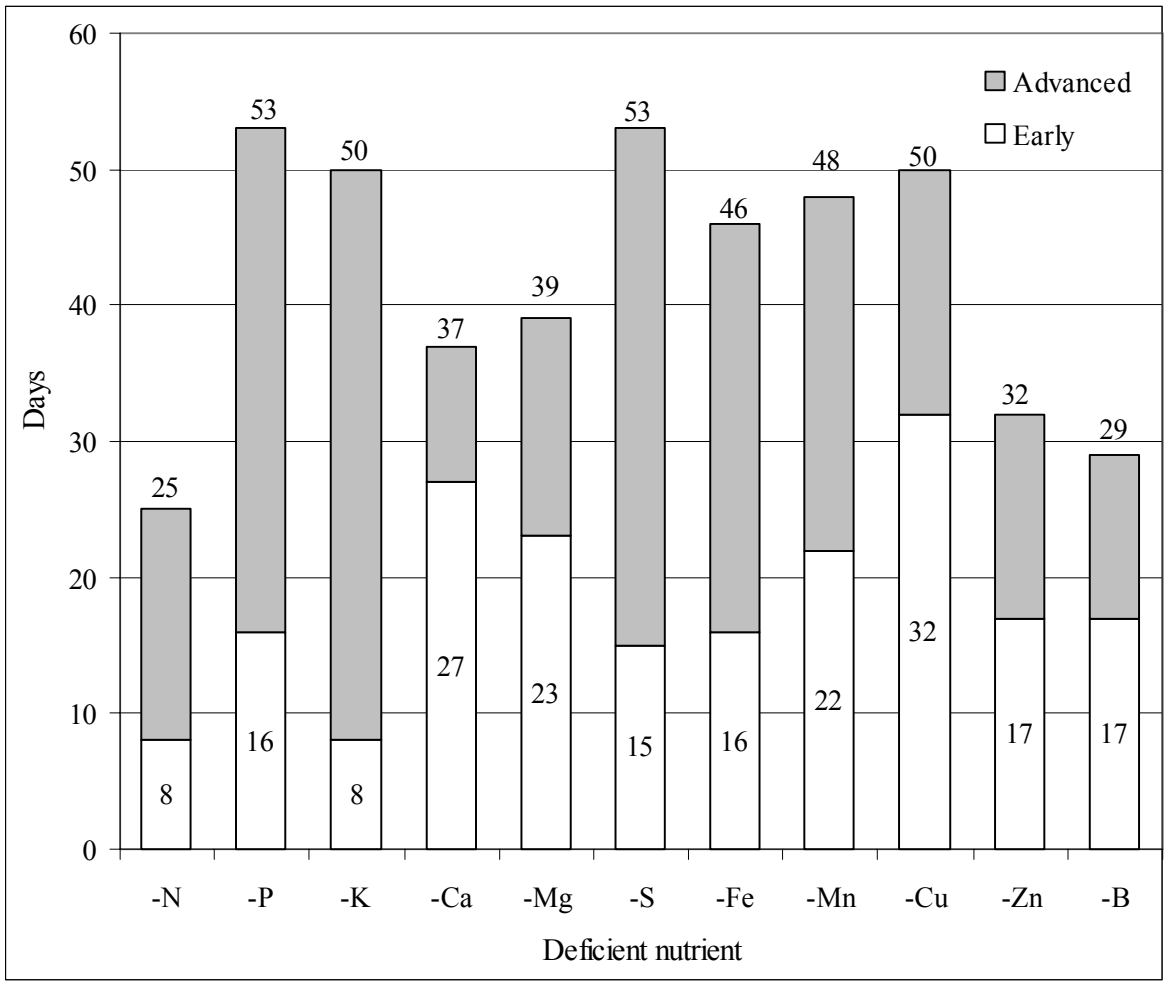

Figure 1. Days from initiation of treatments to leaf sampling for nutrient analysis for celosia at early and advanced stages of deficiency.

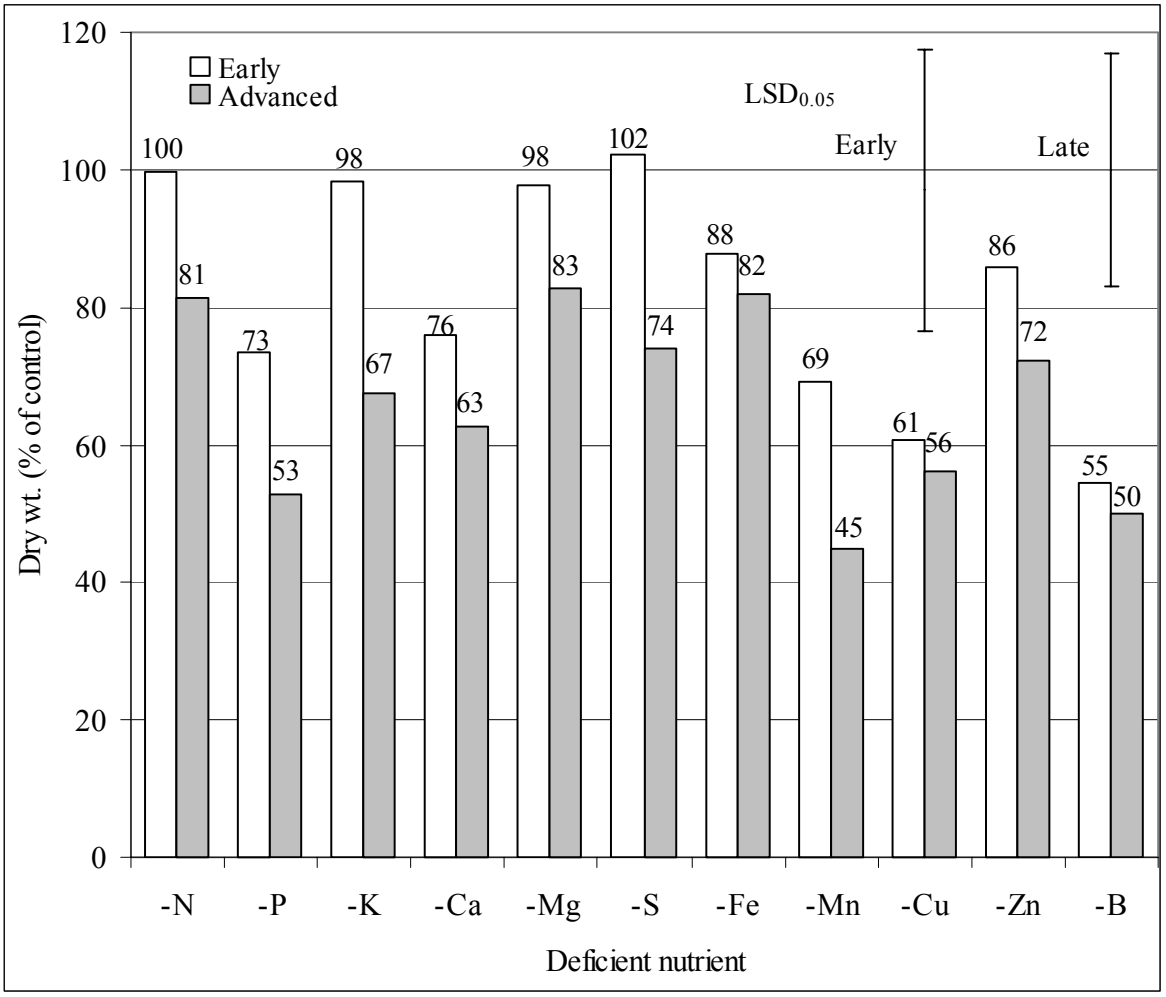

Figure 2. Celosia dry weight of nutrient deficient plants expressed as a percentage of control plants at early and advanced sages.

CHAPTER 6

IMPATIENS FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

James L. Gibson[1], Dharmalingam S. Pitchay[1], Brian E.Whipker[1], Brenda Cleveland[2], Paul V. Nelson[1], and F.R. Walls[2]

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

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

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IMPATIENS FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

James L. Gibson[1], Dharmalingam S. Pitchay[1], Brian E.Whipker[1], Brenda Cleveland[2], Paul V. Nelson[1], and F.R. Walls[2]

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

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

ABSTRACT

Tissue analysis standards and complete visual deficiency symptoms have not been established for Impatiens (*Impatiens walleriana* Hook. f.), which are crucial for monitoring plant nutrient status. Impatiens plants were grown hydroponically in a glass greenhouse. Treatments consisted of a complete nutrient solution, and 11 additional solutions, each devoid of one essential nutrient. Deficiency symptoms and plant size were chronologically recorded for shoots and roots. Fully expanded youngest leaves were analyzed for nutrient concentration. Nutrient dry weight basis concentrations in recently matured leaves at early stages of deficiencies of N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn and B, were 1.21, 0.08, 0.31, 0.34, 0.09, 0.21 (%), 55.4, 10.4, 2.5, 26.1 and 14.1 ($mg \cdot kg^{-1}$) respectively. At the early deficiency stage, shoot dry weight deficit compared to the control plants was most pronounced in deficiencies of Mn (81%), P (79%), Cu (77%), N

and Ca (69%), Zn (65%), B (62%), and K (54%), moderately pronounced in Fe (21%), and least pronounced in Mg (6%), and S (4%). On the other hand, at the advanced deficiency stage a severe deficit of shoot dry weight was observed in plants deficient in P (93%), Mn (91%), K (87%), Ca and B (86%), Zn (84%), N (79%), Cu (68%), Fe (64%), and Mg (44%), and least in S (21%). The order of appearance of visual symptoms at early stage of deficiency from first to last was Ca (9 days), N (10 days), Fe (11 days), P and K (13 days), B (19 days), Mg (21days), Mn (47 days), Zn (57 days), Cu (58 days) and S (61 days). The visual deficiency symptoms are presented in text.

INTRODUCTION

Impatiens (*Impatiens walleriana* Hook. f.), available in a wide range of brilliant colors, is a superb free flowering plant under partial sun or shade. This is an ideal bedding plant in landscapes. It has been rated as the top bedding plant in production value by the USDA (1). The need to produce a premium crop to fulfill consumers demand warrants advanced cultural information. Limited information is available on visual disorder symptoms, and foliar nutritional standards. Current foliar analysis standards guidelines are based on survey ranges for 11 essential elements (2). Values in these ranges are associated with acceptable growth, but do not establish the minimum and maximum critical values. Present fertilizer formulation is solely based on a general recommendation of a 2:1:2 (N: P_2O_5: K_2O) ratio (3). Each elemental disorder has its own characteristic symptoms, which also vary according to species (4). Therefore, growers could use visual symptoms as a diagnostic tool for monitoring nutrient status.

The objectives of this study were to generate visual symptoms of nutrient deficiencies in chronological order as they appear from incipient to advance stages, and to establish foliar analysis standards at early and advanced deficiency stages for N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn and B.

MATERIALS AND METHODS

The protocol for this experiment was reported in Pitchay et al., (5). It was conducted in the same location. Except that, Impatiens 'Super Elfin Lavender' was sown on 21 December 1999 and all treatments were initiated on 29 January 2000. Shoot dry weight and height values are based on measurements while all other size comparisons with control plants are visual assessments.

RESULTS

Following are the progressions of visual symptoms for each element through an advanced stage of deficiency. Early and late leaf sampling was carried out on days as indicated in Figure 1 and the critical foliar analysis standards and tissue concentrations associated with advanced deficiency are shown in Table 1.

Nitrogen

Initially, on day 10, plants appeared stunted due to smaller and fewer leaves, shorter internodes, and poorly developed lateral branching. The young leaves were lighter green and the mature leaves were yellowish green. The tissue N concentration at this point was 1.21%. By day 14, the mature leaves developed acropetal chlorosis (greenish yellow) and by day 18, the entire lamina of these leaves turned chlorotic (yellow). Leaves were drooping. The stems appeared reddish brown compared to that of control (greenish brown). Overall, the plant height and size was 50 and 20% of control, respectively. As the symptoms advanced, the chlorosis of mature leaves intensified to bleached yellow, followed by total disappearance of pigmentation, giving a colorless-like appearance of tissues (day 25). There were more flowers (30% more than the control) and large sized buds without any new bud formation. The buds were pinkish white in color compared to that of the control, which were pinkish. At this point, plants stopped growing and the plant height was 50% of the control. Leaves were fewer (5% of control) and the young and recently matured leaf size declined further. The roots were thinner, shorter (80% of control), and developed reddish pigmentation. The terminal ends of secondary roots were spiral-like. By day 28, entire plants appeared chlorotic (yellowish) and the older leaves began to abscise. Plant height was 42% of the control and the tissue N concentration at this point was 0.82%.

Phosphorus

In the beginning, by day 13, plants appeared slightly darker green and only 20% of the size of control plants. Plants had fewer and shorter lateral shoots, and brownish stems. At this point, the tissue P concentration was 0.08%. By day 15, all leaves appeared to be drooping. Roots were bronze colored while control plants were white. The primary roots were thinner, fewer, and longer, with fewer and shorter secondary roots. As symptoms progressed (day 17), mature leaves developed light brown necrosis starting from the margins, acropetal or basipetal regions. Eventually, by day 25, necrosis progressed to about 30 to 40% of the lamina causing the leaves to abscise. The remaining leaves appeared ovate (lamina length 50% of control) with olive green coloration and a drooped appearance due to downward cupping. Meanwhile, there was no development of flower buds. Plant size and height were 10 and 40% of the control. Roots were less dense with a mass of 40% of the control. The tissue P concentration further dropped to 0.08%, at this point.

Potassium

On day 13, plants appeared slightly compact and foliage color was similar to that of the control. By day 15, young and recently matured leaves developed light chlorosis in the basipetal region at which point the tissue K concentration was 0.31%. Older leaves developed interveinal upward puckering (day 19). Meanwhile, the plants appeared more compact due to shorter internodes and lateral shoots with a plant height 72% of the

control. Stems appeared lighter green and the roots were shorter by 31% of the control. This was followed by downward cupping and drooping of older leaves (day 25). As the symptoms progressed (day 29), the basipetal chlorosis progressed outward in an interveinal pattern on recently matured and older leaves, giving an overall yellowish green appearance. In areas of severe chlorosis, mature leaves developed interveinal whitish gray necrosis (day 35). Finally, by day 41, some of the mature leaves developed brown necrosis in either the acropetal region or margins, which then disintegrated into water-soaked like lesions. Overall, the plants appeared compact and reduced in size. Roots were dark colored and thinner and had fewer secondary roots. Flowers were smaller by 30% of the control and plant height was 71% of the control. Tissue K concentration at this point remained at 0.31%.

Calcium

Initially, by day 9, plants appeared smaller. They had smaller and fewer leaves, and poorly developed lateral branching. The stems were fewer, thinner, and reddish brown compared to the control that was light brownish green. Black necrosis developed on primary root tips. The tissue Ca concentration at this point was 0.34%. Young and recently matured leaves developed black necrotic spots randomly across the basipetal and mid-lamina area. The plant height was 59% of control (day 11). By day 15, the black necrotic spots enlarged and turned brown and finally yellowish white (day 17). The surrounding tissues of these necrotic areas became chlorotic. At this point, plants were less than 20% of the size of control plants. The primary and secondary roots were fewer

and shorter by 85% and 95% of control, respectively. As the symptoms progressed, necrosis also developed on the petioles of young and recently matured leaves, which resulted in leaf abscission. Eventually, by day 27, most of the leaves except the very young leaves and the buds were abscised and plants appeared skeletal looking with a height 42% of the control. Primary and secondary roots had black necrotic tips and dark brown necrosis along their entire axes. The growing points and the very young leaves were free of necrosis. Flower buds were smaller than the control and open flowers was absent. At this point, the tissue Ca concentration was 0.56%.

Magnesium

In the beginning (day 21) plants were shorter (72% of control) due to shorter internodes, and developed yellowish green chlorosis along the acropetal margins of mature leaves as well as on tips of young expanding leaves. The tissue Mg concentration at this point was 0.09%. The chlorosis of mature leaves progressed inward as greenish yellow interveinal chlorosis (day 23). By day 25, mature leaves developed slight interveinal puckering followed by downward cupping. Plants had poorly developed secondary root branching and reduced number of flowers. As the symptoms advanced (day 29), the interveinal chlorosis vanished and the entire lamina of mature leaves except the basipetal area became chlorotic (yellowish). Finally, by day 31, these leaves developed brown necrosis along the margins of acropetal area, which then progressed inwards (day 33). Overall, plants appeared chlorotic. At this point, flowering stopped and plant height was 56% of control. The tissue Mg concentration at this point was 0.07%.

Sulfur

Development of chlorosis along the margins and interveinal area of young leaves, and along the margins of recently matured leaves marked the beginning of deficiency symptoms (day 61). These symptoms occurred when the tissue S concentration was 0.21%. Plant size was equal to the control at this point. By day 69, entire lamina of the young and recently matured leaves developed yellowish green chlorosis, which then turned greenish yellow by day 73. Flowering was similar to the control. By day 81, mature leaves also developed chlorosis along the margins. As the symptoms advanced (day 93), necrosis developed on the acropetal region of recently matured leaves and along the margins of mature leaves. Stems were reddish with short internodes. Overall, the plants including the petioles appeared greenish yellow with faded flowers and buds. Primary roots were shorter (78% of control) and had reddish pigmentation, more so in the basal region (control roots were light greenish white). Plant height was 85% of the control. At this point, the tissue S concentration dropped further to 0.10%.

Iron

A tinge of light chlorosis on the margins of recently matured leaves was the first indication of Fe deficiency (day 11). At this point, the tissue Fe concentration was 55.4 $mg \cdot kg^{-1}$. The symptoms then progressed inward as yellowish green interveinal chlorosis (day 13). Meanwhile, young leaves developed chlorosis on the margins. Plants had light pinkish colored stems and poorly developed lateral shoots. The primary roots were

shorter (49% of control) and had spiral shaped terminals. Plant height at this point was 85% of control. The interveinal chlorosis of recently matured leaves turned greenish yellow by day 17, and yellowish by day 19. As the symptoms advanced (day 21), recently matured leaves appeared yellowish white, and by day 25, these leaves developed water soaked-like necrotic lesions along the margins, tips, and mid-lamina area. This syndrome progressed down the plants to older leaves. Eventually, the spiral shaped primary root terminals uncoiled. Overall, the plants were slightly smaller than the control and the plant height declined to 64% of the control. Flowers were lightly colored and fewer (30% of control). At this point, the tissue Fe concentration dropped further to 43.8 $mg{\cdot}kg^{-1}$.

Manganese

Development of acropetal chlorosis (yellowish green) on young and recently matured leaves marked the beginning of initial deficiency symptoms (day 47). At this point, the tissue Mn concentration was 10.4 $mg.kg^{-1}$. Plants were shorter and smaller with overall size 20% of the control, and had poorly developed lateral branching and root system. There were only about four primary roots with fewer short secondary roots. By day 51, chlorosis of young and recently matured leaves turned greenish yellow as it progressed inward covering about 30 to 40% of the lamina including the veins. Eventually, by day 55, chlorosis of these leaves intensified to yellowish pigmentation as it further progressed inward covering the entire lamina. This syndrome progressed down the plants to older leaves. Finally, chlorosis on the upper portion of the plants intensified to bleached yellow by day 63 and bleached white by day 67. Plants remained smaller at

less than 20% of the control. Leaves on upper portion of the plants were smaller. There was no sign of flower bud development. The tissue Mn concentration at this point was 7.6 $mg.kg^{-1}$

Copper

Initially, on day 58, overall foliage color was darker green and duller than that of the control plants. At this point, the tissue Cu concentration was 2.5 $mg.kg^{-1}$. Plants were shorter (67% of control), and smaller (20% of control) with thinner stems, and smaller leaves (70% of control). Plants had thinner and shorter (30% of control) primary roots with fewer and shorter secondary roots. By day 66, a tinge of bluish pigmentation developed close to the veination of young and recently matured leaves. As the symptoms advanced (day 74), leaves on the upper portion of the plants appeared miniature and the petioles appeared longer than those of control plants due to highly reduced lamina size. There was no flowering although flower buds were present. Flower buds withered and dried up as they began to open. Finally, by day 99, very young leaves developed black necrosis at the tips giving way to deformed appearance. Plants remained darker green and smaller with a height 53% and leaf size 30% of control. Tissue Cu concentration at this point was 2.7 $mg.kg^{-1}$.

Zinc

Initially, on day 57, young and recently matured leaves were considerably smaller (58% of control) with uniform chlorosis (yellowish green). The tissue Zn concentration at this point was 26.1 $mg.kg^{-1}$. Then, these leaves developed interveinal (greenish yellow) chlorosis by day 65. The plants were shorter (67% of control) with brownish red stems and poorly developed buds. Shoot apices appeared brownish red due to significantly smaller lanceolated young leaves and pronounced growth reduction which exposed the terminal end of the stems. As the symptoms advanced (day 73) chlorosis of young leaves intensified. Pigmentation of the stems intensified to more reddish brown. The recently matured and mature leaves turned totally chlorotic (lemon-yellow), while the very young and young leaves remained greenish-yellow. Eventually, by day 91, patches of white necrosis developed on margins and mid-lamina areas of recently matured and mature leaves and progressed inward. As the necrosis formed, there was partial regreening of very young leaves. Flowering was drastically reduced with an absence of new bud development. Flowers were smaller with lanceolated smooth margined petals and gaps in between them. Plant height was 44% of control. Tissue Zn concentration at this point was 23.4 $mg.kg^{-1}$

Boron

Overall, the foliage appeared darker green than the control. By day 19, young leaves developed a glossy green coloration in the basipetal area, while the acropetal area

remained green. The plant height and root length were 70 and 72% of control respectively. At this point, the tissue B concentration was 14.1 $mg.kg^{-1}$. By day 27, uneven leaf expansion was observed between terminal end and basal two thirds of the young leaves, yielding spoon shaped leaves. Upward folding and distortion of young leaves followed this and plant height declined to 59% of the control (day 31). This syndrome progressed to recently matured leaves by day 39. At this point, plants had shorter internodes on the upper portion of the plants. As the symptoms advanced (day 45), the young and the maturing leaves appeared dented and deformed with narrow strap-like leaf blades and distorted margins. Mature leaves developed chlorosis on the margins and acropetal areas. Eventually, by day 57, these leaves developed grayish-white necrosis in the acropetal region. Shoot apices stopped growing and appeared distorted. Leaves were thick and brittle with severely distorted margins. Only a few short and thick trichomes were present. Flowers and buds were absent. The primary and secondary root tips were thick and stubby. The secondary roots were longer than the primary roots located in close proximity to the tips of primary roots and gave the appearance of a witch's broom. The plants appeared stunted with a height 44% of the control. The tissue B concentration at this point was 10 $mg.kg^{-1}$.

DISCUSSION

The rate at which symptoms occurred is an indication of the plant's sensitivity to particular nutrient deficiencies. Ca deficiencies first occurred at 9 days followed by N at 10 days, Fe at 11 days, P and K at 13 days, B at 19 days, and Mg at 21 days. Deficiencies

requiring a longer time to occur were Mn at 47 days, Zn at 57 days, Cu at 58 days, and S at 61 days.

At the early deficiency stage, shoot dry weight deficit compared to the control plants was most pronounced in deficiencies of Mn (81%), P (79%), Cu (77%), N and Ca (69%), Zn (65%), B (62%), and K (54%), moderately pronounced in Fe (21%), and least pronounced in Mg (6%), and S (4%). On the other hand, at the advanced deficiency stage a severe deficit of shoot dry weight was observed in P (93%), Mn (91%), K (87%), Ca and B (86%), Zn (84%), N (79%), Cu (68%), Fe (64%), and Mg (44%), and least in S (21%) deficient plants (Figure 2).

P deficient plants exhibited drastic reduction of shoot dry weight prior to chlorosis, whereas chlorosis was the most obvious initial symptom in deficiencies of N, Ca, Fe, and Mn. Cu and Zn deficient plants took longer to develop early as well as advanced symptoms. However, the shoot dry weight of these plants were significantly lower. This means that neither chlorosis nor delayed development of symptoms is an indication that the plants are least or most stressed to nutrient deficiency.

The minimal drop in shoot dry weight and extended delay in S deficiency symptoms in S deficient plants was consistently noted in several other species (marigold, vinca, petunia, begonia, zinnia, celosia, and salvia – data not shown). This could have been due to the acquisition of S from SO_2 in the atmosphere (6). Moreover, impatiens has a greater ability to tolerate SO_2 (7). On the other hand, minimal reduction in shoot dry weight of Mg deficient plants might have been due to the availability of Mg from Oasis substrate foam.

Spiral-like primary root terminals in Fe deficient plants could be a novel way of acquiring Fe (8) by increasing the root surface area. Although Fe deficient plants developed symptoms much earlier than plants with other deficiencies, the impact on shoot dry weight was minimal. This indicates that impatiens is efficient in adapting to an Fe deficient environment.

The differences in tissue Zn concentration between the control and deficient concentrations at early and advanced stage of deficiency were only 2.6 and 5.1 $mg \cdot kg^{-1}$, respectively. Therefore, regular monitoring of tissue Zn concentration is important to avoid the occurrence of Zn deficiency.

N and S deficiency greatly enhanced flowering, while K, Mg, and Zn deficient plants produced fewer flowers. On the other hand, Cu, P, Mn, and B deficient plants failed to produce flowers for an extended period and finally caused flower abortion.

Nutrient stress dictates the life span of leaves in accordance with leaf zones (9). In most cases, abscission starts with the lower mature leaves and moves upward. In N deficient plants, only lower mature leaves were abscised. On the other hand, in Ca deficient plants the zone of abscission covered all the leaves except the very young leaves. Generally, Ca stress affects the growing points. However, the abscission of recently matured and mature leaves to conserve the limited nutrient, and to reabsorb and reallocate to young tissues (9-11) could have contributed to a certain mobility of Ca and the survival of growing points without developing necrosis even at an advanced stage of Ca deficiency. Moreover, the control and deficient Ca concentrations at early and advanced stages were second highest among all elements, which suggests that impatiens has the ability to load Ca in plant tissue.

Synoptic visual deficiency symptoms were as follows: N – chlorosis on mature leaves, red pigmentation on stems and roots, early flowering, and leaf abscission; P – darker green foliage turned olive green, mature leaves appeared drooped with light brown necrosis; K – interveinal upward puckering, cupping and drooping of older leaves, interveinal chlorosis on recently matured and older leaves turned whitish gray necrosis; Ca – necrosis on primary root tips, young and recently matured leaves developed black necrotic spots which enlarged, turned brown, and finally yellowish white surrounded by chlorotic tissues, and abscised; Mg –recently matured and mature leaves developed yellowish green interveinal chlorosis which turned greenish yellow then totally chlorotic, and finally necrosis and downward cupping; S – chlorosis on young and recently matured leaves and then on mature leaf margins, and finally acropetal necrosis on recently matured and mature leaves, reddish pigmentation on stems and primary roots; Fe – interveinal chlorosis on young and recently matured leaves turned yellowish and then yellowish white, light pinkish stems, and spiral shaped primary root terminals; Mn – yellowish green acropetal chlorosis on young and recently matured leaves turned yellowish and finally bleached yellow to white, poorly developed roots and lateral shoots; Cu –darker green with bluish tinge pigmentation on young and recently matured leaves, with deformed tips, and necrosis on very young leaves; Zn –interveinal chlorosis on young and recently matured leaves, total chlorosis on recently matured and mature leaves, brownish red stems, and partial regreening of young leaves; B –darker glossy green and distorted young and maturing leaves with leathery and brittle texture, distorted shoot apices, thick and stubby root tips and witch's broom-like symptoms.

ACKNOWLEDGMENTS

The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of the products named, nor criticism of similar ones not mentioned. Appreciation is expressed to Tom Abramowski of Rockwell Farms, N.C., the North Carolina Commercial Flower Growers' Association, and Fred C. Gloeckner Foundation for financial support, Fred C. Gloeckner Inc. for providing the seeds and Smithers-Oasis for providing the propagation medium.

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Table 1. Impatiens tissue nutrient concentration at early and advanced stages of deficiency in recently matured leaves of hydroponically grown plants in this study and published standards.

Treatment	**-N**	**-P**	**-K**	**-Ca**	**-Mg**	**-S**	**-Fe**	**-Mn**	**-Cu**	**-Zn**	**-B**
	Percent dry wt.						$mg \cdot kg^{-1}$ of dry wt.				
Element	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn	B
Control early	5.41	0.59	2.83	3.35	0.44	0.30	101.3	133.3	7.6	33.4	68.8
Deficiency early	1.21	0.08	0.31	0.34	0.09	0.21	55.4	10.4	2.5	26.1	14.1
Control advanced	5.84	0.54	2.25	3.22	0.53	0.31	139.1	150.2	8.4	28.5	106.0
Deficiency advanced	0.82	0.07	0.31	0.56	0.07	0.10	43.8	7.6	2.7	23.4	10.0
P value	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.00
Published*	3.64 - 5.83	0.77 - 0.92	1.37 - 2.35	1.75 - 2.40	0.89 - 3.64	0.83 - 0.87	107.0 - 130.0	329.0 - 419.0	20.0 - 37.0	57.0 - 67.0	23.0 - 25.0

*Mills and Jones, 1996. Survey average of concentrations associated with acceptable crop performance, minimum critical concentrations not identified.

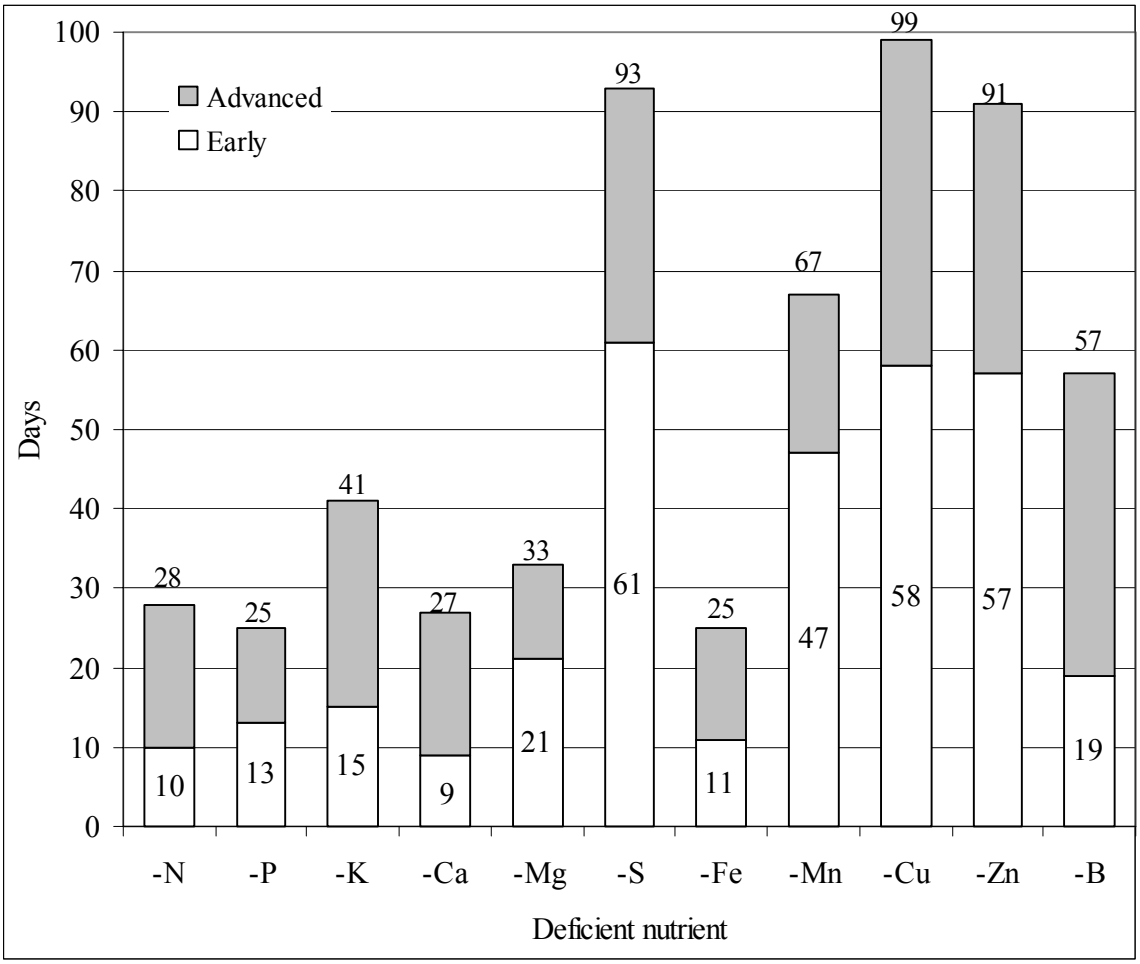

Figure 1. Days from initiation of treatments to leaf sampling for nutrient analysis for impatiens at early and advanced stages of deficiency.

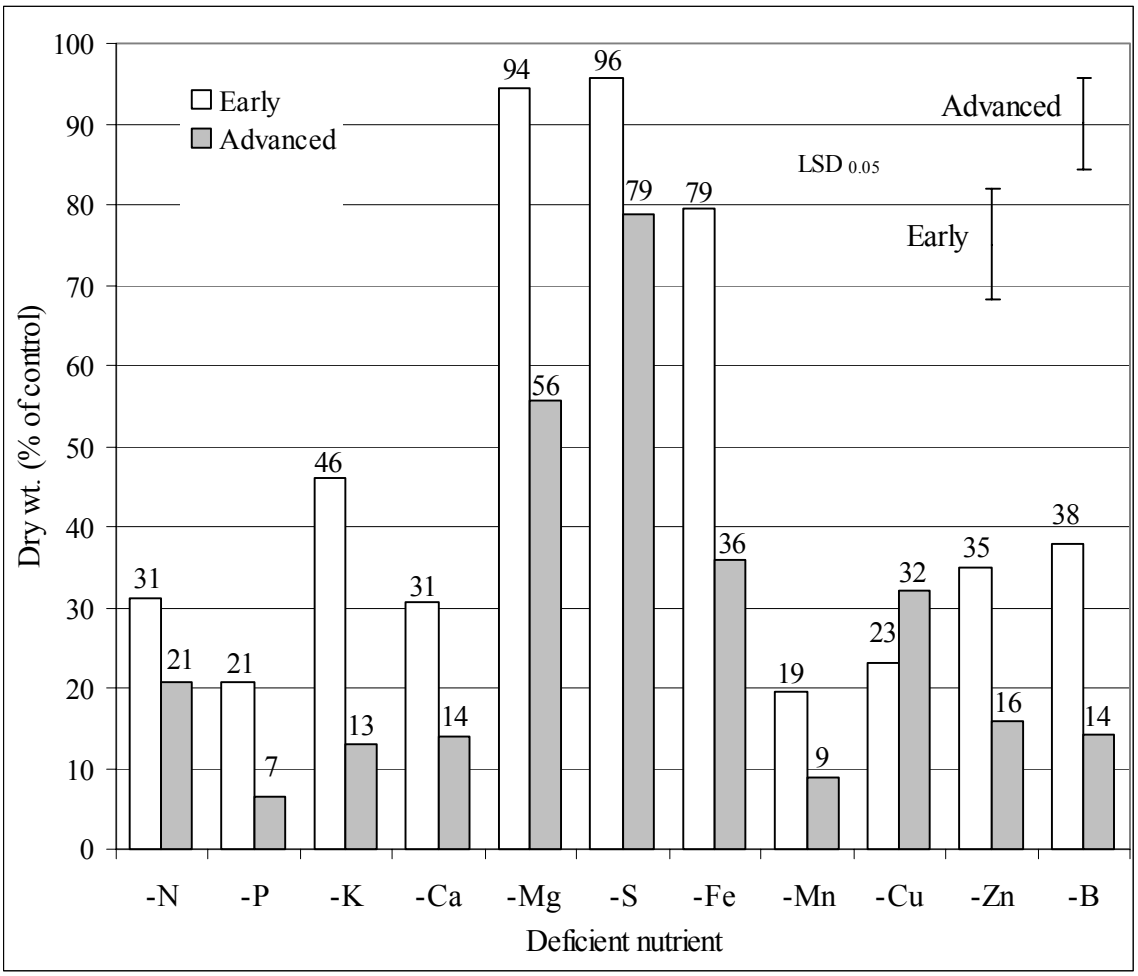

Figure 2. Impatiens dry weight of deficient nutrient plants expressed as a percentage of control plants at early and advanced stages.

CHAPTER 7

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James L. Gibson[1], Dharmalingam S. Pitchay[1], F.R. Walls[2], Brian E.Whipker[1], Brenda Cleveland[2], and Paul V. Nelson[1]

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

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

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

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

ABSTRACT

Tissue analysis standards are crucial for monitoring plant nutrient status for pansy. Complete visual deficiency symptoms have not been established for this crop. Pansy *(Viola x wittrockiana* Gams*)* plants were grown hydroponically in a glass greenhouse. Treatments consisted of a complete nutrient solution, and 11 additional solutions, each devoid of one essential nutrient. Deficiency symptoms and plant size were chronologically recorded for shoots and roots. Fully expanded youngest leaves were analyzed for nutrient concentration. In the absence of N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn and B, tissue concentrations at early deficiency stage were 1.65, 0.17, 1.17, 0.45, 0.14, 0.13 (%), 51.4, 6.7, 2.8, 22.7, and 9.7 ($mg \cdot kg^{-1}$), respectively. At early stage, shoot dry weight reduction was most pronounced in deficiencies of Cu (77%), B (69%), P (68%) and Mn (60%), and moderately pronounced in S (29%), Mg (25%) and Fe (22%). On the

other hand, at advanced stage a severe reduction of shoot dry weight was observed in B (90%), Ca (88%), N (87%), Mn (85%) and Fe (82%) deficient plants. The order of visual symptoms at early stage from first to last was Fe (5 days), Ca (7 days), N (10 days), P (12 days), B (15 days), K (16 days), Mg (17 days), Mn (38 days), Cu (41 days), S (42 days), and Zn (52 days).

INTRODUCTION

Pansy, a cool season crop is considered an ideal flowering bedding plant during fall, winter and spring. Lately, it is being used for food garnishing, and categorized as one of the top three valued bedding and garden flats by USDA, for the year 2001 (1). However, the current foliar analysis standards are limited for this crop. Survey ranges for 11 essential elements (2) have been reported. Values in these ranges are associated with acceptable growth, but do not establish the minimum and maximum critical values. Present fertilizer formulation is solely based on general recommendation of 2:1:2 (N: P: K) ratio (3) and/or on 100 $mg \cdot L^{-1}$ of N concentration (4), and NO_3-N is preferred than NH_4-N (5). Current pansy fertilizers on the market have contained N: K at a ratio of 3:4 and P_2O_5 content equal to 13 to 20% N concentration. Approximately 85% N is in the NH_4 form in these fertilizers. Besides soil testing, foliar analysis and visual diagnosis of nutrient deficiencies are valuable aspect of nutritional management programs (3).

The objectives of this study were to generate visual symptoms of nutrient deficiencies in chronological order as they appear from incipient to advance stages, and

to establish foliar analysis standards that include minimal critical levels and levels associated with late stages of deficiencies for N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn and B.

MATERIALS AND METHODS

The protocol for this experiment was similar to that of vinca (5). It was conducted in the same location. Except that, Pansy 'Crown series - blue' was sown on 17 November 1999 and all treatments were initiated on 16 December 1999. Shoot dry weight and height values are based on measurements while all other size comparisons with control plants are visual assessments.

RESULTS

Following are the progressions of visual deficiency symptoms for each element. Early and late leaf sampling was carried out on days as indicated in Figure 1; and the critical foliar analysis standards and tissue concentration associated with early and advanced deficiencies are shown in Table 1.

Nitrogen

In the beginning (day 10), plants appeared smaller (40% of control) with a height 57% and leaf size 40% of control. Shades of greenish purple pigmentation developed on the cotyledons and lower mature leaves (more so on the abaxial surface) including the petioles. At this point, the tissue N concentration was 1.65%. The greenish purple

pigmentation turned purple in the acropetal area of mature leaves and cotyledons, and moved inwards. The shoot apex and stems appeared pinkish purple. Plant growth was minimal with only a few strands of primary roots (< 10% of control) (day 12). By day 15, the lower petioles appeared shorter (25% of control) and thinner. Then, by day 19, younger leaves appeared lighter green, and the mature leaves developed a distinct purple veination. As the purple pigmentation became more intense on the lower mature leaves, the adaxial surface of upper mature leaves also developed purple pigmentation. The shoot growth was halted, and the plant height and size was 39% and 30% of the control, respectively. The leaves were fewer (28% of control) and smaller with narrow lamina. The root growth was moderately suppressed with a length 70% of control. The lower leaves developed pinkish purple pigmentation on the margins, which then turned to purplish pink. Meanwhile, the recently matured and mature leaves developed pinkish purple pigmentation. Finally, by day 25, acropetal tan necrosis developed on lower mature leaves. The cotyledons shriveled and withered off. The crowns and stems appeared purplish pink. Lateral shoots were absent. At this point, the tissue N concentration dropped further to 1.12%.

Phosphorus

Initially (day 12), plants appeared darker green and smaller with shades of purple pigmentation on the petioles and adaxial surface of lower leaves more so on the cotyledons. At this point, the tissue P concentration was 0.17%. By day 15, lower-most mature leaves developed purple veination. This was followed by development of

basipetal purple pigmentation uniformly across upper and lower surfaces of mature leaves with lower surfaces brighter then upper surfaces (day 19). By day 23, acropetal tips of lower mature leaves developed tan necrosis, while the acropetal area of recently matured leaves developed shades of purple pigmentation giving a darker green coloration. The primary roots were fewer, but slightly longer than the control. As the symptoms advanced, by day 26, whitish-tan necrotic patches developed randomly on mature leaves, which then coalesced and enlarged. At this point, the primary roots were twice the length of control, although, the secondary roots were fewer and shorter. The plant height was 49% of control. The symptoms occurred during the vegetative stage. The tissue P concentration at this point was 0.04%.

Potassium

Initially, on day 16, plants appeared shorter and smaller with intensified darker green foliage. Leaves were smaller and fewer with short petioles. At this point, the tissue K concentration was 1.17%. As the symptoms progressed (day 19), the plants appeared compact due to fewer nodes (45% of control) and shorter internodes. Mature leaves developed puckering at the mid lamina region. By day 23, the acropetal region of these leaves developed chlorosis as the puckering became severe. The chlorosis rapidly turned into black necrosis and progressed inward leaving behind a brown necrotic area (day 27). As these symptoms progressed up the plants (day 32), some of the lower most leaves developed severe necrosis covering almost 80% of the lamina. Meanwhile, some of the mature leaves developed veinal and interveinal purple pigmentation. The plant size was

reduced to 30% of control. Flowering was delayed with reduced number of flowers (20% of control). The tissue K concentration further dropped to 0.59%, at this point.

Calcium

Plants appeared smaller and shorter (31% of control) due to poor growth. Root growth was minimal with few, short (5% of control) primary roots without any secondary roots (day 7). The growing points were visible with few small unfolding young leaves. The petiole bases, stems and crowns developed light purple pigmentation (day 8). By day 9, young leaves developed black necrotic spots in the acropetal area. Plant growth was halted at this point, and the tissue Ca concentration was 0.45%. This was followed by a development of small black spots on the mid-lamina area of recently matured leaves and basipetal margins of mature leaves. Cotyledons and lower mature leaves developed purple pigmentation on the abaxial surface, more so on the veins (day 11). By day 13, the black spots turned to tan necrotic spots, which then coalesced and enlarged to larger necrotic spots and patches (day 16). Meanwhile, the young leaves shriveled and withered. The abaxial surface of mature leaves developed purplish-pink pigmentation and the pigmentation became more intense on the lower matured leaves. The crown also developed purple pigmentation. Necrosis also developed on the growing points. By this time total necrosis occurred on the recently matured leaves. These symptoms occurred during the vegetative stage. The plant height was 28% of control. At this point, the tissue Ca concentration was 0.61%.

Magnesium

Plants were smaller and shorter (87% of control) with slightly lighter green foliage (day 17). By day 19, mature leaves developed silhouette-like chlorosis (yellowish green) on the margins, when the tissue Mg concentration was 0.14%. Chlorosis interveinaly progressed inwards (day 25), and turned greenish yellow, more so on the leaf margins (day 29). At this point, plant height was 78% of control. By day 36, the mature leaf margins turned yellowish, while the interveinal chlorosis vanished on the remaining lamina. As the symptoms progressed (day 42), brown necrosis developed on the margins of mature leaves, which then progressed inwards (day 47). This syndrome progressed up the plants. Plants were smaller than control by 70% with smaller and fewer leaves, proportionate with plant size. Delayed flowering was observed compared to the control.

Sulfur

Foliage color, plant height and size were similar to control during the first five weeks. On day 42, young leaves appeared lighter green when the tissue S concentration was 0.13%. Flowering commenced later than control. By day 47, eighty five percent of the plants flowered, whereas in control, 85% flowering occurred by day 36. At this point, recently matured leaves also appeared lighter green, and the plant size and height remained equal to control. By day 77, entire plants including the stems appeared lighter green with fewer flowers and lateral shoots.

Iron

Initially, on day 5, very young and young leaves developed faint chlorosis in the basipetal area. At this point, the tissue Fe concentration was 51.4 $mg \cdot kg^{-1}$. Plants were shorter (45% of control) and smaller (30% of control). Very young leaves were more chlorotic than the young leaves (day 9). Meanwhile, stems and basal portion of the petioles developed purplish pink pigmentation. As the symptoms progressed, by day 13, the young leaves turned chlorotic (yellowish), while recently matured leaves appeared greenish-yellow. Mild chlorosis developed in the basipetal area of mature leaves. This was followed by total chlorosis of recently matured leaves. Meanwhile, the basipetal chlorosis of mature leaves turned greenish yellow with mild interveinal chlorosis (day 15). At this point, the petioles also turned chlorotic. Root growth was limited. The primary roots were fewer (less than 5% of control) and shorter (20% of control), with fewer and shorter secondary roots compared to that of control. The chlorosis of the leaves, petioles, growing points and stems intensified to bleached yellow by day 19. At the same time, acropetal area of some of the young and recently matured leaves developed bleached tan necrosis. The plants appeared shorter than the control by 64%. The tissue Fe concentration at this point was 64.5 $mg \cdot kg^{-1}$.

Manganese

Plants appeared slightly shorter (90% of control) with slightly lighter green foliage more so along the margins (day 38). At this point, the tissue Mn concentration

was 6.7 $mg \cdot kg^{-1}$. By day 42, mature leaves developed a silhouette of yellowish green chlorosis along the leaf margins. The silhouette then became distinct with yellowish coloration (day 48). As the symptoms progressed, by day 52, yellowish green chlorosis developed in the basipetal area of young leaves, and acropetal area of recently matured and mature leaves. Chlorosis then intensified to greenish-yellow, and dimple-like puckering developed on mature leaves (day 59). By day 65, irregular interveinal chlorosis occurred on young and recently matured leaves. As the symptoms advanced, by day 70, the silhouette of yellow chlorosis gave way to light tan necrosis. Flowering was delayed by 21 days as compare to control (based on 85% flowering). The lateral shoots were fewer and shorter. The plant height was 65% of control. At this point, the tissue Mn concentration was 6.2 $mg \cdot kg^{-1}$.

Copper

Leaves were flimsy thin textured and less turgid, and appeared lighter green with lack of sheen (day 41). At this point, the tissue Cu concentration was 2.8 $mg \cdot kg^{-1}$. Plants were smaller, and shorter by 13% of control with poor root and lateral shoot development. Chlorosis (yellowish green) formed in young leaves without any clear pattern (day 51). Mature leaves appeared olive green (day 55), and developed greenish yellow chlorosis in the acropetal region by day 62. This was followed by light tan necrosis at the tips of newly matured and mature leaves, which spread toward the leaf base. Some of the recently matured leaves developed veinal chlorosis. Flowers were fewer (35% of control) and much smaller, and their purple coloration was considerably

faded (day 68). Meanwhile, upward cupping occurred on young leaves. Eventually, by day 72, necrosis enlarged and covered 30% of the lamina, while wilting-like symptoms occurred on these leaves. At this point, the flowers appeared whitish purple, and the petals rolled upward due to flaccid margins. Plant size and height was considerably reduced by 60% and 40% of control respectively. The tissue Cu concentration at this point was 2.4 $mg \cdot kg^{-1}$.

Zinc

Initially, by day 52, recently matured leaves developed purple pigmentation along the margins, when the tissue Zn concentration was 22.8 $mg \cdot kg^{-1}$. The purple pigmentation then spread inward at the acropetal zone (day 55). By day 59, as the syndrome progressed down the plants to mature leaves, young leaves appeared chlorotic (yellowish green). Plants were smaller and shorter (76% of control) with poorly developed lateral shoots. By day 65, 80% of the lamina of recently matured leaves and 50% of the lamina of mature leaves were covered with purple pigmentation. As the symptoms advanced, by day 70, tan white necrosis developed on the margins of recently matured leaves, which spread inward toward the leaf base. This syndrome moved down the plants to mature leaves (day 80). Eventually, by day 89, young leaves and flower sepals also developed purple pigmentation. Lower-most mature leaves developed basipetal orange colored necrosis. Flowers were fewer and smaller (25% of control) with rounded petals and faded purple pigmentation. The plant height was 60% of control with reduced lateral shoots growth (40% of control). At this point, the tissue Zn concentration was 27.6 $mg \cdot kg^{-1}$

Boron

Initially, by day 15, plants appeared smaller and shorter by 21% of control. The plants had fewer, thicker and shorter primary roots with swollen necrotic tips. Foliage appeared darker green and slightly glossy (day 19). At this point, the tissue B concentration was 9.7 $mg \cdot kg^{-1}$. Young leaves failed to develop fully, giving rise to narrow strap-like or spoon-shaped leaves with smooth distorted margins (day 25). Leaves became thick and leathery. By day 29, Young leaves puckered and cupped upward, and mature leaves cupped downward. Internodes close to the terminal tips were shorter, and lateral branching formed at close intervals giving the shoot a rosette appearance. The shoot meristems stopped developing. By day 36, interveinal purplish red pigmentation developed in the acropetal area of recently matured and mature leaves, more so on the abaxial surface of the leaves. The stems, growing points, and the petioles of young leaves developed purple pigmentation. Most of the flowers aborted, and those survived had partially formed petals. As the symptoms advanced, by day 42, more than 30% of the recently matured and mature leaf lamina was covered with purplish red pigmentation. The remaindure of the lamina turned chlorotic. Meanwhile, the young leaves also developed chlorosis. The plant height was 68% of control. At this point, the tissue B concentration was 10.9 $mg \cdot kg^{-1}$.

DISCUSSION

Fe was the first to develop deficiency symptoms at 5 days followed by Ca at 7 days, N at 10 days, P at 12 days, B at 15 days, K at 16 days, and Mg at 17 days. Mn, Cu, S and Zn were the slowest to develop deficiency at days 38, 41, 42, and 52, respectively. An extended delay in S deficiency symptoms was consistently observed in impatiens, marigold, petunia, salvia, vinca, and zinnia (data not shown). This was probably due to the acquisition of SO_2 in the atmosphere (7). Although Fe was the first to develop initial symptoms, the advance symptoms developed quicker in Ca (within 9 days) and delayed in Fe (14 days).

At early stage, shoot dry weight was considerably reduced in Cu (77%), B (69%), P (68%) and Mn (60%), and moderately in S (29%), Mg (25%) and Fe (22%) deficient plants. At the time of advance deficiency, a significant reduction of shoot dry weight was noted in all nutrient deficient plants. The shoot dry weight reduction was most pronounced in B (90%), Ca (88%), N (87%), Mn (85%) and Fe (82%) at advanced stage (Figure 2).

The rate at which symptoms occur is an indication of the sensitivity of the plant to a particular element. Although Fe was the first deficiency to occur at day 5, the drop in shoot dry weight was moderate, the advanced symptoms were delayed, and the tissue Fe concentration at early and advanced stages were 54 and 57% of maximum critical concentration respectively. This means that it is probable for the plants to recover from Fe deficiency with minimal damage if immediate action is taken. On the other hand, Ca deficiency developed early and progressed rapidly to necrosis within 7 days. It affected

the growing points and shoot dry weight severely. Therefore, constant close monitoring of tissue Ca concentration is vital to avoid the onset of Ca deficiencies in pansy production.

The minimum critical tissue concentration of all deficient treatment except K was below the published survey range value (Table 1).

Mn deficiency occurred when the tissue Mn concentration compared to the control was 3% (at early stage) and 4% (at advanced stage). Pansy is considered a moderate Mn requiring plant since minimum critical level is below 10 $mg \cdot kg^{-1}$ (8). Furthermore, it took about 38 days to develop early visual deficiency symptoms. This indicates that this plant is less prone to Mn deficiency considering the low concentration needed for normal growth. However, considerable decline in shoot dry weight and delayed flowering was noted.

As plants aged the tissue Zn concentration declined in control plants (Table 1). Moreover, the difference in Zn tissue concentration between the control and Zn deficient plants was 16.4 $mg \cdot kg^{-1}$ at early and 6.3 $mg \cdot kg^{-1}$ at advanced stage. Critical concentration of Zn for most crops is below 20 $mg \cdot kg^{-1}$ (9). This could mean that pansy is sensitive to Zn deficiency, and requires Zn in greater quantity during early growth than at later stages of growth or becomes less efficient at accumulating Zn in the leaf tissue as the plant ages. In either situation, pansy appears prone to Zn deficiency as the plant ages.

Visual deficiency symptoms reflect the flower pigmentation instead of the usual chlorosis in N, P, K, Ca, Zn and B deficient plants. Therefore, it is possible to misdiagnose these deficient elements based on the color of pigmentation. The hue saturation, position and pattern of the symptoms on the plants, and specific

morphological changes can be a helpful tool in identifying the nutrient disorder. This further justifies the use of tissue analysis for nutritional diagnosis.

Synoptic visual deficiency symptoms were as follows: N – small size plants, purple pigmentation developed on older leaves, stems, petioles and crowns, and tan necrosis developed on mature leaves; P – darker green foliage, mature leaves developed basipetal purple pigmentation and then whitish-tan necrotic patches; K – compact plants with darker green foliage, mature leaves developed puckering, then black necrosis which turned tan; Ca – smaller plants with poor root system and deformed young leaves, black necrotic spots first developed on young leaves, then on recently matured and mature leaves; Mg – mature leaves developed acropetal interveinal chlorosis from yellowish green to greenish yellow, then yellowish, and finally necrosis on margins; S –uniform lighter green chlorosis turned to yellowish green on young leaves, then on mature leaves including the petioles and stems; Fe – mild interveinal chlorosis of yellowish green turned greenish yellow then yellowish, first on young leaves then on recently matured leaves and finally mature leaves, and acropetal necrosis on young leaves; Mn – silhouette of yellow chlorosis outlined the leaf margins of recently matured leaves, and irregular interveinal chlorosis on young and recently matured leaves, finally light tan necrosis on leaf margins; Cu – leaves lacked sheen, chlorosis and upward cupping of young leaves, acropetal light tan necrosis of recently mature leaves, wilting of petioles, and faded flowers; Zn – acropetal purple pigmentation turned necrotic on recently matured leaves then on mature leaves, and chlorosis of young leaves; B – darker green glossy leaves with thick and leathery texture, distorted and deformed young leaves became chlorotic,

recently matured leaves, petioles and growing points had purple pigmentation, and roots were short, and thick with swollen necrotic tips.

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Table 1. Pansy tissue nutrient concentration at early and advanced stages of deficiency in recently matured leaves of hydroponically grown plants in this study and published standards.

Treatment	**-N**	**-P**	**-K**	**-Ca**	**-Mg**	**-S**	**-Fe**	**-Mn**	**-Cu**	**-Zn**	**-B**
	Percent dry wt.						$mg \cdot kg^{-1}$ of dry wt.				
Element	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn	B
Control early	4.64	0.61	4.64	1.35	0.44	0.28	94.6	237.7	7.6	39.2	45.3
Deficiency early	1.65	0.17	1.17	0.45	0.14	0.13	51.4	6.7	2.8	22.8	9.7
Control advanced	4.62	0.47	4.28	1.70	-	-	113.8	173.0	7.7	33.9	61.8
Deficiency advanced	1.12	0.04	0.59	0.61	-	-	64.5	6.2	2.4	27.6	10.9
P value	0.00	0.00	0.00	0.00	0.03	0.04	0.01	0.00	0.00	0.05	0.00
Published*	3.44 - 4.20	0.37 - 0.64	2.39 - 2.92	0.90 - 1.16	0.36 - 0.49	0.15 - 0.28	80.0 - 398.0	41.0 - 203.0	6.0 - 23.0	44.0 - 137.0	20.0 - 46.0

*Mills and Jones, 1996. Survey average of concentrations associated with acceptable crop performance, minimum critical concentrations not identified.

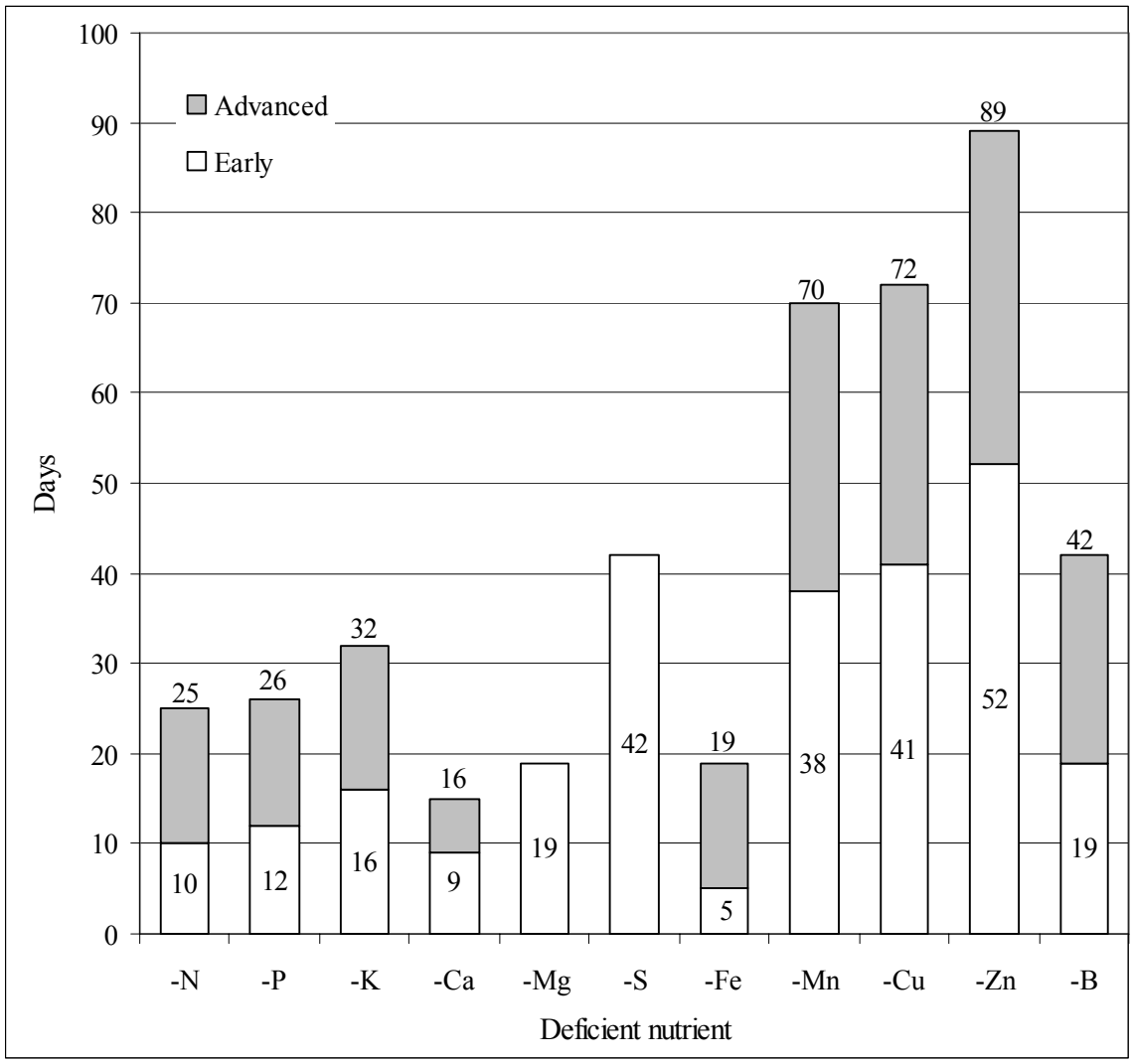

Figure 1. Days from initiation of treatments to leaf sampling for nutrient analysis for pansy at early and advanced stages of deficiency.

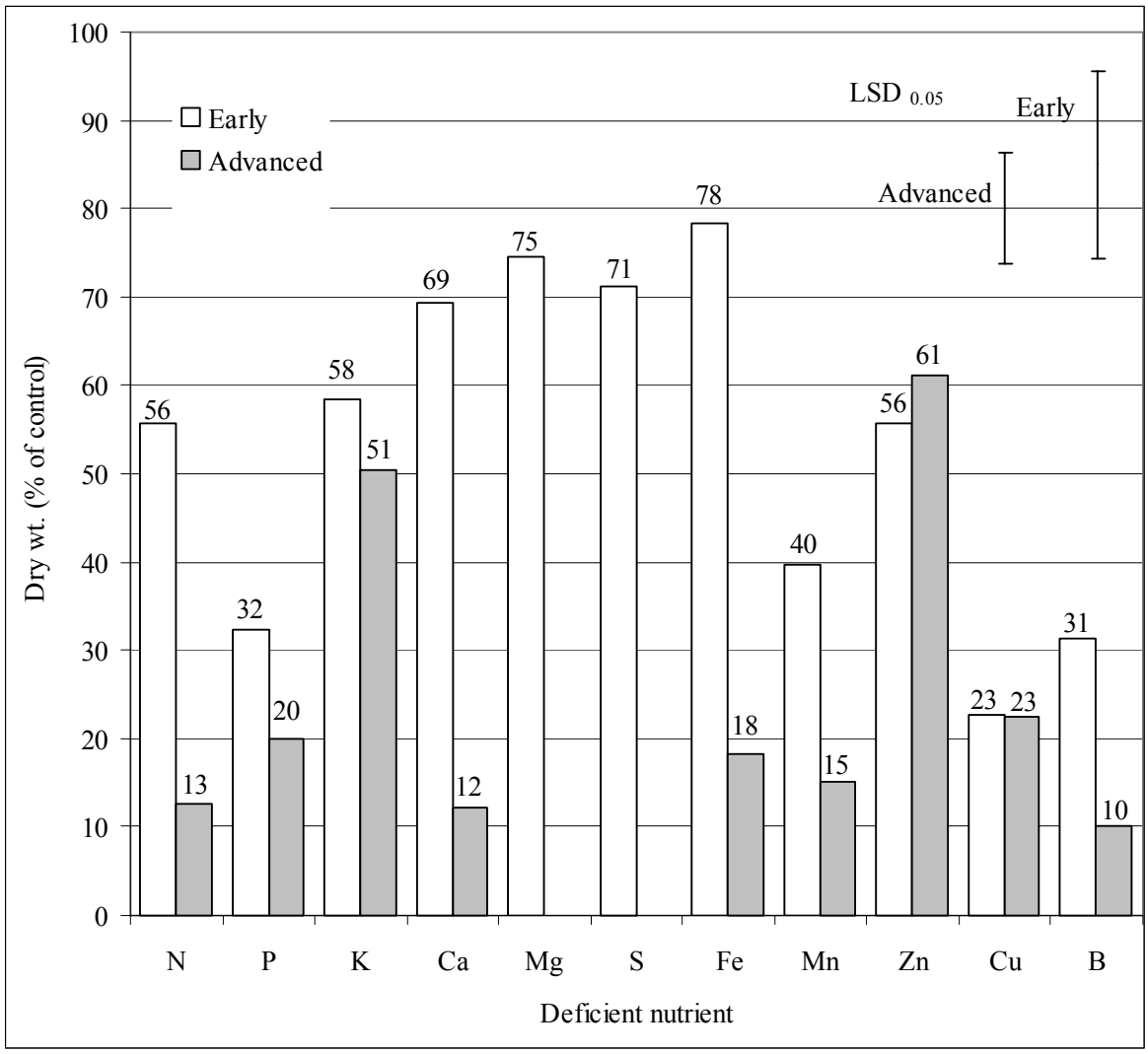

Figure 2. Pansy dry weight of nutrient deficient plants expressed as a percentage of control plants at early and advanced stages.

CHAPTER 8

PETUNIA FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Dharmalingam S. Pitchay[1], James L. Gibson[1], Paul V. Nelson[1], F.R. Walls[2], Brian E.Whipker[1], and Brenda Cleveland[2]

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

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

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Dharmalingam S. Pitchay[1], James L. Gibson[1], Paul V. Nelson[1], F.R. Walls[2], Brian E.Whipker[1], and Brenda Cleveland[2]

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

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

ABSTRACT

Tissue analysis standards are crucial for monitoring plant nutrient status for petunia. Complete visual deficiency symptoms have not been established for this crop. Petunia *(Petunia x hybrida* 'Purple Wave'*)* plants were grown hydroponically in a glass greenhouse. Treatments consisted of a complete nutrient solution, and 11 additional solutions, each devoid of one essential nutrient. Deficiency symptoms and plant size were chronologically recorded for shoots and roots. Fully expanded youngest leaves were analyzed for nutrient concentration. In the absence of N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn and B, tissue concentrations at early deficiency stage were 2.06, 0.07, 0.69, 0.32, 0.08, 0.11 (%), 53.1, 11.4, 3.5, 13.0, and 10.3 ($mg \cdot kg^{-1}$), respectively. At early deficiency stage, shoot dry weight reduction was most pronounced in deficiencies of Cu, and Mn. On the other hand, at advanced stage a severe reduction of shoot dry weight was observed in Cu,

N, P, S and Ca deficient plants. The order of visual deficiency symptoms at early stage from first to last was Fe (4 days), N (6 days), Ca (8 days) and B (8 days), P (9 days), K (10 days) and Mg (10 days), Mn (70 days), Cu (72 days), S (74 days), and Zn (78 days).

INTRODUCTION

Petunia purple wave (*Petunia x hybrida* 'Purple Wave') is a versatile bedding plant that can be used as any conceivable form in landscapes. The current foliar analysis standards are tailored for the standard petunia and not for the 'Purple Wave' petunia. Variation in nutrient requirement exists even among cultivars (1). General guideline ranges for N and Ca (2), and survey ranges for 11 essential elements (3) have been reported for the standard petunia. Values in these ranges are associated with acceptable growth, but do not establish the minimum and maximum critical values. Present fertilizer formulation is solely based on the general recommendation of 2:1:2 (N: P: K) ratio (4). Besides soil testing, foliar analysis and visual diagnosis of nutrient deficiencies are valuable aspect of nutritional management program (4).

The objectives of this study were to generate visual symptoms of nutrient deficiencies in chronological order as they appear from incipient to advance stages, and to establish foliar analysis standards that include minimal critical levels and levels associated with late stages of deficiencies for N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn and B.

MATERIALS AND METHODS

The protocol for this experiment was similar to that of vinca (5). It was conducted in the same location. Except that, petunia *(Petunia x hybrida* 'Purple Wave'*)* was sown on 30 March 2001 and all treatments except Fe were initiated on 2 June 2001 and Fe on 6 June 2001. Shoot dry weight and height values are based on measurements while all other size comparisons with control plants are visual assessments.

RESULTS

Following are the progressions of visual deficiency symptoms through advanced stages for each element. Early and late leaf sampling was carried out on days as indicated in Figure 1; and the critical foliar analysis standards and tissue concentration associated with advanced deficiency is shown in Table 1.

Nitrogen

On day 4, plants appeared smaller, and slightly shorter compared to control. Plants had fewer and smaller leaves with an absence of lateral branching. Young and recently leaves were lighter green than the older leaves. By day 6, the basipetal region of oldest leaves developed purple pigmentation on primary veins. At this point, the tissue N concentration was 2.06%. As these symptoms progressed outward towards terminal tips, secondary veins at basipetal region also developed purple pigmentation by day 9. This

syndrome progressed up the plants to younger leaves. The plant height and leaf size was 46% and 29% of control respectively. The stems and older leaf petioles developed purple pigmentation by day 12. As the symptoms advanced, the purple pigmentation also developed on tertiary and quaternary veins and adjacent cell tissues of oldest leaves. Gradually, the entire lamina of some of the oldest leaves turned purplish and gave way to yellowish pigmentation. Eventually, these leaves developed necrosis (day 14). Again, these symptoms progressed up the plants to younger leaves. The purplish pigmentation on abaxial surface of the leaves was more pronounced than the adaxial surface and to a greater extent on petioles. Leaves were less lanceolated with shorter petioles compared to that of control. The primary and secondary roots were thinner, and shorter (70% of control). The tissue N concentration further dropped to 1.32%.

Phosphorus

Plants were smaller and shorter with a height of 56% of control, and had poorly developed lateral shoots, and fewer and darker green smaller (52% of control) leaves (day 6). By day 9, the base of the main stems, upper portion of lateral shoots, and petioles and primary veins (at basipetal area) on the abaxial surface of oldest leaves developed purple pigmentation. The primary roots were fewer, and longer by 30% of control. At this point, the tissue P concentration was 0.07%. By day 12, older leaves developed chlorosis on the leaf margins, which turned to purple pigmentation. These leaves then developed interveinal chlorosis, which then turned into patches of purple pigmentation (day 17). As the symptoms advanced, 80% of the older leaf lamina was covered with purple

pigmentation. This syndrome moved up the plants. The purple pigmentation of main stems intensified to deep purple (day 19). By day 21, oldest leaves developed acropetal necrosis. Plants remained shorter (37% of control) and smaller with fewer narrow smaller leaves. Younger leaves were darker green compared to that of control. Overall, the roots appeared brownish (control was whitish) with shorter secondary roots. The tissue P concentration at this point was 0.05%.

Potassium

The plants appeared darker green with smaller and fewer leaves (day 8). By day 10, the lower most leaves developed bruised like lesions in the acropetal area. The tissue K concentration at this point was 0.69%. The bruised-like lesions quickly turned into grayish green irregular interveinal patches (day 11). This made the leaves appear darker green than the control. The primary roots were fewer and thinner with a root volume of 80% of control. By day 12, the grayish green pigmentation turned to grayish necrosis in the acropetal area and spread inward toward the basipetal area. The mature leaves had a droopy appearance. Chlorosis (greenish-yellow) developed on some of the older leaves. As the symptoms progressed (day 13), Chlorosis (greenish-yellow) developed on some of the older leaves adjacent to the grayish necrosis, which became a light grayish brown necrosis, and the tissue appeared shriveled and papery. By day 14, several older leaves developed severe necrosis. The described symptoms progressed upwards. Meanwhile, the plant appeared more compact and had short, thin lateral stems with smaller leaf. The

young leaves remained darker green and the plants appeared slightly shorter than the control. The tissue K concentration at this point was 0.63%.

Calcium

Plants appeared darker green with limited growth on the upper portion of plants, and the primary roots were shorter (60% of control) with fewer secondary roots (day 6). Young and recently matured leaf expansion and lateral shoot development was minimal resulting in smaller plants. By day 8, young and recently matured leaves curled downwards in the mid lamina area. Shades of purple pigmentation developed across the lamina of young and recently leaves, more so in the basipetal area. Newly developed leaves had short petioles, and older leaves had a distinct lighter green veination similar to the control. The root tips began developing necrosis. The plant height and leaf size was 44% and 26% of control respectively. These symptoms occurred when the tissue Ca concentrations was 0.32%. By day 9, tiny light black necrotic spots developed sporadically across the basipetal area of recently matured leaves, mid-lamina area of young leaves, and acropetal area of very young leaves. The necrotic spots then turned light brown by day 10. Meanwhile, very young and young leaves became spoon shaped due to uneven leaf expansion in the mid-lamina area. As the symptoms advanced (day 12), 70% of the area of recently matured leaves was covered with light brown necrosis with shades of light purple pigmentation, and a purple silhouette developed on the basipetal margins these leaves. The young leaves rolled downward in the acropetal area, whereas, the very young leaves with narrow leaf blades cupped upward, as the tips curled

inwards giving a hook-like shape. By day 14, older leaves developed black necrotic spots in the basipetal area with yellowish green chlorosis. The margins and tips of young and recently matured leaves developed brown necrosis causing a severe leaf distortion and the shoot apex died. The primary roots were shorter (30% of control) and fewer with necrosis along the axis. The tissue Ca concentration at this point was 0.38%.

Magnesium

Overall, plants appeared shorter (68% of control) and lighter green, more so in the upper portion of the plants. By day 10, the oldest leaves (furthermost leaves from shoot apex) developed basipetal yellowish green interveinal chlorosis, while the acropetal area was similar to that of the control. Margins and acropetal area on the abaxial surface also appeared chlorotic with yellowish green pigmentation. On the other hand, mature leaves immediately below the recently matured leaves developed greenish yellow acropetal chlorosis. Lateral shoots were fewer and shorter, and their leaves were fewer and less lanceolated. The tissue Mg concentration at this point was 0.08%. By day 12, the basipetal chlorosis of oldest leaves moved outward while the acropetal chlorosis of mature leaves moved inward. Meanwhile, recently matured leaves developed mild interveinal chlorosis in the acropetal area. As the symptoms advanced, irregular patches of purplish pigmentation developed in the interveinal area of mature and recently matured leaves. By day 14, mature leaves developed purplish pigmentation along the margins. A few purplish necrotic spots developed interveinaly in the acropetal chlorotic area of mature leaves. Some of the mature leaves on lateral shoots also developed these

symptoms. As the symptoms advanced (day 17), brown to tan-white necrotic patches developed interveinaly on the recently matured and mature leaves. However, the oldest leaves remained chlorotic (greenish yellow) with no signs of necrosis. Mature leaves of lateral shoots also developed tan-white necrotic patches. The primary and secondary roots were fewer by 20% of control. At this point, very young leaves and shoot apex appeared normal similar to that of control, and the tissue Mg concentration was 0.06%

Sulfur

Initially, on day 74, plants appeared shorter with fewer and smaller less lanceolated leaves. Young and recently matured leaves were lighter green. The root volume was 80% of control. At this point, the tissue S concentration was 0.11%. Lateral shoots were shorter and smaller with light purplish-pigmented stems (day 76). Whereas in control, light purplish pigmentation was only found at the terminal tips of lateral shoots. The plant height was 60% of control. By day 81, young and recently matured leaves developed uniform basipetal chlorosis (yellowish green), which progressed outward. The yellowish green basipetal chlorosis turned greenish yellow, while the purple pigmentation of lateral shoot stems intensified to darker purple by day 87. The plant height and leaf size was 45% and 64% of the control, respectively. By day 89, lower leaves developed chlorosis (light greenish yellow) in the basipetal area including the veins. At this point, the tissue S concentration dropped further to 0.06%.

Iron

Plants were smaller and shorter. Young and very young leaves developed basipetal interveinal chlorosis (yellowish green) day 4. The tissue Fe concentration at this point was 53.1 $mg \cdot kg^{-1}$. By day 7, chlorosis turned greenish yellow as it progressed outward. The margins of these leaves developed a yellowish silhouette. Meanwhile, recently matured leaves developed acropetal light interveinal chlorosis (yellowish green). Chlorosis then intensified to greenish yellow (day 8). As symptoms progressed (day 10), a distinct interveinal chlorosis developed on recently matured leaves. Meanwhile, the interveinal chlorosis of young and very young leaves, including the shoot apex, vanished and the entire lamina appeared chlorotic (yellowish). The lateral shoots were chlorotic, more so at the terminal apex, and had light purple pigmentation close to the apex. By day 13, these symptoms progressed down the plants to mature leaves. As the symptoms advanced (day 15), the young leaves cupped upwards along the mid lamina as the margins rolled inwards. Entire lamina of young and recently matured leaves became bleached yellow. Eventually, translucent necrotic patches developed randomly on margins and across the lamina of young and recently matured leaves by day 17, and then turned tan white. At this point, chlorosis on lateral shoots intensified and progressed toward the basal portion of the stems. The plant height was 63% of control. The tissue Fe concentration at this point was 30.0 $mg \cdot kg^{-1}$.

Manganese

Plants appeared smaller and shorter (44% of control) with lighter green foliage (day 70). Yellowish green uniform basipetal chlorosis developed on young and recently matured leaves and the tissue Mn concentration at this point was 11.4 $mg \cdot kg^{-1}$. This then turned greenish yellow by day 72. These symptoms progressed down the plants to older leaves. By day 73, interveinal chlorosis developed on mature leaves (leaves immediately below the recently matured leaves). As the symptoms progressed, the entire plants except the lower most matured leaves appeared greenish yellow. Meanwhile, shoot apex growth was halted, and leaves were soft textured with wavy lamina and obtuse tips. Lateral shoot stems appeared chlorotic (greenish yellow). As the symptoms progressed (day 76), black necrotic stippling (very fine spots) developed in the basipetal area of mature leaves, mid lamina area of recently matured leaves and acropetal area of young leaves. By day 78, some of this stippling coalesced to form light brown necrosis, which eventually turned to small tan-white necrotic patches. The entire lamina of some of the mature leaves were covered with this necrotic stippling. This occurred when the tissue Mn concentration dropped to 9.2 $mg \cdot kg^{-1}$.

Copper

Severely limited shoot and root growth (10% and 5% of control respectively) with the absence of lateral and axillary shoot development was the initial symptoms of Cu deficiency (day 72). The plants appeared skeletal with only about 10 to 12 dark green

leaves per plant (control with >90 leaves). The leaves were smaller (27% of control) with a lack of sheen. The tissue Cu concentration at this point was 3.5 $mg \cdot kg^{-1}$. As the symptoms advanced (day 76), desiccation developed on mature leaf margins and interveinal areas, as light olive green patches. These symptoms turned into light brown necrotic patches along the leaf margins and interveinal areas. In some plants the young leaves severely cupped upward and rolled inwards with desiccation along margins, which appeared as light olive green non-glossy lamina (day 81). The growing point appeared dormant and there was an absence of new leaf development and leaf expansion. The plant height was 16% of control. The tissue Cu concentration at this point was 3.3 $mg \cdot kg^{-1}$.

Zinc

On day 78, plants had darker green leaves with a network of veinal chlorosis, more so on the basipetal area of young and recently matured leaves. The plants were taller than the control. The tissue Zn concentration at this point was 13.0 $mg \cdot kg^{-1}$. Due to well developed veinal chlorosis, the secondary and tertiary veins were clearly visible. Meanwhile, the very young leaves had light basipetal chlorosis. By day 80, the secondary veins of recently matured leaves developed brownish green necrosis, which spread to the adjacent tissues. There was puckering in the basipetal area. The lateral shoots were shorter (30% of control) and developed purple pigmentation at the terminal (day 82). The primary roots were shorter, fewer and thinner and had fewer secondary roots. By day 85, necrosis turned tan-white and spread even more. These symptoms progressed up and down the plants to other leaves. Plants appeared slightly smaller due to shorter lateral

shoots (30% of control) and a height 61% of control. The tissue Zn concentration at this point was 10.2 $mg \cdot kg^{-1}$.

Boron

Initially on day 8, plants appeared shorter (52% of control) and compact with darker green foliage. The tissue B concentration at this time was 10.3 $mg \cdot kg^{-1}$. By day 12, recently matured leaves were distorted with downward curling along the margins, and wavy lamina. Leaf texture was thick and brittle. Young and recently matured leaves appeared narrower. The acropetal tips of very young and young leaves were rounded (control was acute) and appeared glossy and darker green (day 17). Shoot growth was halted, deformed and clustered together with the immediate axillary shoots. Upper portion of the internodes were shorter and stacked together forming a rosette appearance (day 23). The secondary roots had swollen tips and were very short (1 to 3 mm) along the basipetal end of the primary roots and abnormally longer closer to the tips of the primary roots. The secondary roots near the tip of primary roots resembled a witches' broom-like growth. As the symptoms advanced, by day 28, severe crinkling, twisting, dents and dimples occurred in the basipetal area of young and mature leaves, giving a distorted appearance. The petioles of these leaves were twisted and distorted as well. Lateral branching was severely stunted and longitudinal corking of tissue occurred at the base of stems and axillary shoots. The plant height declined to <14% of control. At this point, the tissue B concentration dropped further to 5.4 $mg \cdot kg^{-1}$.

DISCUSSION

Fe was the first to develop deficiency symptoms at 4 days followed by N at 6 days, Ca and B at 8 days, P at 9 days, and K and Mg at 10 days. Mn, Cu, S and Zn were the slowest to develop deficiency at days 70, 72, 74, and 78 respectively. An extended delay in S deficiency symptoms was consistently observed in marigold, salvia, vinca, and zinnia. This was probably due to the acquisition of SO_2 in the atmosphere (6). Although Ca and B developed symptoms at the same time, the advance symptoms developed quicker in Ca (6 days) than in B (20 days). All the macronutrients except S, and micronutrients Fe and B developed early symptoms within 10 days (Figure 1).

A significant reduction in shoot dry weight was noted in N, P, Ca, Mn, Cu, Zn, and B during the early stage of deficiency (Figure 2). However, dry weight of K, Mg, S, and Fe deficient plants were equal to control. By the time of advance deficiency, reduction in dry weight was noted in all deficient plants. Shoot dry weight was most pronounced in Cu (6%), N (11%), P (17%), S (20%), and Ca (22%) at advanced stage.

Based on the speed at which deficiency occurred (Figure 1), petunia was more prone to Fe deficiency. Early deficiency occurred when the tissue Fe content was 53.1 $mg \cdot kg^{-1}$ while at advanced stage the control tissue Fe concentration was greater than the early deficient plant tissue concentration by only 3.6 $mg \cdot kg^{-1}$ (Table 1). This could be due to poor uptake of Fe as a result of high rhizosphere pH (7). Also, petunia has the facility to tolerate increased Fe concentration as was noted in *Petunia x hybrida* cv. 'Ultra Crimson Star', which failed to develop toxicity symptoms even when 6mM nutrient

solution was provided (8). Therefore, one has to be vigilant about Fe deficiency when growing petunia.

Purple pigmentation is generally associated with P deficiency. In petunia 'Purple Wave', purple pigmentation (flower color) manifested in the leaves, petioles, shoots, and stems of N, P, Ca, Mg, S, and Fe deficient plants. This may be mistaken for just P deficiency. However, the misdiagnosis could be avoided by determining the specific areas of the plant tissue, pattern of the symptoms, and the intensity of purple coloration.

The minimum critical tissue concentration of all deficient treatment except Cu was below the published survey range value (Table 1). Based on tissue concentration, N has been generally considered as the largest required element for plant growth and development (9). However, in petunia (control plants), the tissue K concentration was higher than N. Nevertheless, the visual symptoms of K deficiency developed only when the tissue K concentration was 9% of control (0.69% K in deficient plant), without affecting the plant height and shoot dry weight (Figure 2). This could mean that petunia has the tendency to accumulate K as the plant develops. The early stage of deficiency could be considered critical for an immediate recovery plan because the advanced symptoms developed within 4 days (from early deficiency stage) when the tissue K concentration dropped by only 0.03%.

Unlike marigold, vinca and salvia, petunia developed deficiency symptoms before flowering in all treatments. This points out that nutrient stress does not necessarily promote early flowering. However, in N and Ca free environments, *Petunia x hybrida* 'Coral sea' had flowered (2).

Synoptic visual deficiency symptoms were as follows: N – development of purple pigmentation starting on the veins and then the lamina of oldest leaves which turned yellowish and finally necrotic; P – development of purple pigmentation on the leaf margins of oldest leaves followed by interveinal chlorosis, which turned to purple pigmentation and finally necrotic; K – lower leaves developed darker green to bruised like lesions which then turned to a grayish green to gray necrosis; Ca – downward curling of young and recently matured leaves then development of shades of purple pigmentation with tiny black necrotic spots, which turned light brown, severe leaf distortion with dead shoot apex; Mg – lower leaves developed interveinal chlorosis with irregular patches of purple pigmentation on the interveinal area which then turned to brown to tan-white necrotic patches; S – lighter green young and recently matured leaves developed yellowish green basipetal chlorosis which turned greenish yellow, lateral shoot stems developed purplish pigmentation; Fe – young and very young leaves developed interveinal chlorosis from yellowish green to greenish yellow and then to yellowish to bleached yellow, and finally to white necrosis; Mn – young and recently matured leaves developed yellowish green uniform chlorosis which turned greenish yellow, and mature leaves with black necrotic stippling turned light brown necrosis; Cu - leaves light olive green lack sheen and developed desiccation on mature leaf margins and interveinal areas to light brown necrosis; Zn - young and recently matured leaves darker green leaves, with a fine network of veinal chlorosis turned to brownish green necrosis, and necrosis on adjacent tissues turned tan-white; B - darker green matured leaves thick and brittle distorted by crinkling, twisting, dimples and wavy basipetal lamina, longitudinal corking of shoot and stem tissue, and witches' broom-like root appearance.

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Table 1. Petunia tissue nutrient concentration at early and advanced stages of deficiency in recently matured leaves of hydroponically grownin plants this study and published standards.

Treatment	**-N**	**-P**	**-K**	**-Ca**	**-Mg**	**-S**	**-Fe**	**-Mn**	**-Cu**	**-Zn**	**-B**
	Percent dry wt.						$mg \cdot kg^{-1}$ of dry wt.				
Element	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn	B
Control early	6.49	0.41	7.36	1.48	0.39	0.30	73.2	74.2	8.5	28.0	28.7
Deficient early	2.06	0.07	0.69	0.32	0.08	0.11	53.1	11.4	3.5	13.0	10.3
Control advanced	7.13	0.33	8.30	1.57	0.37	0.29	56.7	71.3	6.1	18.0	48.6
Deficient advanced	1.32	0.05	0.63	0.38	0.06	0.06	30.0	9.2	3.3	10.2	5.4
P value	0.00	0.01	0.02	0.02	0.00	0.00	0.03	0.03	0.00	0.00	0.00
Published*	3.85 - 7.60	0.47 - 0.93	3.13 - 6.65	1.20 - 2.81	0.36 - 1.37	0.33 - 0.80	84.0 - 168.0	44.0 - 177.0	3.0 - 19.0	33.0 - 85.0	18.0 - 43.0

*Mills and Jones, 1996. Survey average of concentrations associated with acceptable crop performance, minimum critical concentrations not identified.

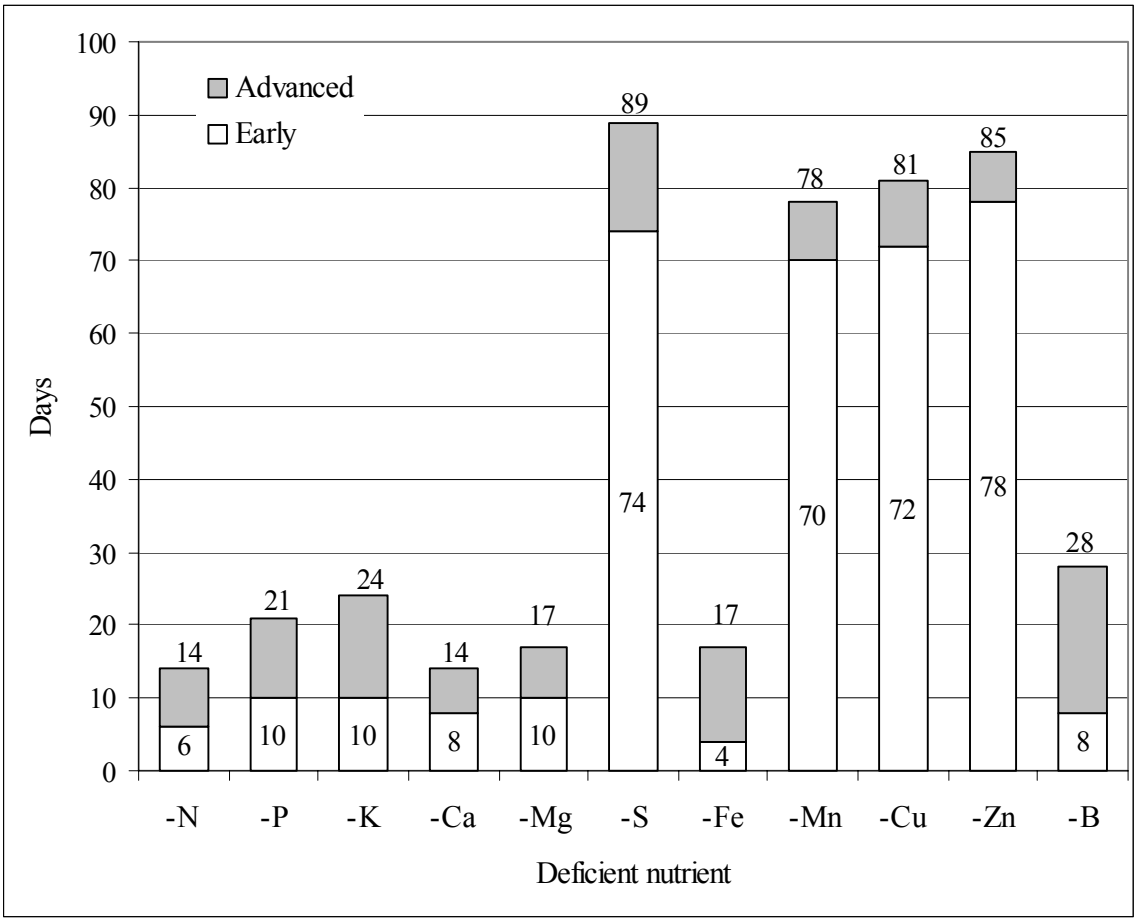

Figure 1. Days from initiation of treatments to leaf sampling for nutrient analysis for petunia at early and advanced stages of deficiency.

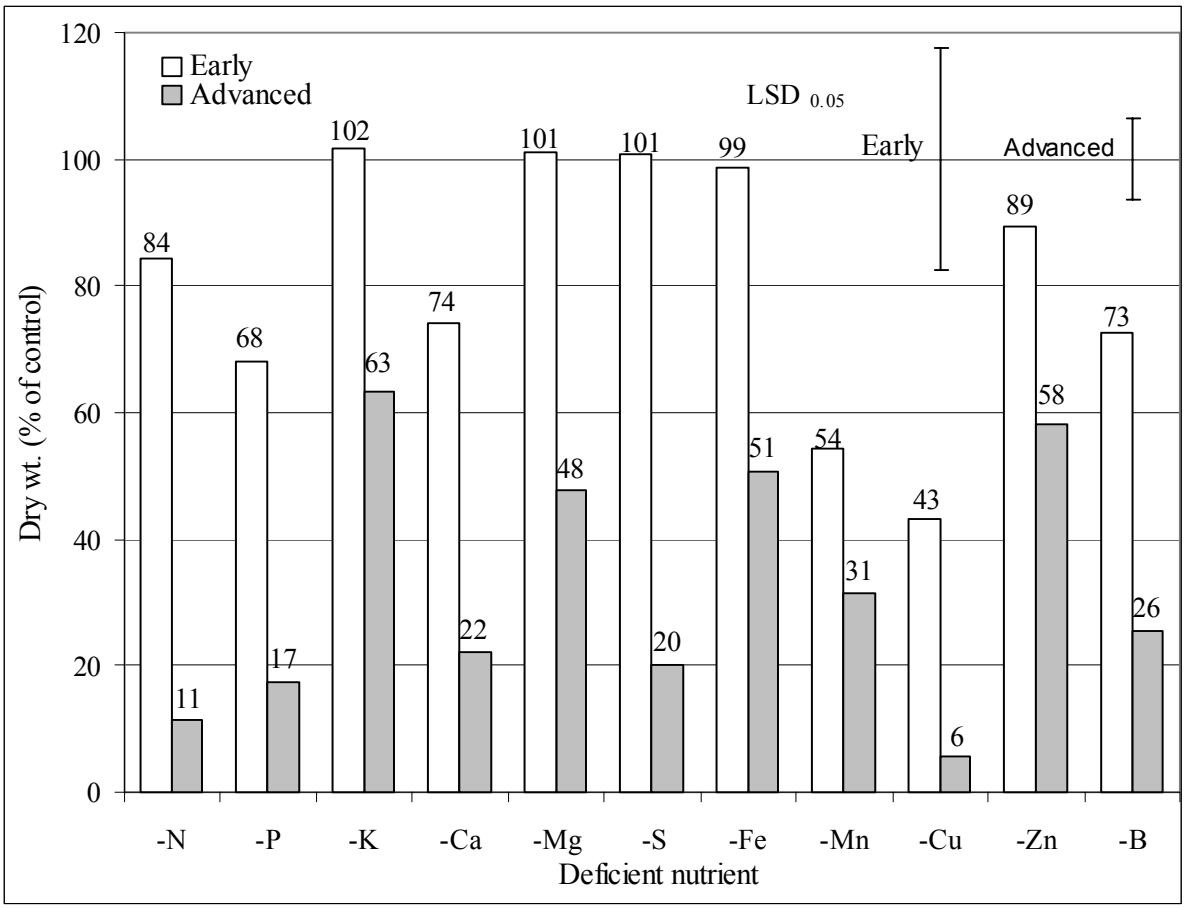

Figure 2. Petunia dry weight of deficient nutrient plants expressed as a percentage of control plants at early and advanced stages.

CHAPTER 9

BEGONIA FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

James L. Gibson[1], Dharmalingam S. Pitchay[1], Brenda Cleveland[2], Paul V. Nelson[1], F.R. Walls[2], and Brian E.Whipker[1]

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

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

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James L. Gibson[1], Dharmalingam S. Pitchay[1], Brenda Cleveland[2], Paul V. Nelson[1], F.R. Walls[2], and Brian E.Whipker[1]

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

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

ABSTRACT

Tissue analysis standards are crucial for monitoring plant nutrient status for begonia (*Begonia x semperflorens-cultorum* Hort). Complete visual deficiency symptoms have not been established for this crop. Begonia plants were grown hydroponically in a glass greenhouse. Treatments consisted of a complete nutrient solution, and 11 additional solutions, each devoid of one essential nutrient. Deficiency symptoms and plant size were chronologically recorded for shoots and roots. Fully expanded youngest leaves were analyzed for nutrient concentration. In the absence of N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn and B, tissue concentrations at early deficiency stage were 1.50, 0.10, 0.75, 0.55, 0.10, 0.13 (%), 62.6, 9.4, 1.7, 21.8, and 9.1 ($mg \cdot kg^{-1}$), respectively. At early deficiency stage, reduction of shoot dry weight was most pronounced in deficiencies of N (51%), Cu (33%), moderate in B (30%), S (27%), Mn (20%), and Ca (14%). On the other hand, at

advanced stage a severe reduction of shoot dry weight was observed in N (92%), P (76%), B (66%), S (58%), Ca (55%), K (47%). The order of visual deficiency symptoms at early stage from first to last was N and Fe (6 days), B (7 days), Ca (8 days), P and K (11 days), Mg (15 days), Mn (77 days), S (78 days), and Cu and Zn (85 days). Descriptions of deficiency symptoms are presented in the text.

INTRODUCTION

Begonia with glossy green foliage is an excellent choice of bedding plant for partial shade planting. This crop has been widely used in landscapes across North America. In spite of its significant role in landscaping, limited information is available on foliar nutritional standards and critical levels, and visual deficiency symptoms. Critical foliar levels and visual symptoms of N, P, K, Ca, Mg, Fe and B are available for elatior begonia. However, the results may differ due to differences between species (1). General guidelines of survey ranges for 11 essential elements (2) have been reported. Values in these ranges are associated with acceptable growth, but do not establish the minimum and maximum critical values. Present fertilizer formulation is solely based on the general recommendation of 2:1:2 (N: P: K) ratio (3). Besides soil testing, foliar analysis and visual diagnosis of nutrient deficiencies are valuable aspects of nutritional management program (3).

The objectives of this study were to generate visual symptoms of nutrient deficiencies in chronological order as they appear from incipient to advance stages, and

to establish foliar analysis standards that include minimal critical levels and levels associated with late stages of deficiencies for N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn and B.

MATERIALS AND METHODS

The protocol for this experiment was similar to that of vinca (4). It was conducted in the same location. Except that, Begonia 'Super Olympia Red' was sown on 26 March 2001 and all treatments except Fe were initiated on June 1, 2001, and Fe was initiated on 6 June 2001. Shoot dry weight and height values are based on measurements while all other size comparisons with control plants are visual assessments.

RESULTS

Following are the progressions of visual deficiency symptoms through advanced stages for each element. Early and late leaf sampling was carried out on days as indicated in Figure 1 and the critical foliar analysis standards and tissue concentration associated with advanced deficiency is shown in Table 1.

Nitrogen

Initially, on day 6, recently matured leaves developed chlorosis (yellowish green) in acropetal area. Young and recently matured leaves developed a distinct broad silhouette of reddish pigmentation as compared to that of control, which had a thin

silhouette of reddish pigmentation. At this point, the tissue N concentration was 1.50%. By day 9, older leaves developed interveinal reddish-pigmented spots. Meanwhile, on the abaxial surface of recently matured leaves there was a broader area of reddish pigmentation along the margins, which was moving inward. Chlorosis (greenish yellow) was also more distinct on the abaxial surface of recently matured leaves. Plants were smaller and shorter (59% of control) with smaller and fewer leaves, and fewer lateral shoots. The petioles were reddish in color as compared to that of the control, which were green. As the symptoms advanced (day 11), almost 90% of the abaxial area and 60% of the adaxial area of young leaves had reddish pigmentation. Meanwhile, recently matured leaves developed basipetal necrosis covering 80% of the lamina. Older leaves developed acropetal necrosis along the margins and randomly across the lamina. By day 13, necrosis also developed on margins of lateral shoot leaves. Flowering was absent. Primary roots were thinner, and shorter by 30% of the control. Secondary roots were also thinner and shorter, and emerged close to the terminals of the primary roots. By this time, tissue N concentration dropped to 1.3%.

Phosphorus

Plants were slightly smaller and shorter (93% of control), and had darker green foliage (day 10). The leaves were fewer with a lack of sheen. The lateral shoots were fewer and shorter. Plants developed a silhouette of reddish pink pigmentation, which were broader than the control, when the tissue P concentration was 0.10% (day 11). By day 13, reddish pink pigmentation also developed on the abaxial surface of the leaves,

more so on young leaves. This pigmentation progressed inward towards the basipetal area (day 18). The primary roots were fewer, but longer than the control by 10%. By day 24, almost 60% of the lamina including the petioles was covered with the pigmentation. The primary roots were fewer (40% of control), but longer than the control by 10%. The secondary roots were fewer and shorter than control. As the symptoms advanced (day 26), lower mature leaves developed tan-brown necrosis on the tips and mid lamina areas. Meanwhile, the axillary shoots also developed irregular tan necrotic patches. The plants were stunted with a height 45% of control, and smaller leaves (40% of control). These symptoms occurred at tissue P concentration of 0.06%.

Potassium

Initially, on day 11, plants appeared slightly shorter (93% of control) with darker green and smaller leaves (60% of control). Mature leaves developed numerous tiny black spots in the acropetal area adjacent to the margins, which accentuated the darker green pigmentation. These symptoms occurred at a tissue K concentration of 0.75%. By day 13, the tiny black spots enlarged and coalesced (1 to 2 mm diameter) to form chains of black necrotic circles with tan necrosis in the center of each. These chains of circles enlarged and coalesced further (3 to 5 mm diameter) and formed necrotic patches (day 15). This syndrome spread to recently matured and young leaves. The leaves lacked glossiness as compared to that of control. As the symptoms progressed (day 17), necrosis in the acropetal area closer to margins of older leaves intensified and progressed inward as bands covering about 30% of the lamina. Primary roots were shorter and fewer with

proportionately smaller secondary roots. As the symptoms advanced (day 18), young leaves developed black necrotic spots. On the abaxial surface, the necrotic spots appeared as black gray necrosis. The plant height declined further to 73% of control due to shorter internodes, and the leaf size reduced to 30% of control. The tissue concentration dropped further to 0.52%.

Calcium

By day 7, plants were very small with a size 30% of control. The plants had fewer (20% of control) smaller leaves (60% of control) that curved inwards along the margins, and poorly developed lateral shoots due to minimal growth. Primary and secondary roots were fewer and shorter (30% of control) and with the absence of new growth. By day 8, brown necrosis first developed in the basipetal area of recently matured leaves and acropetal margins of young and very young leaves when the tissue Ca concentration was 0.55%. At this point, there were no signs of new leaf development. As the symptoms advanced, by day 11, the necrosis became severe affecting the growing points including the lateral shoots at which point the plants stopped growing. By day 13, very young leaves developed necrosis covering about 80% of the lamina. Necrosis also developed on the acropetal area of older leaves. Primary and secondary roots were shorter and fewer (5% of control) with an absence of new growth and the secondary roots appeared closer to the root tips. The roots appeared light tan due to necrosis along the root axis. At this point, the tissue Ca concentration was 0.43%.

Magnesium

In the beginning, on day 15, plants appeared slightly smaller and lighter green than the control. Mature leaves developed yellowish green interveinal chlorosis, at which point the tissue Mg concentration was 0.10%. By day 16, chlorosis turned greenish yellow, more so along the margins adjacent to the red-pigmented silhouette. The reddish-pigmented silhouette along the margins appeared distinct due to the chlorosis adjacent to it. As the symptoms advanced (day 17), the mature leaves developed bruised-like lesions along the margins in the acropetal area. These lesions rapidly turned into black necrosis and spread inward leaving behind tan necrosis by day 18. A similar syndrome was then observed on young leaves. On some of the older leaves, necrosis was interveinal. Meanwhile, very young leaves appeared chlorotic. Overall, the plant height and leaf size was 89 % and 70% of control respectively. The tissue Mg concentration at this point was 0.11%.

Sulfur

Initially, on day 78, plants appeared smaller and shorter (81% of control) with uniform light green basipetal chlorosis on young leaves. At this point, the tissue S concentration was 0.13%. By day 81, the basipetal chlorosis of young leaves appeared yellowish green. Meanwhile, recently matured leaves appeared lighter green with a more distinct silhouette of reddish pigmentation on leaf margins compared to control. By day 86, young leaves appeared greenish yellow and recently matured leaves appeared

yellowish green. Very young leaves were chlorotic (greenish yellow) with shades of reddish pigmentation in the acropetal area progressing inward. As the symptoms advanced (day 93), the very young leaves became yellowish with shades of intensified reddish pigmentation in the basipetal region and on the abaxial surface, while the petioles and veins developed reddish pigmentation. Meanwhile, the chlorosis of young and recently matured leaves also intensified and the red-pigmented margins appeared distinct. On the other hand, young leaves on lateral shoots developed necrosis along the margins. These symptoms occurred at tissue S concentration of 0.06%. The plant height and size was 59% and 50% of control. Primary and secondary roots were fewer by 30% of control.

Iron

In the beginning, at day 5, plants were slightly smaller with a height 89% of control. Young and very young leaves developed light green pigmentation in the basipetal area, which then turned yellowish green by day 6. Meanwhile, recently matured leaves developed light green interveinal chlorosis when the tissue Fe concentration was 62.6 $mg \cdot kg^{-1}$. By day 10, the young and very young leaves developed greenish yellow interveinal chlorosis, more so in the basipetal area, while the interveinal chlorosis of recently matured leaves turned yellowish green. By day 13, the glossiness of the leaves vanished. As the symptoms advanced (day 24), the red silhouette of young leaves disappeared as the chlorosis along the margins intensified to yellow pigmentation. The interveinal chlorosis of young and recently matured leaves became distinct leaving a

green network of veins. By day 27, the margins of recently matured leaves became bleached yellow. At this point, the tissue Fe concentration was 63.9 $mg \cdot kg^{-1}$. These symptoms also progressed to older leaves. Flowers had bleached white sepals with light red-pigmented petals. The primary roots were fewer, shorter (80% of control) and slightly thicker than the control. Plant height remained at 89% of control.

Manganese

Development of basipetal chlorosis (yellowish green) of young and recently matured leaves was the initial Mn deficiency symptom (day 77). At this point, the tissue Mn concentration was 9.4 $mg \cdot kg^{-1}$. Plants appeared slightly shorter by 93% of control. By day 79, chlorosis turned greenish yellow, more so on the leaf margins. Primary roots were fewer (30% of control) and shorter. By day 80, the basipetal area of young and recently matured leaves developed small patches (2 x 5 mm) of water-soaked like translucent light-tan necrosis, which rapidly enlarged and coalesced covering about 10% of the lamina. These necrotic patches progressed outward toward the acropetal area leaving behind tan papery necrosis. By day 81, 40% of the recently matured leaves and 80% of the young leaves were covered with these necrotic patches. Meanwhile, some of the recently matured and mature leaves developed whitish yellow stippling along the acropetal area, which then moved inward. As the symptoms advanced (day 82), the entire lamina of some of the young leaves and petioles was affected by these necrotic symptoms. These symptoms occurred when the tissue Mn concentration was 8.2 $mg \cdot kg^{-1}$. Plant height further declined to 70% of control.

Copper

Plants were smaller and shorter (72% of control) with shorter and smaller lateral branching. Leaves were slightly smaller than control. Overall, foliage lacked glossiness compared to the control. Flowers were smaller and fewer. At this point, the tissue Cu concentration was 1.7 $mg \cdot kg^{-1}$ (day 85). By day 88, pale olive green coloration developed in the basipetal area of young leaves. As this coloration progressed outward toward the acropetal area, the red silhouette along the margins vanished (day 91). This was followed by development of brown to black scorch-like necrosis along the acropetal margins of recently matured leaves (day 95), which then spread inward and along the margins (day 101). This resulted in extensive degradation of leaf tissues and reduction of leaf area as the leaves matured. Meanwhile, the expanding young leaves began curling downward along the margins. The young leaf size was reduced to 50% of the control. Flowers turned pale reddish white. At this point, the tissue Cu concentration remained at 1.7 $mg \cdot kg^{-1}$.

Zinc

Uniform light yellowish green chlorosis developed in the basipetal area of very young and young leaves, and then progressed outward toward acropetal area (day 85). At this point, the tissue Zn concentration was 21.8 $mg \cdot kg^{-1}$. Chlorosis then turned greenish yellow (day 87). Meanwhile, shades of light reddish pigmentation developed on stems and the abaxial surface and petioles of young leaves. A ring-like deep reddish

pigmentation appeared on petioles close to the young leaf blades. As the symptoms advanced (day 90), recently matured leaves developed a distinct interveinal chlorosis while the young leaves appeared yellowish. By day 91, the young leaves turned bleached yellow, and by day 93, bleached white. The plant height was 93% of the control. At this point, the tissue Zn concentration was 22.0 $mg \cdot kg^{-1}$.

Boron

Initially, by day 7, plants appeared smaller with darker green foliage, more so the young leaves. Plants also appeared stunted due to shorter internodes. At this point, the tissue concentration was 9.1 $mg \cdot kg^{-1}$. By day 10, young leaves cupped upwards with limited leaf expansion. Recently matured leaves also slightly cupped upwards. The mid lamina region of young and recently matured leaves developed dented and uneven morphology with greater green pigmentation. Leaf texture was thick and brittle. By day 14, the adaxial surface of young leaves appeared deformed with sunken upper epidermal layer tissues where gray colored callus tissues formed. As the symptoms progressed (day 17), necrosis appeared along the margins of young and very young leaves. Plants had poor lateral branching. Lateral branching were also subject to the above symptoms. The terminal growth was halted. At this point, all the leaves except the oldest leaves appeared severely distorted. Meanwhile, oldest leaves developed reddish pigmentation on the abaxial surface and reddish spots in the acropetal area. The primary roots were fewer shorter by 70 and 60% of control respectively. The described symptoms occurred when the tissue concentration dropped to 6.7 $mg \cdot kg^{-1}$.

DISCUSSION

N and Fe were the first to develop early deficiency symptoms at 6 days, followed by B at 7 days, Ca at 8 days, P and K at 11 days, and Mg at 15 days. Mn, S, and, Cu and Zn were the slowest to develop deficiency at days 77, 78, and 85 respectively. An extended delay in S deficiency symptoms was consistently observed in petunia, marigold, salvia, vinca, and zinnia. This was probably due to the acquisition of SO_2 in the atmosphere (5).

Calcium deficiency symptoms developed earlier than B in petunia, marigold, salvia, celosia, vinca and zinnia. However, in begonia B deficiency symptoms developed earlier than Ca (Figure 1). Although P and K deficient plants developed early symptoms at the same time, P deficient plants took longer to develop advanced symptoms than K deficient plants. Ca, Mg, N, K, and Mn rapidly developed advanced symptoms within 7 days from early stage.

Although N and Fe deficient plants were the first to develop symptoms, Fe was the slowest to progress to advanced symptoms (22 days) with minimal drop in dry weight at early and advanced stages of deficiency (Figure 2). In addition, the symptoms developed when the tissue Fe concentration declined by only 37% (at early stage) and 34% (at advanced stage) compared to tissue Fe concentration of control. The minimum critical tissue Fe concentration was also highest compared to other bedding plant species (vinca, marigold, zinnia, petunia and impatiens - data not shown). Although plants were sensitive to Fe deficiency at early stage, the plants acclimated to the Fe deprived environment as they grew.

At the early stage of deficiency, decline in shoot dry weight was observed in all except Mg (Figure 2). Reduction of shoot dry weight was most pronounced in N (51%), and Cu (33%), moderate in B (30%), S (23%), Mn (20%), and Ca (14%), and least pronounced in K and Zn (9%), P (5%), and Fe (4%). At the advanced stage, shoot dry weight reduction was severe in N (91%), P (76%), B (64%), S (58%), Ca (55%), K (47%), Cu (41%), and Mn (37%), moderate in Mg (30%), and Zn (20%), and least in K (10%).

Red pigmentation in N and P deficient plants (6) may contribute to misdiagnosis of these elements. In P, the symptoms began with darker green leaves, and the reddish pigmentation was lighter colored. On the other hand, in N, symptoms began with chlorosis followed by intense red pigmentation. Besides visual diagnosis, the deficient element could be identified through tissue analysis.

The critical tissue concentration of N, P, K, Mg, and B in *Begonia x semperflorens-cultorum* is lower than in the Rieger Elatior Begonia (7,8). This may be due in part to species difference. Since variations do occur between cultivars (1).

Synoptic visual deficiency symptoms were as follows: N - chlorosis on recently matured leaves, broad reddish silhouette on young and recently matured leaves, reddish pigmented spots on older leaves followed by reddish pigmentation on abaxial surface of young leaves and petioles, and finally necrosis on mature leaves; P - dark green foliage with broad reddish pink silhouette, reddish pigmentation on abaxial surface, and tan brown necrosis on mature leaves; K - Darker green foliage with tiny black spots on mature leaves that turned to chains of necrotic circles and finally necrotic patches; Ca- small plants, with necrosis on young leaves and growing points, and severe reduction of

root growth with necrosis along the axis; Mg - interveinal chlorosis, and bruised-like lesions which then turned black and finally tan necrosis on mature leaves; S - uniform light green chlorosis turned greenish yellow and finally yellowish on young and recently matured leaves, red pigmentation on veins and petioles; Fe - light green chlorosis on young leaves, then light interveinal chlorosis on recently matured leaves, which finally became distinct leaving a fine green network of veins, and flowers with bleached white sepals and light red-pigmented petals; Mn - chlorosis of young and matured leaves that turned to a water-soaked-like light tan necrosis, which rapidly enlarged and coalesced to tan papery necrosis, and stippling on margins of recently matured leaves; Cu - Foliage lacked glossiness, pale olive green young leaves, black necrosis along the margins of recently matured leaves, and pale reddish white flowers; Zn - Light reddish pigmentation on stems and abaxial surface and petioles of young leaves, and distinct interveinal chlorosis on recently matured leaves; B - Stunted plants with darker green, thick and brittle foliage, severe leaf distortion, and finally necrosis along the margins of young leaves, reddish spots on oldest leaves, and halted growth.

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Table 1 . Begonia tissue nutrient concentration at early and advanced stages of deficiency in recently matured leaves of hydroponically grown plants in this study and published standards.

Treatment	**-N**	**-P**	**-K**	**-Ca**	**-Mg**	**-S**	**-Fe**	**-Mn**	**-Cu**	**-Zn**	**-B**
	Percent dry wt.						$mg \cdot kg^{-1}$ of dry wt.				
Element	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn	B
Control early	3.95	0.36	3.74	1.76	0.55	0.20	101.4	75.2	8.0	36.3	67.5
Deficiency early	1.50	0.10	0.75	0.55	0.10	0.13	62.6	9.4	1.7	21.8	9.1
Control advanced	3.66	0.35	5.00	1.62	0.53	0.21	97.6	77.9	5.4	47.1	35.5
Deficiency advanced	1.39	0.06	0.52	0.43	0.11	0.06	63.9	8.2	1.7	22.0	6.7
P value	0.00	0.03	0.00	0.00	0.03	0.02	0.00	0.00	0.00	0.00	0.00
Published*	2.00 - 6.00	0.29 - 0.75	2.25 - 6.00	1.00 - 3.10	0.30 - 0.88	0.22 - 0.70	50.0 - 200.0	45.0 - 200.0	7.0 - 33.0	25.0 - 100.0	20.0 - 75.0

*Mills and Jones, 1996. Survey average of concentrations associated with acceptable crop performance, minimum critical concentrations not identified.

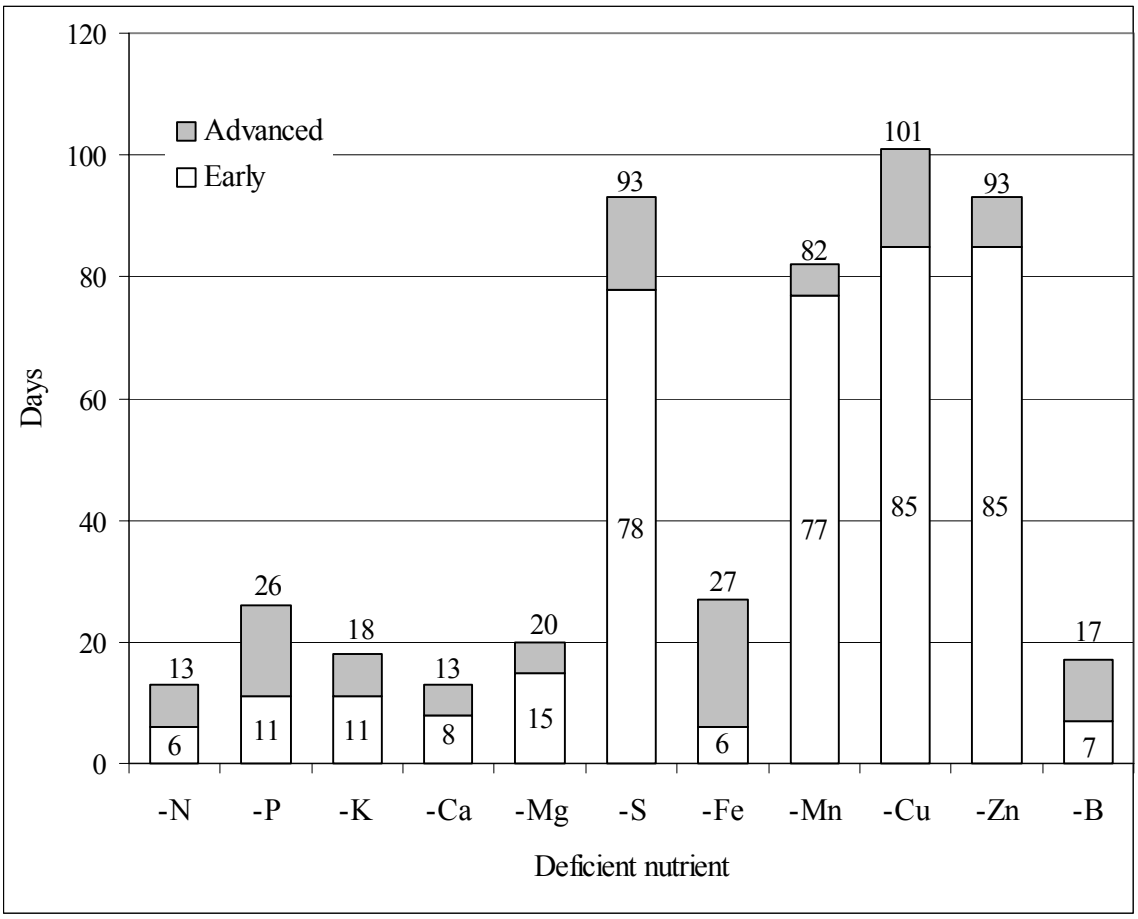

Figure 1. Days from initiation of treatments to leaf sampling for nutrient analysis for begonia at early and advanced stages of deficiency.

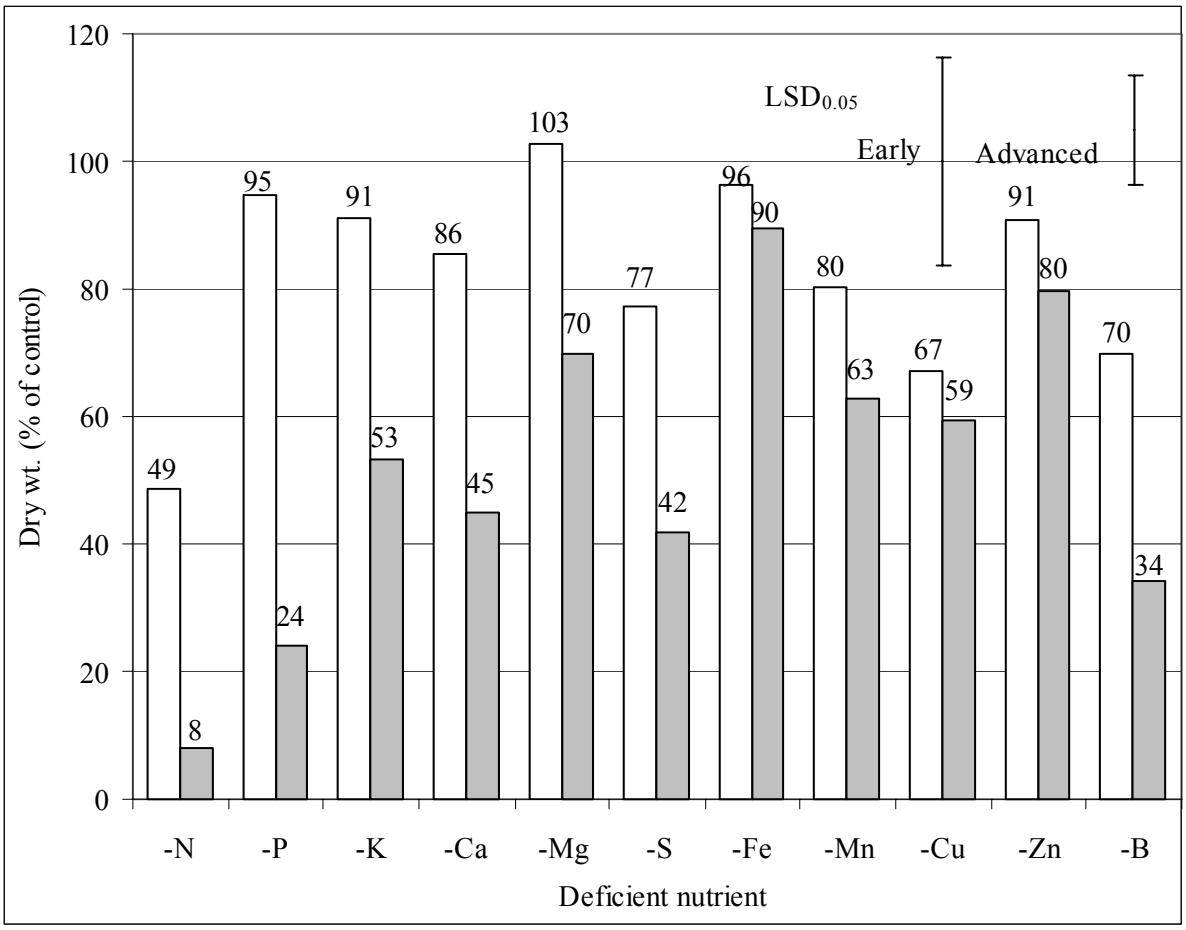

Figure 2. Begonia dry weight of deficient plants expressed as a percentage of control plants at early and advanced stages.

CHAPTER 10

ORNAMENTAL CABBAGE FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

James L. Gibson[1], Dharmalingam S. Pitchay[1], Brian E.Whipker[1], C. Ray Campbell[2], and Paul V. Nelson[1]

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

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

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James L. Gibson[1], Dharmalingam S. Pitchay[1], Brian E.Whipker[1], C. Ray Campbell[2], and Paul V. Nelson[1]

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

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

ABSTRACT

Tissue analysis standards and complete visual deficiency symptoms have not been established for ornamental cabbage (*Brassica oleracea* var. acephala L.), which are crucial for monitoring plant nutrient status. Ornamental cabbage plants were grown hydroponically in a glass greenhouse. Treatments consisted of a complete nutrient solution, and 11 additional solutions, each devoid of one essential nutrient. Deficiency symptoms and plant size were chronologically recorded for shoots and roots. Fully expanded youngest leaves were analyzed for nutrient concentration. In the absence of any one of the elements N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn and B, the minimum critical levels were 2.58, 0.06, 0.41, 0.74, 0.11, 0.20 (%), 47.3, 7.0, 1.7, 7.1 and 26.8 ($mg \cdot kg^{-1}$) respectively. At the early deficiency stage, compared to the control, shoot dry weight declined in all treatments except Mg. On the other hand, at the advanced deficiency stage

deficit of shoot dry weight was observed in all treatments. The order of appearance of initial visual symptoms was Ca and Fe (5 days), N and P (7 days), K (15 days), B (18 days), S (19 days), Mg (30 days), Mn (39 days), Zn (42 days), and Cu (55 days). The visual deficiency symptoms are presented in the text.

INTRODUCTION

Ornamental cabbage (*Brassica oleracea* var. acephala L.) is a popular cool season crop grown in North America. Visual symptoms as well as tissue concentrations of certain cruciferous crops under the category of vegetables have been documented (1,2). In addition, survey ranges of 11 essential elements for vegetables under the genus Brassica species have been established (3). However, visual disorder symptoms and foliar nutritional standards for ornamental cabbage have not been established. Present fertilizer formulation is solely based on general recommendation of 2:1:2 (N: P: K) ratio (4, 5).

The objectives of this study were to generate visual symptoms of nutrient deficiencies in chronological order as they appear from incipient to advance stages, and to establish foliar analysis standards for early and late stages of deficiencies for N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn and B.

MATERIALS AND METHODS

The protocol for this experiment was similar to that of vinca (6). It was conducted in the same location. For this experiment, Ornamental cabbage 'Osaka white' was sown

on 28 August 1999 and all treatments were initiated on 20 September 1999. Shoot dry weight and height values are based on measurements while all other size comparisons with control plants are visual assessments.

RESULTS

Following are the progressions of visual deficiency symptoms for each element that includes early and advanced stage. Early and late leaf sampling was carried out on days as indicated in Figure 1; and the critical foliar analysis standards and tissue concentration associated with advanced deficiency is shown in Table 1.

Nitrogen

Development of light green pigmentation on the adaxial surfaces and shades of purple pigmentation on the abaxial surfaces of the lower-most mature leaves and the cotyledons were the first indication of N deficiency (day 7). At this point, the tissue N concentration was 2.58%. Plants were shorter (70% of control) and smaller (75% of control) with olive green foliage. By day 11, the purple pigmentation intensified on the abaxial surface of lower most mature leaves, and spread to upper mature leaves including petiole bases, and stems. Meanwhile, cotyledons developed chlorosis on the adaxial surfaces and pinkish purple pigmentation on the abaxial surfaces. The leaf size and plant height was 40 and 58% of control respectively. By day 21, young and recently matured leaves developed purple veination on the abaxial surfaces and lighter green coloration on

the adaxial surfaces. The leaf orientation of recently matured leaves and the leaves below them were upright. At this time, the cotyledons appeared shriveled. As the symptoms advanced (day 26), the purple pigmentation intensified to deep purple coloration, and greenish yellow chlorosis developed on the adaxial surface of mature leaves. Necrosis developed at terminal ends of older leaves as a gray discoloration that turned tan and spread toward the leaf base. Plant growth was halted with a height 40% and size 48% of control. Primary roots were thinner, shorter (60%), and fewer (40%) compared to that of the control. The tissue N concentration further dropped to 1.27% at this point.

Phosphorus

Plants were shorter and smaller (82% and 60% of the control) with slightly darker green foliage (day 7). Primary roots were shorter and appeared slightly brown (day 9). By day 11, lower mature leaves developed purple pigmentation on the abaxial surfaces. The tissue P concentration at this point was 0.06%. Young and recently matured leaves' orientation was upright. By day 19, the leaf size was considerably reduced to 25% of control. The purple pigmentation intensified while the veins were free of purple pigmentation. Veinal chlorosis developed in the mid lamina area and acropetal region of the adaxial surface of the lowest leaves, and recently matured leaves appeared dull bluish green. By day 21, cotyledons developed uniform chlorosis and the veinal chlorosis (yellow) of the lower-most leaves spread to adjacent tissues in mid-lamina zones (day 22). As the symptoms progressed (day 24), chlorosis enlarged and changed to pinkish yellow and this syndrome moved up the plants to upper mature leaves. Primary roots

were longer and necrosis developed from the basal portion of the roots. Eventually by day 32, the entire lamina of lower leaves developed orange yellow chlorosis with sporadic and random green-pigmented tissue patches on adaxial surfaces. The purple pigmentation on the abaxial surfaces gave way to purplish pink pigmentation on lower mature leaves and pink pigmentation on further-most mature leaves. Some of these leaves developed necrosis. This syndrome moved up the plants. Plant height and leaf size were 41 and <20% of control respectively. Although the primary roots were longer than in control plants by 20%, they were fewer (<10% of control) and had fewer secondary roots (<5% of control). At this point, the tissue P concentration was 0.05%.

Potassium

Plants were shorter (82 % of the control) and smaller (day 15). By day 18, plant height was 77% and leaf size was 70% of control. The plants had shorter internodes and appeared compact. At this point, the tissue K concentration was 0.41%. By day 22, light greenish gray smooth sunken necrotic spots developed on the areas adjacent to the terminal margins of lower-most leaves. At this point, tissues along the terminal margins of these leaves also developed greenish gray necrosis causing the tissues to collapse. By day 24, acropetal chlorosis (yellowish green) developed on lower mature leaves and moved inward, while the upper mature leaves remained darker green. The greenish gray necrosis turned gray and enlarged as patches. Plants remained compact with a height 50% of the control. Chlorosis of mature leaves turned greenish yellow. This syndrome progressed up the plants by day 27. Eventually (day 32), chlorosis also developed on

margins of young leaves. Areas between the necrotic patches of lower-most leaves turned chlorotic, as the patches of necrosis increased in size and number towards the leaf bases. Finally, these leaves abscised. Plant size and height was 41% of control and root growth proportionate with shoot. At this point, the tissue K concentration was 0.34%.

Calcium

Plants appeared smaller and shorter (75 and 74% of the control) with darker green foliage. Primary and secondary roots were fewer and shorter than in the control by 92% (day 5). By day 7, shades of purple pigmentation developed on the abaxial surface of lower mature leaves and necrosis developed on the shoot apex. At this point, the tissue Ca concentration was 0.74%. Leaves were fewer and smaller (65 and 40% of control). Young leaves failed to develop lateral margins and became strap-like. The terminal ends of these leaves were broader then the remaindure of the lamina and cupped upward giving a spoon-like appearance (day 9). Recently matured leaves had shorter petioles, and cupped downward at terminal ends. Overall, the shoot apices were deformed. The purple pigmentation became distinct on the abaxial surfaces of mature leaves (day 14). At this point, the plant height was 45% of the control. As the symptoms advanced (day 19) primary and secondary roots with necrotic tips developed light brown coloration along the axis. The secondary roots were abnormally short (0.2mm). Finally, by day 21, the necrotic shoot apex died while the young leaves developed severe necrotic symptoms. At this point, the plant height was 35% of the control and the tissue Ca concentration was 0.99%.

Magnesium

Initially, plants were slightly smaller and shorter with lighter green foliage (day 30). By day 32, light yellowish green interveinal chlorosis developed on the lower-most mature leaves when the tissue Mg concentration was 0.11%. The light yellowish green interveinal chlorosis turned greenish yellow (day 38). By day 44, the interveinal chlorosis became yellowish covering 30% of the lamina, and the adjoining tissues became greenish yellow. Eventually, by day 46, some of these leaves developed sporadic necrotic patches which enlarged, coalesced, and spread inwards by day 50. This syndrome moved up the plants to mature leaves. The necrosis was not restricted to the leaf margins. It formed in large areas such as one entire side of a leaf beginning at the mid-vein area or as a larger v-shaped area at the terminal ends of the leaves. The color of necrotic tissues advanced from whitish-yellow to light beige. At this point, the tissue Mg concentration was 0.28%.

Sulfur

Initially, plants were slightly shorter and smaller than in the control (day 19). By day 22, patches of chlorosis developed on the adaxial surface of lower mature leaves. Meanwhile the roots developed yellowish coloration. At this point, the tissue S concentration was 0.20%. By day 24, upper leaves became lighter green and lower mature leaves developed interveinal chlorosis. Uniform yellowish green chlorosis developed on young leaves and shoot apices (day 32). By day 38, interveinal chlorosis and shades of reddish pink pigmentation developed on young leaves. As the symptoms

progressed, by day 44, yellow patches formed as crescent patterns, a short distance from the margins of the terminal half of older leaves and progressed to white coloration by day 49. Eventually, by day 50, upper mature leaves developed reddish-pink tinges. Meanwhile, young leaves developed acropetal chlorosis, and mature leaves developed interveinal chlorosis. Finally (day 56), yellowish-white necrosis formed along the margins of older leaves, which eventually turned white. On the other hand, mid-lamina zones of recently matured leaves developed yellowish-white necrotic patches surrounded by greenish white chlorotic tissues, when the tissue S concentration was 0.58%.

Iron

In the beginning, recently matured leaves appeared lighter green (day 5). By day 7, young and recently matured leaves developed acropetal interveinal chlorosis (yellowish green). The chlorosis was more pronounced on the recently matured leaves. Plant height was 84% of control. At this point, the tissue Fe concentration was 47.3 $mg \cdot kg^{-1}$. By day 9, chlorosis intensified to greenish yellow pigmentation with a light purple cast in the basipetal zone of very young leaves. Chlorosis progressed down to lower leaves as interveinal chlorosis (day 11). By day 19, purple pigmentation developed on the very young and young leaves masking the chlorosis. Meanwhile, the interveinal chlorosis progressed to uniform yellow on some of the recently matured leaves. Finally, by day 22, these leaves developed whitish-yellow necrosis on the tips and margins of leaves that progressed inward. Primary roots were shorter and thicker with fewer

secondary roots. The plant height and leaf area was 65 and 61% of the control respectively. At this point, the tissue Fe concentration was 17.1 $mg \cdot kg^{-1}$.

Manganese

Plants were shorter and smaller (70 and 60% of control respectively), with slightly light green foliage over the entire plant (day 39). By day 42, a faint pattern of light green interveinal chlorosis developed on recently matured and mature leaves. At this point, the tissue Mn concentration was 7.0 $mg \cdot kg^{-1}$. The chlorosis spread to young and lower mature leaves (day 45). By day 47, the interveinal chlorotic pattern of mature leaves became distinct with greenish yellow pigmentation. Meanwhile, the chlorosis of very young and young leaves intensified to greenish yellow coloration. Primary roots were fewer, thinner and shorter with severe reduction in secondary root growth (day 53). By day 55, light yellow stippling developed on lower mature leaves scattered across the blade between veins. Eventually, by day 61, stippling gave way to white tan necrotic spots as they spread to upper mature leaves as yellow stippling. Meanwhile, young and recently matured leaves developed very pronounced chlorosis. Finally (day 67), the necrotic spots coalesced and enlarged on mature leaves. The primary and secondary roots were fewer (30% of control). The tissue Mn concentration at this point was 3.6 $mg \cdot kg^{-1}$.

Copper

Initially, by day 55, plants were shorter (78% of control) and smaller (70% of control) with uniformly lighter green foliage. Root development was poor with fewer primary roots (15% of control). Margins of young leaves had more numerous serrations. At this point, the tissue Cu concentration was 1.7 $mg \cdot kg^{-1}$. The leaves lacked sheen. By day 67, leaf veins appeared thinner and secondary veins were visible only at the base of the leaves. The tertiary veins were not visible at all. Petioles were thinner and narrower (control petioles were thick and flat). As the symptoms progressed (day 73), large patches of desiccated tissue occurred along the margin and randomly across the lamina of upper mature leaves. Eventually, by day 79, the desiccated areas turned to irregular tan necrotic patches along margins and in interveinal areas. These symptoms were less pronounced on recently matured leaves. The necrotic pattern moved to leaves above and below the upper mature leaves. Meanwhile, desiccation also developed in the acropetal zones of young leaves. Plant size and height were 40 and 60% of control, respectively, and leaf size was 70% of control. At this point, the tissue Cu concentration was 1.3 $mg \cdot kg^{-1}$.

Zinc

Initially on day 42, uniform light purple pigmentation appeared between the primary and secondary veins on the abaxial surface of recently matured leaves. At this point, the tissue Zn concentration was 7.1 $mg \cdot kg^{-1}$. By day 45, purple pigmentation spread to the upper portion of plants and the mature leaves became abnormally thick. Plants

were smaller and shorter by 10% and 16 % of control respectively. By day 47, purple pigmentation on the abaxial surfaces of recently matured leaves intensified, while the adaxial surface of these leaves developed shades of purple pigmentation. The purple pigmentation was less pronounced on young leaves. As the symptoms advanced (day 55), the shoot apex and young stems were unusually exposed due to reduced leaf expansion. Plant height and leaf size was 63 and 34% of control respectively. Young leaf laminas were narrow, wavy and cupped upward. Eventually (day 67), purple pigmentation on the abaxial surfaces of mature leaves faded and appeared as interveinal purple pigmentation while the adaxial surfaces developed patches of interveinal purple pigmentation in the acropetal region. Meanwhile, the young leaves also developed purple pigmentation on the margins. Finally, by day 73, the interveinal purple pigmentation gave way to chlorosis followed by necrosis (brownish purple) on the adaxial surface and the margins of mature leaves. The abaxial surfaces of these leaves developed grayish purple pigmentation. Tertiary veins of young leaves were not visible although primary and secondary veins were easily recognized by their thick and raised form. The lower-most mature leaves were free from purple pigmentation. At this point, the tissue Zn concentration was 8.0 $mg \cdot kg^{-1}$.

Boron

Plants appeared smaller with darker green foliage (day 18). By day 20, young and recently matured leaves cupped downward. Leaf size reduced to 60% and plant height to 74% of the control. At this point, the tissue B concentration was 12.9 $mg \cdot kg^{-1}$. Primary veins of very young and young leaves were raised and twisted in mid lamina area. The

lamina developed slight puckering, which resulted in spoon shaped upward cupping of leaves. Leaf texture was thick, leathery, and brittle. Lateral lamina expansion of young and recently matured leaves was partial. The margins of these leaves were smooth and less serrated. Secondary roots were short and stubby (day 22). Internodes were shorter, more so close to shoot apices. Plant height was 52% of control (day 32). As the symptoms advanced, the young and recently matured leaves were deformed and appeared strap-like due to narrow and puckered lamina with halted leaf expansion. Notches of missing tissues appeared in rows along and perpendicular to the axis of the mid-rib of these leaves. Loss of shoot apical dominance and proliferation of deformed lateral shoots closer to the terminal shoot gave rise to a rosette-like appearance (day 35). By day 45, the arrangement of secondary roots near the terminal end of primary roots developed a witches' broom-like appearance. Necrosis developed in the shoot apex and the margins of young leaves. Meanwhile, abaxial surfaces of recently matured and mature leaves developed pinkish purple pigmentation along the margins that progressed inward between the veins. At the same time, the adaxial surfaces of these leaves developed interveinal chlorosis (greenish yellow). Overall, leaves were severely distorted. At this point, the tissue B concentration was 26.8 $mg \cdot kg^{-1}$.

DISCUSSION

The rate at which symptoms occurred is an indication of the plant's sensitivity to a particular element. Ca and Fe deficiencies first occurred at 5 days followed by N and P at 7 days, K at 15 days, B at 18 days, and S at 19 days. Deficiencies requiring a long time

to occur were Mg at 30 days, Mn at 39 days, Zn at 42 days, and Cu at 55 days. Similar to other bedding plants (marigold, salvia, vinca, and zinnia –data not shown) Ornamental kale appeared to be most sensitive to Ca and Fe, and least sensitive to Cu and Zn deficiencies. The progression of symptoms from early to advanced stage occurred within 25 days in Ca, Fe, N, K, Mg, and P, and required more than 30 days in S and Zn deficient plants.

At the time of early deficiency, shoot dry weight compared to the control was very low in P (69%), Cu (66%), N (53%), Mn (45%), K (44%), Ca (37%), moderately low in B (24%), Fe (23%), Zn (22%), and slightly low in S (11%) deficient plants. However, shoot dry weight of Mg deficient plants was equal to the control. At the time of advanced deficiency, shoot dry weight reduction was severe in N (89%), Ca (87%), B (79%), P (77%), Mn (76%), Fe (64%), K (63%), Cu and Zn (54%), and least in S (9%) and Mg (8%) deficient plants (Figure 2).

The minimum critical tissue concentration of all deficient treatment except Ca and B was below the published survey range value (Table 1). B deficiency occurred when the tissue B concentration was 83% of control plant B concentration. Additionally, the early deficiency tissue B concentration was higher in ornamental cabbage than in marigold, vinca, zinnia, salvia, celosia, impatiens, pansy, and begonia (data not shown). This could mean that ornamental kale requires more B for normal growth.

Generally, purple pigmentation is associated with P deficiency (7). In ornamental kale, purple pigmentation developed on the mature leaves of N, P, Ca, and Zn deficient plants in the early stage of deficiency and on the mature leaves of B and young leaves of Fe deficient plants in the advanced stage. In the advanced stage, S deficient plants

developed reddish pink coloration on young leaves. Therefore, it is possible to misdiagnose deficiency symptoms based on the pigmentation color. However, confusion could be avoided by determining the specific areas of the plant tissues, pattern of the symptoms, and the intensity of the pigmentation.

Number of days to develop symptoms is an indication of the plant's sensitivity to the element. Although Ca and Fe deficient plants developed symptoms at the same period, the need for Ca seems to be more crucial than Fe, based on the following criteria: a) decline in shoot dry weight was greater and quicker in Ca than in Fe, b) development of necrosis at the shoot apex within 7 days caused an extensive damage in Ca deficient plants, and c) necrosis developed on roots of Ca and not Fe deficient plants.

S deficiency developed much quicker in ornamental cabbage than in vinca, zinnia, marigold, salvia, petunia, begonia, pansy and impatiens (data not shown) in spite of the atmospheric contribution of S to all of these crops (8). The progression of symptoms from chlorosis to necrosis was rapid. The early and late deficiency tissue S concentrations were higher than associated tissue P and Mg concentrations within ornamental kale. Because this was not the case in the above listed crops (data not shown) it infers that ornamental kale needs a greater amount of S for normal growth. This mandates for a special S fertilizer application program.

Synoptic visual deficiency symptoms were as follows: N- mature leaves developed greenish yellow pigmentation which turned yellowish green and finally to tan necrosis on the adaxial surfaces, and purple pigmentation on the abaxial surface; P- dark green mature leaves developed purple pigmentation on the abaxial surface and veinal chlorosis on the adaxial surface, which finally turned necrosis, young and recently

matured leaves were upright; K- light greenish-gray necrotic spots adjacent to the margins of lower-most mature leaves enlarged, and the tissues collapsed, and finally the leaves abscised, acropetal chlorosis of mature leaves; Ca- shades of purple pigmentation on the abaxial surface of mature leaves, necrosis on shoot apex and tips of roots, spoon shaped young leaves; Mg – lower-most mature leaves developed interveinal chlorosis with whitish-yellow sporadic necrotic patches, which enlarged and coalesced to light beige coloration; S – mature leaves developed interveinal chlorosis, young leaves developed shades of reddish pink pigmentation and interveinal chlorosis, and finally recently matured leaves developed necrosis; Fe- interveinal chlorosis more so on recently matured leaves became uniformly chlorotic (yellow) and finally turned to whitish yellow necrosis starting from the tips and margins, purple pigmentation on very young and young leaves; Mn – mature leaves developed interveinal chlorosis followed by light yellow stippling which then turned to white tan necrotic spots that enlarged and coalesced; Cu – young leaves had numerous serrated margins, mature leaves developed tissue desiccation which turned into tan necrosis on margins and in interveinal areas; Zn – purple pigmentation of recently matured leaves first developed on abaxial, and then on adaxial surface and became interveinal, and then chlorosis and finally necrosis, leaves were deformed; B - darker green thick, leathery and deformed foliage, notches of missing tissues on mid-rib, purple pigmentation on abaxial and interveinal chlorosis on adaxial surface of recently matured and mature leaves.

ACKNOWLEDGEMENTS

The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Research Service of the products named, nor criticism of similar ones not mentioned. Appreciation is expressed to Tom Abramowski of Rockwell Farms, N.C., the North Carolina Commercial Flower Growers' Association, and Fred C. Gloeckner Foundation for financial support, Fred C. Gloeckner Inc. for providing the seeds and Smithers-Oasis for providing the propagation medium.

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Table 1. Ornamental cabbage tissue nutrient concentration at early and advanced stages of deficiency in recently matured leaves of hydroponically grown plants in this study and published standards.

Treatment	**-N**	**-P**	**-K**	**-Ca**	**-Mg**	**-S**	**-Fe**	**-Mn**	**-Cu**	**-Zn**	**-B**
	Percent dry wt.						$mg \cdot kg^{-1}$ of dry wt.				
Element	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn	B
Control early	6.41	0.41	4.69	2.99	0.43	0.93	82.6	77.5	3.1	25.2	54.3
Deficiency early	2.58	0.06	0.41	0.74	0.11	0.20	47.3	7.0	1.7	7.1	26.8
Control advanced	6.07	0.46	4.57	2.95	0.54	0.98	65.6	59.7	4.3	17.0	66.2
Deficiency advanced	1.27	0.05	0.34	0.99	0.28	0.58	17.1	3.6	1.3	8.0	12.9
P value	0.01	0.01	0.00	0.00	0.00	0.04	0.04	0.00	0.00	0.02	0.03
Published*	3.50 - 4.50	0.20 - 0.60	3.00 - 4.00	0.50 - 1.00	0.20 - 0.40	0.20 - 1.00	50.0 - 300.0	-	3.0 - 10.0	20.0 - 75.0	20.0 - 40.0

*NCDA. Normal leaf tissue ranges for ornamental cabbage, minimum critical concentrations not identified.

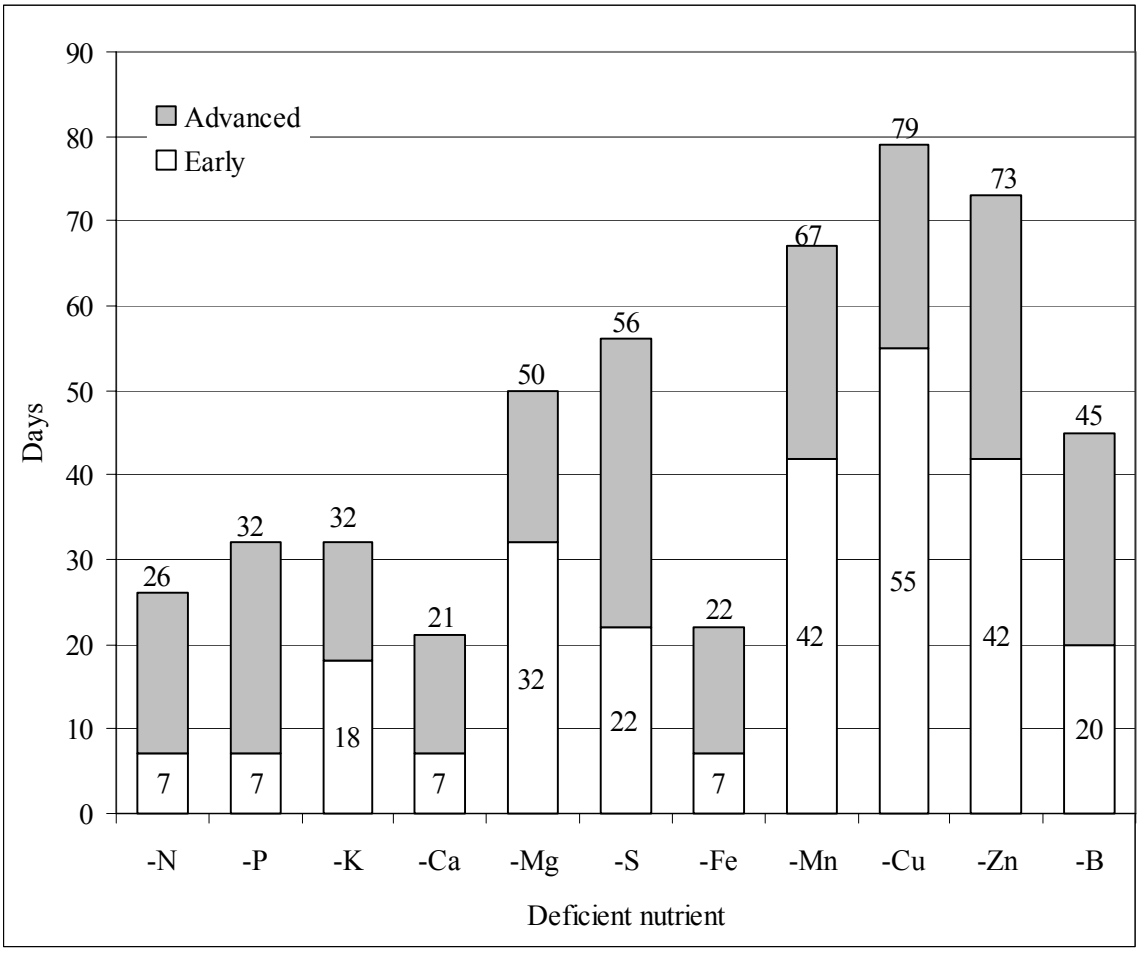

Figure 1. Days from initiation of treatments to leaf sampling for nutrient analysis for ornamental cabbage at early and advanced stages of deficiency.

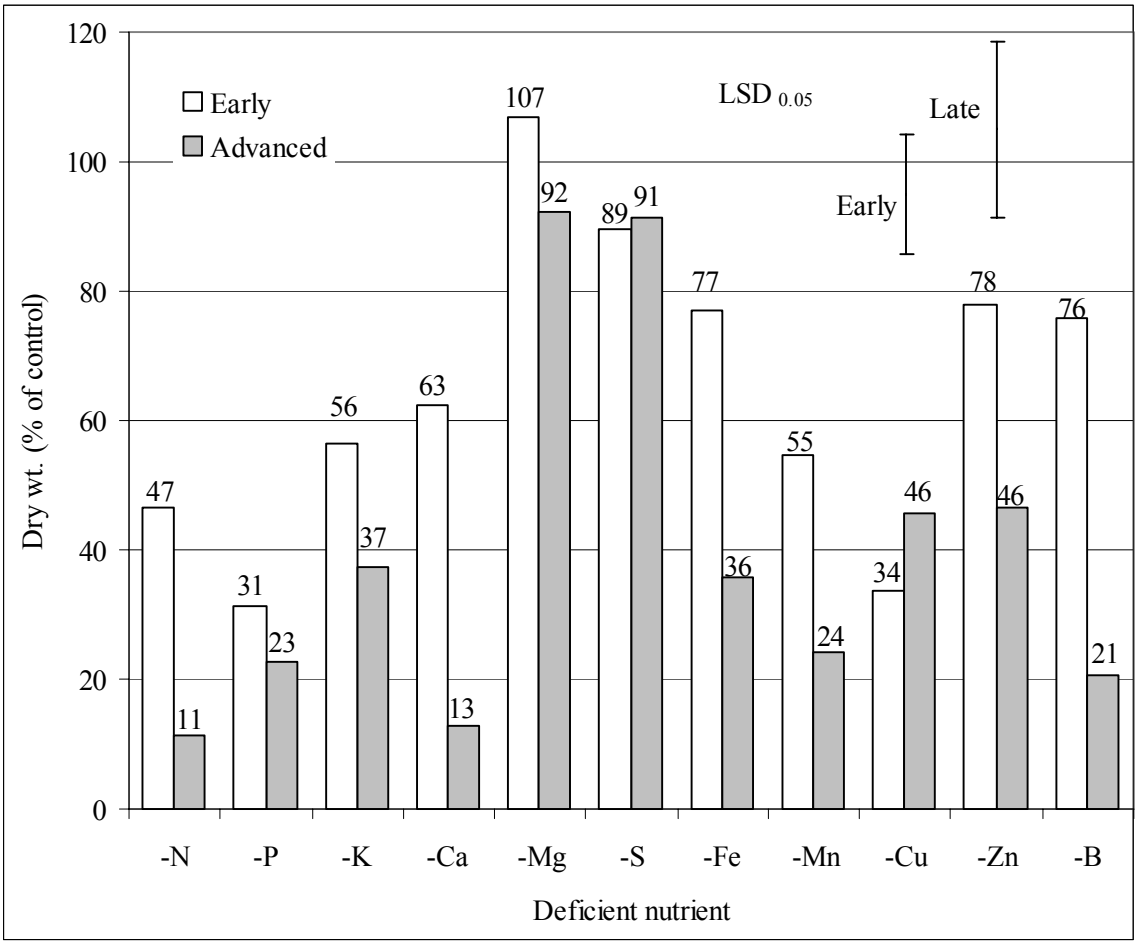

Figure 2. Ornamental cabbage dry weight of deficient nutrient plants expressed as a percentage of control plants at early and advanced stages.

CHAPTER 11

SNAPDRAGON FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF N, P, Ca, Fe, Mn, Cu, Zn and B.

James L. Gibson[1], Dharmalingam S. Pitchay[1], Brian E.Whipker[1], Brenda Cleveland[2], Paul V. Nelson[1], and F.R. Walls[2]

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

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

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SNAPDRAGON FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF N, P, Ca, Fe, Mn, Cu, Zn and B.

James L. Gibson[1], Dharmalingam S. Pitchay[1], Brian E.Whipker[1], Brenda Cleveland[2], Paul V. Nelson[1], and F.R. Walls[2]

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

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

ABSTRACT

Foliar nutrient analysis standards and characteristics of visual deficiency symptoms are indispensable monitoring tools for precision crop production system. A comprehensive study of snapdragon (*Antirrhinum majus* L.) encompassing nutritional deficiency stress environment is vital for the floriculture industry as well as for research and development. Snapdragon plants were grown hydroponically in a glass greenhouse. Treatments consisted of a complete nutrient solution and 11 additional solutions, each devoid of one essential nutrient. Deficiency symptoms and plant size were chronologically recorded for shoots and roots. Fully expanded youngest leaves were analyzed for nutrient concentration. In the absence of N, P, Ca, Fe, Mn, Cu, Zn and B, tissue concentrations at the early deficiency stage were 1.88, 0.07, 0.67 (%), 51. 9, 4.2, 1.4, 12.4 and 4.6 ($mg \cdot kg^{-1}$), respectively. At the early deficiency stage, shoot dry weight

was considerably reduced in Ca (81%), P (67%), N and Mn (62%), and B (43%), moderately in Fe (28%) and Zn (79%), and least in Cu (4%) deficient plants. At the advanced deficiency stage, a severe reduction of shoot dry weight was observed in all deficient plants except Cu. Ca was the first deficiency to occur at 9 days followed by N (11 days), Fe (12 days), P (17 days), and B (20 days). Mn, Zn, and Cu were the slowest to develop symptoms at days 43, 65, and 69 respectively.

INTRODUCTION

Snapdragon (*Antirrhinum majus* L.) captures attention when used as cut flowers or in landscapes as hanging baskets, potted plants, or ground cover. Additionally, snapdragon is a preferred plant for genetic research purposes. Snapdragon is a member of the family Scrophulariaceae. Results of a general nutritional study for N, P, Mg, S, Fe, and B (1-8), as well as survey ranges for 11 essential elements (9) have been documented for this crop. Present fertilizer formulation is solely based on the general recommendation of 2:1:2 (N: P: K:) ratio (10). Nutritional requirement varies within cultivars (11). Each element plays a unique role in plant growth and development. Thus, each elemental disorder has its own characteristic symptoms, which also vary according to species and within cultivars.

The objectives of this study were to characterize visual symptoms of nutrient deficiencies in chronological order as they appear from incipient to advance stages, and to establish foliar analysis standards that include minimal critical levels and levels associated with early and late stages of deficiencies for N, P, Ca, Fe, Mn, Cu, Zn and B.

MATERIALS AND METHODS

The protocol for this experiment was described in Pitchay et al., (12). Exceptions included: the test crop snapdragon 'Animation Red'; the sowing date on 11 November 2000; and the initiation of all treatments on 11 December 2001. Shoot dry weight and height values are based on measurements while all other size comparisons with control plants are visual assessments.

RESULTS

Following are the progressions of visual deficiency symptoms from early to late stages for each element. Early and late leaf sampling was carried out on days as indicated in Figure 1 and the resulting recently matured leaf concentrations are presented in Table 1.

Nitrogen

Initial symptoms began on day 11 when plants were smaller (48% of control) and shorter (79% of control) with thinner stems. Plants had fewer nodes with no axillary shoots compared to control. By day 13, shades of purplish-red pigmentation developed on the abaxial surfaces of lower-most leaves when the tissue N concentration was 1.88%. By day 15, symptoms progressed up the plants. Young and recently matured leaf margins were distinguished by a silhouette of darker red pigmentation. Leaf shape was more ovular and leaves became reflexed. The purplish red pigmentation slowly spread to the

adaxial surfaces (day 16). Chlorosis was not apparent, possibly due to masking of the purplish-red pigmentation. By day 19, reddish pigmentation developed on primary veins and petioles of mature leaves, and stems. Shades of purplish-red pigmentation also developed in the basipetal area of recently matured leaves. As the symptoms advanced (day 21), the pigmentation intensified to deep red coloration, more so on the abaxial surfaces. Plant height further declined to 43% of control and flowering was reduced. Tissue N concentration at this point was 0.95%.

Phosphorus

Initially, on day 17, foliage appeared deeper green, and plant size and height were 40% and 69% of the control, respectively. Deficient plants had five nodes (control had 6 nodes) and smaller leaves (51% of control) that were less lanceolated. Recently matured leaves had a thin silhouette of reddish pigmentation on the margins and drooped downward (day 19). The tissue P concentration at this point was 0.07%. By day 23, shades of reddish purple pigmentation developed on the abaxial surfaces of leaves, more so on the lower mature leaves. The primary roots were longer than the control. As the symptoms progressed (day 26), leaves, more so the recently matured leaves, curled downward like wood shavings. Pigmentation intensified to a deep reddish-purple color. Plant growth was arrested at only six nodes (12 nodes in the control) and plant height was considerably suppressed to 25% and size less than 10% of the control with thin stems. Axillary shoots and flowering were absent. Eventually, by day 31, the adaxial surfaces of leaves appeared reddish green. The tissue P concentration at this point was 0.03%.

Calcium

Plants had fewer nodes and shorter internodes that resulted in a plant height 55% and size 50% of control (day 9). Tan necrotic streaks developed along the primary root axis. Leaves were slightly curled downwards. Tissues in the basipetal area of recently matured leaves appeared bruised like. The tissue Ca concentration at this point was 0.67%. By day 10, a few tan white necrotic spots developed near the leaf base of young and recently matured leaves, which then became more numerous and spread toward the mid-lamina. Areas between the necrotic spots turned reddish. Necrotic spots enlarged and coalesced and formed necrotic patches covering about 50% and 15% of the lamina of young and recently matured leaves, respectively (day 12). Necrosis also spread toward the leaf tips of very young leaves. Primary roots were fewer and shorter (15% of control) with light brown coloration. The secondary roots were shorter (2-3 mm long) and fewer, but appeared normal. As the symptoms advanced (day 14), young leaves became severely distorted and necrotic. The shoot tips died. Plant height declined to 40% of control with five nodes (control had 7 nodes). The tissue Ca concentration at this point was 0.74%.

Iron

Development of basipetal interveinal chlorosis (yellowish green) on very young and young leaves was the initial symptom on day 12. Chlorosis was more pronounced along the basipetal margins. The tissue Fe concentration at this point was 51.9 $mg \cdot kg^{-1}$. By day 14, chlorosis turned greenish yellow as it progressed outward. As the recently

matured leaves developed basipetal interveinal chlorosis (day 16), the very young and young leaves became uniformly chlorotic and shades of red pigmentation appeared on the shoot apex. The leaves were smaller (80% of control) and plant height was 85% of the control. As the symptoms advanced (day 18), tan brown necrosis developed on terminal tips of young leaves and as it spread inward, mature leaves developed interveinal chlorosis starting from the basipetal region (day 20). Eventually, by day 22, the entire lamina of very young and young leaves including the shoot apex became totally necrotic and died. Primary roots were fewer and shorter 40% and 50% of the control respectively. The plant size and height was substantially reduced (40% and 70% of control respectively) due to fewer nodes (5 vs. 6 in control) and shorter internodes. The tissue Fe concentration at this point was 51.3 $mg \cdot kg^{-1}$.

Manganese

In the beginning (day 43), plants were shorter (86% of control). Very young and young leaves developed basipetal veinal chlorosis on the adaxial surfaces, and reddish pigmentation on the main veins of abaxial surfaces. At this point, the tissue Mn concentration was 4.2 $mg \cdot kg^{-1}$. By day 47, basipetal veinal chlorosis spread to the minor veins as the symptoms progressed outward. The upper portion of the stems and the growing points developed red pigmentation. Eventually, by day 53, tissues adjacent to the veins became chlorotic (greenish yellow) starting from the basipetal area. These symptoms spread down the plants to recently matured leaves with a similar pattern (day 57). As the symptoms progressed (day 61), leaves began to appear twisted. The tips and

terminal margins of recently matured leaves developed desiccated-like necrosis. The leaves were fewer and smaller (47% of control). The plant height further declined to 49% of control. Primary roots were considerably fewer (20% of control), thinner and shorter with fewer and shorter secondary roots. The tertiary roots were not visible. At this point, the tissue Mn concentration was 3.1 $mg \cdot kg^{-1}$.

Copper

Initially, on day 69, plants were slightly shorter (93% of control) with slightly smaller leaves (92% of control). Recently matured and mature leaves on the upper portion of the plants appeared reflexed. At this point, the tissue Cu concentration was 1.4 $mg \cdot kg^{-1}$. Dark pigmentation formed on the recently matured leaves (3rd node from shoot apex) that had a bluish-green appearance, particularly, over the veins (day 70). Then, dark reddish-brown necrotic patches formed on the mid-ribs of these leaves and spread lengthwise. This was followed by development of desiccation on petioles (4th node from shoot apex) that spread toward the leaf bases (day 72). By day 73, the petioles shriveled and became totally necrotic and the green leaves hanged down. This syndrome rapidly progressed down the plants. Finally, by day 74, the stems at the internodes of the affected leaves became necrotic and collapsed. Plant size and height was 55% and 65% of control respectively. There were no signs of lateral shoot development and flower bud formation as compared to the control. The tissue Cu concentration at this point was 2.2 $mg \cdot kg^{-1}$.

Zinc

Initially, young and recently matured leaves developed a faint silhouette of pinkish purple pigmentation on the adaxial surface margins, and reddish purple pigmentation on the abaxial surfaces when the tissue Zn concentration was 12.4 $mg \cdot kg^{-1}$ (day 65). The very young leaves appeared normal. By day 73, the silhouette became distinct on these leaves, and reddish purple pigmentation on the abaxial surfaces of these leaves intensified. This syndrome progressed down the plants to mature leaves (day 79). As the symptoms advanced (day 87), upper mature leaves developed reddish pigmentation on primary veins. Basipetal chlorosis developed on very young and young leaves. The young leaf lamina was narrow and appeared elongated with rounded tips. The stems were purplish-pink with poorly developed lateral shoots. The internodes were shorter, more so on the upper portion of the plants giving the shoot a rosette-like appearance. Although the number of nodes was equal to that of the control, the plants appeared shorter (45% of control) due to shorter internodes. Flower buds developed, however they were aborted. By day 94, rosette-like appearance became severe as a result of loss of apical dominance and proliferated lateral shoot growth. The abaxial surfaces of young and mature leaves turned purplish brown. Finally, by day 109, acropetal necrosis developed on young leaves and progressed inward. The tissue Zn concentration at this point was 9.4 $mg \cdot kg^{-1}$.

Boron

In the beginning, on day 20, plants were smaller and shorter (77% of the control). The foliage was darker green, mainly in the tissues closer to primary and secondary veins, and slightly glossy. Leaves were thick, leathery, and more ovular shaped and curled downward. The tissue B concentration at this point was 4.6 $mg \cdot kg^{-1}$. Young leaves were partially expanded and appeared narrow and strap-like with slight twisting (day 24). Meanwhile, some of the recently mature leaves developed mild puckering and indentation at leaf bases. As the symptoms advanced (day 28), young and recently matured leaves developed severe crinkling, puckering, and indentations that gave way to distorted spoon-shaped lamina. By day 32, the shoot meristem growth stopped and apical dominance was lost. Lateral branches only emerged from the lower portion of the plants. Lateral shoots developed similar but more severe symptoms of leaf and shoot distortion. The primary and secondary roots were thick, short (25% of control), and stiff. However, numerous lateral roots developed closer to terminal tips of primary roots giving a witches' broom-like appearance. There was no sign of flower bud formation unlike the control, and necrosis developed at the shoot apex (day 38). Plant height was 56% of the control and leaf size was 60% of the control. The tissue B concentration at this point was 4.7 $mg \cdot kg^{-1}$.

DISCUSSION

Ca was the first deficiency to occur at 9 days followed by N (11 days), Fe (12 days), P (17 days), and B (20 days). Mn, Zn, and Cu were the slowest to develop symptoms at days 43, 65, and 69, respectively. The order at which the advance symptoms occurred was more or less similar to the order of early symptoms, except for Zn and Cu. The progression of symptoms from early to advance was quicker in Ca and Cu, which took only 4 days. Compared to all the elements, Zn was the slowest to develop advanced symptoms.

At the early stage of deficiency, shoot dry weight was considerably reduced in Ca (81%), P (67%), N and Mn (62%), and B (43%), moderately in Fe (28%), and Zn (79%), and least in Cu (4%) deficient plants. At the advanced deficiency stage, a severe reduction of shoot dry weight was observed in all deficient plants except Cu (Figure 2).

The deficient tissue Fe concentration at early and advanced stages of deficiency were only less by 15.7 and 17.5 $mg.kg^{-1}$ of control plants, respectively. The tissue Fe concentration in control plants was lower in this crop than salvia, marigold, vinca, and zinnia (data not shown). The Fe deficiency symptoms developed quickly and affected the dry weight moderately at early deficiency and severely at advanced deficiency. This could mean that the plant is Fe sensitive or inefficient.

Shoot dry weight is not always an indication that the plant is severely affected by the deficient element. The main stems of Cu deprived plants quickly and drastically collapsed, although, the decline in shoot dry weight was minimal at early as well as advanced stages. This might have been due to the following reasons. Cu has been

implicated as being recycled when senescence of leaves occurs (13). In Cu deficient plants, this process might have taken place at a very early stage of symptoms. Since, Cu is important in lignification (14), the tissues from where Cu was extracted quickly and drastically collapsed. This would render it almost impossible to recover from most of the stages of Cu deficiency. Moreover, in most instances, Cu deficiency symptoms could be easily misdiagnosed for disease.

N and P deficiency symptoms may be easily misdiagnosed due to close resemblance of the symptoms. In both deficiencies, the expression of red pigmentation masking the chlorosis can be correlated with the flower color. This could be avoided by observing the hue saturation, extent of the rolling of leaf margins, the difference in foliage color, and the length of primary roots.

Synoptic visual deficiency symptoms were as follows: N - abaxial surfaces of mature leaves developed reddish purple pigmentation that spread to adaxial surfaces, darker red pigmentation on leaf margins, ovular shaped leaves; P – leaves developed deep reddish purple pigmentation on the abaxial surfaces, leaves had reddish silhouette margins and curled downward, severe reduction of plant size and height, longer primary roots; Ca – necrotic spots on young and recently matured leaves enlarged and coalesced to large necrotic patches, severe distortion of leaves, and shoot tips died; Fe - very young and young leaves developed basipetal interveinal chlorosis, then uniform chlorosis and finally necrosis, and died together with the shoot apex; Mn - very young and young leaves developed veinal chlorosis on the adaxial surfaces that spread to the adjacent tissues, and reddish veins on the abaxial surfaces, and finally necrosis developed on the tips and margins of mature leaves; Cu - midribs of recently mature leaves developed

reddish brown necrotic patches, petioles became necrotic causing the leaves to hang down, and finally, the stems at the internodes of the affected leaves became necrotic and collapsed; Zn – young leaf margins had a pinkish-purple silhouette on adaxial surfaces and reddish purple pigmentation on abaxial surfaces that progressed down the plants, purplish pink stems, shorter internodes, finally, acropetal necrosis of leaves; B - Leaf shape was more ovular than lanceolated, leaf texture was thick and leathery, young leaves were partially developed, then became spoon-shaped, roots were thick, short, and stiff.

ACKNOWLEDGMENTS

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Table 1. Snapdragon tissue nutrient concentration at early and advanced stages of deficiency in recently matured leaves of hydroponically grown plants in this study and published standards.

Treatment	**-N**	**-P**	**-Ca**	**-Fe**	**-Mn**	**-Cu**	**-Zn**	**-B**
	Percent dry wt.			$mg \cdot kg^{-1}$ of dry wt.				
Element	N	P	Ca	Fe	Mn	Cu	Zn	B
Control early	5.16	0.50	1.13	67.65	71.73	5.42	20.58	23.77
Deficiency early	1.88	0.07	0.67	51.89	4.20	1.39	12.39	4.60
Control advanced	5.10	0.39	1.25	68.87	65.72	4.25	26.57	17.23
Deficiency advanced	0.95	0.03	0.74	51.28	3.05	2.16	9.40	4.73
P value	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.12
Published*	3.80 - 5.00	0.30 - 0.50	1.00 - 1.50	75.0	139.0	9.0	56.0	30.0

*Mills and Jones, 1996. Survey average of concentrations associated with acceptable crop performance, minimum critical concentrations not identified.

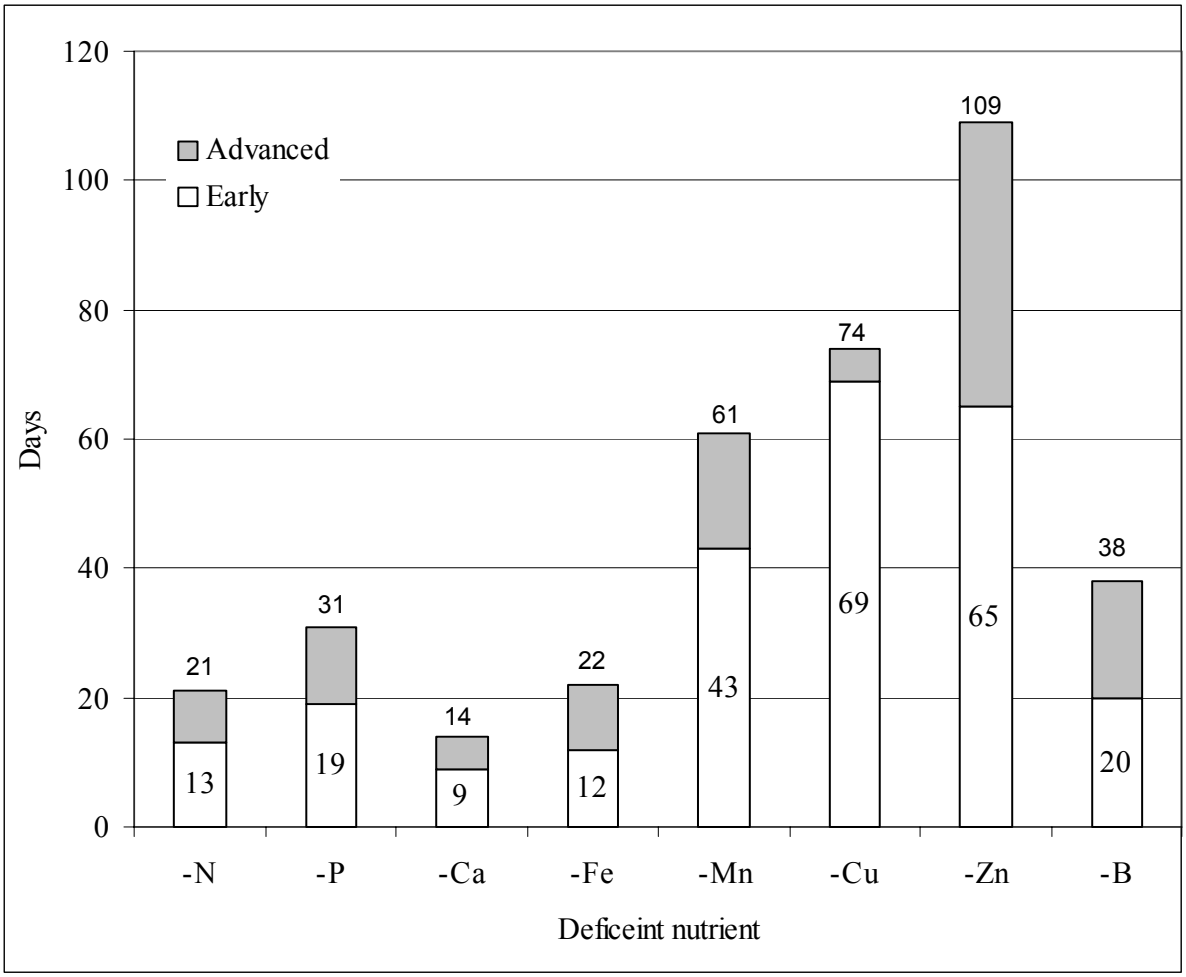

Figure 1. Days from initiation of treatments to leaf sampling for nutrient analysis for snapdragon at early and advanced stages of deficiency.

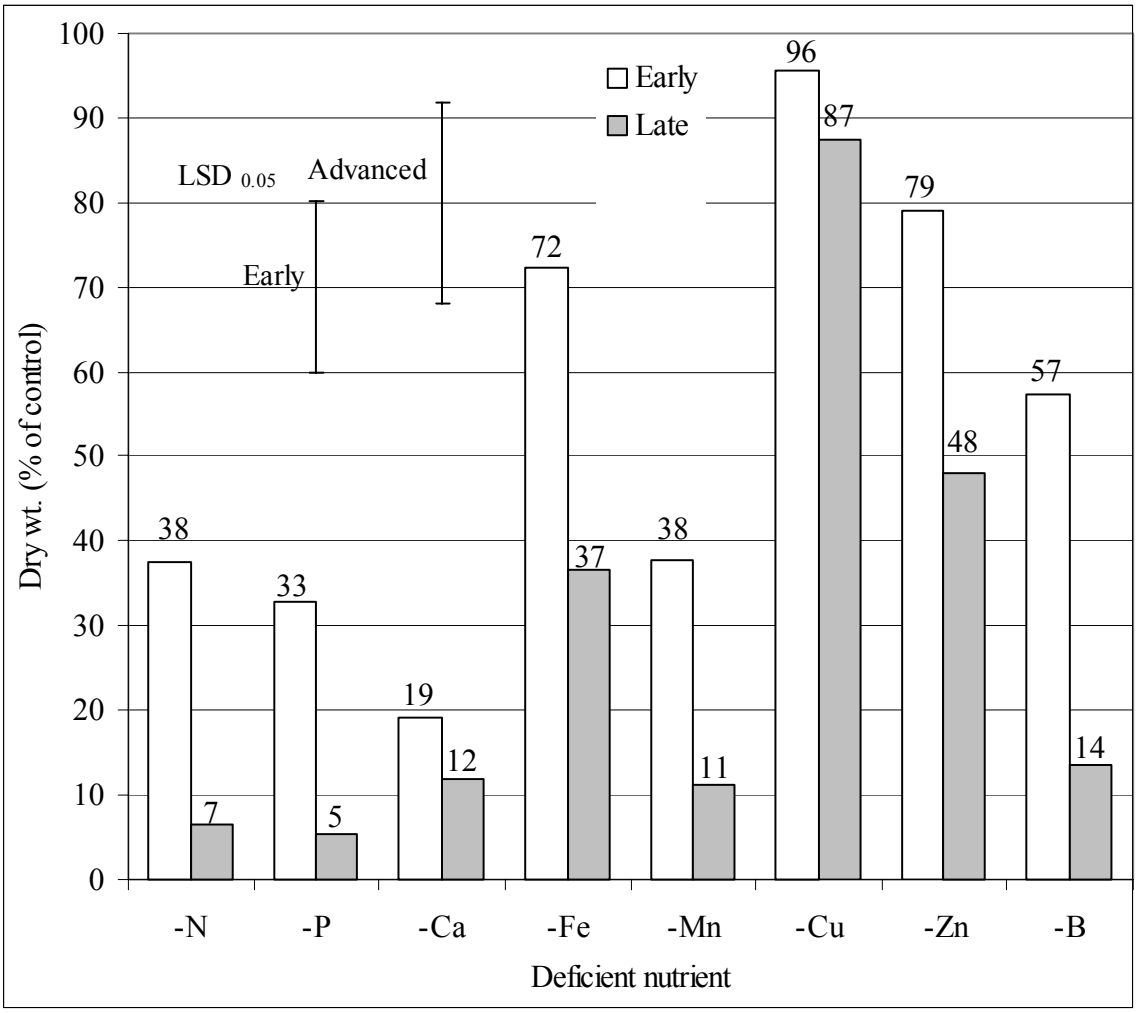

Figure 2. Snapdragon dry weight of nutrient deficient plants expressed as a percentage of control plants at early and advanced stages.

CHAPTER 12

NEW GUINEA IMPATIENS FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Dharmalingam S. Pitchay[1], James L. Gibson[1], Brenda Cleveland[2], Paul V. Nelson[1], F.R. Walls[2], and Brian E.Whipker[1]

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

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

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NEW GUINEA IMPATIENS FOLIAR ANALYSIS STANDARDS AND DEFICIENCY SYMPTOMS OF 11 NUTRIENTS

Dharmalingam S. Pitchay[1], James L. Gibson[1], Brenda Cleveland[2], Paul V. Nelson[1], F.R. Walls[2] and Brian E.Whipker[1]

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

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

ABSTRACT

To produce a premium crop on commercial scale, precise nutritional standards are indispensable. These values have not been established for New Guinea impatiens cultivars 'Grenada' and 'Timor' (*Impatiens hawkeri Bull*). Experiments were conducted by growing the plants hydroponically in a glass greenhouse. Treatments consisted of a complete modified Hoagland nutrient solution and 11 additional solutions, each devoid of one essential nutrient (N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn and B). Deficiency symptoms and plant size were chronologically recorded for shoots. Fully expanded youngest leaves were analyzed for nutrient content. At the early stage of deficiency, shoot dry weight reduction was minimal in Mg, S, and Fe deficient plants of both cultivars compared to the control. In addition, minimal decrease in shoot dry weight was observed in B deficient

plants of ‘Timor’, and P and Zn deficient plants of ‘Grenada’. Cu deficient plants of ‘Timor’ (55% of control) and K deficient plants of ‘Grenada’ (65% of control) had the lowest dry weight. At the advanced stage of deficiency, reduction of shoot dry weight was greater in K, Ca, S, Fe, Mn, Cu, and B deficient plants of ‘Grenada’. N and Ca deficiencies were the first to occur at 16 and 10 days, respectively suggesting that New Guinea impatiens is prone to these deficiencies. Delayed development of symptoms was observed in S, Mn, Cu, and Zn deficient plants of both cultivars. At the early stage of deficiency, tissue N, K, and Zn concentrations were slightly lower in ‘Grenada’ than ‘Timor’. On the other hand, in control plants, tissue K, Fe, Mn, Zn, and B concentrations of ‘Grenada’ were higher than ‘Timor’. There were only slight differences in shoot dry weight and days to develop visual symptoms between the two cultivars. Tissue nutrient concentrations were very similar.

INTRODUCTION

New Guinea impatiens is an excellent hanging basket plant, which has gained considerable popularity among consumers. To grow premium crops, precise nutritional standards are indispensable. In spite of its significant role in floriculture production, limited information is available on foliar nutritional standards. General guideline ranges for 10 essential elements (1), and survey ranges for 11 essential elements (2) have been reported. Values in these ranges are associated with acceptable growth, but do not establish the minimum and maximum critical values. Variation in nutrient requirement exists even among cultivars (3). Present fertilizer formulation is solely based on general

recommendations of a 2: 1: 2 (N: P_2O_5: K_2O) ratio (4) and a N fertilization rate of 100 $mg \cdot L^{-1}$.

The objectives of this study were to characterize visual symptoms of nutrient deficiencies from incipient to advance stages and to establish foliar analysis standards for early and advanced deficiency stages for N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, and B.

MATERIALS AND METHODS

The protocol for this experiment was reported in Pitchay et al., (5). Exceptions included: the treatment solutions consisted of ▯ strength of complete modified Hoagland's solution consisted of the following mM concentrations of macronutrients, 5.0 NO_3-N, 0.33 PO_4-P, 2.0 K, 1.67 Ca, 0.67 Mg, and 0.67 SO_4-S (7), plus µM concentrations of micronutrients, 72 Fe, 9.0 Mn, 1.5 Cu, 1.5 Zn, 45.0 B, and 0.1 Mo; the test crops New Guinea impatiens 'Grenada' and 'Timor'; rooting date 24 January 2001; and initiation of all treatments on 1 March 2001. Shoot dry weight and height values are based on measurements while all other size comparisons with control plants are visual assessments.

RESULTS

Following are the progressions of visual deficiency symptoms for each element that includes early and advanced stage. Early and late leaf sampling was carried out on

days as indicated in Figures 1a and 1b, and the resulting recently matured leaf concentrations are presented in Tables 1a and 1b.

Nitrogen

'Timor' - Plants were smaller (40% of control) and shorter (60% of control) with lighter green foliage (day 16). Overall, plant growth was reduced drastically, and light red pigmentation developed on stems, petioles and primary and secondary veins, more so on abaxial surfaces of lower mature leaves. At this point, the tissue N concentration was 1.95%. Lateral shoots were smaller (40% of control). Uniform basipetal chlorosis developed on lower mature leaves (day 21). By day 25, chlorosis progressed outward as a band close to the acropetal area. Meanwhile, the red pigmentation extended as red patches on both adaxial and abaxial surfaces of lower mature leaves. This was followed by a development of necrosis on terminal tips of these leaves. This syndrome then moved up the plants. As the symptoms advanced (day 29), plant size and height declined further (30% and 40% of control respectively). Leaf size was 50% of control. The basal portion of roots also developed red pigmentation. Eventually, by day 35, young leaves became uniformly chlorotic (greenish yellow) with light random patches of red pigmentation on tissues adjacent to primary and secondary veins. Flowers and buds also developed red pigmentation (control was brownish green). Lateral branching was drastically reduced in size and length (in control the lateral branching was equal to the main stems). The tissue N concentration at this point declined to 1.09%.

'Grenada' - Initial symptoms began on the same day as 'Timor'. However, advanced symptoms developed six days earlier than 'Timor'. The symptoms were similar to 'Timor' with the following exceptions. The red pigmentation was less intense on the stems, petioles, and veins. The red pigmentation had a purple cast in it. The growth of stems and lateral branching was significantly reduced compared to that of 'Timor'. Young leaves developed shades of reddish purple pigmentation in the basipetal areas. 'Timor' was more robust growing than 'Grenada'. The tissue N concentrations were 1.73% at the early stage and 1.14% at the advanced stage of deficiency.

Phosphorus

'Timor' - Plants appeared smaller with slightly darker green foliage (day 10). The darker green foliage was due in part to the development of purple pigmentation over the green lamina. At this point, the tissue P concentration was 0.07%. Lower mature leaves developed shades of purplish pink coloration in the mid lamina areas (day 14). Stems and veins of lower mature leaves appeared more pinkish compared to that of control (light greenish pink). Flower buds were absent at this point. As the symptoms progressed (day 23), purplish pink pigmentation was gradually masked by chlorosis (yellowish green). The stems were dark maroon. By day 27, these symptoms moved up the plants. The abaxial surfaces of the affected leaves were light reddish. Eventually, by day 30, brown necrosis developed along the acropetal margins of lower mature leaves, when the tissue P concentration was 0.05%.

'Grenada' – Initially, the shoot apex appeared darker green and the basipetal zones of mature leaves had a purplish black appearance (day 12). At this point, the tissue P concentration was 0.10%. By day 15, purplish black pigmentation intensified. However, the very ends of leaf tips were darker green. This could be due to this darker foliage cultivar. Light green necrotic patches developed on leaf bases of mature leaves (day 21), and gradually turned to brown necrosis (day 26). Eventually, the necrotic patches enlarged and coalesced, as the symptoms moved up the plants (day 29). The stems and veins appeared blackish. The tissue P concentration at this point further declined 0.06%

Potassium

'Timor' – Initially, on day 12, plants appeared darker green due to darker green leaves (more so the upper leaves) including the veins (control had pinkish green veins). The young and upper mature leaves developed upward puckering and curled downward, while lower mature leaves developed a few tiny black interveinal necrotic spots in mid lamina areas. At this point, the tissue K concentration was 0.53%. By day 14, the number of necrotic spots increased on these lower leaves. As the symptoms progressed (day 17), the necrotic spots enlarged. This syndrome progressed up the plants to other leaves except the young leaves. Meanwhile, the tissues adjacent to the necrotic spots turned chlorotic, and some of the lower mature leaves developed necrosis on tips and margins, and began to move inward (day 19). At this point, stems also appeared darker colored.

The plants appeared compact due to shorter internodes especially at the base of the plants (length <10% of control). The tissue K concentration at this point was 0.50%.

'Grenada' – Most of the symptoms were similar to 'Timor', although the development of initial symptoms were delayed in 'Grenada' (day 25), at which point the tissue K concentration was 0.43%. Plants had fewer internodes and lateral shoots. The internodes were shorter, thinner and black colored. Leaf puckering was severe, and the leaves were oval shaped. Initially, black necrotic spots were not clearly visible due to darker colored foliage. However, as the symptoms advanced, they were visible as large brown necrotic spots in the acropetal areas of lower mature leaves. On the other hand, terminal tips and margins of lower mature leaves developed black necrosis, which then turned to brown and spread inward as the symptoms advanced rapidly (day 30). Meanwhile, the remaindure of the lamina turned chlorotic. The tissue K concentration at this point was 0.44%.

Calcium

'Timor' – Small streaks of black necrosis developed along the mid-veins in basipetal areas of recently matured leaves (day 10). At this point, the tissue Ca concentration was 0.72%. By day 13, the necrosis spread to the adjacent tissues as irregular black necrotic patches which was visible on both surfaces of the lamina. The patches then enlarged and spread outward (day 15). By day 19, the symptoms spread to younger leaves except the very young leaves and terminal apices. Eventually, by day 23,

recently matured leaves, and some of the mature leaves just below the recently matured leaves abscised. The tissue Ca concentration further dropped to 0.42%.

'Grenada' – Initially, by day 10, symptoms developed on roots as black necrotic streaks along the primary roots, when the tissue Ca concentration was 0.86%. By day 11, the necrotic symptoms spread to secondary roots, as the necrosis on primary roots became severe. At this point, the mature leaves wilted. The wilting symptoms were pronounced on sunny days, however, and the plants recovered from wilting during the night and early morning. The period of wilting period gradually increased, and by day 15, all the leaves wilted without recovery. Finally, by day 16, roots began to disintegrate. Although there were fewer flowers (compared to control), flowering was slightly earlier by 2 days than control. At this point, the Ca concentration was 0.74%.

Magnesium

'Timor' – Initially, on day 19, bruised-like interveinal patches developed randomly across the acropetal zones of lower mature leaves. At this point, the tissue Mg concentration was 0.07%. The symptoms turned to light black necrosis and enlarged. By day 24, the symptoms progressed up the plants to recently matured and young leaves. As the symptoms progressed, by day 30, the necrosis of mature leaves turned brown and the abaxial surfaces of these leaves developed red pigmented patches. The tissue Mg concentration remained at 0.07% at this point.

'Grenada' – Similar symptoms were observed in 'Grenada' cultivar. However, there was no development of red pigmentation and the progression of symptoms from

early (day 19) to advanced stage (day 25) was quicker compared to 'Timor'. The tissue Mg concentration was 0.09% at early, and 0.06% at advanced deficiency stages.

Sulfur

'Timor' – In the beginning on day 50, young and recently matured leaves developed uniform chlorosis in the basipetal areas, at a tissue S concentration of 0.08%. By day 57, chlorosis spread outward toward acropetal area. The petioles and veins were yellowish green unlike the control, which were pinkish green (day 62). By day 69, chlorosis spread down the plants to mature leaves. Streaks of black necrosis developed on the primary veins of upper mature leaves. Flowering was earlier than in the control. By day 72, chlorosis along the margins of lower mature leaves intensified to yellow and spread inward. The acropetal and basipetal zones were free from this chlorosis. Finally, as the symptoms progressed (day 77), regular sunken necrotic spots developed randomly along the mid-veins of recently matured and upper mature leaves. These symptoms were also visible on the undersurface of the lamina. The tissue S concentration at this point was 0.05%

'Grenada' – the symptoms were similar to 'Timor' except that chlorosis appeared less intense due to red pigment in this cultivar. The abaxial surfaces appeared faded red. The flower petals developed faded pigmentation and gradually turned to pinkish white (control was reddish orange). Tissue S concentration was 0.09% at the early stage (day 53), and 0.07% at the advanced stage (day 78).

Iron

'Timor' – Initially, on day 8, faint basipetal chlorosis (yellowish green) developed on young leaves. At this point, the tissue Fe concentration was 43.9 $mg \cdot kg^{-1}$. By day 15, recently matured leaves developed mild light green interveinal chlorosis, which then became distinct as greenish yellow chlorosis by day 19. Meanwhile, petioles and stems appeared faded pink. With prolonged deprivation of Fe, the symptoms spread to mature leaves immediately below the recently matured leaves (day 35). In the meantime, chlorosis on the young leaves appeared in a distinct interveinal pattern except at the margins which were yellowish. Eventually, by day 48, the interveinal chlorosis gave way to total chlorosis (yellowish) covering the entire lamina including the mid-veins and petioles on some of the young leaves. Pedicels, sepals, and flower buds developed a faded greenish yellow color. The flowers also appeared light colored compared to the control. Eventually by day 65, streaks of black markings developed sporadically on mid-veins and the adjacent tissues of recently matured leaves and the upper mature leaves. The entire growing points were chlorotic without any interveinal pattern. At this point, the tissue Fe concentration was 55.6 $mg \cdot kg^{-1}$.

'Grenada' - The symptoms were similar to 'Timor', except that the growing points, mid-veins of young leaves, petioles, and buds developed light pink pigmentation. The interveinal pattern was less distinct due to the dark leaf color of this cultivar. Leaf margins, and tips had distinct chlorosis (yellowish) compared to the interior part of the lamina. The tissue Fe concentration was 47.8 $mg \cdot kg^{-1}$ at the early stage (day 11), and 52.8 $mg \cdot kg^{-1}$ at the advanced stage (day 72).

Manganese

'Timor' – Initially, on day 50, light green basipetal chlorosis developed on young leaves. The tissue Mn concentration at this point was 5.8 $mg \cdot kg^{-1}$. Chlorosis turned to greenish yellow pigmentation and spread to recently matured leaves (day 56). Chlorosis then moved down to the mid-lamina areas of mature leaves just below the recently matured leaves in an interveinal pattern (day 67). The margins and acropetal areas that were directly exposed to light became tan green. By day 75, the tan green pigmentation intensified to interveinal brown coloration. The epidermal layer appeared normal. Eventually, by day 94, young leaves developed brown patches and spots along the margins and tips. Meanwhile, these symptoms appeared severe on the mature leaves just below the recently matured leaves. The bases of these leaves were free from these symptoms. The tissue Mn concentration at this point was 4.0 $mg \cdot kg^{-1}$.

'Grenada' – Chlorosis was distinct on recently matured and mature leaves and moderate on young leaves (day 60). The tissue Mn concentration was 5.8 $mg \cdot kg^{-1}$ at this point. Margins and acropetal zones of recently matured leaves and the leaves just below them developed brown pigmentation (day 75). This was followed by development of brown stippling on young and mature leaves (day 79), which enlarged to sporadic sunken circular and necrotic spots across the lamina (day 85). In some instances, young leaves developed necrosis along the margins. As the symptoms advanced (day 96) young leaves also developed numerous fine stippling and puckering at acropetal areas. Flowers were fewer. The tissue Mn concentration was 4.2 $mg \cdot kg^{-1}$ at this point.

Copper

'Timor' - Initially, on day 64, young and recently matured leaves developed yellowish green chlorosis along the margins of basipetal area, when the tissue Cu concentration was 1.6 $mg \cdot kg^{-1}$. By day 69, chlorosis progressed inward while the acropetal areas of these leaves appeared darker green. Chlorosis then progressed outward towards the acropetal tips and slight puckering occurred along the mid-vein areas (day 74). This syndrome moved down the plants to mature leaves just below the recently matured leaves (day 79). Meanwhile, young leaves began to curl upward along the longitudinal margins making the lamina narrow. The stems were thinner with shorter internodes. As the symptoms advanced (day 84) puckering along the mid veins became severe, more so on young leaves. The rolling of leaf margins gradually increased in young leaves giving a canoe-like appearance. Eventually, very young and some of the young leaves developed necrosis along the rolled margins and veins (day 90). Flower buds including the pedicels began to shrivel and dry up just before anthesis. This syndrome of shriveling, withering, and drying of flower buds also occurred on young buds resulting in bud abortion (day 96). Leaf primordial and the mid-lamina areas and margins of recently matured and upper mature leaves withered, shriveled, and dried up. The tissue Cu concentration remained at 1.6 $mg \cdot kg^{-1}$ at this point.

'Grenada' – Chlorosis appeared mild due to dark leaf cultivar. However, along the mid-lamina areas chlorosis was severe. Other than that, the visual symptoms appeared the same in both cultivars. The tissue Cu concentration was 2.2 $mg \cdot kg^{-1}$ at the early stage (day 74), and 2.0 $mg \cdot kg^{-1}$ at the advanced stage (day 96).

Zinc

'Timor' – In the beginning, on day 55, plants developed basipetal chlorosis on young leaves. The tissue Zn concentration at this point was 18.6 $mg \cdot kg^{-1}$. The chlorosis gradually spread to recently matured leaves (day 57). Shades of black spots developed sporadically and randomly over veins and the adjoining tissues (day 59). By day 62, the spots enlarged and coalesced as long streaks of black necrosis along the veins, and as patches on the adjacent tissues of young and recently matured leaves. Eventually, by day 64, the black pigmented tissues began to disintegrate and gave way to black outlined irregular tan-black necrotic patches. The adjacent tissues close to these necrotic patches appeared chlorotic. On the abaxial surfaces, these symptoms appeared as light black shades over veins and tissues adjacent to it. Margins of very young and young leaves rolled upward, more so in the basipetal areas giving a bottleneck-like appearance. At this point, the tissue Zn concentration was 17.7 $mg \cdot kg^{-1}$.

'Grenada' – The symptoms began on day 74, when the tissue Zn concentration was 15.4 $mg \cdot kg^{-1}$. Chlorosis was less distinct due to dark colored leaves. Prominent leaf puckering along the mid-veins and the absence of dark colored patches prior to necrosis was observed. The necrotic spot-like patches were sporadic and developed randomly across the lamina of young and recently matured leaves. On the abaxial surfaces of these leaves, numerous smaller brown necrotic spots were observed across the lamina which were not visible on the adaxial surfaces. At the advanced stage on day 78, the tissue Zn concentration was 13.7 $mg \cdot kg^{-1}$.

Boron

'Timor' – young and recently matured leaves were less lanceolated with slightly chlorotic margins (day 17), when the tissue B concentration was 6.1 $mg \cdot kg^{-1}$. The leaves were thick and glossy with red-pigmented thick and raised prominent veins. Petioles were shorter, thicker, and broader with bright red pigmentation. Upper portion of the stems was thicker with shorter internodes. As the symptoms progressed (day 29) numerous lateral shoots were clustered together giving an overall bonsai-like dwarf appearance. Hydothodes secreted highly viscous fluids which solidified as white crystals. As the symptoms advanced (day 34) young and recently matured leaves developed acropetal desiccation. The symptoms progressed inward, as the desiccated areas became withered (day 42). Flower buds were tightly enclosed by thick and uneven sepals and appeared glossy. Pedicels were thick and short. Flowers were faded and partially formed with missing tissues. The petals were wavy and wrinkled with jagged margins. The tissue B concentration further dropped to 4.9 $mg \cdot kg^{-1}$ at this point.

'Grenada' – The symptoms were similar to those of 'Timor' with the following exceptions. Development of initial symptoms was delayed (day 25) and occurred at tissue B concentration of 9.8 $mg \cdot kg^{-1}$. The tissue B concentration was 7.6 $mg \cdot kg^{-1}$ at the advanced stage (day 43). Severe leaf twisting and crinkling were noted. Stems and shoots darker and glossier.

DISCUSSION

The rate of development of deficiency symptoms is a good indication of the sensitivity of New Guinea impatiens to a nutrient deficiency. The order of occurrence of deficiencies for the two cultivars collectively from first to last was Ca = Fe, P, N, K, B = Mg, S, Mn, Cu, Z. The main difference between the cultivars involved K. K deficiency occurred rapidly in 'Timor' and late in 'Grenada' indicating a sensitivity to this deficiency in 'Timor'. Overall, New Guinea impatiens appear to be prone to Ca, Fe, and P deficiencies.

Tissue concentrations of nutrients at the early stage of each deficiency were similar in both cultivars. Using the highest value of the two cultivars for each nutrient the following set of early deficiency standards emerge for N 1.95%, P 0.10%, K 0.53%, Ca 0.86%, Mg 0.09%, S 0.09%, Fe 48 $mg \cdot kg^{-1}$, Mn 5.8 $mg \cdot kg^{-1}$, Cu 2.2 $mg \cdot kg^{-1}$, Zn 18.6 $mg \cdot kg^{-1}$, and B 9.8 $mg \cdot kg^{-1}$.

Reduction in dry weight gain was very minimal (10% or less) in both cultivars for Fe, S and Mg deficiency and B in 'Timor' and P and Zn in 'Grenada'.

Time to expression of Ca deficiency symptoms was 2 days earlier in 'Grenada'. The shoots of 'Grenada' wilted before developing other symptoms due to severe necrotic injury of the roots. This suggests that 'Grenada' is more sensitive to Ca deficiency. Generally, Ca deficiency targets the shoot apex. However, in both cultivars, the shoot apex did not develop necrotic symptoms. This might have been due to characteristics of species within the family Balsaminaceae where Ca is mobilized to shoot apex.

Fe was the first deficiency to occur in 'Timor' and the second in 'Grenada'. However, advanced symptoms of Fe deficiency occurred very slowly over 57 and 61 days in 'Timor' and 'Grenada', respectively. Additionally, the progression of Fe symptoms occurred over a longer period in New Guinea impatiens than in marigold, salvia, celosia, vinca, zinnia, impatiens (data not shown). On top of that, Fe deficient plants failed to develop necrotic symptoms. This suggests that while New Guinea impatiens is initially sensitive to Fe deficiency, it quickly develops efficiency in utilizing the element at later stages.

Synoptic visual symptoms were as follows: N- 'Timor' basipetal uniform chlorosis of mature leaves turned red on adaxial and abaxial surface, and petioles, primary and secondary veins of mature leaves and stems developed red pigmentation. 'Grenada' similar symptoms except the red pigmentation had red cast; P- 'Timor' darker green foliage, stems were maroon, lower mature leaves developed purple pigmentation on the petioles and in the mid lamina area which turned light reddish and finally brown necrosis along the acropetal margins. 'Grenada' the symptoms were similar except that the pigmentation was purplish black and necrosis turned from light green to brown; K – 'Timor' compact plants, upper foliage was darker green, lower mature leaves puckered and curled downward with necrotic spots. 'Grenada' similar symptoms, but delayed with severe puckering. Ca – 'Timor' streaks of black necrosis along the primary veins and adjacent tissues of recently matured leaves, and abscission of recently matured and mature leaves. 'Grenada' streaks of black necrosis on roots and gradual wilting of recently matured and mature leaves; Mg – 'Timor' interveinal patches of black necrosis of lower mature leaves turned brown, abaxial surface of mature leaves developed red

pigmented patches. ‘Grenada’ similar symptoms except the absence of red pigmented patches and delayed progression to advanced symptoms; S – ‘Timor’ uniform chlorosis of recently matured leaves spread to mature leaves, streaks of black necrosis develop of primary veins of upper mature leaves. ‘Grenada’ less intensive chlorosis and abaxial surface appeared faded red; Fe – ‘Timor’ faint basipetal chlorosis turned interveinal first on young leaves then on recently matured leaves, faded flowers. ‘Grenada’ – similar symptoms except light pigmentation developed on shoot apex, mid-veins of young leaves, petioles and buds; Mn – ‘Timor’ chlorosis on young leaves spread to recently matured and mature leaves, brown patches and spots developed on these leaves. ‘Grenada’ – chlorosis was distinct on recently matured and mature leaves, and brown stippling developed on these leaves; Cu – ‘Timor’ young and recently matured leaves developed chlorosis along the margins, then puckering and upward rolling of leaf margins, withering, shriveling, and drying up of margins of recently matured and upper mature leaves and flower buds. ‘Grenada’ – similar symptoms except mild chlorosis; Zn – ‘Timor’ young and mature leaves developed chlorosis then shades of black spots, which turned tan-black necrotic patches. ‘Grenada’ mild chlorosis with puckering and necrosis spot-like patches on young and recently matured leaves; B – ‘Timor’ – ‘bonsai’-like dwarf plants, thick and glossy leaves with red-pigmented thick and raised prominent veins, desiccation of young and recently matured leaves. ‘Grenada’ – similar symptoms except that stems and shoots were darker and glossier.

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Table 1a. 'Timor' tissue nutrient concentration at early and advanced stages of deficiency in recently matured leaves of hydroponically grown plants.

Treatment	**-N**	**-P**	**-K**	**-Ca**	**-Mg**	**-S**	**-Fe**	**-Mn**	**-Cu**	**-Zn**	**-B**
	Percent dry wt.						$mg \cdot kg^{-1}$ of dry wt.				
Element	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn	B
Control early	4.28	0.43	2.45	1.69	0.32	0.23	79.0	78.4	4.3	27.6	57.0
Deficiency early	1.95	0.07	0.53	0.72	0.07	0.08	43.9	5.8	1.6	18.6	6.1
Control advanced	4.12	0.54	2.56	2.13	0.34	0.23	75.1	129.1	3.7	22.3	70.9
Deficiency advanced	1.09	0.05	0.50	0.42	0.07	0.05	55.6	4.0	1.6	17.7	4.9
P value	0.01	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.02	0.03	0.00

Table 1b. 'Grenada' tissue nutrient concentration at early and advanced stages of deficiency in recently matured leaves of hydroponically grown plants in this study and published standards.

Treatment	**-N**	**-P**	**-K**	**-Ca**	**-Mg**	**-S**	**-Fe**	**-Mn**	**-Cu**	**-Zn**	**-B**
	Percent dry wt.						$mg \cdot kg^{-1}$ of dry wt.				
Element	N	P	K	Ca	Mg	S	Fe	Mn	Cu	Zn	B
Control early	4.03	0.24	3.38	1.24	0.28	0.19	83.4	101.4	5.8	31.0	77.7
Deficiency early	1.73	0.10	0.43	0.86	0.09	0.09	47.8	5.8	2.2	15.4	9.8
Control advanced	3.90	0.41	3.64	1.36	0.27	0.21	81.4	179.0	4.9	36.8	92.2
Deficiency advanced	1.14	0.06	0.44	0.74	0.06	0.07	52.8	4.2	2.0	13.7	7.6
P value	0.00	0.01	0.00	0.00	0.01	0.01	0.01	0.00	0.00	0.04	0.0.01
Published*	2.00 - 4.50	0.20 - 0.80	1.50 - 4.50	0.50 - 2.00	0.30 - 0.80	-	75.0 - 300.0	50.0 - 250.0	5.0 - 15.0	25.0 - 100.0	20.0 - 60.0
Published**	2.50 - 4.50	0.30 - 0.80	1.90 - 2.70	1.00 - 2.00	0.30 - 0.80	-	150.0 - 300.0	100.0 - 250.0	5.0 - 10.0	40.0 - 85.0	50.0 - 60.0

*Mills and Jones, 1996. Survey average of concentrations associated with acceptable crop performance, minimum critical concentrations not identified.

**Erwin et al., 1992. Normal leaf tissue ranges for New Guinea impatiens, minimum critical concentrations not identified.

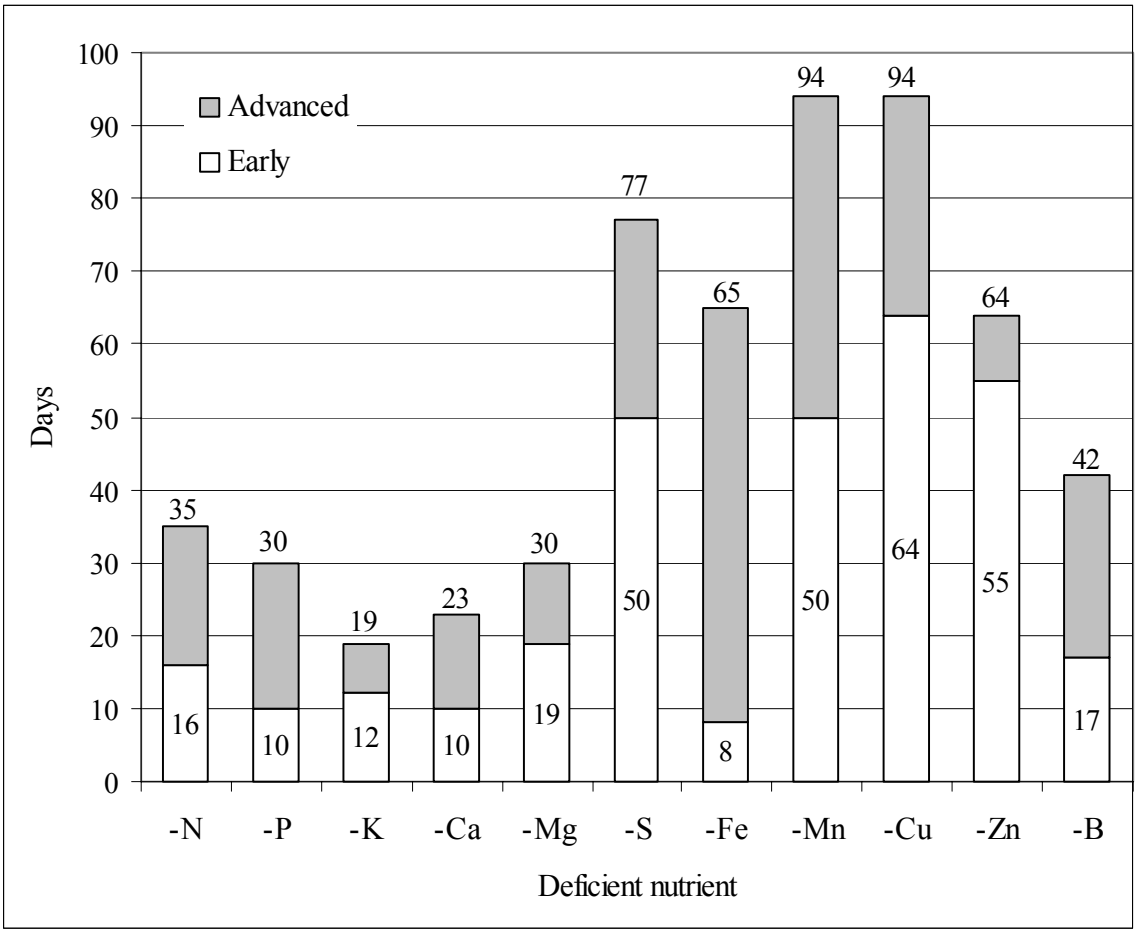

Figure 1a. Days from initiation of treatments to leaf sampling for nutrient analysis for 'Timor' at early and advanced stages of deficiency.

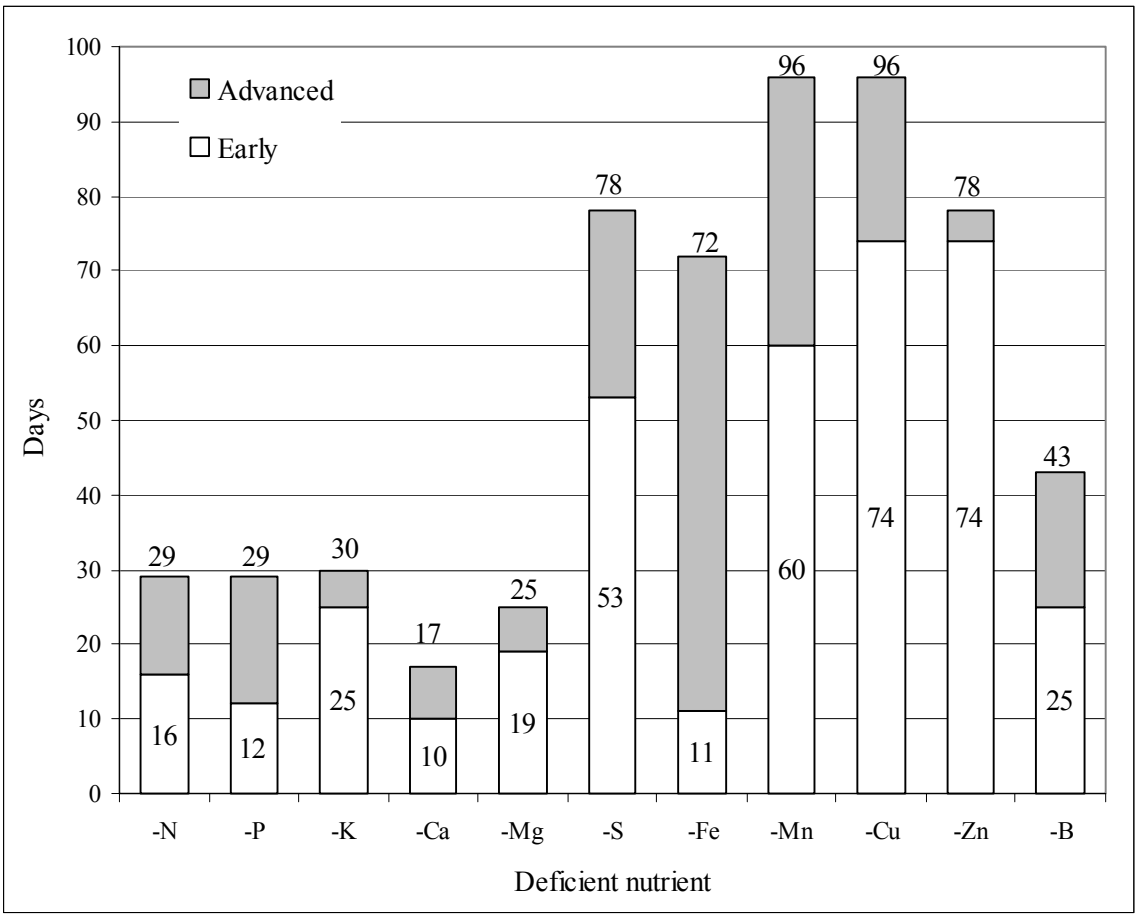

Figure 1b. Days from initiation of treatments to leaf sampling for nutrient analysis for 'Grenada' at early and advanced stages of deficiency.

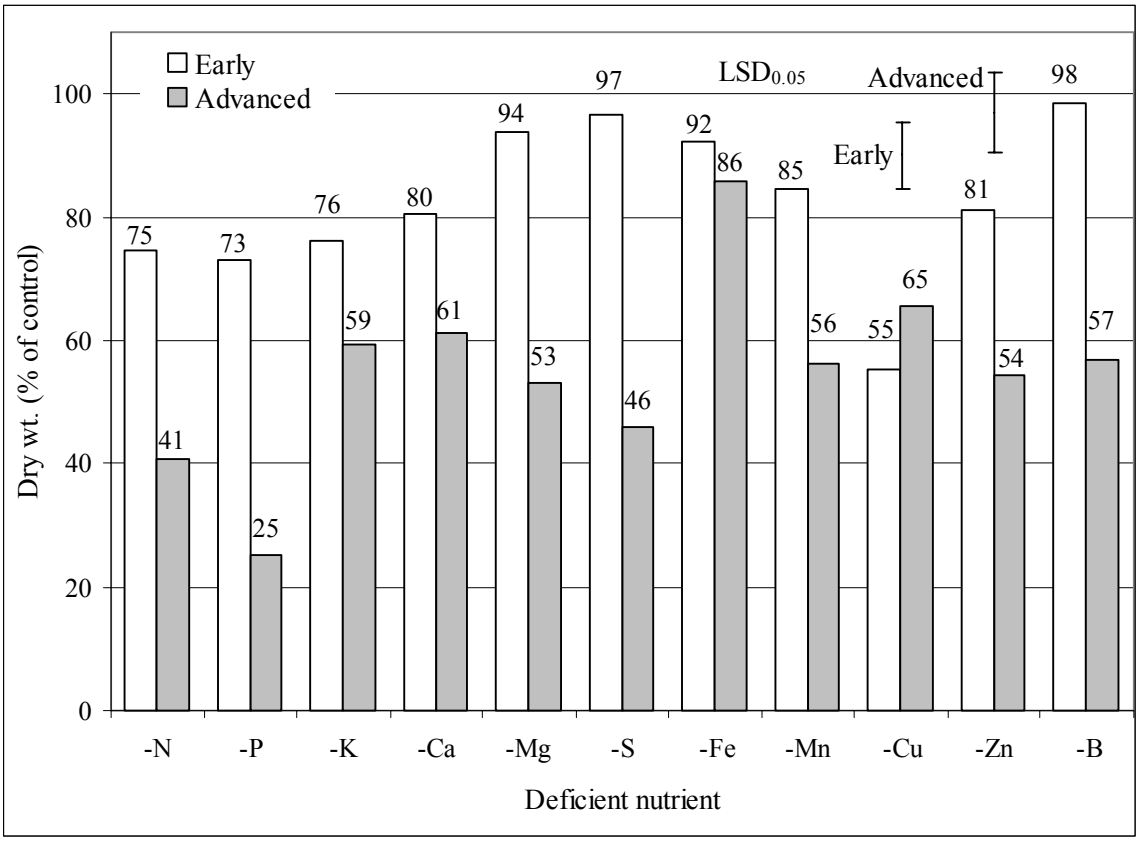

Figure 2a. 'Timor' dry weight of nutrient deficient plants expressed as a percentage of control plants at early and advanced stages.

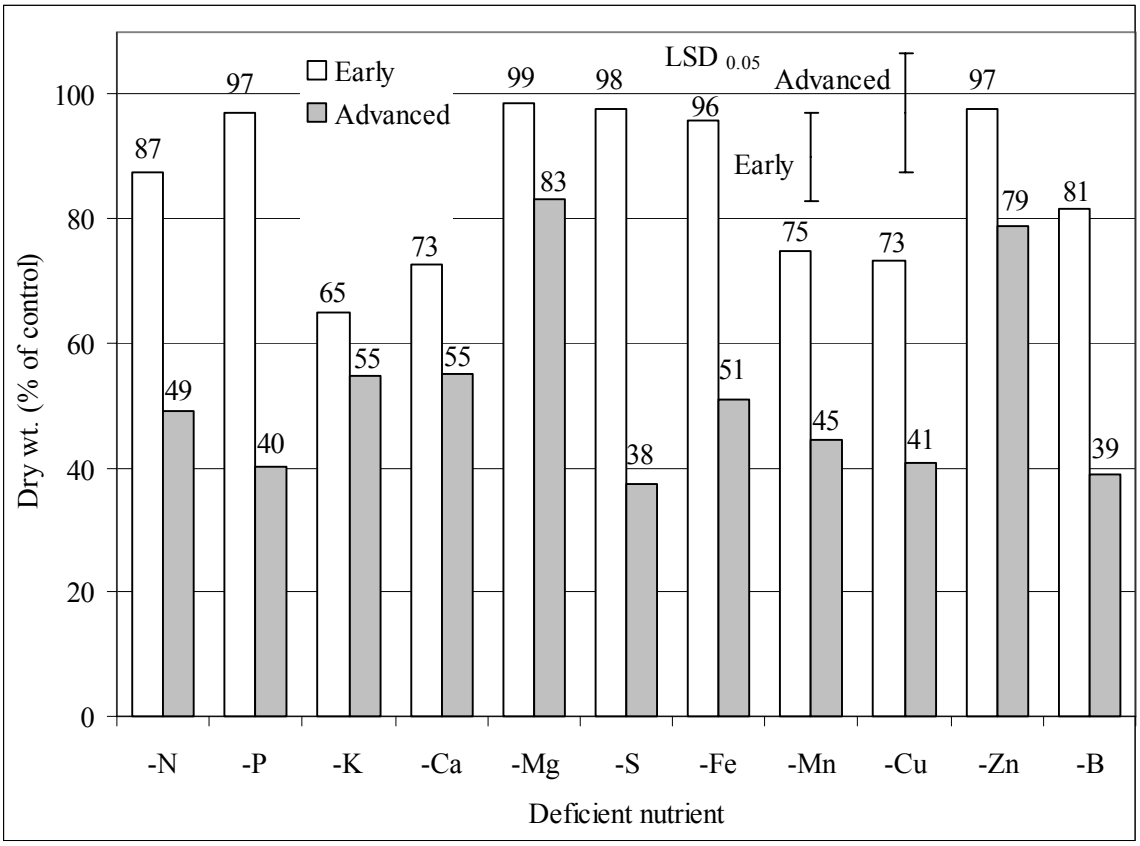

Figure 2b. 'Grenada' dry weight of nutrient deficient plants expressed as a percentage of control plants at early and advanced stages.

CHAPTER 13

CELLULAR ANALYSIS OF Ca, B, AND Ca AND B DEPRIVATION

Dharmalingam S. Pitchay[1], Paul V. Nelson[1], Chad V. Jordan[2], Udo Blum[2] , Eva Johannes[2], and Dominique Robertson[2]

[1]Department of Horticultural Science, North Carolina State University, Raleigh, North Carolina 27695-7609, USA

[2]Department of Botany, North Carolina State University, Raleigh, North Carolina 27695-7612, USA

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[1]Department of Horticultural Science, North Carolina State University, Raleigh, North Carolina 27695-7609, USA

[2]Department of Botany, North Carolina State University, Raleigh, North Carolina 27695-7612, USA

ABSTRACT

Root and shoot apical meristems are specialized areas where cell proliferation and organogenesis take place. The role of Ca and B in the root development, and the interactions between Ca and B deficiency in nuclear morphology, cell proliferation and primary root growth were investigated. The absence of Ca, B, or both Ca and B greatly reduced primary root growth relative to control root growth within 12 hours. Nuclei in both Ca and Ca\B deficiency treatments showed a significant decrease in volume. Boron deficiencies initially resulted in an increased mitotic index (MI), concurrent with root apical meristem (RAM) distortion. Most of the increase in root diameter could be explained by an increase in the number of cell files in the cortex. Ca and B deficiencies together reduced both the MI and root tip distortion. Because calcium increased cell death

but did not promote cell division, while boron increased cell division, the combination of Ca and B deficiencies appeared to attenuate symptoms of nutrient deficiency.

INTRODUCTION

Numerous specific interaction of biotic and abiotic factors are involved in the complex process of cell division, elongation and differentiation. Generally, cell cycle progression in the meristematic region is disrupted when nutrient resources are limiting. Carbon, nitrogen, phosphorus, vitamins, calcium, and calcium related proteins are directly involved in the cell cycle process. Cells undergo a stationary phase when nutrients are limiting (Nagata et al., 2001).

Calcium is a unique essential element based on its structural, chemical and signaling roles (Marshner, 1995, and Sanders et al., 1999,). It is vital in the regulation of many cellular processes in higher plants. This includes cell division, cell expansion (Kirby and Pilbeam, 1984), and cytoplasmic streaming (Bush, 1995). The requirement of Ca is specific and it ranges from mM concentrations in cell wall and organelles to μM concentrations in the cytoplasm (Marshner, 1995). The chemistry of Ca^{2+} itself provides special features for signal transduction (Hepler and Wayne, 1985) and protein conformation, allowing remote domains to be involved in calcium binding (Sanders et al., 1999).

The effects of Ca deficiency develop first in growing tissues, such as the shoot and root apical meristem (Sorokin and Sommer, 1929 and 1940), young leaves, fruits, and seeds. Mature and older leaves are least affected by Ca deficiency. This is mainly due

to the absence of Ca in the rhizosphere, and the inability of plants to retranslocate Ca within the plant tissues and organs. In a Ca deprived environment, root tips become swollen and covered with dead cells (Davis, 1949).

Boron is an essential element in plants for cell wall formation and stabilization, lignification, xylem differentiation, membrane integrity, auxin activity, and inhibition of callose formation (Gupta, 1993 and Welch, 1995), and apical meristem function (Kouchi and Kumazawa, 1975 and 1976). The optimal B concentration varies among species. The plant cell wall is modified in differentiated tissues, and B is an important element of differentiated cell morphology and function (Hirsch and Torrey. 1980). Almost 90% of cellular B is contained in the cell wall (Loomis and Durst, 1992).

Like Ca, B disorders develop in the shoot and root meristem, and on young leaves. Visual symptoms of B deficiency first occur in roots rather than shoots (Kouchi and Kumazawa, 1975). Symptoms include short and thick roots with an enlarged and malformed root meristem and root cap. In most instances, the root tips are stiff, stubby and swollen with necrosis developing on primary and secondary root tips. Lateral roots develop close to the root apex and have a witches' broom-like appearance. In some instances, root elongation was affected as early as 6 hours under a B deprived environment, while total root arrest occurred by 24 hours (Cohen and Albert. 1974). Roots failed to elongate prior to a decline in RNA content in root tips within 24 to 48 hours when B was deprived (Albert, 1965).

The study reported here examined the effects of Ca, B, and a combination of Ca and B deprivation on tomato *Lycopersicon esculentum,* Mill. 'Better Boy' root growth. Previous work showed that absence of Ca resulted in dead roots and the absence of B

caused stubby and necrotic roots in tomato plants (Haynes and Robbins, 1948). However, the interaction of Ca and B deficiencies were not tested. By using hydroponic culture, Ca and B levels in nutrient medium were easily modified. Although both shoot apical meristem (SAM) and root apical meristem (RAM) affected, this study used the RAM to avoid potential differences is translocation of these elements to the shoot. Root and shoot apical meristems are specialized areas where cell proliferation and organogenesis take place. Therefore, this study was conducted to investigate the role of Ca and B in the root cell cycle, and to determine the synergistic/antagonistic effects of Ca and B deficiency in cell proliferation and elongation in the root meristem.

MATERIALS AND METHODS

Tomato 'Better Boy' was sown on 6 December 2001 in Oasis LC1 foam cubes (Smithers Oasis, Kent, OH.) containing only Ca and Mg from dolomitic limestone. The experiment was conducted in a glass greenhouse in Raleigh, NC at 35°N latitude that was set at night/cloudy day/clear day temperatures of 17/21/24 ^{o}C. During the establishment phase, seedlings were fertilized at each irrigation with complete 1/3 strength modified Hoagland's solution (N-source all nitrate) using reagent grade chemicals. Seedlings were grown with this nutrient regime until the first set of true leaves developed.

Tomato seedlings were transplanted into 1.5 liter white plastic tubs covered with a lid containing a single hole (one plant/tub). The interiors of the tubs and lids were lined with black polyethylene sleeves. Three replications for each time interval (3, 4, 6, 8, 12, 24, 48, 72, 96 and 120) were assigned to four treatments that

included complete nutrient formula, and complete minus Ca, B, or the combination Ca and B nutrients. The treatments were induced on 27 January 2002.

The complete modified Hoagland's solution consisted of the following mM concentrations of macronutrients, 15 NO_3-N, 1.0 PO_4-P, 6.0 K, 5.0 Ca, 2.0 Mg, and 2.0 SO_4-S (7), plus μM concentrations of micronutrients, 72 Fe, 9.0 Mn, 1.5 Cu, 1.5 Zn, 45.0 B, and 0.1 Mo. Solutions were formulated with the following reagent grade chemicals KNO_3, $Ca(NO_3)_2 \cdot 4H_2O$, KH_2PO_4 , $MgSO_4 \cdot 7H_2O$, $CaCl_2$, KCl, $NaNO_3$, NaH_2PO_4, Na_2SO_4, $MgCl_2$, FeDTPA, $MnCl_2 \cdot 4H_2O$, $ZnCl_2 \cdot 7H_2O$, $CuCl_2 \cdot 5H_2O$, H_3BO_3, and $Na_2 2MoO_4 \cdot 2H_2O$ in deionized water of 18-mega ohms purity.

Root Growth Measurement

At the time of plant transfer into the treatment solution the primary roots were marked with black India ink at a point 1 cm from the tips. The marked root tips were measured at 3, 4, 6, 8, 12, 24, 48, 72, 96, and 120-hour intervals for root elongation.

Fixation and Sectioning of Plant Tissues

1 to 1.5 cm of the root tips were cut and fixed for 48 hr at room temperature in 10 mM PBS, pH 7.2, containing 4% formaldehyde prepared from powdered Para-formaldehyde (Fisher). Tissues were then stored in the fixative at 4^0C until sectioned. Fixed tissues were embedded in 5% low-gelling-temperature agarose (Type XI; Sigma) in 10 mM PBS, pH 7.2, before sectioning. Thick tissue sections (50 to 60 μM) were cut

using a Vibratome 1000 (Technical Products International, Inc., St. Louis, MO). Tissue sections were then transferred to 10 mM PBS, pH 7.2, 0.1% BSA and stored at 4^0C (Nagar et al., 1995). Sections were mounted in 90% glycerol made in 1xPBS, with one μg·ml DAPI (4,6-diaminodino-2-phenylindole) and imaged using a Nikon and Optiphot microscope, and Nomarski differential interference contrast optics and Scanning Confocal Microscope (Model DM IRBE) (Nagar et al., 1995). Nuclear dimension (μ^2) in the cortex area (1 mm from the quiescent center) was measured using the Adobe Photoshop 5.0 – by placing the micrometer ruler pasted on to the grid tools with same magnification. For each treatment 10 root sections were sampled, and for each section, 10 nuclei were measured to calculate the mean. Sampling of cells in mitosis and the total number of cells were counted in the area as shown in Figure 1 to calculate the mitotic index (MI).

$$\text{Mitotic index (MI)} = \frac{\text{Cells in Mitosis}}{\text{Total \# of cells}} \times 100$$

RESULTS

Root Growth and Morphology

Root growth was minimal in all treatments, including the control, until 12 hr when the controls resumed normal rates of growth (Figure 2). By hour 12, new secondary roots were visible in all treatments. Root tips of plants grown in B deficient medium

appeared slightly swollen at 12 h. By hour 24, root elongation was not detectable in plants grown in Ca and B deficient medium and greatly reduced in treatments lacking B (0.16 cm), or Ca (0.27 cm). Plants grown in Ca and B deficient medium developed small brown spots (necrosis) along the upper regions of the root, distal to the root elongation zone (control plants were free from necrosis). By hour 48, root elongation of plants grown in the following media: –Ca, -B, and –Ca\-B was 3%, 2%, and 3% of control plants respectively. Root tips appeared swollen in all treatments and swelling was most noticeable in plants grown in B deficient medium. Primary root growth ceased by hour 72 in all treatments, except the control (Figure 2). Lateral roots continued to elongate, especially in plants grown in medium lacking both B and Ca.

By 120 hours, root tips of plants in the -Ca\-B medium became brown, but lateral roots continued to elongate. In B deficient medium, the primary root tip was swollen with brown coloration on the terminal tips, while lateral roots continued to elongate. In plants grown in -Ca medium, lateral roots did not elongate and the terminal tips turned brown.

Effects of B, Ca and, Ca and B Deficiency on the Root Apical Meristem (RAM)

Primary root tips of plants from all four treatments were examined by fluorescence microscopy following sectioning and staining with DAPI, which allows visualization of DNA. The intensity of fluorescence is directly proportional to the amount of DNA present (Maruo, 1990). At hour 3, nuclear morphology in some cells of the quiescent center and procambium was abnormal. The number of abnormal nuclei decreased in the order of Ca > Ca\B > B deficient treatments.

At 8 hour, abnormally shaped nuclei were found at the procambium zone adjacent to the quiescent center especially in the -Ca treatment. At hour 12, zones devoid of cells were observed in the area immediately distal to the quiescent center. These cells may have disappeared during the sectioning process, possibly due to weaker cell walls. The abnormally puckered and punctate shaped nuclei were scattered extending to the cortex close to the quiescent center and columella, especially in -Ca followed by -Ca/-B plants. Light brown coloration developed in the procambial zone at 5 mm from the quiescent center in B-D (Figure 4) is never seen in control RAM.

Cells around the quiescent area appeared scattered. By hour 24, the cells were not able to form as single files at the cortex adjacent to the procambium in -Ca /-B medium. Moreover, there were numerous missing cells. Meanwhile, the nuclei in the procambium did not align as single files and instead the meristem attained an abnormal shape (Figure 3). Furthermore, the cellular files were further apart compared to that of controls. At hour 48, cell death was observed in the procambium. It appeared that cell death occurred in the upper area of the procambium. However, the procambial area adjacent to the quiescent zone was free from cell death in –Ca medium (Figure 4). At this point, the number of cellular files were significantly greater in B deprived RAM than the rest of the treatments (Figure 5). In addition, nuclear dimensions in B deprived root tip cortical cells were significantly larger than in -Ca and -Ca\-B roots, but were not significantly different from the control RAM (Figure 6). We conclude that the increase in number of cellular files is determining factor in the swollen appearance of B deprived root tips.

At 42 and 72 hour, Ca deprived RAM developed a brown coloration in the procambium and cortex, which is an indication of cell death (necrosis or programmed cell

death) (Figure 4). These symptoms were found to a lesser extent in Ca and B deprived RAM. On the other hand, in B deprived RAM, cell death occurred was less extensive at earlier time points but was noticeable after 48 hr.

Cell Counts

When the tomato plants were transferred to Ca, B, or Ca and B deficient nutrient media, cells began to reveal abnormal nuclear shapes and root morphology within 12 hours. Cell counts for each treatment were taken from the cortex, 5 mm from the quiescent center (Figure 1). The number of cells observed for each treatment ranged from 90 to 240 in the cortical area of RAM.

Mitotic Index (MI)

A trend of non-significant in mitotic index (MI) of -Ca, -B, -Ca/B as well as control treatments at 6 hours was noted (Figure 7). A fluctuation in MI was observed -B, -Ca/B treatments. This could be due to the adjustment of the plants from complete nutrient to deprived conditions. Ca deprivation had the first significant reduction in the MI from 12 hours onwards and it became stationary as compare to the control and -B. On the other hand, prolonged B deprivation caused a higher MI at 24 to 48 hours compared to the Ca, and Ca\B deprived roots. Although, -B followed a declining trend from 24 hours onwards, the MIs remained significantly greater than -Ca and -Ca\-B. At this point, there was no difference in the MIs between the control and -B. However, at 96

hour B deprivation reduced MI dramatically. The Ca and B deprived mitotic index was slightly greater than the Ca deprived cells during the 72 period.

Meristem Integrity

Empty areas of cells and nuclei at the procambium and cortex may result from a loss of cell integrity. This was more evident in Ca deprived RAM than -B and the effect was moderate in Ca\B deprived RAM.

A dramatic effect was the puckered shaped nuclei in Ca deprived cells and the loss of plane division. These symptoms were milder, but still present in B, and both Ca\B deprived cells. The whole syndrome of puckering, empty nuclear area, loss of cell division and alignment was severe and occurred sooner in Ca deprived cells. B deprived and both Ca\B deprived root also showed this syndrome to a lesser extent, with -B plants more severe than -Ca/-B plants. At hour 72, there was nearly complete disintegration of cell structure and nuclei in the RAM of plants grown in -Ca, and combination of Ca and B deficient medium. However, plants grown in B deprived medium had less severe impact on the cell structure at hour 72 than -Ca, and -Ca\-B.

The occurrence of cell death was severe in the procambial area of plants grown in Ca deficient medium, which developed at $\pm$ 10 mm from the procambium initials. At hour 12 and at hour 72 cell death took place at procambium initials. A similar effect was observed, but the degree of cell death was less than 25% of cells and in -Ca\-B, the cell death effect was 75% of Ca deprived cells. Cell death occurred later in B deficient medium. All death occurred acropetally in -Ca and basipetally in -B.

Chromatin Morphology

Distinct changes in chromatin structure, including chromatin condensation, cells with an increase of chromatin at the periphery of their nucleus, and punctate or knobby regions of chromatin were noted in nuclei of cells distal to the procambium in plants grown in B deficient medium and, to a lesser extent, in -Ca\-B medium. Interestingly, chromatin condensation was not detected in plants grown in Ca deficient medium. At hour 72, cell death was distinct in and around the procambium and quiescent center in all three treatments. Initiation of new lateral roots was still observed in plants grown under both B, and Ca\B deficient medium Lateral root initiation and elongation was still observed and appeared to increase at later time periods in plants grown in -B medium.

DISCUSSION

Both B and Ca play important roles in cell division alignment during the mitotic phase at the cell initials, and at procambial and cortical areas 0-5 mm from the cell initials. However, there were significant differences between roots grown in complete Hoagland's medium, and the same medium lacking Ca or B. In plants grown in -Ca, morphological effects occurred earlier by 3 to 4 hours and were more pronounced compared to plants grown in -B, a difference that persisted for 12 hours. Deleterious effects of nutrient depletion in plants grown in -Ca/-B were intermediate between those grown in -Ca and -B alone, suggesting that there may be interactions between developmental and cellular pathways requiring Ca and B. In Ca\B deprived condition the

impact was delayed compared to -Ca but earlier than -B. This implies that there is a critical role of Ca as compared to B in cell division alignment. On the other hand, the effect of -Ca\-B deficiency was less drastic than -Ca.

The orientation of cell walls is determined at pre and late prophase of mitosis (Wick and Rogers, 2001). Ca may be directly involved in cell wall alignment and B in a post-cell alignment process. The tomato root apical meristem is characterized as a closed meristem based on the clear demarcation of cells between the root cap and root meristem. Interestingly, the absence of B and -Ca/-B in nutrient media resulted in less organized cells at the columella resembling meristems with an open structure (Groot and Rost, 2001). There was an apparent loss of apical dominance, probably due to abnormalities in the meristem. This may have promoted compensatory growth leading to initiation of an increased number of lateral roots and greater elongation of lateral roots in B and Ca\B deficient plants at 72 hours from the onset of deprivation, which shortens the indeterminate root growth of primary root. Compared to the -Ca/-B treatment, the absence of B alone produced the most severe effect.

The absence of B caused additional abnormal anticlinal cell divisions in the peripheral region of the root caps, increased in number of cellular files, and abnormal alignments (scattered cells), which initiated the 'pseudo-lateral' roots at outer adjacent areas of the cortex giving rise to root tips with abnormally increased diameters within 72 hours. The absence of B triggered cell division in the peripheral lateral root cap. However, perhaps due to the limitation of nutrients, nuclear form was disturbed. To counter cell death in the procambium, cell division may have occurred at the peripheral root cap. The proliferative cells in this zone led to epidermal layers that sloughed off. The

effect of B deprivation had an overriding effect on Ca and B deprived cells. This indicates that B may help to mitigate Ca deprivation effects in a Ca deficient environment.

CONCLUSION

Overall, the absence of Ca and B or B or Ca greatly reduced primary root growth relative to control within 24 and 96 hours, respectively. Boron deficiencies resulted in an increased MI, consistent with RAM distortion. Compensatory divisions as a result of loss of RAM integrity may have occurred to reestablish meristematic activity. Ca and B deficiencies reduced both MI and distortion. In this treatment, the RAM morphology remained intact during the 96 hours because a lack of B delayed cell death, and lack calcium arrested cell division.

ACKNOWLEDGMENT

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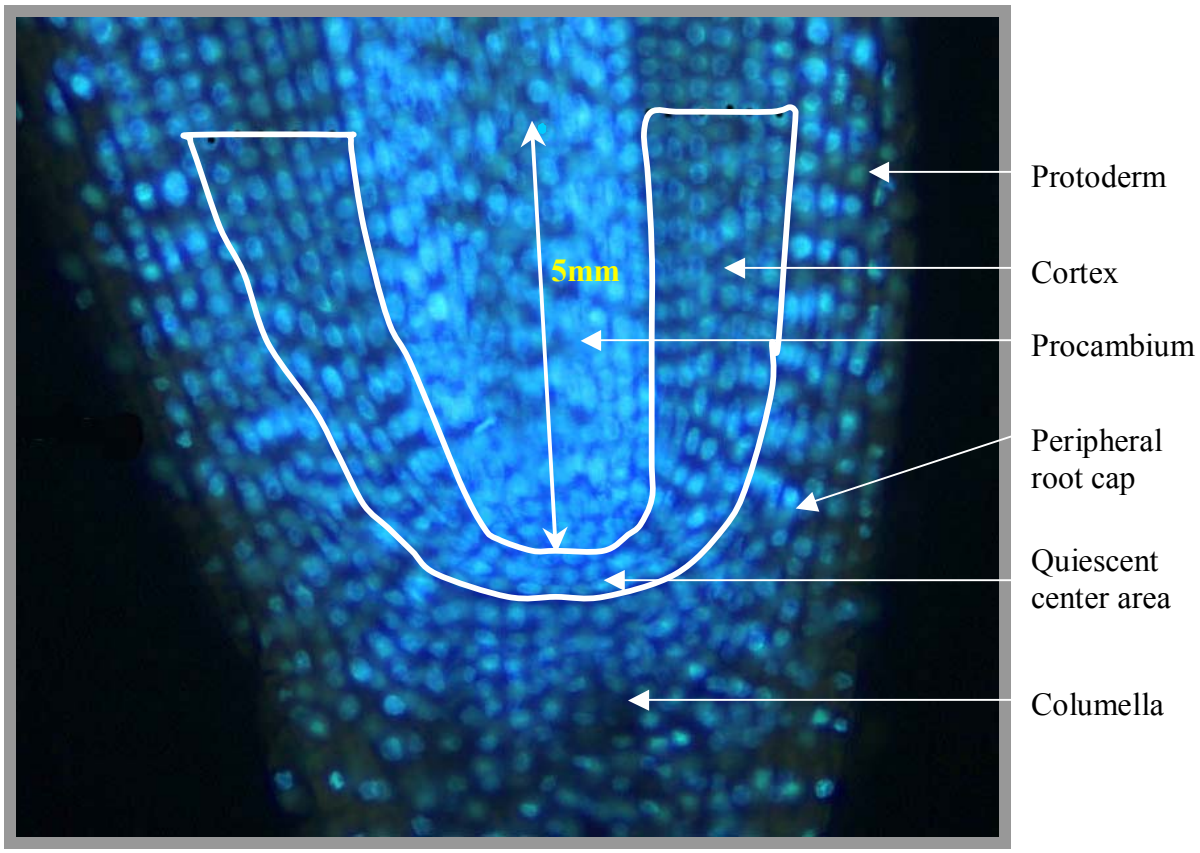

Figure 1. Longitudinal section of tomato root apical meristem (RAM) grown in complete nutrients. The marked area 5 mm from quiescent center is where mitotic index (MI) calculations were done.

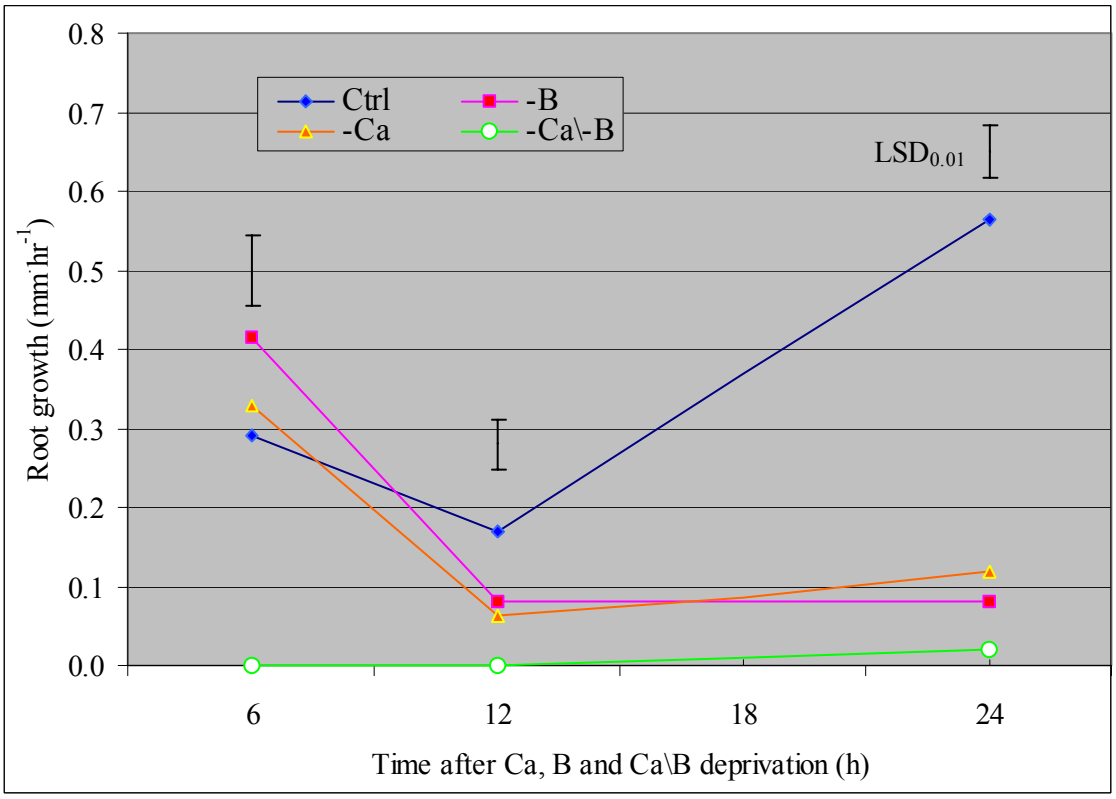

Figure 2. Root growth of Ca, B, and Ca\B deprived treatments.

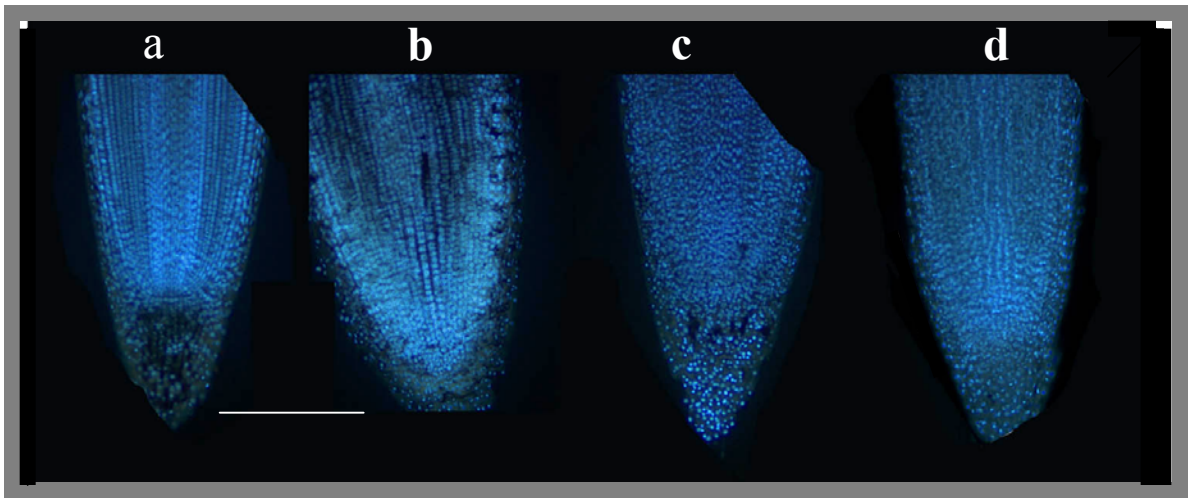

Figure 3. Alterations in RAM morphology 24 hrs after Ca, B, and Ca and B deprivation. (a) Control treatment, (b) B deprived treatment, (c) Ca deprived treatment, and (d) Ca and B deprived treatment. Bar, 5 mm.

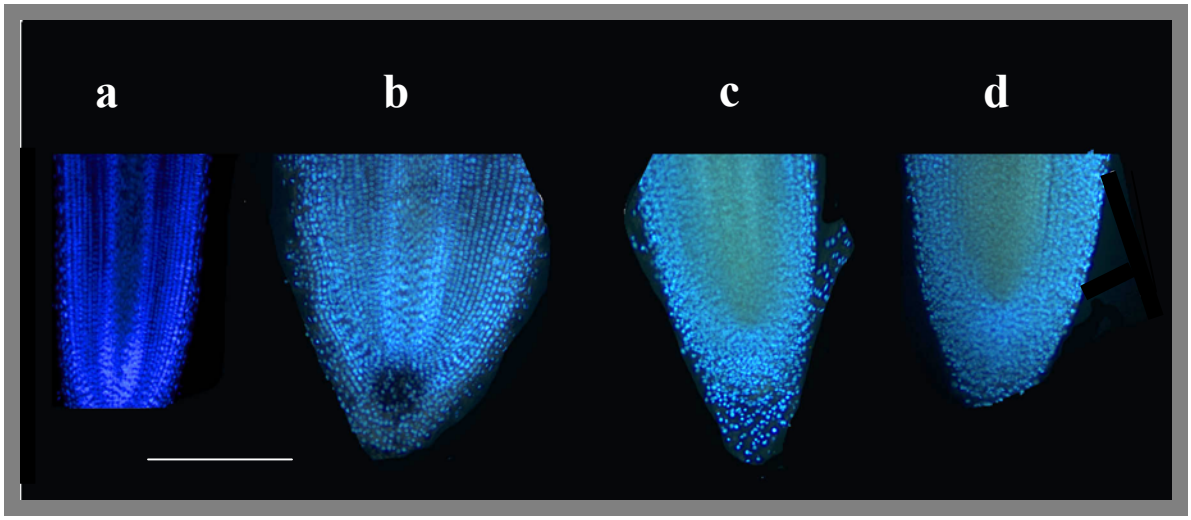

Figure 4. RAM morphology at 48 hrs after Ca, B, or Ca and B deprivation. (a) Control treatment, (b) B deprived treatment, (c) Ca deprived treatment and (d) Ca and B deprived treatment. Bar, 5 mm.

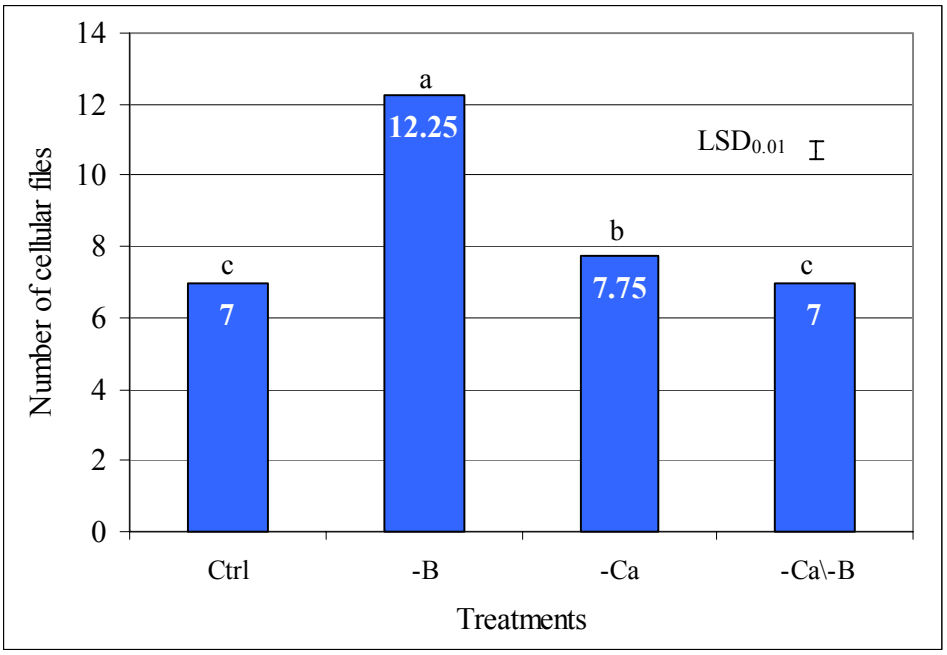

Figure 5. Number of cellular files at 48 hr in control and Ca, B and Ca\B deprived RAM.

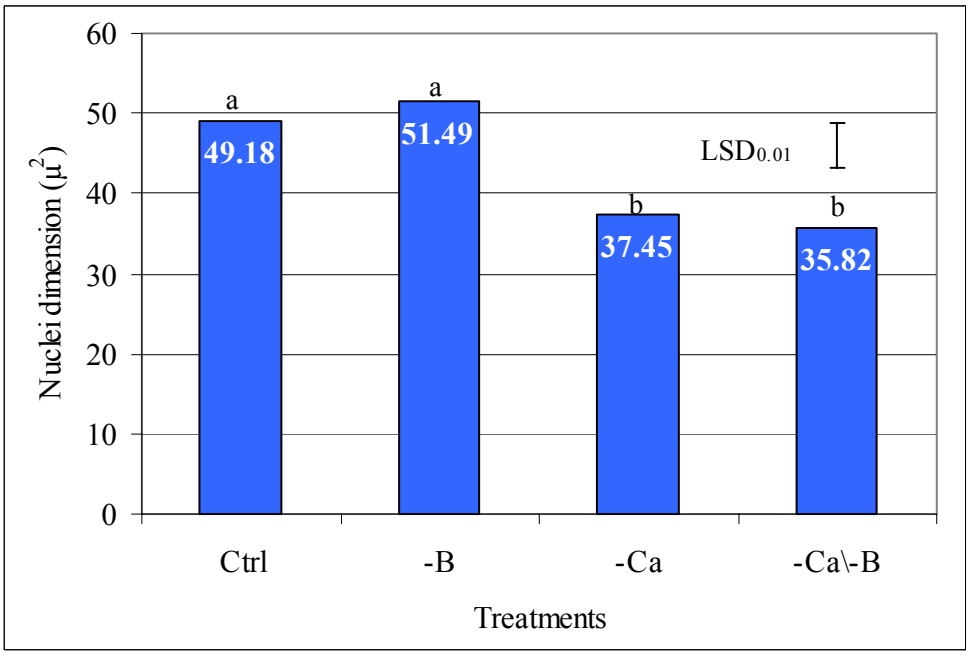

Figure 6. Nuclear dimensions at 48 hr in control, and Ca, B and Ca\B deprived RAM. Mean separation by least significant difference (LSD) at *P<0.01* is indicated by small cap letters.

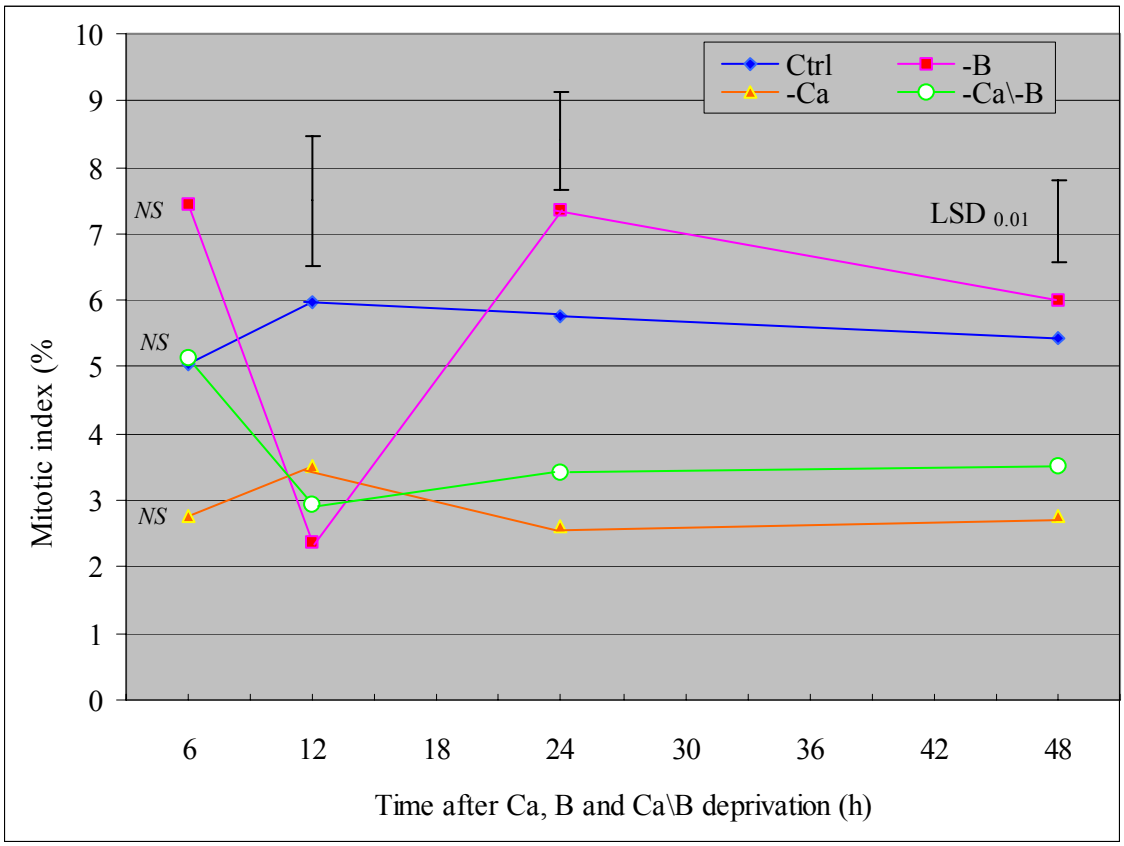

Figure 7. Mitotic index (MI) of Ca, B, and Ca\B deprived treatments.

Note: *NS non significant at P<0.05*

CHAPTER 14

THE IMPACT OF Ca, B, AND Ca\B DEPRIVED NUTRIENT SOLUTION ± SUBSTRATES ON ROOT GROWTH AND DEVELOPMENT

THE IMPACT OF Ca, B, AND Ca\B DEPRIVED NUTRIENT SOLUTION ± SUBSTRATES ON ROOT GROWTH AND DEVELOPMENT

INTRODUCTION

Our earlier nutrient deficiency studies of 17 ornamental taxa demonstrated that visual symptoms of B deficiency first occurred in roots, two to three days before appearing on shoots. The symptoms included short, stunted and thick roots with swollen apices. At times the primary root tips had deformed or missing root caps. In most instances, the roots were stiff and brittle, followed by necrotic tips. Lateral roots developed close to root apex resembling a witches' broom like appearance.

Like B, Ca deficiency symptoms first occurred on roots rather than shoots. Symptoms included poorly developed short and stunted roots. However, unlike in B deficient plants, necrosis first developed along the root axis and not the tips. Lateral roots were fewer, shorter and thinner.

Since B and Ca deficiency symptoms first occur on roots rather than shoots, early diagnosis of root symptoms is critical for corrective measures. Therefore, this study was conducted to investigate tomato root morphology of Ca, B, and a combination of Ca and B deprived plants grown in nutrient solution ± various substrates.

MATERIALS AND METHODS

Tomato (*Lycopersicon esculentum,* Mill.) cv. 'Better Boy' was sown on 6 December 2001 in Oasis LC1 foam cubes (Smithers Oasis, Kent, OH.) containing only Ca and Mg from dolomitic limestone. The experiment was conducted in a glass

greenhouse in Raleigh, NC at 35°N latitude that was set at night/cloudy day/clear day temperatures of 17/21/24 °C. During the establishment phase, seedlings were fertilized at each irrigation with complete 1/3 strength modified Hoagland's solution (N source all nitrates) using reagent grade chemicals. Seedlings were grown under this nutrient regime until the first set of true leaves developed.

Tomato seedlings were transplanted into 1.5 liters white plastic tubs covered with a lid containing a single hole (one plant/tub). The interiors of the tubs and lids were lined with black polythene sleeves. Sixteen treatments consisting of a factorial combination of four nutrient formulations and four substrates were used (Table 1) in a randomized complete block design with four single tub replications per treatment. The four nutrient treatments included complete nutrient formula, and complete minus Ca, B, or the combination of Ca and B. Substrates consisted of only nutrient solution, sand saturated with nutrient solution, sand periodically moistened with nutrient solution, and peat\perlite periodically moistened with nutrient solution. Substrate sand was first washed with deionized water and soaked in 0.5 N HCl for five days. Then the sand was rinsed six times with deionized water and soaked in nutrient solution (Hoagland's solution devoid of Ca and B) over night. Finally, the nutrient solution was drained and the sand was soaked with treatment nutrient solutions for six hours for pH stabilization. The peat substrate consisted of 3 sphagnum peat moss: 1 perlite by volume, deionized water (60% by weight) was added to moisten the peat substrate. The nutrient solution\sand combination consisted of 1nutrient solution: 2 sand by volume. The pH of nutrient solution and substrate was adjusted to 5.6 using 1N H_2S_4 or 1N KOH. Nutrient solution treatments (control, -Ca, -B and -Ca\-B) for peat and sand substrates were pumped from

tanks of single strength solution in sufficient volumes to allow 20% to leach from the bottom of the tubs. The substrates were fertigated 6 times during the daytime. The treatments were induced on 27 January 2002.

The complete modified Hoagland's solution consisted of the following mM concentrations of macronutrients, 15 NO_3-N, 1.0 PO_4-P, 6.0 K, 5.0 Ca, 2.0 Mg, and 2.0 SO_4-S (7), plus μM concentrations of micronutrients, 72 Fe, 9.0 Mn, 1.5 Cu, 1.5 Zn, 45.0 B, and 0.1 Mo. Solutions were formulated with the following reagent grade chemicals KNO_3, $Ca(NO_3)_2 \cdot 4H_2O$, KH_2PO_4 , $MgSO_4 \cdot 7H_2O$, $CaCl_2$, KCl, $NaNO_3$, NaH_2PO_4, Na_2SO_4, $MgCl_2$, FeDTPA, $MnCl_2 \cdot 4H_2O$, $ZnCl_2 \cdot 7H_2O$, $CuCl_2 \cdot 5H_2O$, H_3BO_3, and $Na_2 2MoO_4 \cdot 2H_2O$ in deionized water of 18-mega ohms purity.

Plants were harvested at the onset of visual deficiency symptoms on the shoot. Measurements of shoot height, number of nodes, stem diameter (3rd node from the base), and leaf area using LI- COR 3100 Area Meter (LI- COR, Inc., Lincoln, NE) were obtained. Root lengths and root surface areas were measured using a Monochrome AgVision System 286 image analyzer (Decagon Devices Inc., Pullman, WA). Shoot and root tissue dry weight measurements were taken after drying in the oven at 70° for 24 hours.

All the data were subjected to ANOVA using PROC GLM SAS program (SAS Inst., Cary, NC). Where the F test indicated evidence of significant difference among the means, LSD ($P \leq 0.05$) was used to establish differences between means.

Table 1. Summary of a factorial combination of four nutrient formulations and four substrates tested in this study.

	Substrates			
Nutrient	**Nutrient Solution**	**Nutrient solution + Sand**	**Sand**	**Peat/Perlite**
Complete (control)	Treatment 1	Treatment 2	Treatment 3	Treatment 4
-Ca	Treatment 5	Treatment 6	Treatment 7	Treatment 8
-B	Treatment 9	Treatment 10	Treatment 11	Treatment 12
-Ca\-B	Treatment 13	Treatment 14	Treatment 15	Treatment 16

RESULTS

Root Observations

All plants including the control, grown in nutrient solution had thinner roots than plants grown in other substrates. Root thickness and waviness in descending order by substrates was nutrient solution > peat > nutrient solution + sand > sand and root thickness in descending order by nutrient was -Ca\-B > control > -Ca > -B. The development of lateral roots close to the root tips resembling witches' broom–like symptoms was more obvious in B deprived plants than in Ca deprived plants, which had laterals root slightly away from the root tip. Ca deprived roots had necrosis along the root axis, whereas in B deprived roots the necrosis was only at the root tips. On the other hand, -Ca\-B deprived roots had necrosis along the axes of primary and lateral roots

including the tips. The -Ca\-B deprived roots had numerous and short lateral roots close together just above the primary root tips.

Plant Height

Overall, a reduction of plant height was observed in the nutrient deficient plants compared to the control (Table 2). There was no difference in plant height between -Ca, and -Ca/-B plants irrespective of type of substrate. However, the plant height of -Ca and -Ca/-B plants varied with substrates. Plants grown in nutrient solution + sand substrate were the tallest. However, there was no difference in plant height of B deprived plants irrespective of substrate (Table 4).

Stem Diameter

-Ca, -B, and –Ca/-B deprived plants grown in nutrient solution, and sand substrates had thinner stems compared to the control. On the other hand, there was no difference in stem diameter between nutrient deprived plants and control grown in peat substrate (Table 2). The greatest effect on stem diameter was caused by nutrient solution + sand substrate (Table 5).

Leaf Area

Significant reduction of leaf area irrespective of substrates was observed in all nutrients deficient plants compared to the control. Nutrient solution + sand substrates had a greater impact on leaf surface area. Substrates had no impact on B deficient plants. Plants grown in solution substrate had smaller leaf area while plants grown in solution + sand had greater leaf area (Table 6). Leaf area of control plants was significantly greater than –Ca, -B, and –Ca/-B deprived plants irrespective of substrates (Table 2). In solution and solution + sand substrates, –B deficient plants had larger leaf area than – Ca, and – Ca/-B deficient plants, while –Ca deficient plants had larger leaf area than –B, and -Ca/-B deficient plants grown in peat.

Number of Nodes

In all substrates except peat, the control plants had greater number of nodes than the nutrient deprived plants (Table 2). The nutrient deprived plants grown in nutrient solution + sand substrate had more nodes. B deprived plants had a fewer nodes grown in nutrient solution, and nutrient solution + sand substrate than -Ca and -Ca\-B deprived plants (Table 7).

Shoot Dry Weight

The shoot dry weight of plants grown in solution + sand substrate was greater than plants grown in other substrates. Shoot dry weight of the control plants was greater than the nutrient deprived plants in all substrates except sand (Table 8). There was a

severe reduction in shoot dry weight of –Ca/-B deprived plants compared to –Ca, and –B deprived plants grown in nutrient solution, nutrient solution + sand and peat substrates (Table 2).

Root Dry Weight

The root dry weight of plants grown in nutrient solution + sand was greater than of plants grown in other substrates. The root dry weight of control plants was significantly greater than that of nutrient deprived plants (Table 9). There was no significant difference in the root dry weight of nutrient deprived plants within each substrate except nutrient solution + sand whereby the –B deprived plants had higher root dry weight (Table 3).

Root Surface Area

The root surface area of nutrient solution + sand was greater than other substrates. While, plants grown in nutrient solution had the smallest root surface area. Ca deprived plants had higher root surface area than –B and –Ca/-B deprived plants (Table 10). –Ca/-B deprived plants had lower root surface area grown in nutrient solution, nutrient solution ± sand and sand (Table 3).

Root Length

Plants grown in nutrient solution + sand had a greater root length than plants grown in nutrient solution, sand and peat (Table 11). However, only in nutrient solution

and sand substrates the control plants had greater root length than the nutrient deprived plants (Table 3).

Shoot/Root Dry Weight Ratio

Plants grown in nutrient solution + sand substrate had significantly high shoot to root dry weight ratio, and plants grown in nutrient solution had low shoot to root dry weight ratio (Table 12). The control plants had a lower shoot to root dry weight ratio than the nutrient deprived plants. –Ca/-B deprived plants had a higher shoot to root dry weight ratio in sand and solution + sand substrates while Ca deprived plants had a higher shoot to root dry weight ratio in nutrient solution substrate (Table 3).

Shoot/Root Surface Area Ratio

Plants grown in nutrient solution + sand had a lower shoot to root surface area ratio than the rest of the substrates (Table 13). Overall, control plants had low shoot to root surface area ratio grown in nutrient solution + sand and sand substrates. In solution and sand substrates –Ca/-B deprived plants and -B in nutrient solution + sand substrate had a greater shoot to root ratio (Table 3).

DISCUSSION

Plant growth was severely affected by the absence of Ca, B, and both Ca\B. The impact was significantly greater in the root system than in the shoot. This indicates that

the unavailability of these nutrients first affects the root growth and development. In addition to that, physical and chemical factors determine the root morphology. B deprived plants were more compact due to the fewer nodes in nutrient solution substrate.

Overall, a higher shoot\root dry weight, and surface area ratio of nutrient deprived plant indicates a greater photosynthate allocation to the root system than shoot. Reduction in root length of sand substrate indicates a greater mechanical resistance than in peat and nutrition solution. On the other hand, root length and surface area of peat grown plants were greater due to lower mechanical resistance than sand. The reduction in root surface area and shoot/dry weight ratio in nutrient solution indicates the lesser mechanical impedance than sand and peat. The degree of thickness and waviness also indicates the difference in mechanical resistance.

There was no difference in root variables of Ca and B deprived plants irrespective of nutrient solution or substrate, although there was a significant difference between the control and nutrient deprived plants. The significant increase in root, shoot variables, and delayed development of symptoms of solution + sand treatments indicate the release of Ca and B from the sand, which interferes with the results. A rigorous purification of sand may not be helpful in absolute removal Ca and B. Therefore, it is advisable to use absolute inert substrate to study the impact of Ca, B, and Ca\B on root growth and development.

Table 2. Effects of -Ca, -B, and -Ca\-B on shoot variables of tomato plants grown in four different substrates.

Treatment		**Plant**	**Caliper**	**Leaf area**	**No. of**	**Shoot**
Substrate	**Nutrient**	**ht. (cm)**	**diameter**	**(cm^2)**	**nodes**	**dry wt. (g)**
Solution	Control	9.98 a	0.31 a	39.4 a	4 a	0.137 a
Solution	-B	7.35 c	0.18 c	16.9 c	2 c	0.097 c
Solution	-Ca	8.93 ab	0.19 b	25.7 b	3 b	0.061 b
Solution	-Ca\-B	7.83 bc	0.17 c	15.5 c	2 c	0.047 c
	$LSD_{0.05}$	1.256	0.012	6.757	0.000	0.028
Solution.+ Sand	Control	28.38 a	0.84 a	1079.0 a	10.8 a	5.41 a
Solution.+ Sand	-B	24.50 b	0.84 a	919.5 b	8.5 b	4.89 b
Solution.+ Sand	-Ca	17.45 c	0.55 c	138.1 c	7.0 c	2.44 d
Solution.+ Sand	-Ca\-B	19.70 c	0.65 b	208.2 c	7.3 c	3.10 c
	$LSD_{0.05}$	2.779	0.056	71.993	1.033	0.398
Sand	Control	10.63	0.40 a	43.9 a	0.2	0.14
Sand	-B	8.83	0.21 bc	22.7 b	2 c	0.08
Sand	-Ca	9.95	0.28 b	26.5 b	3 b	0.09
Sand	-Ca\-B	8.20	0.18 c	19.3 b	3 b	0.07
	$LSD_{0.05}$	NS	0.080	10.720	0.000	NS
Peat	Control	12.43	0.32 ab	67.2 a	4.0	0.18 a
Peat	-B	11.50	0.31 b	49.3 b	3.3	0.17 ab
Peat	-Ca	11.83	0.33 a	45.4 b	4.0	0.14 bc
Peat	-Ca\-B	10.63	0.31 b	27.1 c	3.5	0.11 c
	$LSD_{0.05}$	NS	0.0107	12.467	NS	0.0338

Table 3 . Effects of -Ca, -B, and -Ca\-B on root variables and shoot to root ratios of tomato plants grown in four different substrates.

Treatments		**Root**	**Root**	**Root**	**Shoot/root**	**Shoot/root**
Substrate	**Nutrient**	**dry wt. (mg)**	**surface area (cm^2)**	**Length (cm)**	**dry wt. ratio**	**surface area ratio**
Solution	Control	0.0037 a	5.8 a	125.4 a	9.96 c	6.94 b
Solution	-B	0.0007 b	1.3 bc	19.0 bc	9.96 c	14.4 b
Solution	-Ca	0.0009 b	1.8 b	32.4 b	114.60 a	14.80 b
Solution	-Ca\-B	0.0007 b	0.6 c	6.3 c	64.60 b	26.18 a
	$LSD_{0.05}$	1.200	0.980	15.086	24.427	10.020
Solution.+ Sand	Control	0.735 a	928.3 a	32945.8	7.42 b	1.17 c
Solution.+ Sand	-B	0.479 b	263.6 b	3143.5	10.25 b	3.50 a
Solution.+ Sand	-Ca	0.084 c	64.4 c	822.0	33.13 a	2.14 b
Solution.+ Sand	-Ca\-B	0.110 c	142.5 d	1666.8	28.69 a	1.49 c
	$LSD_{0.05}$	0.077	61.339	NS	12.821	3.325
Sand	Control	0.008 a	8.5 a	123.9 a	17.89 c	0.088 c
Sand	-B	0.001 b	1.1 b	14.2 b	76.75 b	0.73 b
Sand	-Ca	0.002 b	0.8 bc	11.8 b	50.08 bc	0.86 b
Sand	-Ca\-B	0.001 b	0.6 c	7.2 b	705 a	1.18 a
	$LSD_{0.05}$	0.002	0.474	18.4 b	52.500	0.277
Peat	Control	0.015 a	10.9	133.8	12.71	6.73
Peat	-B	0.001 b	6.9	91.8	17.33	8.06
Peat	-Ca	0.002 b	8.8	125.3	0.59	5.16
Peat	-Ca\-B	0.001 b	4.8	62.0	19.86	6.74
	$LSD_{0.05}$	0.003	NS	NS	NS	NS

Table 4. Summary of analysis of variance of substrate types [a) solution, b) sand, c) solution + sand, d) peat] and nutrition [control, -Ca, -B and -Ca/-B], and their interactions on plant height.

Source	DF	Mean Square	Pr > F
Solution	1	402.5039	<.0001
Sand	1	556.370156	<.0001
Solution+Sand	1	1047.331406	<.0001
-Ca	1	90.963906	<.0001
Solution-Ca	1	45.731406	<.0001
Sand-Ca	1	56.062656	<.0001
Solution+Sand-Ca	1	58.713906	<.0001
-B	1	30.387656	<.0001
Solution-B	1	0.026406	0.8793
Sand-B	1	0.113906	0.7525
Solution+Sand-B	1	3.106406	0.1045
-Ca-B	1	13.782656	0.0011
Solution-Ca-B	1	15.503906	0.0006
Sand-Ca-B	1	6.063906	0.0252
Solution+Sand-Ca-B	1	4.568906	0.0505
Error	45	1.131351	

Table 5. Summary of analysis of variance of substrate types [a) solution, b) sand, c) solution + sand, d) peat] and nutrition [control, -Ca, -B and -Ca/-B], and their interactions on stem diameter.

Source	DF	Mean Square	Pr > F
Solution	1	0.4900	<.0001
Sand	1	0.8235	<.0001
Solution+Sand	1	1.2488	<.0001
-Ca	1	0.1444	<.0001
Solution-Ca	1	0.0552	<.0001
Sand-Ca	1	0.0663	<.0001
Solution+Sand-Ca	1	0.0095	0.0020
-B	1	0.0333	<.0001
Solution-B	1	0.0189	<.0001
Sand-B	1	0.0000	0.8673
Solution+Sand-B	1	0.0676	<.0001
-Ca-B	1	0.0248	<.0001
Solution-Ca-B	1	0.0045	0.0281
Sand-Ca-B	1	0.0030	0.0711
Solution+Sand-Ca-B	1	0.0036	0.0497
Error	45	0.0008	

Table 6. Summary of analysis of variance of substrate types [a) solution, b) sand, c) solution + sand, d) peat] and nutrition [control, -Ca, -B and -Ca/-B], and their interactions on leaf area.

Source	DF	Mean Square	Pr > F
Solution	1	1172775	<.0001
Sand	1	1205159	<.0001
Solution+Sand	1	1379144	<.0001
-Ca	1	728679	<.0001
Solution-Ca	1	622102	<.0001
Sand-Ca	1	631119	<.0001
Solution+Sand-Ca	1	668727	<.0001
-B	1	11219	<.0001
Solution-B	1	1699	0.0776
Sand-B	1	1370	0.1118
Solution+Sand-B	1	1994	0.0566
-Ca-B	1	13290	<.0001
Solution-Ca-B	1	10333	<.0001
Sand-Ca-B	1	10681	<.0001
Solution+Sand-Ca-B	1	7899	0.0003
Error	45	521.0	

Table 7. Summary of analysis of variance of substrate types [a) solution, b) sand, c) solution + sand, d) peat] and nutrition [control, -Ca, -B and -Ca/-B], and their interactions on nodes.

Source	DF	Mean Square	Pr > F
Solution	1	78.7656	<.0001
Sand	1	97.5156	<.0001
Solution+Sand	1	159.3906	<.0001
-Ca	1	8.2656	<.0001
Solution-Ca	1	9.7656	<.0001
Sand-Ca	1	4.5156	<.0001
Solution+Sand-Ca	1	3.5156	<.0001
-B	1	17.0156	<.0001
Solution-B	1	0.7656	0.0216
Sand-B	1	0.0156	0.7353
Solution+Sand-B	1	0.7656	0.0216
-Ca-B	1	8.2656	<.0001
Solution-Ca-B	1	0.3906	0.0959
Sand-Ca-B	1	2.6406	<.0001
Solution+Sand-Ca-B	1	0.0156	0.7353
Error	45	0.1350	

Table 8. Summary of analysis of variance of substrate types [a) solution, b) sand, c) solution + sand, d) peat] and nutrition [control, -Ca, -B and -Ca/-B], and their interactions on shoot dry weight.

Source	DF	Mean Square	Pr > F
Solution	1	57.6160	<.0001
Sand	1	58.1371	<.0001
Solution+Sand	1	61.8310	<.0001
-Ca	1	6.1465	<.0001
Solution-Ca	1	5.4416	<.0001
Sand-Ca	1	5.4171	<.0001
Solution+Sand-Ca	1	5.6595	<.0001
-B	1	0.0025	0.6769
Solution-B	1	0.0051	0.5558
Sand-B	1	0.0134	0.3453
Solution+Sand-B	1	0.0249	0.2003
-Ca-B	1	0.3718	<.0001
Solution-Ca-B	1	0.3526	<.0001
Sand-Ca-B	1	0.3630	<.0001
Solution+Sand-Ca-B	1	0.3009	<.0001
Error	45	0.0147	

Table 9. Summary of analysis of variance of substrate types [a) solution, b) sand, c) solution + sand, d) peat] and nutrition [control, -Ca, -B and -Ca/-B], and their interactions on root dry weight.

Source	DF	Mean Square	Pr > F
Solution	1	0.4606	<.0001
Sand	1	0.4599	<.0001
Solution+Sand	1	0.5163	<.0001
-Ca	1	0.2679	<.0001
Solution-Ca	1	0.2619	<.0001
Sand-Ca	1	0.2603	<.0001
Solution+Sand-Ca	1	0.2518	<.0001
-B	1	0.0158	<.0001
Solution-B	1	0.0102	<.0001
Sand-B	1	0.0128	<.0001
Solution+Sand-B	1	0.0142	<.0001
-Ca-B	1	0.0189	<.0001
Solution-Ca-B	1	0.0196	<.0001
Sand-Ca-B	1	0.0220	<.0001
Solution+Sand-Ca-B	1	0.0182	<.0001
Error	45	0.0005	

Table 10. Summary of analysis of variance of substrate types [a) solution, b) sand, c) solution + sand, d) peat] and nutrition [control, -Ca, -B and -Ca/-B], and their interactions on root surface area.

Source	DF	Mean Square	Pr > F
Solution	1	466454	<.0001
Sand	1	468506	<.0001
Solution+Sand	1	496743	<.0001
-Ca	1	251076	<.0001
Solution-Ca	1	238729	<.0001
Sand-Ca	1	242260	<.0001
Solution+Sand-Ca	1	238266	<.0001
-B	1	92416	<.0001
Solution-B	1	83131	<.0001
Sand-B	1	84201	<.0001
Solution+Sand-B	1	84506	<.0001
-Ca-B	1	141846	<.0001
Solution-Ca-B	1	136493	<.0001
Sand-Ca-B	1	139278	<.0001
Solution+Sand-Ca-B	1	133974	<.0001
Error	45	346.7	

Table 11. Summary of analysis of variance of substrate types [a) solution, b) sand, c) solution + sand, d) peat] and nutrition [control, -Ca, -B and -Ca/-B], and their interactions on root length.

Source	DF	Mean Square	Pr > F
Solution	1	364640507	0.0939
Sand	1	363647260	0.0943
Solution+Sand	1	373470602	0.0901
-Ca	1	286687967	0.1361
Solution-Ca	1	281377559	0.1397
Sand-Ca	1	281831487	0.1394
Solution+Sand-Ca	1	279125102	0.1412
-B	1	214762063	0.1957
Solution-B	1	208375359	0.2023
Sand-B	1	207849528	0.2029
Solution+Sand-B	1	207590103	0.2031
-Ca-B	1	237331355	0.1741
Solution-Ca-B	1	234753341	0.1765
Sand-Ca-B	1	235517747	0.1758
Solution+Sand-Ca-B	1	231655629	0.1793
Error	45	124469046	

Table 12. Summary of analysis of variance of substrate types [a) solution, b) sand, c) solution + sand, d) peat] and nutrition [control, -Ca, -B and -Ca/-B], and their interactions on shoot/root dry weight ratio.

Source	DF	Mean Square	Pr > F
Solution	1	98758	<.0001
Sand	1	113064	<.0001
Solution+Sand	1	207914	<.0001
-Ca	1	185503	<.0001
Solution-Ca	1	51602	<.0001
Sand-Ca	1	75017	<.0001
Solution+Sand-Ca	1	151425	<.0001
-B	1	115281	<.0001
Solution-B	1	152995	<.0001
Sand-B	1	138867	<.0001
Solution+Sand-B	1	105127	<.0001
-Ca-B	1	74643	<.0001
Solution-Ca-B	1	109222	<.0001
Sand-Ca-B	1	99591	<.0001
Solution+Sand-Ca-B	1	74436	<.0001
Error	45	396	

Table 13. Summary of analysis of variance of substrate types [a) solution, b) sand, c) solution + sand, d) peat] and nutrition [control, -Ca, -B and -Ca/-B], and their interactions on shoot/root surface area.

Source	DF	Mean Square	Pr > F
Solution	1	682.77	<.0001
Sand	1	66.17	0.1394
Solution+Sand	1	3818.62	<.0001
-Ca	1	906.91	<.0001
Solution-Ca	1	124.43	0.0449
Sand-Ca	1	179.82	0.0169
Solution+Sand-Ca	1	1132.99	<.0001
-B	1	402.60	0.0006
Solution-B	1	0.01	0.9861
Sand-B	1	2.92	0.7533
Solution+Sand-B	1	251.85	0.0052
-Ca-B	1	47.54	0.2088
Solution-Ca-B	1	54.68	0.1782
Sand-Ca-B	1	121.66	0.0472
Solution+Sand-Ca-B	1	14.13	0.4904
Error	45	29.23	

APPENDICES

APPENDIX I

Potential Factors Confounding Nutrient Accumulation and Deficiency Symptoms Expression in This Study

Given the large number of crops and treatments, it was not possible to add additional treatments to eliminate confounding factors. Potentially confounding factors in this study included the following.

- Deficiency occurred at different times due to the differential susceptibility of a crop for each of the 11 nutrient deficiencies.
- The availability of nutrient differs between hydroponic system and solid substrates.
- N source provided in this study was totally in NO_3-N form. N form (NO_3/NH_4/Urea) and N concentration have great influence on the mineral composition of plants, which influence the different nutrient uptake. N is a macronutrient of high metabolic demand that affects the balance of anions and cations in plants. Nitrogen in the form of NO_3 or NH_4 has distinct effect on biochemical and physiological processes in plants. Both at higher concentrations can result in different growth responses.
- Large number of nutrients and crops studied render it impossible to apply more than one treatment to the deficiency of each nutrient within species.
- The 13 species crops were not grown at the same time.
- Environmental factors may have contributed to nutrient uptake.

- One source of variation was that dolomitic limestone was used to discharge the hydrogen ions during the manufacturing process of oasis cubes. However, if residual nutrient problem were to occur it would have only affected the Ca and Mg deficiency.
- Some of the Mn deficiency symptoms appeared to be similar to ozone injury. Therefore, Mn deficiency symptoms could have been manifested under high ozone conditions.
- Part of the observation was affinity of flower pigment /floral color in plant tissues. Flower pigmentation could have masked the chlorotic symptoms.

APPENDIX II

pH of 12 treatment nutrient solutions before and after replacement.

A complete replacement of nutrient solutions was done weekly for all crops during this study. The pH of various treatment nutrient solutions was measured before and after (day 6) replacement.

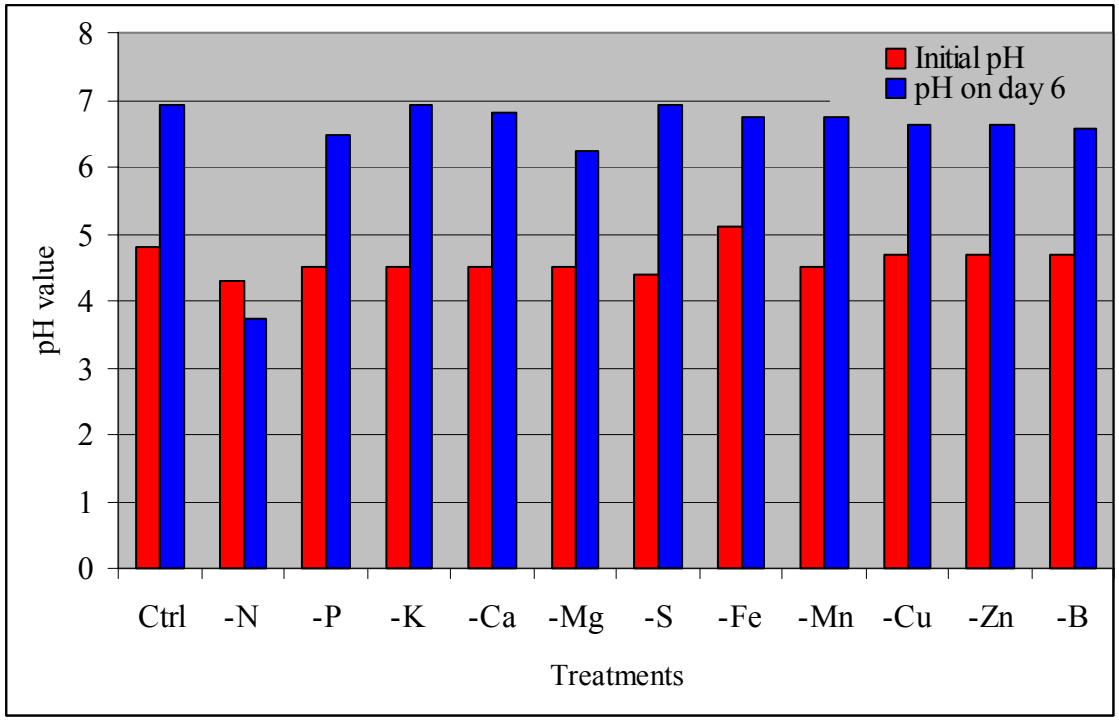

Figure 1. pH of nutrient solution at initial (day zero) and on day 6 for marigold.

APPENDIX III

Analysis of 11 nutrient elemental concentrations for each nutrient deficient treatment

Within each of first 12 chapters, only the concentration of deficient nutrient at early and advanced stages has presented for each treatment and the corresponding control. The detail analysis of other nutrient elemental concentration for each nutrient deficient treatment as well as the corresponding control is included in this appendix. The concentration values are means of three samples. There are total of 26 tables and for each crop there are two set of nutrient analysis, which is for early and advanced stage of deficiency.

Table 1. Vinca mean tissue nutrient concentration (n = 3) at early stages of deficiency in recently matured leaves of hydroponically grown plants.

	Percent dry wt.						mg·kg^{-1} of dry wt.				
Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
-B	5.11	0.61	4.08	1.74	0.43	0.21	54.1	172.3	16.5	6.6	10.5
Ctrl	4.70	0.70	3.64	1.65	0.42	0.16	50.9	143.0	15.6	5.1	55.8
-Ca	5.66	0.79	3.91	0.32	0.80	0.25	51.0	196.4	22.3	8.4	44.9
Ctrl	5.99	0.83	4.22	1.74	0.44	0.24	57.5	174.0	18.6	8.1	43.1
-Cu	5.67	0.84	3.30	1.63	0.44	0.24	51.4	80.6	17.0	1.4	53.0
Ctrl	5.76	0.79	3.86	1.76	0.44	0.23	55.0	103.7	16.3	7.5	52.4
-Fe	5.82	0.70	4.11	1.61	0.40	0.33	29.0	231.0	45.9	8.5	50.7
Ctrl	5.76	0.79	3.86	1.76	0.44	0.23	55.0	103.7	16.3	7.4	52.4
-K	4.99	0.70	0.73	2.13	0.59	0.23	69.9	90.2	19.3	7.7	70.7
Ctrl	5.21	0.78	3.64	1.69	0.42	0.19	56.5	97.4	17.4	7.2	45.1
-Mg	5.71	0.77	4.70	1.77	0.11	0.26	58.4	188.7	22.4	7.9	49.5
Ctrl	5.99	0.83	4.22	1.74	0.40	0.24	57.5	174.0	18.6	8.1	43.1
-Mn	5.73	0.92	5.08	1.75	0.44	0.26	48.8	9.3	17.5	8.0	67.4
Ctrl	4.66	0.77	3.83	1.74	0.39	0.19	60.9	122.0	17.6	5.4	53.1
-N	2.66	0.55	2.83	1.36	0.29	0.17	68.2	140.7	17.1	6.6	46.5
Ctrl	5.99	0.83	4.22	1.74	0.44	0.24	57.5	174.0	18.6	8.1	43.1
-P	3.86	0.07	3.49	1.46	0.33	0.16	67.6	151.0	19.1	8.1	47.0
Ctrl	4.70	0.70	3.64	1.65	0.42	0.16	50.9	143.0	15.6	5.1	55.8
-S	4.22	0.62	3.09	1.72	0.37	0.09	37.9	121.0	14.5	4.5	64.0
Ctrl	4.61	0.91	3.41	2.15	0.53	0.20	60.5	147.0	16.5	5.9	101.0
-Zn	4.99	1.20	3.16	2.24	0.50	0.23	79.9	123.0	12.3	7.4	81.6
Ctrl	5.21	0.78	3.64	1.69	0.42	0.19	56.5	97.4	17.4	7.2	45.1

Table 2. Vinca mean tissue nutrient concentration (n = 3) at advanced stages of deficiency in recently matured leaves of hydroponically grown plants.

	Percent dry wt.						mg·kg^{-1} of dry wt.				
Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
-B	4.44	0.82	3.17	2.28	0.48	0.19	52.0	195.5	16.8	6.1	7.9
Ctrl	5.17	0.99	3.47	2.19	0.54	0.22	62.2	108.0	14.2	6.8	134.3
-Ca	5.10	0.71	3.80	0.28	0.83	0.25	61.1	214.8	26.3	9.6	51.9
Ctrl	5.29	0.64	4.21	1.59	0.39	0.21	55.1	152.0	14.7	7.2	44.0
-Cu	5.73	1.06	3.96	2.15	0.53	0.29	59.2	117.3	18.8	1.0	136.3
Ctrl	5.17	0.99	3.47	2.19	0.54	0.22	62.2	108.0	14.2	6.8	134.3
-Fe	6.22	0.55	6.07	2.10	0.46	0.52	16.4	596.7	48.5	8.3	89.8
Ctrl	5.10	0.82	3.56	1.80	0.45	0.18	54.5	139.5	12.1	5.7	67.1
-K	5.03	0.63	0.28	1.72	0.86	0.24	60.8	215.3	23.6	7.7	114.3
Ctrl	5.10	0.82	3.56	0.18	0.45	0.18	54.5	139.5	12.1	5.7	67.1
-Mg	5.41	0.67	4.98	1.84	0.09	0.23	44.5	.262.67	19.4	6.9	51.4
Ctrl	5.10	0.82	3.56	1.80	0.45	0.18	54.5	139.5	12.1	5.7	67.1
-Mn	6.33	1.02	5.79	2.52	0.62	0.32	58.6	5.2	24.0	8.7	140.0
Ctrl	5.17	0.99	3.47	2.19	0.54	0.22	62.2	108.0	14.2	6.8	134.3
-N	1.04	0.26	1.94	1.10	0.18	0.09	49.4	140.7	11.9	3.0	47.7
Ctrl	5.10	0.82	3.96	1.80	0.45	0.18	54.5	139.5	12.1	5.7	67.2
-P	4.64	0.04	2.91	1.83	0.45	0.16	70.0	187.3	25.9	9.3	90.3
Ctrl	5.17	0.99	3.47	2.19	0.54	0.22	62.2	108.0	14.2	6.8	134.3
-S	4.04	0.60	2.93	1.63	0.42	0.07	34.0	81.5	10.6	4.1	92.1
Ctrl	5.17	0.99	3.47	2.19	0.54	0.22	62.2	108.0	14.2	6.8	134.3
-Zn	4.04	1.15	3.19	1.93	0.44	0.15	53.5	251.0	6.4	6.3	149.7
Ctrl	4.61	0.91	3.41	2.15	0.33	0.20	60.5	147.0	12.2	5.9	101.0

Table 3. Marigold mean tissue nutrient concentration (n = 3) at early stages of deficiency in recently matured leaves of hydroponically grown plants.

	Percent dry wt.						mg·kg^{-1} of dry wt.				
Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
-B	4.12	0.30	3.20	1.82	0.28	0.18	43.0	178.3	21.2	6.0	10.9
Ctrl	5.53	0.37	4.33	2.12	0.35	0.23	104.3	229.0	29.3	11.6	43.9
-Ca	3.78	0.24	3.75	0.30	0.65	0.14	60.1	195.3	21.6	7.0	34.2
Ctrl	5.53	0.37	4.33	2.12	0.35	0.23	104.3	229.0	29.3	11.6	43.9
-Cu	5.85	0.46	4.27	2.55	0.46	0.31	244.0	306.0	38.3	2.1	65.1
Ctrl	4.35	0.19	3.60	2.10	0.38	0.21	91.3	208.7	22.3	9.3	50.2
-Fe	4.82	0.55	4.87	2.33	0.44	0.28	40.9	476.7	102.5	17.7	56.3
Ctrl	5.53	0.37	4.33	2.12	0.35	0.23	104.3	229.0	29.3	11.6	43.9
-K	5.50	0.44	0.55	3.04	1.04	0.35	112.0	313.0	29.5	13.0	77.7
Ctrl	4.35	0.19	3.60	2.10	0.38	0.21	91.3	208.7	22.3	9.3	50.2
-Mg	5.11	0.37	3.62	2.01	0.11	0.25	83.5	169.0	22.5	9.3	53.5
Ctrl	4.35	0.19	3.60	2.10	0.38	0.21	91.3	208.7	22.3	9.3	50.2
-Mn	6.23	0.49	5.24	2.67	0.48	0.30	99.6	11.3	36.5	12.5	65.7
Ctrl	4.35	0.19	3.60	2.10	0.38	0.21	91.3	208.7	22.3	9.3	50.2
-N	1.52	0.34	2.50	1.33	0.18	0.17	56.8	174.3	25.0	6.9	39.1
Ctrl	5.53	0.37	4.33	2.12	0.35	0.23	104.3	229.0	29.3	11.6	43.9
-P	3.96	0.07	2.72	1.51	0.26	0.19	80.2	215.0	29.2	9.6	40.1
Ctrl	5.53	0.37	4.33	2.12	0.35	0.23	104.3	229.0	29.3	11.6	43.9
-S	3.50	0.31	2.22	2.05	0.23	0.09	73.0	221.7	23.2	7.9	47.7
Ctrl	4.35	0.19	3.60	2.10	0.38	0.21	91.3	208.7	22.3	9.3	50.2
-Zn	3.06	0.80	2.02	2.73	0.71	0.25	82.0	237.7	9.7	7.2	68.5
Ctrl	5.38	0.40	5.16	2.79	0.56	0.28	151.0	276.5	25.7	9.7	72.5

Table 4. Marigold mean tissue nutrient concentration (n = 3) at advanced stages of deficiency in recently matured leaves of hydroponically grown plants.

	Percent dry wt.						mg·kg^{-1} of dry wt.				
Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
-B	3.61	0.47	2.77	2.09	0.37	0.19	60.3	259.0	26.5	6.2	9.3
Ctrl	5.78	0.59	3.34	2.66	0.58	0.29	105.0	245.0	45.5	13.6	72.7
-Ca	3.78	0.25	4.63	0.30	0.76	0.14	73.0	234.3	24.2	6.7	42.8
Ctrl	4.35	0.19	3.60	2.10	0.38	0.21	91.3	208.7	22.3	9.3	50.2
-Cu	5.06	0.72	4.17	2.74	0.72	0.32	401.3	302.3	32.4	2.1	79.1
Ctrl	4.84	0.41	3.85	3.07	0.61	0.24	150.5	275.5	36.6	18.5	86.0
-Fe	5.39	0.66	4.63	2.95	0.62	0.37	34.1	417.3	79.6	15.8	100.7
Ctrl	5.38	0.40	5.16	2.79	0.56	0.28	151.0	276.5	25.7	9.7	72.5
-K	6.02	0.63	0.40	3.32	1.61	0.58	316.7	456.7	50.9	40.9	108.0
Ctrl	5.78	0.59	3.34	2.66	0.58	0.29	105.0	245.0	45.5	13.6	72.7
-Mg	3.56	0.33	3.52	1.88	0.06	0.18	108.0	138.7	17.6	16.9	54.3
Ctrl	4.84	0.41	3.85	3.07	0.61	0.24	150.5	275.5	36.6	18.5	86.0
-Mn	6.25	0.86	6.18	2.57	0.59	0.35	238.0	6.7	51.2	13.5	78.1
Ctrl	5.40	0.59	3.56	2.73	0.60	0.29	129.5	212.0	31.0	24.2	77.0
-N	0.91	0.28	1.98	1.59	0.19	0.14	57.5	166.3	22.8	5.6	49.4
Ctrl	5.38	0.40	5.16	2.79	0.56	0.28	151.0	276.5	25.7	9.7	72.5
-P	4.21	0.06	2.69	1.75	0.29	0.22	95.9	258.0	43.9	10.3	49.6
Ctrl	4.35	0.19	3.60	2.10	0.38	0.21	91.3	208.7	22.3	9.3	50.2
-S	3.43	0.48	2.60	2.94	0.46	0.10	140.0	337.3	44.3	34.5	73.4
Ctrl	5.40	0.59	3.56	2.73	0.60	0.29	129.5	212.0	31.0	24.2	77.0
-Zn	2.88	1.11	1.79	3.04	0.95	0.29	59.1	267.3	8.1	6.9	76.6
Ctrl	4.84	0.41	3.85	3.07	0.61	0.24	150.5	275.5	36.6	18.5	86.0

Table 5. Zinnia mean tissue nutrient concentration (n = 3) at early stages of deficiency in recently matured leaves of hydroponically grown plants.

	Percent dry wt.						mg·kg^{-1} of dry wt.				
Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
-B	4.58	0.78	4.19	0.95	0.48	0.27	88.5	135.7	31.3	7.2	23.4
Ctrl	5.00	0.60	4.77	1.14	0.42	0.23	54.2	147.0	35.1	6.2	56.6
-Ca	4.78	0.74	5.36	0.53	1.08	0.30	86.7	137.0	31.1	5.0	72.9
Ctrl	5.55	0.72	4.89	1.43	0.57	0.28	66.1	128.0	30.0	5.4	57.3
-Cu	5.50	0.96	5.39	1.44	0.78	0.34	118.7	128.7	29.9	0.9	84.1
Ctrl	4.70	0.82	4.39	1.34	0.63	0.26	79.6	144.0	20.0	4.1	72.2
-Fe	5.30	0.70	5.36	1.32	17.35	0.27	55.1	177.7	31.4	5.8	64.6
Ctrl	5.02	0.57	6.94	1.49	0.54	0.27	55.7	591.7	122.0	15.3	92.4
-K	4.98	1.10	4.56	2.20	0.96	0.26	86.9	202.0	27.1	4.6	135.0
Ctrl	4.36	0.81	5.65	1.93	0.10	0.20	81.9	192.7	22.0	4.0	113.7
-Mg	5.55	0.72	4.89	1.43	0.57	0.28	66.1	128.0	30.0	5.4	57.3
Ctrl	5.68	1.10	6.03	1.27	0.74	0.30	108.1	10.8	43.1	7.2	75.2
-Mn	5.00	0.60	4.77	1.14	0.42	0.23	54.2	147.0	35.1	6.2	56.6
Ctrl	1.66	0.82	5.02	1.11	0.43	0.14	84.4	97.7	25.5	7.1	108.3
-N	5.00	0.60	4.77	1.14	0.42	0.23	54.2	147.0	35.1	6.2	56.6
Ctrl	4.04	0.10	4.78	1.36	0.46	0.25	92.9	180.0	36.3	6.9	74.9
-P	5.00	0.60	4.77	1.14	0.42	0.23	54.2	147.0	35.1	6.2	56.6
Ctrl	5.04	1.12	0.89	2.04	1.79	0.38	376.7	449.0	52.7	14.4	111.0
-S	4.98	1.10	4.56	2.20	0.96	0.26	86.9	202.0	27.1	4.6	135.0
Ctrl	3.66	0.87	4.70	1.79	0.54	0.11	62.5	194.0	22.3	3.1	116.3
-Zn	4.70	0.82	4.39	1.34	0.63	0.26	79.6	144.0	20.0	4.1	72.2
Ctrl	4.02	1.06	4.16	1.87	0.75	0.27	160.7	154.0	9.2	4.7	91.2

Table 6. Zinnia mean tissue nutrient concentration (n = 3) at advanced stages of deficiency in recently matured leaves of hydroponically grown plants.

	Percent dry wt.						mg·kg^{-1} of dry wt.				
Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
-B	4.30	1.06	3.74	0.88	0.55	0.24	72.1	132.0	32.7	6.9	15.1
Ctrl	4.70	0.82	4.39	1.34	0.63	0.26	79.6	144.0	20.0	4.1	72.2
-Ca	4.26	0.79	4.97	0.44	1.01	0.26	108.3	155.7	36.6	8.6	86.3
Ctrl	5.00	0.60	4.77	1.14	0.42	0.23	54.2	147.0	35.1	6.2	56.6
-Cu	4.93	1.58	4.67	2.53	1.04	0.31	137.0	290.0	39.3	1.2	139.3
Ctrl	4.53	1.01	5.02	1.69	0.76	0.22	59.7	172.0	16.8	3.9	111.5
-Fe	4.53	1.01	5.02	1.69	0.76	0.22	59.7	172.0	16.8	3.9	111.5
Ctrl	4.89	0.57	5.42	2.46	0.89	0.28	63.3	328.7	62.3	12.3	120.3
-K	4.53	1.01	5.02	1.69	0.76	0.22	59.7	172.0	16.8	3.9	111.5
Ctrl	4.28	1.05	5.71	2.12	0.10	0.22	90.8	220.0	23.6	4.5	114.0
-Mg	4.98	1.10	4.56	2.20	0.96	0.26	86.9	202.0	27.1	4.6	135.0
Ctrl	5.28	1.44	5.91	1.12	0.65	0.30	102.9	9.2	53.9	7.7	103.4
-Mn	4.70	0.82	4.39	1.34	0.63	0.26	79.6	144.0	20.0	4.1	72.2
Ctrl	1.23	0.89	4.68	1.02	0.43	0.09	80.9	87.6	21.8	4.6	168.0
-N	4.70	0.82	4.39	1.34	0.63	0.26	79.6	144.0	20.0	4.1	72.2
Ctrl	3.83	0.07	4.47	1.74	0.62	0.29	177.7	267.3	46.1	7.5	122.0
-P	4.70	0.82	4.39	1.34	0.63	0.26	79.6	144.0	20.0	4.1	72.2
Ctrl	5.06	1.02	0.37	1.54	2.19	0.36	419.0	713.0	59.3	19.8	175.3
-S	4.53	1.01	5.02	1.69	0.76	0.22	59.7	172.0	16.8	3.9	111.5
Ctrl	3.68	1.16	4.67	2.50	0.68	0.12	73.7	274.3	28.8	3.4	146.7
-Zn	4.98	1.10	4.56	2.20	0.96	0.26	86.9	202.0	27.1	4.6	135.0
Ctrl	3.71	1.90	4.33	2.29	1.15	0.27	247.0	287.0	8.1	4.8	148.0

Table 7. Salvia mean tissue nutrient concentration (n = 3) at early stages of deficiency in recently matured leaves of hydroponically grown plants.

	Percent dry wt.						mg·kg^{-1} of dry wt.				
Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
-B	3.48	0.26	4.18	2.18	0.41	0.17	56.1	189.9	22.8	4.4	9.3
Ctrl	4.81	0.38	5.27	2.98	0.59	0.24	101.0	268.3	30.3	6.4	68.7
-Ca	3.44	0.28	3.08	0.66	0.70	0.17	98.9	116.2	22.4	4.3	41.2
Ctrl	5.06	0.43	4.96	2.66	0.54	0.27	75.0	242.1	40.5	7.7	45.2
-Cu	5.51	0.45	5.85	2.97	0.56	0.30	575.0	307.4	72.4	1.8	75.3
Ctrl	4.81	0.38	5.27	2.98	0.59	0.24	101.0	268.3	30.3	6.4	68.7
-Fe	5.01	0.43	5.69	2.85	0.60	0.27	52.0	282.3	45.3	7.9	54.5
Ctrl	5.06	0.43	4.96	2.66	0.54	0.27	75.0	242.1	40.5	7.7	45.2
-K	5.28	0.52	0.22	2.37	0.93	0.24	84.8	313.0	43.3	6.6	62.2
Ctrl	4.81	0.38	5.27	2.98	0.59	0.24	101.0	268.3	30.3	6.4	68.7
-Mg	4.48	0.38	4.72	2.46	0.09	0.24	90.1	248.5	51.7	9.7	48.4
Ctrl	5.06	0.43	4.96	2.66	0.54	0.27	75.0	242.1	40.5	7.7	45.2
-Mn	6.52	0.75	8.33	2.56	0.64	0.41	297.1	5.1	79.1	19.6	55.9
Ctrl	5.06	0.43	4.96	2.66	0.54	0.27	75.0	242.1	40.5	7.7	45.2
-N	2.22	0.44	3.34	2.29	0.53	0.32	121.6	229.3	47.1	8.0	53.4
Ctrl	5.06	0.43	4.96	2.66	0.54	0.27	75.0	242.1	40.5	7.7	45.2
-P	4.06	0.07	4.32	3.14	0.51	0.31	111.1	398.1	73.3	7.7	79.0
Ctrl	4.81	0.38	5.27	2.98	0.59	0.24	101.0	268.3	30.3	6.4	68.7
-S	4.30	0.44	3.98	3.47	0.55	0.10	92.3	338.3	32.0	5.4	75.6
Ctrl	4.81	0.38	5.27	2.98	0.59	0.24	101.0	268.3	30.3	6.4	68.7
-Zn	4.75	1.26	4.85	3.87	0.72	0.22	313.1	542.5	12.0	10.4	60.0
Ctrl	4.81	0.38	5.27	2.98	0.59	0.24	101.0	268.3	30.3	6.4	68.7

Table 8. Salvia mean tissue nutrient concentration (n = 3) at advanced stages of deficiency in recently matured leaves of hydroponically grown plants.

	Percent dry wt.						mg·kg^{-1} of dry wt.				
Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
-B	3.60	0.26	4.44	2.18	0.42	0.15	49.5	210.8	21.3	3.8	8.8
Ctrl	4.90	0.40	5.30	3.17	0.55	0.22	114.0	349.5	32.7	6.0	79.7
-Ca	3.45	0.34	2.94	0.46	0.58	0.23	100.7	93.4	25.6	6.2	45.2
Ctrl	4.81	0.38	5.27	2.98	0.59	0.24	101.0	268.3	30.3	6.4	68.7
-Cu	5.11	0.42	5.12	2.90	0.50	0.24	479.0	246.8	61.5	1.8	78.0
Ctrl	4.90	0.40	5.30	3.17	0.55	0.22	114.0	349.5	32.7	6.0	79.7
-Fe	4.70	0.41	5.37	3.21	0.61	0.23	69.6	364.6	69.0	10.9	87.8
Ctrl	4.90	0.40	5.30	3.17	0.55	0.22	114.0	349.5	32.7	6.0	79.7
-K	5.44	0.59	0.20	2.17	0.82	0.23	92.2	314.0	48.1	9.8	68.9
Ctrl	4.90	0.40	5.30	3.17	0.55	0.22	114.0	349.5	32.7	6.0	79.7
-Mg	4.88	0.40	4.90	2.53	0.07	0.22	104.7	377.2	54.1	12.6	64.1
Ctrl	4.81	0.38	5.27	2.98	0.59	0.24	101.0	268.3	30.3	6.4	68.7
-Mn	6.33	0.68	7.36	2.08	0.55	0.45	229.5	4.2	79.0	23.1	55.1
Ctrl	4.81	0.38	5.27	2.98	0.59	0.24	101.0	268.3	30.3	6.4	68.7
-N	2.01	0.62	4.17	3.34	0.72	0.54	243.2	377.3	49.7	8.8	115.9
Ctrl	4.81	0.38	5.27	2.98	0.59	0.24	101.0	268.3	30.3	6.4	68.7
-P	3.97	0.07	4.07	3.09	0.48	0.25	89.4	415.5	54.8	6.0	86.8
Ctrl	4.85	0.38	5.37	3.44	0.61	0.23	152.7	361.5	34.4	6.8	86.3
-S	4.37	0.45	4.55	3.82	0.53	0.13	97.9	462.2	33.2	5.7	81.1
Ctrl	4.90	0.40	5.30	3.17	0.55	0.22	114.0	349.5	32.7	6.0	79.7
-Zn	4.57	1.36	5.16	3.25	0.60	0.18	240.3	591.0	11.3	13.5	65.9
Ctrl	4.90	0.40	5.30	3.17	0.55	0.22	114.0	349.5	32.7	6.0	79.7

Table 9. Celosia mean tissue nutrient concentration (n = 3) at early stages of deficiency in recently matured leaves of hydroponically grown plants.

	Percent dry wt.						mg·kg^{-1} of dry wt.				
Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
-B	4.30	0.44	6.21	1.61	0.60	0.19	43.0	324.3	23.6	8.7	8.9
Ctrl	5.42	0.46	8.18	2.17	0.77	0.26	72.7	258.3	27.9	10.4	28.4
-Ca	5.28	0.38	8.07	0.64	1.86	0.22	80.6	517.3	22.5	8.5	42.9
Ctrl	5.19	0.47	9.14	2.45	0.84	0.24	74.2	398.0	26.5	9.7	38.6
-Cu	6.08	0.68	8.89	2.50	0.86	0.26	129.0	528.0	48.5	1.8	63.9
Ctrl	5.19	0.47	9.14	2.45	0.84	0.24	74.2	398.0	26.5	9.7	38.6
-Fe	5.31	0.43	8.15	2.30	0.81	0.24	54.9	509.7	55.9	12.5	30.4
Ctrl	5.42	0.46	8.18	2.17	0.77	0.26	72.7	258.3	27.9	10.4	28.4
-K	5.73	0.60	4.81	3.29	1.31	0.26	91.5	330.7	30.4	11.0	36.1
Ctrl	5.72	0.47	7.96	2.17	0.73	0.27	54.1	221.0	31.7	10.2	25.2
-Mg	5.32	0.46	7.97	2.37	0.11	0.25	86.9	356.0	24.3	10.1	32.2
Ctrl	5.42	0.46	8.18	2.17	0.77	0.26	72.7	258.3	27.9	10.4	28.4
-Mn	6.30	0.64	9.97	1.80	0.70	0.26	81.8	13.7	29.8	11.7	49.7
Ctrl	5.42	0.46	8.18	2.17	0.77	0.26	72.7	258.3	27.9	10.4	28.4
-N	3.71	0.72	7.35	2.22	0.77	0.30	110.7	353.3	39.1	13.1	41.4
Ctrl	5.72	0.47	7.96	2.17	0.73	0.27	54.1	221.0	31.7	10.2	25.2
-P	4.19	0.09	6.01	1.95	0.74	0.21	84.0	362.0	38.3	9.7	35.4
Ctrl	5.14	0.44	8.16	2.26	0.80	0.25	67.5	348.0	25.2	9.1	31.1
-S	5.66	0.48	7.68	2.25	0.74	0.17	72.1	288.3	29.5	10.2	26.1
Ctrl	5.72	0.47	7.96	2.17	0.73	0.27	54.1	221.0	31.7	10.2	25.2
-Zn	5.12	1.14	6.78	2.41	0.83	0.17	119.0	508.3	7.6	13.5	26.5
Ctrl	5.42	0.46	8.18	2.17	0.77	0.26	72.7	258.3	27.9	10.4	28.4

Table 10. Celosia mean tissue nutrient concentration (n = 3) at advanced stages of deficiency in recently matured leaves of hydroponically grown plants.

	Percent dry wt.						mg·kg^{-1} of dry wt.				
Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
-B	3.57	0.44	5.37	1.45	0.51	0.17	40.3	384.0	23.9	8.9	7.9
Ctrl	5.19	0.47	9.14	2.45	0.84	0.24	74.2	398.0	26.5	9.7	38.6
-Ca	4.94	0.27	8.74	0.47	1.80	0.20	85.1	570.7	23.4	7.9	66.6
Ctrl	4.56	0.34	9.45	2.57	0.86	0.19	96.4	545.7	25.2	8.1	52.4
-Cu	5.64	0.66	9.75	2.45	0.82	0.23	127.3	626.7	43.1	1.8	92.1
Ctrl	4.56	0.34	9.45	2.57	0.86	0.19	96.4	545.7	25.2	8.1	52.4
-Fe	4.86	0.40	9.85	2.57	0.90	0.22	46.8	654.3	46.9	17.7	65.1
Ctrl	4.56	0.34	9.45	2.57	0.86	0.19	96.4	545.7	25.2	8.1	52.4
-K	4.93	0.56	1.81	4.15	2.35	0.23	106.7	790.3	29.9	9.3	63.0
Ctrl	4.40	0.31	9.54	2.47	0.72	0.20	114.9	532.0	25.9	8.6	53.7
-Mg	4.47	0.45	7.41	2.09	0.08	0.22	94.8	398.0	22.7	10.0	39.4
Ctrl	5.19	0.47	9.14	2.45	0.84	0.24	74.2	398.0	26.5	9.7	38.6
-Mn	5.95	0.58	10.57	1.67	0.63	0.24	77.1	14.2	34.4	12.1	65.6
Ctrl	4.56	0.34	9.45	2.57	0.86	0.19	96.4	545.7	25.2	8.1	52.4
-N	2.82	0.83	7.28	2.37	0.86	0.30	168.7	588.7	56.7	19.0	92.6
Ctrl	5.19	0.47	9.14	2.45	0.84	0.24	74.2	398.0	26.5	9.7	38.6
-P	4.23	0.05	6.96	2.28	0.91	0.22	89.8	613.0	56.7	11.9	69.2
Ctrl	4.40	0.31	9.54	2.47	0.72	0.20	114.9	532.0	25.9	8.6	53.7
-S	5.35	0.55	7.95	2.59	0.87	0.14	74.0	624.0	30.7	7.3	55.6
Ctrl	4.56	0.34	9.45	2.57	0.86	0.19	96.4	545.7	25.2	8.1	52.4
-Zn	5.06	1.60	7.11	2.96	1.14	0.17	133.1	683.3	7.7	17.0	32.6
Ctrl	5.19	0.47	9.14	2.45	0.84	0.24	74.2	398.0	26.5	9.7	38.6

Table 11. Impatiens mean tissue nutrient concentration (n = 3) at early stages of deficiency in recently matured leaves of hydroponically grown plants.

	Percent dry wt.						$mg \cdot kg^{-1}$ of dry wt.				
Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
-B	5.20	0.57	3.14	3.07	0.47	0.24	49.2	153.6	35.5	7.9	14.1
Ctrl	5.84	0.54	2.83	3.22	0.53	0.32	109.4	150.2	28.6	7.6	68.8
-Ca	3.28	0.35	2.29	0.34	0.52	0.13	108.6	72.7	32.4	7.2	52.7
Ctrl	5.41	0.59	3.48	3.35	0.44	0.30	101.3	133.3	40.4	9.0	63.5
-Cu	5.92	0.76	3.95	3.41	0.54	0.33	469.4	144.8	62.4	2.5	105.1
Ctrl	5.84	0.54	2.83	3.22	0.53	0.32	109.4	150.2	28.6	7.6	68.8
-Fe	4.93	0.77	4.14	3.68	0.62	0.52	55.4	401.0	81.4	27.9	83.7
Ctrl	5.41	0.59	3.48	3.35	0.44	0.30	101.3	133.3	40.4	9.0	63.5
-K	6.72	1.00	0.31	3.68	1.09	0.28	101.3	203.1	56.9	10.0	79.9
Ctrl	5.84	0.54	2.83	3.22	0.53	0.32	109.4	150.2	28.6	7.6	68.8
-Mg	4.65	0.50	3.51	2.75	0.09	0.17	243.4	146.6	37.4	9.2	51.9
Ctrl	5.41	0.59	3.48	3.35	0.44	0.30	101.3	133.3	40.4	9.0	63.5
-Mn	6.30	0.90	5.55	3.44	0.62	0.31	104.4	10.4	79.0	19.0	78.3
Ctrl	5.41	0.59	3.48	3.35	0.44	0.30	101.3	133.3	40.4	9.0	63.5
-N	1.21	0.38	2.16	2.27	0.29	0.16	337.4	129.9	66.7	9.7	67.6
Ctrl	5.41	0.59	3.48	3.35	0.44	0.30	101.3	133.3	40.4	9.0	63.5
-P	4.32	0.08	3.06	3.00	0.61	0.25	270.8	114.7	84.3	15.0	81.9
Ctrl	5.41	0.59	3.48	3.35	0.44	0.30	101.3	133.3	40.4	9.0	63.5
-S	5.52	0.62	3.65	3.63	0.44	0.21	157.2	146.7	74.5	10.4	61.0
Ctrl	5.41	0.59	3.48	3.35	0.44	0.30	101.3	133.3	40.4	9.0	63.5
-Zn	5.88	0.68	3.02	3.84	0.61	0.25	265.7	292.9	27.9	11.9	65.9
Ctrl	5.84	0.54	2.83	3.22	0.53	0.32	109.4	150.2	28.6	7.6	68.8

Table 12. Impatiens mean tissue nutrient concentration (n = 3) at advanced stages of deficiency in recently matured leaves of hydroponically grown plants.

	Percent dry wt.						$mg \cdot kg^{-1}$ of dry wt.				
Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
-B	4.50	0.48	3.00	2.94	0.40	0.23	45.3	194.0	52.7	10.0	10.0
Ctrl	5.29	0.53	2.00	4.37	0.59	0.31	85.1	223.6	30.9	8.4	106.0
-Ca	3.41	0.31	1.85	0.56	0.65	0.16	122.7	78.0	42.4	9.9	68.8
Ctrl	5.84	0.54	2.83	3.22	0.53	0.32	109.4	150.2	28.6	7.6	68.8
-Cu	5.39	0.72	3.44	3.55	0.51	0.35	438.1	200.1	57.0	5.0	120.2
Ctrl	5.29	0.53	2.00	4.37	0.59	0.31	85.1	223.6	30.9	8.4	106.0
-Fe	4.85	0.78	4.00	3.54	0.68	0.67	43.8	602.2	104.0	28.6	89.1
Ctrl	5.84	0.54	2.83	3.22	0.53	0.32	109.4	150.2	28.6	7.6	68.8
-K	6.13	0.82	0.31	3.72	1.26	0.21	106.4	174.7	45.2	9.1	76.2
Ctrl	5.63	0.60	2.25	4.00	0.58	0.33	94.5	200.7	28.5	9.1	93.6
-Mg	3.51	0.35	3.05	2.16	0.07	0.11	156.8	104.9	45.7	8.5	49.6
Ctrl	5.84	0.54	2.83	3.22	0.53	0.32	109.4	150.2	28.6	7.6	68.8
-Mn	6.21	1.15	6.38	4.14	0.61	0.32	117.5	7.6	65.0	19.6	122.0
Ctrl	5.84	0.54	2.83	3.22	0.53	0.32	109.4	150.2	28.6	7.6	68.8
-N	0.82	0.23	1.42	1.44	0.15	0.11	170.9	88.8	42.2	6.4	63.9
Ctrl	5.84	0.54	2.83	3.22	0.53	0.32	109.4	150.2	28.6	7.6	68.8
-P	4.01	0.07	2.88	2.36	0.46	0.23	222.2	97.0	68.5	14.8	101.1
Ctrl	5.84	0.54	2.83	3.22	0.53	0.32	109.4	150.2	28.6	7.6	68.8
-S	4.16	0.65	2.05	4.87	0.50	0.10	97.5	240.7	38.8	7.8	136.2
Ctrl	5.29	0.53	2.00	4.37	0.59	0.31	85.1	223.6	30.9	8.4	106.0
-Zn	5.78	0.76	2.63	4.27	0.62	0.25	362.5	358.2	28.8	15.4	71.1
Ctrl	5.63	0.60	2.25	4.00	0.58	0.33	94.5	200.7	28.5	9.1	93.6

Table 13. Pansy mean tissue nutrient concentration (n = 3) at early stages of deficiency in recently matured leaves of hydroponically grown plants.

	Percent dry wt.						mg kg-1 of dry wt.				
Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
-B	3.21	0.34	3.01	1.15	0.34	0.19	85.3	67.9	80.6	8.0	9.7
Ctrl	3.96	0.41	4.64	1.52	0.38	0.21	78.5	173.0	39.2	7.6	45.3
-Ca	2.78	0.36	3.67	0.45	0.45	0.16	81.1	70.6	73.2	9.1	51.1
Ctrl	4.64	0.55	4.54	1.35	0.43	0.22	94.6	146.4	70.5	10.4	44.2
-Cu	5.59	0.51	6.17	1.71	0.52	0.24	106.7	236.1	60.9	2.8	89.5
Ctrl	3.96	0.41	4.64	1.52	0.38	0.21	78.5	173.0	39.2	7.6	45.3
-Fe	3.31	0.35	5.06	1.58	0.60	0.21	51.4	450.6	85.3	13.6	62.9
Ctrl	4.64	0.55	4.54	1.35	0.43	0.22	94.6	146.4	70.5	10.4	44.2
-K	3.77	0.40	1.17	1.28	0.62	0.17	110.3	108.8	39.3	7.9	57.2
Ctrl	3.96	0.41	4.64	1.52	0.38	0.21	78.5	173.0	39.2	7.6	45.3
-Mg	4.03	0.39	4.61	1.59	0.14	0.19	85.5	154.6	50.0	8.1	72.2
Ctrl	4.22	0.41	4.28	1.41	0.44	0.28	84.8	175.7	33.9	7.7	61.8
-Mn	5.60	0.65	6.78	1.33	0.40	0.24	127.8	6.7	76.3	12.7	59.5
Ctrl	4.62	0.47	5.36	1.54	0.38	0.20	103.1	237.7	60.1	9.6	44.4
-N	1.65	0.35	3.39	1.21	0.40	0.17	147.5	99.1	88.1	14.0	63.4
Ctrl	4.64	0.55	4.54	1.35	0.43	0.22	94.6	146.4	70.5	10.4	44.2
-P	2.55	0.17	6.87	1.71	0.67	0.34	273.6	497.4	113.4	17.8	101.9
Ctrl	4.70	0.61	5.06	1.70	0.45	0.20	113.8	212.9	74.9	10.7	44.3
-S	4.11	0.44	3.84	1.52	0.42	0.13	82.1	207.8	37.8	9.0	72.4
Ctrl	4.22	0.41	4.28	1.41	0.44	0.28	84.8	175.7	33.9	7.7	61.8
-Zn	2.88	1.49	4.29	1.85	0.53	0.14	115.1	364.5	64.1	7.8	59.5
Ctrl	3.96	0.41	4.64	1.52	0.38	0.21	78.5	173.0	39.2	7.6	45.3

Table 14. Pansy mean tissue nutrient concentration (n = 3) at advanced stages of deficiency in recently matured leaves of hydroponically grown plants.

	Percent dry wt.						mg kg-1 of dry wt.				
Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
-B	3.45	0.42	3.91	1.51	0.41	0.27	163.8	76.9	41.1	11.2	10.9
Ctrl	4.22	0.41	4.28	1.41	0.44	0.28	84.8	175.7	33.9	7.7	61.8
-Ca	2.85	0.31	3.42	0.61	0.48	0.19	138.6	63.9	99.6	8.5	50.1
Ctrl	4.70	0.61	5.06	1.70	0.45	0.20	113.8	212.9	74.9	10.7	44.3
-Cu	4.91	0.49	5.55	1.75	0.47	0.19	98.7	240.8	52.1	2.4	74.6
Ctrl	4.22	0.41	4.28	1.41	0.44	0.28	84.8	175.7	33.9	7.7	61.8
-Fe	5.09	0.63	7.96	1.86	0.81	0.36	64.5	710.7	142.3	22.6	131.7
Ctrl	4.70	0.61	5.06	1.70	0.45	0.20	113.8	212.9	74.9	10.7	44.3
-K	3.63	0.41	0.59	0.85	0.56	0.15	66.1	87.6	47.1	7.2	49.3
Ctrl	4.22	0.41	4.28	1.41	0.44	0.28	84.8	175.7	33.9	7.7	61.8
-Mn	5.56	0.67	6.83	1.44	0.45	0.25	148.9	6.2	74.9	13.3	72.6
Ctrl	3.96	0.41	4.64	1.52	0.38	0.21	78.5	173.0	39.2	7.6	45.3
-N	1.12	0.30	3.10	1.15	0.38	0.11	124.4	110.2	60.9	7.1	59.1
Ctrl	4.62	0.47	5.36	1.54	0.38	0.20	103.1	237.7	60.1	9.6	44.4
-P	3.03	0.04	1.45	0.34	0.13	0.08	64.1	92.6	32.3	4.0	25.2
Ctrl	4.62	0.47	5.36	1.54	0.38	0.20	103.1	237.7	60.1	9.6	44.4
-Zn	3.04	1.52	3.86	1.45	0.47	0.15	95.0	319.9	39.4	7.8	64.2
Ctrl	4.22	0.41	4.28	1.41	0.44	0.28	84.8	175.7	33.9	7.7	61.8

Table 15. Petunia mean tissue nutrient concentration (n = 3) at early stages of deficiency in recently matured leaves of hydroponically grown plants.

	Percent dry wt.						mg·kg^{-1} of dry wt.				
Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
-B	5.03	0.46	5.69	1.28	0.30	0.28	75.7	38.8	24.1	6.7	10.3
Ctrl	6.34	0.45	7.59	1.48	0.35	0.29	73.2	74.2	29.6	8.5	28.7
-Ca	4.21	0.37	3.99	0.32	0.56	0.23	67.0	57.6	22.9	5.4	31.1
Ctrl	6.34	0.45	7.59	1.48	0.35	0.29	73.2	74.2	29.6	8.5	28.7
-Cu	7.12	0.60	7.22	1.53	0.36	0.41	117.0	52.8	47.1	3.5	39.6
Ctrl	6.34	0.45	7.59	1.48	0.35	0.29	73.2	74.2	29.6	8.5	28.7
-Fe	6.58	0.47	7.49	1.52	0.35	0.30	53.1	68.0	64.4	14.7	27.7
Ctrl	6.34	0.45	7.59	1.48	0.35	0.29	73.2	74.2	29.6	8.5	28.7
-K	6.20	0.51	0.69	1.77	0.99	0.27	74.7	88.5	29.2	10.9	34.4
Ctrl	6.21	0.41	7.36	1.69	0.39	0.30	76.2	76.0	34.4	10.1	27.8
-Mg	5.93	0.47	6.88	1.73	0.08	0.29	73.8	37.7	27.9	9.1	31.4
Ctrl	6.21	0.41	7.36	1.69	0.39	0.30	76.2	76.0	34.4	10.1	27.8
-Mn	7.33	0.62	8.17	1.86	0.43	0.36	112.5	11.4	37.2	10.2	37.1
Ctrl	6.34	0.45	7.59	1.48	0.35	0.29	73.2	74.2	29.6	8.5	28.7
-N	2.06	0.37	4.33	1.24	0.28	0.27	112.0	45.9	45.1	6.8	53.0
Ctrl	6.49	0.48	7.17	1.73	0.38	0.32	87.3	54.3	35.6	8.5	30.8
-P	4.13	0.07	5.61	1.20	0.30	0.20	79.2	44.2	26.6	5.2	34.4
Ctrl	6.21	0.41	7.36	1.69	0.39	0.30	76.2	76.0	34.4	10.1	27.8
-S	4.30	0.55	6.29	1.55	0.33	0.11	60.3	51.6	23.3	6.8	29.6
Ctrl	6.21	0.41	7.36	1.69	0.39	0.30	76.2	76.0	34.4	10.1	27.8
-Zn	6.78	0.76	8.19	1.69	0.44	0.27	93.6	89.9	13.0	7.0	30.1
Ctrl	7.13	0.49	8.30	1.57	0.36	0.29	68.1	71.3	28.0	8.6	29.2

Table 16. Petunia mean tissue nutrient concentration (n = 3) at advanced stages of deficiency in recently matured leaves of hydroponically grown plants.

	Percent dry wt.						mg·kg^{-1} of dry wt.				
Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
-B	4.36	0.41	5.34	1.48	0.33	0.33	59.0	63.6	25.1	7.8	5.4
Ctrl	5.77	0.22	7.88	1.59	0.48	0.31	97.1	60.5	32.5	6.5	48.6
-Ca	5.52	0.39	5.35	0.38	0.95	0.32	80.9	113.4	31.4	10.7	39.9
Ctrl	7.13	0.49	8.30	1.57	0.36	0.29	68.1	71.3	28.0	8.6	29.2
-Cu	7.42	0.66	8.32	1.86	0.43	0.55	125.0	82.6	57.6	3.3	49.2
Ctrl	6.23	0.33	8.44	1.53	0.37	0.32	56.7	61.0	22.7	6.1	26.6
-Fe	7.54	0.69	10.29	1.88	0.48	0.52	30.0	165.5	95.3	22.3	41.9
Ctrl	6.23	0.33	8.44	1.53	0.37	0.32	56.7	61.0	22.7	6.1	26.6
-K	6.89	0.55	0.63	1.56	0.94	0.29	96.3	102.6	42.5	12.0	37.2
Ctrl	7.13	0.49	8.30	1.57	0.36	0.29	68.1	71.3	28.0	8.6	29.2
-Mg	5.18	0.42	6.69	1.34	0.06	0.22	74.0	51.7	27.1	9.6	27.1
Ctrl	6.23	0.33	8.44	1.53	0.37	0.32	56.7	61.0	22.7	6.1	26.6
-Mn	7.95	0.81	9.92	1.83	0.45	0.40	102.4	9.2	47.3	17.8	45.7
Ctrl	7.13	0.49	8.30	1.57	0.36	0.29	68.1	71.3	28.0	8.6	29.2
-N	1.32	0.47	5.10	1.92	0.44	0.37	127.1	128.6	50.7	8.7	74.3
Ctrl	7.13	0.49	8.30	1.57	0.36	0.29	68.1	71.3	28.0	8.6	29.2
-P	4.89	0.05	6.48	1.46	0.39	0.25	86.6	75.7	28.9	6.6	47.3
Ctrl	6.70	0.33	8.47	1.69	0.43	0.34	76.1	73.5	18.0	5.3	32.8
-S	4.33	0.62	5.75	1.90	0.45	0.06	51.6	86.1	22.0	6.0	46.1
Ctrl	5.61	0.23	7.63	1.53	0.44	0.29	96.4	66.4	16.3	5.8	39.9
-Zn	5.39	0.75	8.13	1.89	0.49	0.23	73.6	96.8	10.2	6.3	32.4
Ctrl	6.70	0.33	8.47	1.69	0.43	0.34	76.1	73.5	18.0	5.3	32.8

Table 17. Begonia mean tissue nutrient concentration (n = 3) at early stages of deficiency in recently matured leaves of hydroponically grown plants.

	Percent dry wt.						$mg \cdot kg^{-1}$ of dry wt.				
Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
-B	3.36	0.37	3.63	2.34	0.64	0.20	117.0	75.7	50.5	7.4	9.1
Ctrl	3.95	0.37	4.08	2.10	0.61	0.21	102.6	99.6	44.7	8.6	67.5
-Ca	2.96	0.38	2.96	0.55	0.78	0.19	91.9	78.5	43.0	8.4	41.8
Ctrl	3.54	0.36	3.53	1.76	0.60	0.18	85.6	91.9	35.1	7.1	36.7
-Cu	4.19	0.39	5.38	1.63	0.57	0.23	136.0	106.6	37.2	1.7	44.1
Ctrl	3.92	0.39	5.00	1.42	0.53	0.21	75.3	86.8	36.3	8.0	38.7
-Fe	3.16	0.32	3.29	1.51	0.54	0.20	62.6	109.5	52.4	11.9	42.7
Ctrl	3.53	0.36	3.74	1.62	0.55	0.20	101.4	74.5	34.0	7.5	38.4
-K	3.59	0.37	0.75	1.58	0.94	0.19	97.7	85.0	40.8	8.3	41.4
Ctrl	3.53	0.36	3.74	1.62	0.55	0.20	101.4	74.5	34.0	7.5	38.4
-Mg	3.73	0.36	3.88	1.76	0.10	0.20	93.2	86.0	39.1	9.4	46.2
Ctrl	3.86	0.40	3.73	1.62	0.55	0.20	102.8	77.9	40.2	9.7	35.5
-Mn	3.58	0.41	3.49	1.81	0.56	0.20	128.3	9.4	93.2	8.8	45.2
Ctrl	3.66	0.39	3.73	1.80	0.55	0.20	156.0	75.2	33.3	7.4	38.6
-N	1.50	0.31	3.04	2.03	0.48	0.16	114.2	48.0	46.2	5.6	42.8
Ctrl	3.95	0.37	4.08	2.10	0.61	0.21	102.6	99.6	44.7	8.6	67.5
-P	2.26	0.10	2.26	0.99	0.44	0.15	66.4	58.5	32.3	6.6	34.5
Ctrl	3.53	0.36	3.74	1.62	0.55	0.20	101.4	74.5	34.0	7.5	38.4
-S	3.11	0.34	3.39	1.87	0.56	0.13	91.9	78.8	67.6	10.0	36.5
Ctrl	3.53	0.36	3.74	1.62	0.55	0.20	101.4	74.5	34.0	7.5	38.4
-Zn	3.56	0.42	4.81	1.56	0.57	0.19	133.7	116.7	21.8	8.5	39.8
Ctrl	3.92	0.39	5.00	1.42	0.53	0.21	75.3	86.8	36.3	8.0	38.7

Table 18. Begonia mean tissue nutrient concentration (n = 3) at advanced stages of deficiency in recently matured leaves of hydroponically grown plants.

	Percent dry wt.						$mg \cdot kg^{-1}$ of dry wt.				
Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
-B	3.45	0.35	2.88	2.22	0.62	0.21	106.3	89.8	46.7	7.9	6.7
Ctrl	3.86	0.40	3.73	1.62	0.55	0.20	102.8	77.9	40.2	9.7	35.5
-Ca	2.62	0.35	2.17	0.43	0.72	0.18	81.1	89.1	41.6	7.7	47.0
Ctrl	3.86	0.40	3.73	1.62	0.55	0.20	102.8	77.9	40.2	9.7	35.5
-Cu	4.49	0.35	5.18	1.91	0.54	0.24	140.0	146.0	46.6	1.7	57.1
Ctrl	4.15	0.29	3.74	2.09	0.45	0.21	94.5	112.5	38.3	5.4	38.0
-Fe	4.00	0.35	4.93	1.56	0.61	0.22	63.9	178.7	54.7	14.6	51.7
Ctrl	4.15	0.34	5.34	1.84	0.59	0.20	97.6	123.3	43.7	6.6	50.6
-K	3.68	0.43	0.52	1.53	1.27	0.21	108.7	103.4	47.8	8.4	48.1
Ctrl	3.92	0.39	5.00	1.42	0.53	0.21	75.3	86.8	36.3	8.0	38.7
-Mg	3.63	0.40	4.49	1.64	0.11	0.21	102.5	104.3	52.1	9.7	45.9
Ctrl	3.92	0.39	5.00	1.42	0.53	0.21	75.3	86.8	36.3	8.0	38.7
-Mn	3.62	0.44	3.45	1.93	0.57	0.21	151.7	8.2	63.8	10.1	46.5
Ctrl	3.86	0.40	3.73	1.62	0.55	0.20	102.8	77.9	40.2	9.7	35.5
-N	1.39	0.29	3.21	2.81	0.71	0.17	149.0	81.3	68.2	7.7	65.8
Ctrl	3.66	0.39	3.73	1.80	0.55	0.20	156.0	75.2	33.3	7.4	38.6
-P	3.14	0.06	3.10	1.37	0.64	0.21	104.6	92.5	41.4	7.0	65.6
Ctrl	4.20	0.35	5.31	1.61	0.57	0.21	109.5	110.0	47.1	10.2	41.4
-S	2.63	0.38	3.00	1.51	0.60	0.06	62.5	73.1	56.8	8.7	64.9
Ctrl	4.20	0.35	5.31	1.61	0.57	0.21	109.5	110.0	47.1	10.2	41.4
-Zn	2.93	0.78	3.89	1.70	0.68	0.17	119.3	132.7	22.0	9.8	42.6
Ctrl	4.20	0.35	5.31	1.61	0.57	0.21	109.5	110.0	47.1	10.2	41.4

Table 19. Ornamental cabbage mean tissue nutrient concentration (n = 3) at early stages of deficiency in recently matured leaves of hydroponically grown plants.

	Percent dry wt.						mg·kg^{-1} of dry wt.				
Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
-B	5.48	0.43	5.13	2.87	0.56	1.26	87.0	72.6	36.8	6.3	26.8
Ctrl	6.41	0.50	5.17	2.99	0.54	1.05	82.6	85.4	29.2	4.7	41.8
-Ca	5.73	0.44	4.64	0.74	1.23	1.05	82.2	146.3	31.2	6.2	72.8
Ctrl	6.41	0.50	5.17	2.99	0.54	1.05	82.6	85.4	29.2	4.7	41.8
-Cu	6.01	0.53	4.84	3.55	0.45	1.05	65.2	85.0	26.2	1.7	79.2
Ctrl	5.26	0.34	4.43	2.95	0.45	0.87	57.0	56.2	15.9	3.1	66.2
-Fe	5.97	0.61	6.05	3.29	0.69	1.54	47.3	289.7	219.3	30.7	80.9
Ctrl	6.41	0.50	5.17	2.99	0.54	1.05	82.6	85.4	29.2	4.7	41.8
-K	5.74	0.77	0.41	2.45	0.54	1.01	74.3	105.0	24.1	6.6	65.9
Ctrl	6.07	0.41	4.69	2.95	0.53	0.93	65.6	77.5	25.2	4.6	56.6
-Mg	5.17	0.51	4.46	2.81	0.11	0.97	62.4	71.8	22.9	3.9	82.9
Ctrl	5.40	0.46	4.57	2.60	0.43	0.95	57.9	69.7	16.4	2.8	80.1
-Mn	6.28	0.53	4.72	3.20	0.54	1.17	56.9	7.0	31.3	4.6	59.4
Ctrl	6.07	0.41	4.69	2.95	0.53	0.93	65.6	77.5	25.2	4.6	56.6
-N	2.58	0.65	5.71	3.13	0.56	1.76	173.3	130.3	76.3	23.4	100.0
Ctrl	6.41	0.50	5.17	2.99	0.54	1.05	82.6	85.4	29.2	4.7	41.8
-P	3.96	0.06	2.93	2.21	0.38	0.62	56.0	78.9	24.5	4.6	49.8
Ctrl	6.07	0.41	4.69	2.95	0.53	0.93	65.6	77.5	25.2	4.6	56.6
-S	6.05	0.49	4.39	3.07	0.48	0.20	61.8	95.2	28.3	4.5	70.6
Ctrl	6.07	0.41	4.69	2.95	0.53	0.93	65.6	77.5	25.2	4.6	56.6
-Zn	4.75	0.49	4.25	2.72	0.49	1.02	85.2	82.4	7.1	2.8	50.7
Ctrl	6.07	0.41	4.69	2.95	0.53	0.93	65.6	77.5	25.2	4.6	56.6

Table 20. Ornamnetal cabbage mean tissue nutrient concentration (n = 3) at advanced stages of deficiency in recently matured leaves of hydroponically grown plants.

	Percent dry wt.						mg·kg^{-1} of dry wt.				
Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
-B	3.79	0.37	4.13	2.35	0.35	0.77	44.3	63.1	24.1	2.3	12.9
Ctrl	5.26	0.34	4.43	2.95	0.45	0.87	57.0	56.2	15.9	3.1	66.2
-Ca	3.20	0.25	2.87	0.99	0.76	0.59	101.9	83.5	32.0	8.3	70.0
Ctrl	6.07	0.41	4.69	2.95	0.53	0.93	65.6	77.5	25.2	4.6	56.6
-Cu	6.91	0.66	5.21	2.91	0.47	1.21	74.7	83.4	28.6	1.3	86.1
Ctrl	5.14	0.38	4.10	2.52	0.54	0.98	78.4	59.7	17.0	4.3	79.2
-Fe	6.91	0.65	7.24	3.06	0.69	1.72	17.1	132.7	96.3	29.0	82.4
Ctrl	6.07	0.41	4.69	2.95	0.53	0.93	65.6	77.5	25.2	4.6	56.6
-K	5.73	0.74	0.34	2.29	0.38	0.91	66.8	88.7	31.2	5.3	63.3
Ctrl	5.40	0.46	4.57	2.60	0.43	0.95	57.9	69.7	16.4	2.8	80.1
-Mg	5.02	0.36	3.62	2.44	0.28	1.06	76.1	48.0	16.7	4.2	73.8
Ctrl	5.14	0.38	4.10	2.52	0.54	0.98	78.4	59.7	17.0	4.3	79.2
-Mn	6.80	0.68	6.07	3.20	0.51	1.22	49.5	3.6	28.3	3.4	79.4
Ctrl	5.14	0.38	4.10	2.52	0.54	0.98	78.4	59.7	17.0	4.3	79.2
-N	1.27	0.48	3.85	2.55	0.46	1.26	107.9	126.3	58.8	14.9	84.3
Ctrl	6.07	0.41	4.69	2.95	0.53	0.93	65.6	77.5	25.2	4.6	56.6
-P	3.64	0.05	2.45	2.06	0.33	0.62	53.3	73.0	23.7	4.6	51.6
Ctrl	5.40	0.46	4.57	2.60	0.43	0.95	57.9	69.7	16.4	2.8	80.1
-S	5.24	0.36	3.87	2.53	0.59	0.58	73.0	57.7	16.1	4.4	77.3
Ctrl	5.14	0.38	4.10	2.52	0.54	0.98	78.4	59.7	17.0	4.3	79.2
-Zn	5.24	0.93	4.70	2.72	0.54	1.27	565.3	208.3	8.0	7.2	78.7
Ctrl	5.14	0.38	4.10	2.52	0.54	0.98	78.4	59.7	17.0	4.3	79.2

Table 21. Snapdragon mean tissue nutrient concentration (n = 3) at early stages of deficiency in recently matured leaves of hydroponically grown plants.

	Percent dry wt.						mg·kg^{-1} of dry wt.				
Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
-B	3.35	0.36	2.68	0.89	0.37	0.21	64.9	40.4	26.1	4.9	4.6
Ctrl	5.16	0.50	3.59	1.13	0.57	0.32	67.7	71.7	26.5	5.4	23.8
-Ca	4.18	0.43	3.19	0.67	0.90	0.31	110.1	64.1	40.2	6.5	29.6
Ctrl	5.16	0.50	3.59	1.13	0.57	0.32	67.7	71.7	26.5	5.4	23.8
-Cu	5.58	0.57	4.10	1.55	0.71	0.39	71.7	80.2	37.6	1.4	28.6
Ctrl	5.16	0.50	3.59	1.13	0.57	0.32	67.7	71.7	26.5	5.4	23.8
-Fe	6.78	0.62	4.84	1.96	0.83	0.54	51.9	141.5	82.6	14.4	30.8
Ctrl	5.16	0.50	3.59	1.13	0.57	0.32	67.7	71.7	26.5	5.4	23.8
-Mn	6.75	0.67	5.50	1.29	0.75	0.35	140.4	4.2	47.8	8.5	35.5
Ctrl	5.16	0.50	3.59	1.13	0.57	0.32	67.7	71.7	26.5	5.4	23.8
-N	1.88	0.29	1.83	1.10	0.41	0.22	102.5	54.0	40.3	5.2	16.5
Ctrl	5.16	0.50	3.59	1.13	0.57	0.32	67.7	71.7	26.5	5.4	23.8
-P	3.17	0.07	2.11	1.03	0.45	0.24	80.0	84.2	49.8	5.7	17.0
Ctrl	5.16	0.50	3.59	1.13	0.57	0.32	67.7	71.7	26.5	5.4	23.8
-Zn	3.95	0.79	3.32	1.90	0.89	0.27	53.1	146.2	12.4	4.3	19.1
Ctrl	4.45	0.39	3.38	1.25	0.68	0.26	54.8	77.5	20.6	4.3	17.2

Table 22. Snapdragon mean tissue nutrient concentration (n = 3) at advanced stages of deficiency in recently matured leaves of hydroponically grown plants.

	Percent dry wt.						mg·kg^{-1} of dry wt.				
Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
-B	2.95	0.31	2.27	0.88	0.34	0.18	58.1	48.6	23.2	3.2	4.7
Ctrl	4.45	0.39	3.38	1.25	0.68	0.26	54.8	77.5	20.6	4.3	17.2
-Ca	4.02	0.41	3.13	0.74	0.84	0.29	156.0	73.6	63.8	11.7	38.8
Ctrl	5.16	0.50	3.59	1.13	0.57	0.32	67.7	71.7	26.5	5.4	23.8
-Cu	5.16	0.48	3.67	1.27	0.63	0.31	81.9	80.5	42.4	2.2	29.9
Ctrl	4.96	0.42	3.30	1.07	0.65	0.28	53.6	65.7	22.2	4.2	20.8
-Fe	6.43	0.59	4.20	1.58	0.75	0.48	51.3	129.4	69.8	14.9	32.6
Ctrl	5.16	0.50	3.59	1.13	0.57	0.32	67.7	71.7	26.5	5.4	23.8
-Mn	7.45	0.85	7.81	1.58	0.87	0.38	105.7	3.0	59.2	10.5	60.1
Ctrl	4.96	0.42	3.30	1.07	0.65	0.28	53.6	65.7	22.2	4.2	20.8
-N	0.95	0.30	2.50	1.34	0.41	0.26	696.1	136.5	42.0	3.6	24.1
Ctrl	5.10	0.45	3.70	1.95	0.78	0.31	127.2	127.5	28.1	6.0	30.9
-P	2.96	0.03	1.91	1.08	0.46	0.18	149.3	173.5	59.6	6.9	19.4
Ctrl	4.45	0.39	3.38	1.25	0.68	0.26	54.8	77.5	20.6	4.3	17.2
-Zn	4.70	1.06	3.65	2.53	1.28	0.30	49.4	280.7	9.4	4.1	33.4
Ctrl	4.95	0.46	4.02	1.58	0.78	0.26	68.9	109.5	26.6	4.9	26.2

Table 23. Timor mean tissue nutrient concentration (n = 3) at early stages of deficiency in recently matured leaves of hydroponically grown plants.

	Percent dry wt.						mg·kg^{-1} of dry wt.				
Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
-B	3.48	0.40	2.20	1.70	0.29	0.22	80.8	108.4	29.2	5.3	6.1
Ctrl	4.17	0.43	2.54	1.69	0.31	0.23	79.0	113.3	28.0	4.9	57.0
-Ca	3.57	0.33	2.53	0.72	0.50	0.18	68.8	117.5	21.3	4.2	60.7
Ctrl	4.17	0.43	2.54	1.69	0.31	0.23	79.0	113.3	28.0	4.9	57.0
-Cu	4.54	0.62	3.23	1.75	0.35	0.26	88.9	154.0	23.6	1.6	98.1
Ctrl	4.37	0.54	2.67	1.93	0.34	0.25	79.2	155.6	22.3	4.3	85.2
-Fe	3.90	0.41	3.02	1.62	0.31	0.26	43.9	87.0	38.6	7.3	49.9
Ctrl	4.17	0.43	2.54	1.69	0.31	0.23	79.0	113.3	28.0	4.9	57.0
-K	3.71	0.45	0.53	2.74	0.71	0.22	82.3	136.8	31.4	5.1	52.9
Ctrl	3.61	0.36	2.45	1.29	0.26	0.21	75.4	73.6	37.7	5.5	60.9
-Mg	3.87	0.42	2.70	1.89	0.07	0.21	76.2	145.3	27.0	4.6	57.7
Ctrl	4.28	0.41	2.56	1.80	0.32	0.24	83.8	129.2	27.6	5.2	65.5
-Mn	4.38	0.55	3.23	1.58	0.30	0.25	78.4	5.8	27.7	5.5	64.0
Ctrl	3.77	0.38	2.45	1.33	0.27	0.23	89.1	78.4	30.5	5.4	67.2
-N	1.95	0.31	1.57	1.30	0.24	0.20	65.7	98.8	24.4	5.4	68.5
Ctrl	4.28	0.41	2.56	1.80	0.32	0.24	83.8	129.2	27.6	5.2	65.5
-P	2.39	0.07	1.72	1.10	0.22	0.17	87.7	55.2	35.9	6.3	48.0
Ctrl	3.91	0.43	2.61	1.68	0.31	0.26	98.6	99.4	42.1	6.3	58.2
-S	3.43	0.48	2.43	1.55	0.28	0.08	64.3	101.4	29.5	5.3	57.0
Ctrl	3.77	0.38	2.45	1.33	0.27	0.23	89.1	78.4	30.5	5.4	67.2
-Zn	3.72	0.60	2.48	1.49	0.29	0.25	74.8	117.1	18.6	4.9	64.9
Ctrl	4.28	0.41	2.56	1.80	0.32	0.24	83.8	129.2	27.6	5.2	65.5

Table 24. Timor mean tissue nutrient concentration (n = 3) at advanced stages of deficiency in recently matured leaves of hydroponically grown plants.

	Percent dry wt.						mg·kg^{-1} of dry wt.				
Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
-B	3.09	0.35	1.80	1.80	0.27	0.18	71.0	136.7	26.0	3.8	4.9
Ctrl	4.12	0.52	2.19	2.13	0.36	0.23	74.7	187.2	22.4	4.0	70.9
-Ca	3.31	0.34	2.26	0.42	0.61	0.16	64.6	134.9	17.3	3.6	72.8
Ctrl	4.12	0.52	2.19	2.13	0.36	0.23	74.7	187.2	22.4	4.0	70.9
-Cu	4.20	0.48	2.64	1.79	0.32	0.22	89.8	191.8	30.7	1.6	104.4
Ctrl	3.91	0.43	1.98	2.15	0.35	0.23	72.3	129.1	19.7	3.7	87.7
-Fe	3.70	0.29	1.49	2.58	0.69	0.34	55.6	174.1	44.7	10.7	127.1
Ctrl	4.00	0.47	1.80	2.43	0.39	0.25	75.1	161.6	22.1	3.8	90.6
-K	4.37	0.53	0.50	3.47	0.81	0.26	88.2	187.9	39.0	5.3	57.0
Ctrl	4.28	0.41	2.56	1.80	0.32	0.24	83.8	129.2	27.6	5.2	65.5
-Mg	3.67	0.47	2.86	1.62	0.07	0.17	67.9	139.4	29.4	4.5	66.8
Ctrl	4.37	0.54	2.67	1.93	0.34	0.25	79.2	155.6	22.3	4.3	85.2
-Mn	4.21	0.44	2.73	1.89	0.31	0.20	65.4	4.0	27.9	4.9	68.0
Ctrl	3.91	0.43	1.98	2.15	0.35	0.23	72.3	129.1	19.7	3.7	87.7
-N	1.09	0.28	1.50	1.45	0.25	0.16	57.5	156.2	27.7	5.2	134.9
Ctrl	4.12	0.52	2.19	2.13	0.36	0.23	74.7	187.2	22.4	4.0	70.9
-P	2.39	0.05	1.61	1.32	0.26	0.25	99.3	95.3	36.4	6.6	96.1
Ctrl	4.37	0.54	2.67	1.93	0.34	0.25	79.2	155.6	22.3	4.3	85.2
-S	3.37	0.64	3.21	1.69	0.29	0.05	51.6	138.5	24.6	5.2	92.0
Ctrl	4.12	0.52	2.19	2.13	0.36	0.23	74.7	187.2	22.4	4.0	70.9
-Zn	3.99	0.86	2.70	1.80	0.34	0.25	73.5	163.3	17.7	5.3	74.6
Ctrl	4.37	0.54	2.67	1.93	0.34	0.25	79.2	155.6	22.3	4.3	85.2

Table 25. Grenada mean tissue nutrient concentration (n = 3) at early stages of deficiency in recently matured leaves of hydroponically grown plants.

	Percent dry wt.						mg·kg^{-1} of dry wt.				
Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
-B	3.13	0.28	2.70	1.28	0.26	0.15	65.3	70.2	34.7	5.7	9.8
Ctrl	3.95	0.35	3.38	1.36	0.27	0.17	55.6	101.4	25.1	5.2	77.7
-Ca	3.20	0.26	2.75	0.86	0.44	0.15	103.2	81.0	46.4	6.8	51.7
Ctrl	3.11	0.34	3.35	1.24	0.27	0.17	83.4	62.5	37.1	6.3	59.3
-Cu	4.54	0.42	3.30	1.67	0.31	0.22	71.6	153.0	37.8	2.2	111.7
Ctrl	4.29	0.45	3.22	1.93	0.33	0.21	65.7	174.2	31.0	5.8	91.6
-Fe	3.48	0.32	2.92	1.37	0.27	0.16	47.8	67.5	50.7	11.0	54.6
Ctrl	3.11	0.34	3.35	1.24	0.27	0.17	83.4	62.5	37.1	6.3	59.3
-K	3.90	0.39	0.43	2.84	0.90	0.16	67.0	170.7	33.2	6.0	71.0
Ctrl	3.95	0.35	3.38	1.36	0.27	0.17	55.6	101.4	25.1	5.2	77.7
-Mg	3.64	0.35	3.41	1.32	0.09	0.15	68.1	80.7	29.6	6.0	63.0
Ctrl	4.03	0.36	3.45	1.17	0.28	0.18	62.1	67.2	30.3	6.2	65.3
-Mn	4.06	0.42	3.92	1.31	0.27	0.17	67.0	5.8	30.1	6.7	84.9
Ctrl	3.95	0.35	3.38	1.36	0.27	0.17	55.6	101.4	25.1	5.2	77.7
-N	1.73	0.24	1.85	1.04	0.23	0.12	84.4	48.5	41.2	7.5	59.1
Ctrl	4.03	0.36	3.45	1.17	0.28	0.18	62.1	67.2	30.3	6.2	65.3
-P	2.50	0.10	2.00	1.08	0.24	0.13	99.0	39.0	48.9	7.3	43.3
Ctrl	2.18	0.24	1.25	1.75	0.35	0.16	192.0	27.0	110.3	9.7	23.0
-S	2.86	0.31	2.62	1.05	0.24	0.09	90.9	46.7	33.1	6.9	55.3
Ctrl	4.03	0.36	3.45	1.17	0.28	0.18	62.1	67.2	30.3	6.2	65.3
-Zn	4.53	0.77	2.75	1.90	0.33	0.22	59.2	188.3	15.4	6.5	101.0
Ctrl	4.29	0.45	3.22	1.93	0.33	0.21	65.7	174.2	31.0	5.8	91.6

Table 26. Grenada mean tissue nutrient concentration (n = 3) at advanced stages of deficiency in recently matured leaves of hydroponically grown plants.

	Percent dry wt.						mg·kg^{-1} of dry wt.				
Treatment	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
-B	3.14	0.34	3.10	1.53	0.28	0.16	64.5	100.7	34.1	5.9	7.6
Ctrl	4.23	0.42	3.12	2.09	0.34	0.21	66.9	192.3	36.8	5.5	92.2
-Ca	3.09	0.24	2.31	0.74	0.47	0.15	76.5	98.6	36.8	5.6	54.7
Ctrl	3.95	0.35	3.38	1.36	0.27	0.17	55.6	101.4	25.1	5.2	77.7
-Cu	4.65	0.35	3.10	2.04	0.34	0.23	71.9	203.7	41.9	2.0	135.7
Ctrl	4.26	0.43	2.79	2.31	0.41	0.23	60.2	179.0	28.7	4.9	116.0
-Fe	3.63	0.37	2.10	2.43	0.53	0.32	52.8	303.3	67.8	15.9	123.0
Ctrl	4.28	0.52	2.36	2.32	0.40	0.25	81.4	171.7	33.0	5.3	112.3
-K	4.39	0.47	0.44	3.29	1.06	0.18	66.5	204.7	37.1	6.2	75.3
Ctrl	4.07	0.41	3.64	1.50	0.28	0.19	53.5	133.0	28.1	5.4	84.3
-Mg	3.21	0.32	3.21	1.32	0.06	0.13	55.6	100.8	26.9	5.5	64.8
Ctrl	3.95	0.35	3.38	1.36	0.27	0.17	55.6	101.4	25.1	5.2	77.7
-Mn	4.39	0.45	3.52	2.07	0.31	0.23	64.7	4.2	48.4	7.1	105.3
Ctrl	4.26	0.43	2.79	2.31	0.41	0.23	60.2	179.0	28.7	4.9	116.0
-N	1.14	0.19	1.65	0.87	0.19	0.09	57.1	48.6	28.1	5.2	77.4
Ctrl	3.90	0.41	3.65	1.46	0.27	0.18	53.3	122.7	26.3	5.3	82.2
-P	2.29	0.06	2.11	0.87	0.19	0.13	68.3	44.6	30.8	5.9	72.4
Ctrl	3.90	0.41	3.65	1.46	0.27	0.18	53.3	122.7	26.3	5.3	82.2
-S	3.43	0.58	3.51	1.54	0.27	0.07	48.9	107.3	28.2	6.8	112.0
Ctrl	4.23	0.42	3.12	2.09	0.34	0.21	66.9	192.3	36.8	5.5	92.2
-Zn	4.57	0.83	2.84	2.01	0.33	0.23	56.3	194.3	13.7	7.1	107.3
Ctrl	4.23	0.42	3.12	2.09	0.34	0.21	66.9	192.3	36.8	5.5	92.2