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MANGANESE TOXICITY IN MARIGOLD
AS
AFFECTED BY CALCIUM AND MAGNESIUM

A Dissertation Presented

by

TOURIA EL JAOUAL

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2002

Department of Plant and Soil Sciences

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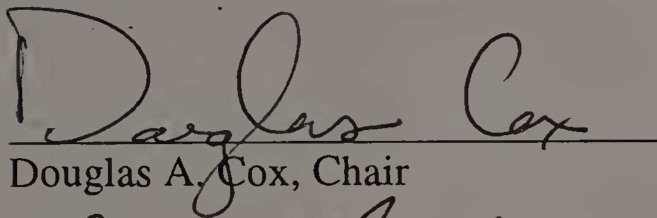
Approved as to style and content by:



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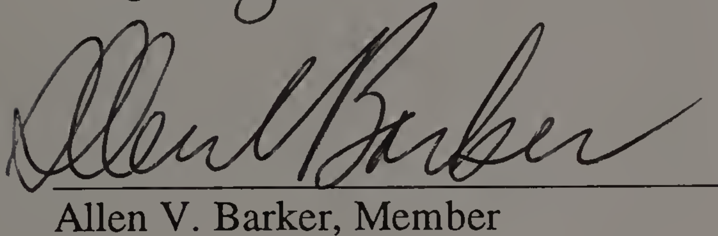

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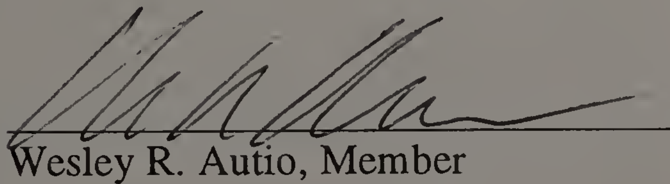

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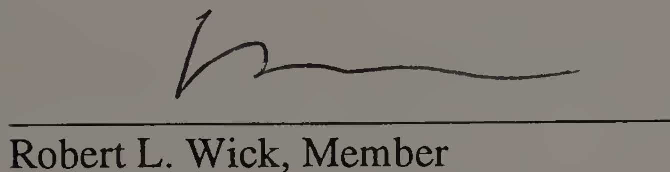

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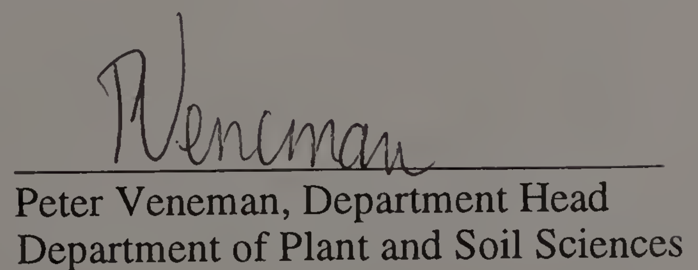

Peter Veneman, Department Head
Department of Plant and Soil Sciences

To my husband Robert Willis Eaton

To my son Adam Jalal Eaton

In memory of my son Ryan Moraad Eaton

with all my love

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ABSTRACT

MANGANESE TOXICITY IN MARIGOLD
AS
AFFECTED BY CALCIUM AND MAGNESIUM

MAY 2002

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Directed by: Professor Douglas A. Cox

Iron/manganese toxicity disorder in marigold (*Tagetes erecta* L.) has been related to high concentrations of Mn and low concentrations of Ca and Mg in the affected leaves. Preplant addition of micronutrients in the media combined with constant feed program and low medium pH create favorable conditions for the development of Mn toxicity in greenhouse crops. Deficiency of Ca or Mg is due in part to low medium pH and to a lack of Mg and Ca in many of the fertilizers used in greenhouse production. The objectives of this research were to determine the relationship of Mn toxicity to the incidence of Fe/Mn toxicity disorder in marigold, and to evaluate the effect of low Mg supply and/or low Ca supply on the occurrence of the toxicity.

Six experiments were conducted. Plants of *Tagetes erecta* L. 'First Lady' were started from seeds and then grown in solution culture supplying different concentrations of Mn, Ca and Mg depending on the objectives of each experiment. Symptoms were described for each experiment. When the plants were harvested, their dry weights were taken and their tissues were analyzed for Mn, Fe, Ca, and Mg concentrations.

The symptoms of Mg deficiency included stunting, chlorotic and necrotic areas on the leaves. The symptoms of Ca deficiency included chlorosis and curling, especially of the new leaves. The symptoms of Mn toxicity included curled leaves, bleached patches and brown spots on the leaves. These symptoms of Mn toxicity are similar to those related to Fe/Mn toxicity disorder.

The incipient deficiency solution concentration of Mg was 10 mg/l (internal incipient deficiency concentration was 1.5%). The incipient deficiency solution concentration of Ca was 20 mg/l (internal incipient deficiency concentration was 0.54%). The critical toxicity concentration of Mn was 4.5 mg/l (internal critical toxicity concentration was 270 mg/kg DW).

Low Ca in solution (20 mg/liter) increased the sensitivity of marigold to high levels of Mn in solution by reducing the critical toxicity concentration of Mn from 4.5 to 0.5 mg/liter. Similar results were found when both Ca and Mg were low.

Iron/manganese toxicity disorder can be attributed to Mn toxicity. Low Ca supply or low Ca and Mg supplies are factors favoring the occurrence of the disorder. Low Mg supply, alone does not seem to affect Mn toxicity in marigold.

Based on this research, high Ca supply could alleviate the harmful effects of Mn toxicity in marigold. Low Mn supply could prevent the toxicity problems. Agricultural practices and nutritional regimes that reduce the availability of Mn and increase the availability of Ca could reduce the occurrence of Fe/Mn toxicity disorder in marigold and similar physiological disorders in other bedding plants grown in soilless media. Monitoring Mn supply and fertilizing with Ca could prevent or reduce Mn toxicity to floriculture plants.

TABLE OF CONTENTS

Page

ACKNOWLEDGEMENT v

ABSTRACT vi

LIST OF TABLES xiv

LIST OF FIGURES xv

CHAPTER

1. INTRODUCTION 1

1.1 PROBLEM STATEMENT 1
1.2 PREVIOUS WORK AND PRESENT OUTLOOK 2

1.2.1 High Availability of Mn in the Growth Medium 3
1.2.2 Low Level of Ca in the Medium 3
1.2.3 Low Level of Mg in the Medium 4

1.3 OBJECTIVES 5
1.4 CONTRIBUTION 5

2. LITERATURE REVIEW 11

2.1 ABSTRACT 11
2.2 INTRODUCTION 11
2.3 GENERAL EFFECTS 12

2.3.1 Plant Symptoms 12
2.3.2 Physiological and Biochemical Effects 15

2.4 IMPORTANCE AND OCCURANCE 17
2.5 TOLERANCE TO Mn TOXICITY 18

2.5.1 Manganese Absorption 19
2.5.2 Manganese Accumulation and Translocation 21
2.5.3 Internal Tolerance to Excess Mn 22
2.5.4 Genetic Control of Mn Tolerance in Plants 23

2.6 SOIL MANAGEMENT FACTORS 24

2.6.1 Fertilizer and N Form 24
2.6.2 Liming 26
2.6.3 Moisture Level and Growth Medium 27

2.7 ENVIRONMENTAL INTERACTIONS 28

2.7.1 Temperature 29
2.7.2 Light 30

2.8 NUTRITIONAL INTERACTIONS 30

2.8.1 Interaction with Fe 31

2.8.1.1 Manganese-induced Fe deficiency 32
2.8.1.2 The effectiveness of Fe supply 35
2.8.1.3 The effectiveness of Mo and P supply 36
2.8.1.4 Manganese toxicity induced by Fe deficiency 36
2.8.1.5 Manganese-iron toxicity 37

2.8.2 Interaction with Si 38
2.8.3 Interaction with Ca 40
2.8.4 Interaction with Mg 41
2.8.5 Interaction with K 42
2.8.6 Interaction with P 42
2.8.7 Interaction with Mo 43
2.8.8 Other Interactions 45

2.9 CONCLUSIONS 45

3. MAGANESIUM DEFICIENCY IN MARIGOLD 61

3.1 ABSTRACT 61
3.2 INTRODUCTION 61
3.3 MATERIALS AND METHODS 65
3.4 RESULTS AND DISCUSSION 66

3.4.1 Description of Symptoms 66
3.4.2 Effect of Mg Deficiency on Plant Dry Weight 67
3.4.3 Incipient Deficiency Concentration of Mg 69
3.4.4 Effect of Mg Deficiency on the Nutrient Concentrations in Shoots 70

3.4.4.1 Effect of Mg concentration in the nutrient solution on Mn concentration in the shoots 70
3.4.4.2 Effect of Mg concentration in the nutrient solution on Fe concentration in the shoots 72
3.4.4.3 Effect of Mg concentration in the nutrient solution on Ca concentration in the shoots 72
3.4.4.4 Effect of Mg concentration in the nutrient solution on Mg concentration in the shoots 73

3.5 CONCLUSION 73

4. CALCIUM DEFICIENCY IN MARIGOLD 78

4.1 ABSTRACT 78
4.2 INTRODUCTION AND LITERATURE 79

4.2.1 Calcium Uptake and Translocation 79
4.2.2 Role of Ca in Plant Metabolism 80
4.2.3 Calcium: Deficiency: Cause and Impact 82

4.3 MATERIALS AND METHODS 86
4.4 RESULTS AND DISCUSSION 87

4.4.1 Description of Symptoms 87
4.4.2 Effect of Ca Deficiency on the Plant Dry Weight 89
4.4.3 Incipient Deficiency Concentration of Ca 89
4.4.4 Nutrient Distribution 92
4.4.5 Effect of Ca Deficiency on Nutrient Concentrations in the Shoots 92

4.4.5.1 Effect of Ca in solution on Mn in the shoots 94
4.4.5.2 Effect of Ca in solution on Fe in the shoots 94
4.4.5.3 Effect of Ca in solution on Ca in the shoots 94
4.4.5.4 Effect of Ca in solution on Mg in the shoots 98

4.5 CONCLUSION 100

5. MANGANESE TOXICITY IN MARIGOLD 107

5.1 ABSTRACT 107
5.2 INTRODUCTION AND LITERATURE 107

5.2.1 High Level of Mn in the Medium 108
5.2.2 Low Level of Ca in the Medium 109
5.2.3 Low Level of Mg in the Medium 109

5.3 MATERIALS AND METHODS 111
5.4 RESULTS AND DISCUSSION 112

5.4.1 Description of Symptoms 112
5.4.2 Effect of Mn Toxicity on the Plant Dry Weight 113
5.4.3 Critical Toxicity Concentration of Mn 115
5.4.4 Effect of Mn Toxicity on the Nutrient Levels in the Plant 116

5.4.4.1 Effect of Mn in solution on Mn level in the plant 116
5.4.4.2 Effect of Mn in solution on Fe level in the plant 118
5.4.4.3 Effect of Mn in solution on Ca level in the plant 121
5.4.4.4 Effect of Mn in solution on Mg level in the plant 124

5.5 CONCLUSION 125

6. MANGANESE TOXICITY IN MARIGOLD AS AFFECTED BY CALCIUM 132

6.1 ABSTRACT 132
6.2 INTRODUCTION AND LITERATURE 133

6.2.1 High Level of Mn in the Medium 134
6.2.2 Interaction with Ca 134
6.2.3 Interaction with Mg 135

6.3 MATERIALS AND METHODS 136
6.4 RESULTS AND DISCUSSION 137

6.4.1 Symptoms 137
6.4.2 Dry Weight 139
6.4.3 Critical Toxicity Concentration 142
6.4.4 Nutrient Concentrations in the Plant Tissues 142

6.4.4.1 Manganese 142
6.4.4.2 Iron 146
6.4.4.3 Calcium 148
6.4.4.4 Magnesium 150

6.5 CONCLUSION 154

7. MANGANESE TOXICITY IN MARIGOLD AS AFFECTED BY MAGNESIUM 162

7.1 ABSTRACT 162
7.2 INTRODUCTION AND LITERATURE 163

7.2.1 Concentration of Mn in the Growth Medium 164
7.2.2 Concentration of Mg in the Growth Medium 164

7.3 MATERIALS AND METHODS 165
7.4 RESULTS AND DISCUSSION 167

7.4.1 Symptoms 167
7.4.2 Dry Weight 168
7.4.3 Critical Toxicity Concentration of Mn 171
7.4.4 Nutrient Concentration in the Plant Tissue 171

7.4.4.1 Manganese 171
7.4.4.2 Iron 172
7.4.4.3 Calcium 176
7.4.4.4 Magnesium 179

7.5 CONCLUSION 181

8. MANGANESE TOXICITY IN MARIGOLD AS AFFECTED BY CALCIUM AND MAGNESIUM 191

8.1 ABSTRACT 191
8.2 INTRODUCTION AND LITERATURE 192

8.2.1 Concentration of Mn in the Growth Medium 193
8.2.2 Concentration of Ca in the Growth Medium 194
8.2.3 Concentration of Mg in the Growth Medium 196

8.3 MATERIALS AND METHODS 198
8.4 RESULTS AND DISCUSSION 199

8.4.1 Symptoms 199
8.4.2 Dry Weight 201
8.4.3 Critical Toxicity Concentration of Mn 206
8.4.4 Nutrient Concentrations in the Plant Tissues 206

8.4.4.1 Manganese 206
8.4.4.2 Iron 223
8.4.4.3 Calcium 229
8.4.4.4 Magnesium 237

8.5 CONCLUSION 247

9. CONCLUSION 259

9.1 INCIPIENT DEFICIENCY CONCENTRATION 259
9.2 CRITICAL TOXICITY CONCENTRATION OF Mn 259
9.3 NUTRIENT CONCENTRATION IN THE PLANT TISSUE 260

9.3.1 Manganese Concentration in the Plant Tissue 260
9.3.2 Iron Concentration in the Plant Tissue 261
9.3.3 Calcium Concentration in the Plant Tissue 262
9.3.4 Magnesium Concentration in the Plant Tissue 263

APPENDICES

A. TISSUE ANALYSIS BY ATOMIC ABSORPTION FLAME SPECTROPHOTOMETRY 265

B. TISSUE ANALYSIS BY INDUCTIVE COUPLED PLASMA (ICP) 269

C. STATISTICAL ANALYSIS: MAGNESIUM DEFICIENCY IN MARIGOLD 270

D. STATISTICAL ANALYSIS: CALCIUM DEFICIENCY IN MARIGOLD 273

E. STATISTICAL ANALYSIS: MANGANESE DEFICIENCY IN MARIGOLD 277

F. STATISTICAL ANALYSIS: MANGANESE TOXICITY IN MARIGOLD AS AFFECTED BY CALCIUM 281

G. STATISTICAL ANALYSIS: MAGANESE TOXICITY IN MARIGOLD AS AFFECTED BY MAGNESIUM 285

H. STATISTICAL ANALYSIS: MANGANESE TOXICITY IN MARIGOLD AS AFFECTED BY CALCIUM AND MAGNESIUM 289

BIBLIOGRAPHY 297

LIST OF TABLES

Table | Page

1. Critical Toxicity Levels of Mn in the Leaves of Various Plant Species............18

LIST OF FIGURES

Figure		Page
3.1	Effect of Mg level in solution on the plant dry weight	68
3.2	Effect of Mg in solution on the nutrient concentrations in the plant shoots	71
4.1	Effect of Ca level in solution on the plant dry weight	90
4.2	Nutrient distribution in the shoots	93
4.3	Effect of Ca level in solution on the level of Mn in the shoots	95
4.4	Effect of Ca level in solution on the level of Fe in the shoots	96
4.5	Effect of Ca level in solution on the level of Ca in the shoots	97
4.6	Effect of Ca level in solution on the level of Mg in the shoots	99
5.1	Effect of Mn level in solution on the plant dry weight	114
5.2	Effect of Mn level in solution on the level of Mn in the plant tissue	117
5.3	Effect of Mn level in solution on the level of Fe in the plant tissue	119
5.4	Effect of Mn level in solution on the level of nutrients in the plant tissue	122
5.5	Nutrient distribution in the plant	123
6.1	Effect of Mn level in solution on the plant dry weight	140
6.2	Effect of Ca level in solution on the plant dry weight	141
6.3	Effect of Mn level in solution on the level of Mn in the plant tissue	143
6.4	Effect of Ca level in solution on the level of Mn in the plant tissue	144
6.4a	Effect of Mn level in solution on the level of Mn in the plant tissue under low and optimal levels of Ca in solution	145
6.5	Effect of Mn level in solution on the Fe level in the plant tissue under low (L) and optimal (H) levels of Ca	147
6.6	Effect of Mn level in solution on the level of Ca in the plant tissue	149
6.7	Effect of Ca level in solution on the level of Ca in the plant tissue	151
6.8	Effect of Mn level in solution on the level of Mg in the plant tissue	152

6.9 Effect of Ca level in solution on the level Mg in the plant tissue 153

7.1 Effect of Mn level in solution on the plant dry weight 169

7.2 Effect of Mg level in solution on the plant dry weight 170

7.3 Effect of Mn level in solution on the level of Mn in the plant tissue 173

7.3a Effect of Mn level in solution on the level of Mn in the plant tissue under low and optimal levels of Mg in solution 174

7.4 Effect of Mn level in solution on the level of Fe in the plant tissue 175

7.5 Effect of Mg level in solution on the level of Fe in the plant tissue 177

7.6 Effect of Mn level in solution on the level of Ca in the plant tissue 178

7.7 Effect of Mn level in solution on the level of Mg in the plant tissue 180

7.8 Effect of Mg level in solution on the level of Mg in the plant tissue 182

8.1 Effect of Mn level in the solution on the plant dry weight 202

8.2 Effect of Mn level in the solution on the plant dry weight under low and optimal Ca in the solution 203

8.3 Effect of Ca level in the solution on the plant dry weight 204

8.4 Effect of Mg level in the solution on the plant dry weight 205

8.5 Effect of Mn level in the solution on the level of Mn in plant tissue 207

8.6 Effect of Mn level in the solution on the Mn level in the roots under low and optimal levels of Ca 208

8.7 Effect of Mn level in the solution on the Mn level in the stem under low and optimal levels of Ca 209

8.8 Effect of Mn level in the solution on the Mn level in the old leaves under low and optimal levels of Ca 210

8.9 Effect of Mn level in the solution on the Mn level in the new leaves under low and optimal levels of Ca 211

8.10 Effect of Mn level in the solution on the Mn level in the roots under low and optimal levels of Mg 212

8.11 Effect of Mn level in the solution on the Mn level in the stem under low and optimal levels of Mg 213

8.12 Effect of Mn level in the solution on the Mn level in the old leaves under low and optimal levels of Mg 214

8.13 Effect of Mn level in the solution on the Mn level in the new leaves under low and optimal levels of Mg 215

8.14 Effect of Ca level in the solution on Mn level in the plant tissue 217

8.15 Effect of Ca level in solution on Mn level in the roots under low and optimal levels of Mg 218

8.16 Effect of Ca level in solution on Mn level in the stem under low and optimal levels of Mg 219

8.17 Effect of Ca level in solution on Mn level in the old leaves under low and optimal levels of Mg 220

8.18 Effect of Ca level in solution on Mn level in the new leaves under low and optimal levels of Mg 221

8.18a Effect of Mn level in solution on the level of Mn in the plant tissue under different levels of Ca and Mg in solution 222

8.19 Effect of Mg level in the solution on Mn level in the plant tissue 224

8.20 Effect of Mn level in the solution on the level of Fe in the plant tissue 225

8.21 Effect of Mn level in the solution on the Fe level in the roots under low and optimal levels of Ca 226

8.22 Effect of Ca level in solution on the level of Fe in the roots 228

8.23 Effect of Mn level in solution on the level of Ca in the plant tissue 230

8.24 Effect of Mn level in solution on Ca level in the plant tissue under low and optimal levels of Ca 231

8.25 Effect of Mn level in solution on Ca level in the plant tissue under low and optimal levels of Mg 232

8.26 Effect of Mn level in solution on Ca level in the roots under low and optimal levels of Mg 233

8.27 Effect of Mn level in solution on Ca level in the stem under low and optimal levels of Mg 234

8.28 Effect of Mn level in solution on Ca level in the new leaves under low and optimal levels of Mg 235

8.29 Effect of Mn level in solution on Ca level in the old leaves under low and optimal levels of Mg 236

8.30 Effect of Ca level in solution on the level of Ca in the plant tissue 238

8.31 Effect of Mg level in solution on Ca level in the plant tissue 239

8.32 Effect of Mn level in solution on the level of Mg in the plant tissue 240

8.33 Effect of Mn level in solution on Mg level in the plant tissue under low and optimal levels of Mg 242

8.34 Effect of Mn level in solution on Mg level in the plant tissue under low and optimal levels of Ca 243

8.35 Effect of Mn level in solution on Mg level in the roots under low and optimal levels of Ca 244

8.36 Effect of Mn level in solution on Mg level in the stem under low and optimal levels of Ca 245

8.37 Effect of Mn level in solution on Mg level in the new leaves under low and optimal levels of Ca 246

8.38 Effect of Ca level in solution on the level of Mg in the plant tissue 248

8.39 Effect of Mg level in solution on the level of Mg in the plant tissue 249

CHAPTER 1

INTRODUCTION

1.1 PROBLEM STATEMENT

An iron/Manganese physiological disorder affecting marigold (*Tagetes erecta* L.) has been reported in the floriculture industries of the United States and Canada throughout the last decade (Koranski, 1988; Todd, 1994; Albano and Halbrooks, 1991; Albano and Miller, 1993; Biernbaum et al., 1988; Carlson, 1988; halbrooks and Albano, 1990). The disorder is characterized by specific symptomology distinguished visually by speckled patterns of chlorosis and necrosis and downward curling and cupping of the leaves (Albano et al., 1996; Biernbaum et al., 1988; Vetanovetz and Knauss, 1989). Affected leaf tissue has been reported to have excessive concentrations of iron (Fe) and manganese (Mn) (400-2500 mg/kg) (Koranski, 1988; halbrooks and Albano, 1990), and low concentrations of calcium (Ca) and magnesium (Mg) (Koranski, 1988).

Losses in crop quality and production attributed to this disorder have been significant (Biernbaum et al., 1988; Koranski, 1988). Because flowering bedding plants are valued at greater than $750 million annually in the United States (U.S. Dept. of Agr., 1993), economic losses associated with this disorder could be significant.

The relationship of the disorder to Fe toxicity has been investigated (Albano and Miller, 1996; Albano et al., 1996; Vetanovetz and Knauss, 1989, Bachman and Miller, 1995), however its relationship to Mn toxicity has not yet been studied under controlled conditions. Therefore, the relationship of Mn toxicity to the incidence of Fe and Mn-related toxicity disorders in marigold needs to be established.

1.2 PREVIOUS WORK AND PRESENT OUTLOOK

Several trade and extension publications have identified this disorder as an Fe toxicity of some crops including marigold (Albano et al., 1996; Albano and Miller, 1993; Todd, 1994), New Guinea impatiens, common impatiens (Impatiens wallerana), cutting and seedling geraniums, vinca, some species of Brassica, cabbage and tomato transplants, and elatior begonia (Vetanovetz and Knauss, 1989). The disorder may be related to Mn toxicity as well, because the affected plants have large concentrations of Mn in their tissue (Koranski, 1988; Halbrooks and Albano, 1990; Biernbaum et al., 1988). The affected plants also show symptoms similar to those observed on Easter lily, soybean, peanut, muskmelons, water-melons, and sweet potato suffering from Mn toxicity (Holmes and Courts, 1980; Parker et al., 1969; Davis, 1996; Simon et al., 1986; Mortley, 1993). In addition, at low concentrations of Fe in the medium, the occurrence of the disorder is more closely correlated with increased levels of Mn in leaf tissue than Fe (Halbrooks and Albano, 1990).

In many higher plants excessive amounts of Mn disrupt various aspects of plant metabolism, leading to serious physiological and morphological disorders. Manganese toxicity has been associated with reduced enzyme activity (Heenan and Campbell, 1981; Sirkar and Amin, 1974), reduced hormone activity (Morgan et al., 1966), inhibition of ATP synthesis, and reduced respiration rates (Sirkar and Amin, 1974).

Factors that affect the toxicity of Mn in plants include the concentration of Mn in the growth medium, and the concentrations of salts, particularly those of Ca and Mg (Foy et al., 1978; Goh and Haynes, 1978; Bachman and Miller, 1995; Harrison and Bergman, 1981; Le

Mare, 1977). Small changes in these factors can determine the degree of Mn toxicity in a given crop.

1.2.1 High Availability of Mn in the Growth Medium

Most bedding plants are grown in soilless, peat-based media. Increased peat in the medium increases the amount of micropore water and decreases its air capacity, conditions which favor Mn reduction from Mn^{3+} to Mn^{2+} which is more readily taken up by plants (Goh and Haynes, 1978; Mortvedt and Cunningham, 1971; Ponnamperuma et al., 1969).

In some cases, the accumulation of Mn in the leaf tissue is the direct result of applying excessive levels of micronutrients. The continuous use of high levels of peat-lite fertilizers containing micronutrients including Mn may cause problems if salt levels are not monitored carefully (Sheely, 1990). Preplant addition of micronutrients in the media, combined with constant feed program, creates toxicity problems (Sheely, 1990).

1.2.2 Low Level of Ca in the Medium

The second part of the disorder may be a deficiency of Ca due in part to low pH in the growth medium and to the lack of Ca in many of the fertilizers used in greenhouse crop production (Koranski, 1988). In addition, peat-based media commonly used to grow bedding plants, are acidic and contain low concentrations of Ca (Bunt, 1988; Nelson, 1991).

The toxicity effects of high concentrations of Mn in the plant tissue are considerably modified by Ca status. They are much more severe when Ca level in the plant tissue is low (Clark et al., 1981; Galvez et al., 1989; Foy et al., 1981; Horst and Marschner 1978; Keisling and Fuqua, 1979; Wallace et al., 1945). Le Mare (1972, 1977) found that large concentrations of Ca in the medium could alleviate the harmful effects of Mn toxicity. The

plants grown in a soil that supplied little Ca were very sensitive to Mn toxicity (Le Mare, 1972; Le Mare, 1977).

Foy et al. (1978) reported the importance of Ca/Mn ratios in the tolerance of plants to Mn toxicity. Ratios above 80 were found desirable for a balanced nutrition in peanut (Bekker et al., 1994).

Although increasing the concentration of Ca in the medium can alleviate the detrimental effects of high concentrations of Mn, optimal concentration (60 mg/kg) of Ca is crucial for optimal plant growth (Morris and Pierre, 1947). Morris and Pierre (1947) pointed out that high Ca levels (300 mg/kg) in the medium could reduce plant growth regardless of the concentration of Mn in the medium, because of unbalanced nutrition.

1.2.3 Low Level of Mg in the Medium

Another possible aspect of the Fe/Mn toxicity disorder in marigold is low concentration of Mg in the leaf tissue.

Peat-based media commonly used in the production of bedding plants are acidic, contain high levels of available Mn and contain low concentrations of Mg (Goh and Haynes, 1978; Mortvedt and Cunningham, 1971; Ponnamperuma et al., 1969; Bunt, 1988; Nelson, 1991).

Large concentrations of Mn in the medium can induce Mg deficiency in the plant (Mn-induced Mg deficiency) (Heenan and Campbell, 1981). Kazda and Znacek (1989) reported that excess Mn in the medium reduced Mg uptake by 50%.

Manganese toxicity can often be counteracted by large Mg supply (Lohnis, 1960; Elamin and Wilcox, 1986b). It was reported that Mg decreased Mn uptake both by excised

and intact roots of several plant species (Harrison and Bergman, 1981; Lohnis, 1960; Maas et al., 1969)

In some cases, Mg application is not a practical method for the avoidance of Mn toxicity (Davis, 1996). The ability of Mg to reduce Mn uptake depends on the concentration of Mn in the medium. Elamin and Wilcox (1986a) found that at high Mn concentration in the medium, Mg had little effect on Mn uptake and the plants were able to accumulate toxic levels of Mn at all levels of Mg supply. In addition, using Mg to prevent Mn toxicity would require large Mg applications, which could lead to serious nutritional imbalance because Mg would interfere with Ca uptake.

In summary, Fe/Mn toxicity disorder in marigold may be caused by Mn toxicity since the affected plants contain high concentrations of Mn in their tissue. Basic factors affecting Mn toxicity in plants include high levels of Mn in the growth medium, Ca deficiency and Mg deficiency. The relationship of these factors to the incidence of Fe/Mn toxicity in marigold needs to be investigated.

1.3 OBJECTIVES

The objectives of this research are to induce and characterize Fe/Mn toxicity disorder under controlled conditions and high Mn levels in the medium; and to determine the relationship of the disorder to Ca deficiency, Mg deficiency, and a combination of both.

1.4 CONTRIBUTION

The findings of this research will improve the understanding of the relationship of Mn toxicity to the incidence of Fe/Mn toxicity in marigold. They may also help develop better agricultural practices and nutritional regimes to reduce the occurrence of Fe/Mn toxicity disorder in marigold and similar physiological disorders in other bedding plants

grown in soilless media. This information will also allow a better understanding of the effect of Ca deficiency, Mg deficiency or both on Mn toxicity in marigold.

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CHAPTER 2

LITERATURE REVIEW

2.1 ABSTRACT

Manganese toxicity in plants is often not a clearly identifiable disorder. Symptoms of Mn toxicity as well as the concentration of Mn that causes toxicity vary widely among plant species and varieties within species, perhaps because the phytotoxic mechanisms of Mn involve different biochemical pathways in different plant genotypes.

Plant availability of Mn depends on soil adsorption and on root exudates for Mn chelation or reduction. Soils with higher Mn sorption capacity have lower potential for plant absorption of Mn. Manganese tolerance is associated with restricted absorption, restricted translocation of excess Mn to the shoots, or ability to tolerate high Mn levels within the plant tissue. The ability to escape Mn toxicity through limited absorption or translocation is low in plants grown in wet soils rich in organic matter, or grown under high temperature or high light intensity. Manganese toxicity is likely with plants that are fertilized with acid-forming fertilizers, high rates of superphosphate, or nitrate (NO_3^-) as source of nitrogen (N), or plants that are low in silicon (Si) or deficient in calcium (Ca), iron (Fe), or magnesium (Mg). Comparative studies of Mn toxicity among different genotypes of the same species and a multidisciplinary approach are needed for a full understanding of Mn toxicity disorder in plants.

2.2 INTRODUCTION

Manganese is an essential element for plant growth. It can, however, be detrimental when available in excess in the growth medium. Concentrations as low as 1 mg Mn/liter in the solution culture can be toxic to tobacco (*Nicotiana tobacum* L.), potato

(*Solanum tuberosum* L.), or bush clover (*Lespedeza sp.* Michx) (Berger and Gerloff, 1947; Jacobson and Swanback, 1932; Morris and Pierre, 1949).

Excess Mn in the growth medium can interfere with the absorption, translocation, and utilization of other mineral elements such as Ca, Mg, Fe, and P (Clark, 1982). High Mn concentration in plant tissues can alter activities of enzymes and hormones, so that essential Mn-requiring processes become less active or nonfunctional (Epstein, 1961; Horst, 1988).

2.3 GENERAL EFFECTS

2.3.1 Plant Symptoms

Symptoms of Mn toxicity are quite diverse among plant species. They include marginal chlorosis and necrosis of leaves of alfalfa (*Medicago sativa* L.), rape (*Brassica napus* L.), Kale (*Brassica oleracea* L. Acephala Group), or lettuce (*Lactuca sativa* L.)(Foy et al., 1978); interveinal and marginal chlorosis along with brown necrotic spotting in sweet-potato (*Ipomoea batatas* L.), snapbean (*Phaseolus vulgaris* L.), lettuce, barley (*Hordeum vulgare* L.) or cotton (*Gossypium hirsutum* L.) (Foy et al., 1995; Foy et al., 1969); small dark spots surrounded by irregular areas of chlorotic tissues in marigold (*Tagetes erecta* L.)(Albano and Miller, 1996) and geranium (*Pelagonium X hortorum* Bailey) (Bachman and Miller, 1995); dark, reddish-brown leaf spots with chlorotic margins in bush clover (*Lespedeza sp*. Michs); crumbled, distorted small sized leaves with irregular interveinal chlorosis in soybean (*Glycine max* Merr.), small reddish purple spots on the underside of the leaves in cowpeas (*Pisum arvense* L.), and marginal chlorosis and crimping of the leaves with no spotting in sweet-clover (Morris and Pierre,

1949). Loss of apical dominance and enhanced formation of auxiliary shoots ("witches' broom") constitute another symptom of Mn toxicity (Kang and Fox, 1980).

Although expression of Mn toxicity varies considerably among plant species, brown spots on older leaves surrounded by chlorotic zones are typical symptoms of Mn toxicity. The necrotic brown spots were reported to be localized accumulations of oxidized Mn (Horigushi, 1987). Horst (1988) reported that the spots contain precipitated Mn compounds. Quite often, however, Mn-induced symptoms of deficiencies of other mineral nutrients, such as Fe, Mg, and Ca, are dominant (Fleming, 1989; Foy et al., 1981; Lee, 1972).

In severe cases of manganese toxicity, plant roots turn brown (Morris and Pierre, 1949), usually after the shoots have been severely injured (Foy et al., 1978). Aso (1902), on the other hand, found that high concentrations of Mn caused root browning first, then lower-leaf browning. Root browning indicates the presence of oxidized Mn (Horigushi, 1987). Its intensity increases with the severity of the toxicity, is usually darker near the root tips, and progresses upward becoming localized on the older roots (Mortley, 1993). Such roots are inefficient in absorbing nutrients and water (Aso, 1902).

Foy et al. (1978) reported that plant symptoms of Mn toxicity are often detectable at stress levels which produce little or no reduction in vegetative growth. Galvez et al. (1989), however, reported growth restriction of sorghum plants grown with high levels of Mn in the nutrient solution. Morris and Pierre (1949) found that Mn toxicity caused 63% suppression in the dry weight of lespedeza plants. When growth restriction occurs, it is usually more severe in the roots than in the shoots (Mortley, 1993).

Manganese toxicity causes symptoms much before the suppression in dry weight occurs. The concentration at which symptoms occur is usually much lower than the one that causes the suppression in dry weight. For example, the dry weight of soybean and cowpeas was significantly limited at 10 mg/kg Mn but the symptoms occurred at 2.5 mg/kg (Morris and Pierre, 1949).

According to Morris and Pierre (1949), the reduced dry weight may be caused by the shedding of the affected leaves since chlorosis may cover as much as 50% of the leaf area and up to 100% of the leaves may be affected in severe cases of toxicity.

Other reported effects of Mn toxicity include: restricted number and size of nodules (Foy et al., 1978; Vose and Jones, 1963), retarded germination (Brenshley, 1914; Millikan, 1948); retarded growth (Aso, 1902), and delayed ripening (Brenshley, 1914).

Specific physiological disorders such as “bronze speckle” in marigold or geranium, "crinkle leaf" of cotton, "stem streak necrosis" in potato, "internal bark necrosis" of apple (*Malus sylvestris* P. Mill.), "tip burn" in carnation (*Dianthus caryophyllus* L.), and "fruit cracking" in muskmelon (*Cucumis melo* Ser. ‘reticulatus’) have been associated with excess Mn in the tissues (Foy, 1983).

In some Mn-induced disorders, such as leaf marginal chlorosis in mustard, leaf speckling in barley, and internal bark necrosis in apple, the toxicity is related to localized accumulations of Mn rather than high overall Mn concentrations in plant shoots (Fisher et al., 1977; Williams and Vlamis, 1957b; Williams and Vlamis, 1971). For example, Williams et al. (1971) found that the yellow leaf margins of mustard affected by Mn toxicity contained 2300 mg/kg Mn compared to 570 mg/kg for green portions of the same leaves.

2.3.2 Physiological and Biochemical Effects

Epstein (1961) pointed out that an element present in excess can interfere with metabolism through competition for absorption, inactivation of enzymes, or displacement of essential elements from functional sites.

In cotton, Mn toxicity has been associated with an increased activity of indoleacetic acid oxidase, peroxidase, and polyphenol oxidase; lower activities of catalase, ascorbic acid oxidase and glutatione oxidase; lower ATP contents; and lower respiration rates (Morgan et al., 1976; Morgan et al., 1966). In sugar beets, excess Mn restricted leaf cell number and volume (Terry et al., 1975).

Manganese toxicity has been associated with an increase in indoleacetic acid oxidase activity, which results in a destruction of auxin (IAA) (Morgan et al., 1976; Morgan et al., 1966). Bhatt et al. (1976) found that 36 mg/kg Mn was able to reverse the growth inhibition of sorghum roots caused by 100 mg/liter gibberellic acid (GA). Gibberellic acid enhances auxin production. The inhibition of root growth by GA was due to elevated auxin synthesis to a level that was inhibitory for root growth and stimulatory for shoot growth. The promotion of root growth by Mn was thus due to auxin oxidation caused by excess Mn. Manganese toxicity also has been associated with an increase in peroxidase activity (Horigushi and Fukumoto, 1987; Morgan et al., 1966). Horigushi and Fukumoto (1987) demonstrated that the increase in peroxidase activity was followed by the appearance of the necrotic browning (visual symptoms of Mn toxicity). They suggested that the increase of the peroxidase activity is associated with the mechanism of the necrotic browning. Also, they felt that the increase in the peroxidase activity may, however, not be the only factor for the browning because the contact of

peroxidase with the substrates, which are oxidized to form brown substances, is necessary.

It has been reported that peroxidase is localized in the cell walls and plays a significant role in lignification (Swain, 1977). When peroxidase oxidizes the precursors of lignin, the necrotic browning does not occur (Horigushi, 1987). Engelsma (1972) and Brown et al. (1984) reported that Mn stimulates phenolic metabolism. If peroxidase comes into contact with substrates such as caffeic acid or chlorogenic acid, which are easily oxidized to form brown substances, necrotic browning of the tissues appears (Horigushi, 1987).

The brown necrotic spots have also been associated with the accumulation and deposition of oxidized Mn. Horigushi (1987) reported that a peroxidase-malate dehydrogenase system in the root cell walls oxidized Mn. The presence of oxidized Mn in plant tissues has been reported in the stems and leaves of pea (*Pisum sativus* L.) and sunflower (*Helianthus annuus* L.); in the leaves of tobacco and bush bean (*Phaseolus vulgaris* L.); and in the stems, petioles and leaf blades of cucumber (*Cucumis sativus* L.), localized around the trichomes (Horigushi, 1987).

Aso (1902) examined the brown spots of the Mn-affected leaves and found that the membranes of epidermal cells, and in some cases the nuclei, were stained deeply brown.

Excess Mn in plant tissue precipitated nucleic acids (Trim, 1959) and inhibited the nuclear deoxyribonucleic acid (DNA) replication (Baranowska et al., 1977) and protein synthesis (Foy et al., 1978). Guerin (1897) isolated nucleic precipitate of Mn from the woody tissues of the plants, and Foy et al. (1978) reported that excess Mn may act as an

error-producing factor during the mitochondrial replication, which causes mitochondrial mutations that are up to 4500 base-pairs long and that inhibit total protein synthesis by up to 90%.

Excess Mn also may predispose plants to certain diseases. Singh et al. (1974) found that high quantities of Mn (1900 mg/kg) in *Scopolia sinensis*, which is host of the potato spindle tuber virus, increased the replication of the virus ribonucleic acid (RNA).

2.4 IMPORTANCE AND OCCURRENCE

In many parts of the world, Mn toxicity is more important than Mn deficiency in crop plants (Welch et al., 1991). Manganese toxicity is usually encountered in poorly drained, acid soils (below pH 5.5) (Chesworth, 1991; Morris and Pierre, 1949). Such soils usually contain relatively low concentrations of available Ca and Mg and high concentrations of soluble Fe and Mn. Many crops are sensitive to concentrations as low as 1 mg/kg Mn in the medium and 1 mg/kg Mn is within the range found in many acid soils (Morris and Pierre, 1949).

The amount of total Mn in most soils ranges from 200 to 3,000 mg/kg (Mortvedt and Cunningham, 1971). Only a small fraction of the total Mn is in the reduced divalent form, which is available to plants. Studies on the Mn content of some acid soils showed that they sometimes contain as high as 50 mg/kg Mn in the soil solution (Morris and Pierre, 1949).

Manganese toxicity also can occur at high pH levels in soils under reducing conditions created by flooding, compaction, or organic matter accumulation (Kamprath and Foy, 1971).

2.5 TOLERANCE TO Mn TOXICITY

Tolerance to Mn toxicity is affected by several factors including plant genotype (Morris and Pierre, 1949); soil management factors, such as type of fertilizer applied, liming (Labanauskas, 1966), and growth media (Goh and Haynes, 1978); environmental factors, such as temperature (Welch et al., 1991) and light intensity (McCool, 1935); and nutritional interactions (Bachman and Miller, 1995; Foy et al., 1981; Lewin and Reimann, 1969; Ohki, 1975).

The critical Mn concentration associated with injury is quite variable among plant species and varieties within species, due to wide differences in Mn tolerance (Ohki, 1981; Edwards and Asher, 1982). An example of the differences among plant species is given in Table 1.

Table 1: Critical toxicity levels of Mn in the leaves of various plant species[a]

Species	Concentration above which toxicity occurs (mg Mn/kg leaf dry weight)	Reference
Bragg soybean	160	Foy et al., 1978
Corn	200	Horst and Marshner, 1990
Pea	300	"
Soybean	600	"
Cotton	750	"
Sweet potato	1380	"
Sunflower	5300	"
Carrot	7100-9600	Gupta et al., 1970

[a]Critical toxicity levels are associated with a 10% reduction in dry matter production (Ohki, 1981).

Ohki (1981) defined critical toxicity level of Mn as Mn concentration in the leaves associated with 10% restriction in dry weight due to toxicity. Symptoms of Mn toxicity, however, occur at much lower concentrations (Morris and Pierre, 1949). Gupta et al. (1970) reported that 2600 mg/kg Mn in carrot (*Daucus carota* L. 'sativus') shoots caused

symptoms (bronzing) to occur but higher concentrations (7100-9600) were required to limit yields.

Based on the Mn concentration in the medium associated with injury, the critical Mn concentration was found to be 1 mg/kg for tobacco (Jacobson and Swanback, 1932); 3.5 mg/kg for peas; and 10 mg/kg for soybean, peanut (*Arachis hypogaea* L.) (Morris and Pierre, 1949), and barley (Aso, 1902).

So plant species and cultivars within species differ widely in tolerance to excess Mn. In general, water plants are able to accumulate higher amounts of Mn in their tissues without injury, than land plants (Gossl, 1905). Morris and Pierre (1949) compared the differential Mn tolerance among legumes. Lespedeza and sweet-clover (*Melilotus sp.* Mill.) were the most sensitive to Mn toxicity; cowpeas and soybeans were intermediate, and peanuts were the most tolerant. Brenchley (1914) reported that barley is more tolerant to high concentrations of Mn than wheat. Benac (1976) found that in nutrient solution, about 20 times as much Mn was required to produce toxicity in corn as in peanut. Manganese tolerance in plants has been attributed to restricted absorption, restricted translocation of excess Mn to the shoots, and greater tolerance to high Mn levels within plant tissues (Andrew and Hegarty, 1969; Foy et al., 1995; Morris and Pierre, 1949).

2.5.1 Manganese Absorption

Plants can modify the soil chemistry within their root zone (Foy et al., 1978). Certain Mn-tolerant cultivars of wheat (*Triticum sp.* L.), barley, rice (*Oryza sativa* L.), peas, and corn (*Zea mays* L.) increase the pH of their nutrient solutions (Barber, 1974). Such increase in the medium pH reduces the availability of Mn to the plants. In contrast, Mn-sensitive cultivars of the same species decrease or have no effect on the pH of their nutrient

solution (Barber, 1974), and thus are exposed to higher concentrations of available Mn. In some wheat genotypes, these differential pH changes have been demonstrated in thin layers of soil removed directly from the root zone and even in the bulk soil in pot culture (Barber, 1974).

Possible causes of pH lowering in the plant root zone are release of H^+ resulting from excess cation over anion absorption, release of H^+ ions as result of NH_4^+ nutrition, release of H^+ ions from carboxyl groups of polygalacturonic acid residues of pectic acid, or excretion of H^+ protons from microorganisms associated with the roots (Barber, 1974).

The superior Mn tolerance of rice over soybean, coincides with greater oxidizing power of rice roots (Doi, 1952; Engler and Patrick, 1975). The rice roots are able to oxidize Mn from the divalent (available) to the tetravalent (unavailable) form of Mn ($Mn0_2$), which decreases Mn absorption by the plants (Engler and Patrick, 1975). Doi (1952) found that soybean was Mn injured if planted alone in paddy soils, but this injury was reduced if soybean was planted with rice. The beneficial effect was probably due to the oxidation and detoxification of Mn by rice roots.

The superior tolerance of peanut (compared to cowpea and soybean) (Morris and Pierre, 1949), and 'Siam 29' rice (compared to 'Pebifun') (Foy et al., 1978) was attributed to limited Mn absorption.

Robson and Loneragan (1970) found that the greater sensitivity of *Medicago* species of tropical legumes (compared to subterranean clover (*Trifolium subterraneum* L.)) was associated with a higher rate of Mn absorption and less retention of Mn by the roots.

2.5.2 Manganese Accumulation and Translocation

Kamprath and Foy (1971) reported that Mn may be detoxified by accumulating in the woody portions of the azalea plant. High Mn sensitivity in peanut was associated with the accumulation of Mn in its leaves but little or no accumulation in roots, stem, or petioles (Morris and Pierre, 1949).

Morris and Pierre (1949) found that leaves of legumes affected by Mn toxicity contained three to five times the concentrations of Mn in stems.

Manganese tolerance in some plant species is associated with greater retention of excess Mn in the roots. The oxidation of Mn with concomitant deposition of oxidized Mn in the roots reduces Mn translocation to the shoots and enhances the tolerance of the plant to excessive amounts of Mn (Foy et al., 1978). Horiguchi (1987) observed deposition of oxidized Mn on the roots of rice, which displayed a dark brown color and accumulated a large amount of Mn. He also reported that a peroxidase-malate dehydrogenase system in the root cell walls oxidized Mn.

Andrew and Hegarty (1969) reported that the relative tolerance of either tropical or temperate legume species depends in part on the retention of excess amounts of Mn within the root system. The superior Mn tolerance of corn (compared to peanut) was associated with reduced translocation of Mn from the roots and stems to the leaves (Benac, 1976). Ouellette and Dessureaux (1958) reported that the tolerant clones of alfalfa contained lower concentrations of Mn in their shoots and higher concentrations of Mn in their roots than did the more sensitive clones .

Although Mn retention in the roots may contribute to the Mn tolerance of some plant species, this mechanism is not sufficient to account for the Mn tolerance of other species.

2.5.3 Internal Tolerance to Excess Mn

In some species, the Mn tolerance is associated more closely with the internal tolerance of the shoots to excessive amounts of Mn. Horigushi (1987) reported that rice retained less Mn in its roots than other species, and the shoots were able to tolerate a high Mn concentration in the tissues. On the other hand, the shoots of alfalfa were sensitive to a high Mn concentration in the tissues although the roots oxidized Mn and retained it in high concentrations. Foy et al. (1995) reported that the differential Mn tolerance in cotton genotypes [*Gossypium hirsutum* L.](307, 317) was associated with differential internal tolerance to excess Mn.

The higher Mn tolerance of 'Lee' soybean (compared to 'Bragg' and 'Forrest') (Brown and Jones, 1977a), and 'Rex Smooth Leaf' cotton (compared to 'Coher 100-A') (Foy et al., 1969) was attributed to a greater ability to tolerate high levels of Mn in the shoots.

The tolerance of plants to high concentrations of Mn in their shoots varies among species and genotypes within species (Foy et al., 1978; Morris and Pierre, 1949). Winterhalder (1963) showed that seedlings of *Eucalyptus saligna* (Sm.) were more tolerant to excess soil Mn than those of *Eucalyptus gummifera* (Gaertn.) Hochr., although the leaves of the former contained higher concentrations of Mn.

Tea (*Camellia thea* L.) and tomato (*Lycopersicon esculentum* Mill.) are more tolerant to high internal levels of Mn than many other species (Dennis, 1968; Foy et al.,

1978). Rice may tolerate 5 to 10 times as much Mn as those of many other grasses such as oats (*Avena sp.* L.), barley, wheat and ryegrass (*Lolium sp.* L.) (Vlamis and Williams, 1967).

Sorghum (*Sorghum propinguum* (Kunth) Hitoho.) generally tolerates high levels of Mn. However, some genotypes such as NB 9040 tolerate higher concentrations of Mn than others (IS 7173c) (Mgema and Clark, 1995).

A possible explanation for the internal tolerance of some plants to high levels of Mn in the shoots is the oxidation of excess Mn (Horigushi, 1987). Blamey et al. (1986) reported that the shoots of sunflower (*Helianthus sp.* L.) tolerate high Mn concentrations in the tissues through the presence of Mn in a metabolically inactive form. Horigushi (1987) found that when cucumber absorbed an excessive amount of Mn, a part of the Mn absorbed changed to oxidized Mn. They suggested that oxidized Mn is probably metabolically inactive since the dry weight of the plants was preserved up to a relatively high Mn concentration in the tissues. The mechanism of the Mn tolerance of rice does not appear to be associated with Mn oxidation because oxidized Mn could not be detected on the leaves (Horigushi, 1987).

2.5.4 Genetic Control of Mn Tolerance in Plants

Genetic variations in the ability of plants to accumulate Mn have been reviewed by many investigators (Brown et al., 1972; Gerloff and Gabelman, 1983; Graham, 1984). Foy et al. (1978) reported that Mn tolerance in alfalfa was due to additive genes. Lettuce genotypes, *Lactuca sativa* 'Neptune' (Mn-sensitive) and 'Plenos' and 'Troppo' (Mn-tolerant), differ greatly in tolerance to available Mn in steam sterilized soils (Eenink and

Garresten, 1977). Studies of their progenies indicated that Mn tolerance was determined by different genes in the parents (Eenink and Garresten, 1977).

2.6 SOIL MANAGEMENT FACTORS

Because plant roots absorb Mn as the divalent cation, any factors that change its concentration in the soil solution potentially can affect the accumulation of Mn in the plant. These factors include soil physical and chemical properties (Graham, 1988); soil moisture, organic matter, and pH (Christenson et al., 1951); and the concentrations of other elements that interact with Mn absorption, translocation and use (Welch et al., 1991). Small changes in these factors can determine the degree of Mn toxicity in a given crop. Lowering the pH below about 5.5 increases the level of the divalent Mn in the soil solution and increases the likelihood of toxicity, air drying may greatly increase the level of exchangeable Mn in acid soils rich in easily reducible Mn, and flooding and compaction of soils reduce soil aeration and favor the reduction of Mn to the divalent form in which it may reach toxic levels (Kamprath and Foy, 1971).

2.6.1 Fertilizer and N Form

Manganese toxicity can appear on previously productive cropland that has been treated repeatedly with Mn-containing pesticides and fertilizers (Chaney and Giodano, 1977). Excess Mn was reported in certain Florida citrus soils, in which the accumulation resulted from repeated spray and fertilizer applications (Foy et al., 1978).

Manganese toxicity was reported in plants treated with sewage sludge (Chaney and Giodano, 1977). Peterson et al. (1971) found that the Mn content of corn leaves was directly correlated with sludge fertilization.

Application of divalent Mn sources with acid-forming fertilizers results in increased Mn absorption (Hossner and Richards, 1968). Williams et al. (1971) reported that fertilizers increase Mn concentrations in plants in the following order: $(NH_4)_2SO_4$ > NH_4NO_3 > $Ca(NO_3)_2$. This series coincides with the declining order of soil acidification by these fertilizers. There were no symptoms above soil pH 5.5, and yields were inversely correlated with Mn in plants.

Preplant addition of dolomite to a soilless peat-based medium maintained the pH within the range of 5.8 to 6.2 and lowered Fe and Mn concentrations in the tissue of geranium (*Pelagonium X Hortorum* L.H. Bailey 'Aurora') plants suffering from Fe and Mn toxicity. Liquid dolomite drenches during plant growth had little effect on tissue Fe or Mn (Bachman and Miller, 1995).

Larson (1964) reported increased Mn absorption by plants if high rates of superphosphate were applied to neutral and alkaline soils. They suggested chemical mobilization of soil Mn as a result of soil reactions of the superphosphate.

Elamin and Wilcox (1986c) found that N form influenced Mn toxicity in muskmelons. The plants grown with nitrate (NO_3^-) developed Mn toxicity symptoms and had high concentrations of Mn in the shoot tissue when exposed to high concentrations of Mn. In opposition, the plants grown with ammonium (NH_4^+) had less Mn in their shoot tissue and developed no Mn toxicity symptoms when exposed to the same Mn concentration treatments. Ammonium was also found to decrease Mn absorption and toxicity in barley (Arnon, 1937). Reddy and Mills (1991) found that NO_3^- source of N enhanced Mn uptake while NH_4NO_3 reduced it. They also found that the growth of marigold was suppressed with high concentrations of Mn in the nutrient solution (10

mg/liter) and that the suppression was greater with the NO_3^- source than with the NH_4NO_3. Since NO_3^- fertilization increases soil pH, use of this N source should result in a decreased Mn concentration in the soil solution and consequently uptake by plants. However, NO_3^- or NH_4^+ influences not only the rhizosphere pH but also the uptake of other ions by plants (Barker and Mills, 1980) and this latter factor must also be considered independent of Mn availability to plants as a consequence of rhizosphere pH changes.

2.6.2 Liming

The absorption of excessive amounts of Mn and the resultant toxicity in sensitive crops can be reduced by liming acid media to pH 5.5 or above (Fleming, 1989; Jackson et al., 1996). However, Mn toxicity also was observed in calcareous soils and flooded neutral soils (Moraghan and Freeman, 1978). Moraghan and Freeman (1978) reported that flax (*Linum usitatissimum* L.) developed Mn toxicity symptoms at pH 8.1, on a calcareous soil with low available Fe.

It is generally considered that the role of liming in amelioration of Mn toxicity is due primarily to reduced Mn availability at high soil pH. However, such beneficial effects could be, in part, due to increased Ca availability (Bekker et al., 1994).

Bekker et al. (1994) found that liming the soil with coarse corraline lime material containing 31.1% Ca and 1.7% Mg alleviated Mn toxicity in peanut plants. They explained that this alleviation was not likely due to decreased Mn solubility because the lime application increased the soil pH by less than 0.1 unit but that it was due rather to an increase of Ca availability. Calcium availability reduced Mn toxicity through a Ca-Mn antagonism.

Morris and Pierre (1947) attributed the effect of liming in promoting growth of lespedeza on acid soils to removal of toxic concentrations of soluble Mn and not to the addition of Ca to the soil, because lespedeza requires very low Ca. Liming acid soils to pH values of 5.5 to 6.0 eliminates all water-soluble Mn from the soil (Morris and Pierre, 1947).

The availability of Mn to plants generally decreases as soil pH increases. A sharp yield depression may occur when strongly acid soils are limed to pH 6.0 or above, because of micronutrient deficiencies that may occur under limed conditions (Kamprath and Foy, 1971).

2.6.3 Moisture Level and Growth Medium

Waterlogging of soil promotes the reduction of Mn to the divalent (available) form. Tolerances of some plant species to certain wet soils coincide with their tolerances to excess Mn in nutrient cultures. For example, the Mn-tolerant subterranean clover is more tolerant to waterlogging than the Mn-sensitive barrel-clover (*Medicago truncatula* Gaertner) (Robson and Loneragan, 1970). Graven et al. (1965) suggested that the sensitivity of alfalfa to wet soils might be due in part to Mn sensitivity.

Gupta and Chipman (1976) and Gupta et al. (1970) reported Mn toxicity of carrots in a sphagnum peat medium. The toxicity was attributed to an increased solubility of a humic fraction of peat which rendered Mn more available to the plants. Goh and Haynes (1978) found that different proportion of peat resulted in different growth rate of marigold (*Tagetes erecta* L. 'Naughty Marietta'), petunia (*Petunia hybrida nana compacta* Juss 'Rose of heaven') and forget-me-not (*Myosotis alpestris* L.). Generally, poorest growth occurred when peat represented more than 25% of the growth medium. Increased peat in the medium increases the amount of micropore water and decreased its air capacity,

conditions which favor Mn reduction and make it available to plants. Poor growth also occurred if peat was mixed with soil, sawdust, or bark, perhaps because soil, sawdust, and bark contain significant amount of organic matter. Welch (1991) reported that in England Mn toxicity was seen in crops grown in peaty soils and mineral soils with high levels of organic matter.

The biological reduction of Mn in the soil is enhanced by the addition of fresh organic matter (Cotter and Mishra, 1968) and by flooding (Ponnamperuma et al., 1969). The availability of the divalent form of Mn upon flooding is usually greater when soils contain high levels of organic matter (Mortvedt and Cunningham, 1971).

The availability of Mn is closely related to the transformations of organic materials in soils (Kamprath and Foy, 1971). Microorganisms influence the availability of Mn by changing the pH of the medium (Kamprath and Foy, 1971) and by changing the oxidation state (Alexander, 1961).

Soil microorganisms appear to play a major role in determining soil levels of reduced Mn especially at the higher soil pH levels (Douka, 1977; Nambier, 1975).

Christenson et al. (1951) reported that soil pH was of greater importance than organic matter content and moisture level in affecting the status of available soil Mn.

2.7 ENVIRONMENTAL INTERACTIONS

The incidence of Mn toxicity is strongly dependent on environmental and nutritional status of nutrients other than Mn. For example, in some cases concentrations around 700 mg/kg in potato foliage have induced Mn toxicity, but in other cases, levels up to 4,000 mg/kg Mn in field-grown potato foliage did not cause toxicity (Marsh et al., 1989; Ouellette

and Genereaux, 1965). This differential tolerance to Mn appeared to depend on nutritional and environmental interactions.

It is apparent that several of these nutritional and environmental interactions could depend on changes in growth rate. Plant tolerance to Mn toxicity is to some extent dependent on growth rate, cell size, and particularly the size of the vacuole (Rufty et al., 1979), which is the site of Mn accumulation (Horst and Marshner, 1978b; Roby et al., 1987). Any factor that reduces growth might induce Mn toxicity in plants growing under high Mn concentrations.

The relative tolerance of plants to excess Mn is affected by climatic factors such as temperature and light intensity (Rufty et al., 1979; McCool, 1935).

2.7.1 Temperature

Some reports suggest substantial changes in Mn tolerance with temperature (Lohnis, 1951). Plant tolerance to Mn toxicity increases with increasing temperature in tobacco or soybeans despite high Mn absorption (Rufty et al., 1979; Heenan and Carter, 1975). This increased tolerance to Mn toxicity with increasing temperature is attributed mainly to faster growth rate due to high temperature. Fast growing leaves have the opportunity to form large vacuoles, sequestering the potentially toxic Mn. In the case of potato, high temperature reduces the growth rate and increases Mn absorption (Marsh et al., 1989). The combination of these two facts results in accentuated symptoms of Mn toxicity when the plants are grown under high Mn concentration in the medium. Marsh et al. (1989) attributed the severity of Mn toxicity at high temperature to an interaction between growth rate and Mn tolerance.

2.7.2 Light

High light intensity stimulates Mn absorption by plants and accentuates the severity of Mn toxicity (McCool, 1935; Horigushi, 1988b).

Horiguchi (1988b) reported that increased light intensity at high levels of Mn in the nutrient solution, decreased the chlorophyll content of the leaves, causing chlorosis, and precipitated the oxidized Mn in the medium. High light intensity increased the severity of Mn-induced chlorosis even at similar levels of Mn concentration within the leaves.

According to Horiguchi (1988b), the Mn-induced chlorosis in the leaves is due to the oxidation of chlorophyll by the oxidized Mn in the medium. Gerretsen (1950) attributed Mn-induced chlorosis to photooxidation of chlorophyll.

2.8 NUTRITIONAL INTERACTIONS

Several investigators (McCool, 1913; Morris and Pierre, 1947; Somers and Shive, 1942; Williams and Vlamis, 1957a) reported that plant tolerance to soluble Mn was affected by the concentration of other nutrient elements in the medium. Excess Mn can interfere with the absorption, translocation, and utilization of other mineral elements such as Ca, Mg, Fe, and P (Clark, 1982). Galvez et al. (1989) found that high levels of Mn in the nutrient solution decreased shoot concentrations of Si, K, Ca, Mg, Zn, and Cu; increased shoot concentrations of Mn and P; decreased root concentrations of K and Mg; and increased root concentrations of Mn, Si, P, Fe, Zn, and Cu. Under some conditions the addition of Si, Fe, Ca, or Mg alleviates Mn toxicity (Heenan and Carter, 1975; Horst and Marshner, 1978b; Lohnis, 1960; Osawa and Ikeda, 1976).

2.8.1 Interaction with Fe

The interaction between Mn and Fe in their absorption by plants has been known for quite some time (Jahnson, 1917). The nature of the interaction varies among plant species and is not always consistent among studies. The interaction between Mn and Fe was absent in tomato, sorghum, rice, or wheat (Jaurigui and Reisenauer, 1982; Foy et al., 1978; Kuo and Mikkelson, 1981; Sanchez-Raya et al., 1974; Van Der Vorm and Van Diest, 1979); positive in oats, wheat, or barley (Mashhady and El-Damary, 1981; Shuman and Anderson, 1976; Singh and Pathak, 1968) and negative in pineapple (*Ananas comosus* (L.) Merr.), soybean, mustard (*Brassica sp.* L.), tobacco, rice, barley, or flax (Beauchamp and Rossi, 1972; Dekock and Inkson, 1962; Heenan and Campbell, 1983; Hiatt and Ragland, 1963; Jahnson, 1917; Moraghan, 1979; Somers and Shive, 1942; Vlamis and Williams, 1964).

The growth medium used in the studies seems to have a major influence on the nature of the relationship between Fe and Mn. In the same studies conducted by Mashhady and El-Damary (Mashhady and El-Damary, 1981), solution cultures tended to give a negative interaction; sand cultures gave no interaction; and soil-grown plants gave positive interactions. The authors suspected that the dramatic effect of the medium on the nature of the plant Mn-Fe interaction involved the presence or absence of solid phases, and their interaction with plant roots.

To evaluate Mn-Fe interactions for soil-grown plants, Warden and Reisenauer (1991) suggests to determine not only plant Mn and Fe composition but also rhizosphere Mn and Fe levels and root length.

Most of the investigations done on the interaction between Fe and Mn in plants, showed a negative correlation between Fe and Mn accumulation in the shoots of susceptible

cultivars. Ohki (1975) reported reciprocal Fe-Mn relationships in cotton; Mn concentrations 4 and 247 mg/kg in plant shoots were associated with Fe concentrations of 270 and 51 mg/kg, respectively. Kohno and Foy (1983) found that high concentrations of Fe decreased the absorption of Mn by some plants.

2.8.1.1 Manganese-induced Fe deficiency

Osawa and Ikeda (1976) described two types of manganese toxicity in plants. The first type was identical to Fe deficiency symptoms "interveinal chlorosis on young leaves" and was observed in spinach (*Spinacia oleracea* L.), tomato, pepper (*Capsicum annuum* L.) and beans. The second type of Mn toxicity was characterized by marginal chlorosis of old leaves, often with brown necrotic spots, and was observed in lettuce, celery (*Apium graveolens* L.), and cabbage (*Brassica oleracea* Capitata group). In general, increasing the Fe supply prevented or reduced the severity of the interveinal chlorosis, but had little or no effect on reducing the marginal chlorosis and brown spots. Leaf analyses indicated that the Mn-induced interveinal chlorosis was due to Fe deficiency whereas the marginal chlorosis and brown necrotic spots were due to accumulation of excess Mn.

Foy et al. (1978) also described two types of Mn toxicity in chickpeas (*Cicer arietinum* L.). The first type was pronounced in genotype T-1 and was characterized by marginal chlorosis and brown necrotic spots on leaflets of middle and young leaves. The second type of Mn toxicity was pronounced in genotype T-3 and was Mn-induced Fe deficiency chlorosis. Manganese treatment of T-3 decreased Fe concentrations of young leaves causing chlorosis. The reduction of chlorophyll in chlorotic plants was counteracted by high Fe applications.

Millikan (1948) found that flax grown at excess concentrations of Mn (25 mg/kg) developed lower leaf necrosis in addition to chlorosis and necrosis of the top of the plant, which are symptoms identical to those of Fe deficiency. Supplying the plants with Fe eliminated the symptoms of Fe deficiency but did not have any effect on the lower leaf necrosis. Lee (1972) reported that excess Mn in the medium induced Fe deficiency in potato. Manganese-induced Fe deficiency was related to the ratio Fe/Mn (Leach and Taper, 1954) as well as to the concentration of these 2 nutrients in the medium (Beauchamp and Rossi, 1972; Foy et al., 1995). Foy et al. (1995) reported that Fe concentrations and Fe/Mn ratio were higher in the Mn-tolerant cultivar of cotton [*Gossypium hirsutum* L.] (307) than in the Mn-sensitive cultivar (517).

Beauchamp and Rossi (1972) found that high levels of Mn in the solution culture depressed Fe absorption by barley. Somers and Shive (1942) found that Mn toxicity to various plant species decreased by increasing the concentration of Fe in the solution.

Somers and Shive (1942) reported that the Fe/Mn ratio in culture solution, rather than the total amounts of these elements, controlled the growth of soybeans. They found that the optimum ratio was around 2.0 and any appreciable variation in this ratio would result in appearance of Fe or Mn toxicity symptoms. Hophins et al. (1944), however, obtained normal growth of beans in culture solutions with Fe/Mn ratios much higher than 2 and found no toxicity from Mn regardless of the ratio. Morris and Pierre (1947) found that 0.5 mg/liter Fe and 1 mg/liter Mn made excellent growth and that the plants grown with the same ratio (2.0) but with concentrations five times higher than the former treatment resulted in poor growth and severe Mn toxicity symptoms.

Leach and Taper (1954) found that the optimum Fe/Mn ratios in plants ranged from 1.5 to 3 for kidney beans (*Phaseolus vulgaris* L. 'humilis Alef.') and from 0.5 to 5 for tomato. Iron deficiency developed at lower ratios.

Osawa and Ikeda (1976) reported that in spinach the Fe/Mn ratio in nutrient solution was related closely to plant growth. Decreasing this ratio gave Mn-induced chlorosis (Mn-induced Fe deficiency) and decreased growth. They also reported that in tomato, pepper, bean, and eggplant (*Solanum melongena* L.) both Fe/Mn ratio and Mn concentration in nutrient solution affected plant growth and occurrence of Mn-induced chlorosis, and in celery, cabbage, and Welsh onion (*Allium fistulosum* L.) the absolute Mn concentration appeared to be the dominant factor.

The actual means by which Mn induces Fe deficiency in some plant species, is still obscure. Rippel (1923), Chapman (1931), and Millikan (1949) reported that plants manifesting symptoms of Fe deficiency associated with excess Mn may actually contain the same or higher content of Fe than normal plants, indicating that it is not Fe absorption but its action in the tissues that is unfavorably affected by excess Mn. Somers and Shive (1942) suggested that this unfavorable effect is probably due to the Mn catalysis of the oxidation of the physiologically active form of Fe (Fe^{2+}) to the inactive form (Fe^{3+}). They also suggested the probable formation of an insoluble ferric-phosphate-organic complex.

Benette (1945) reported that Mn does not interfere with Fe utilization in the leaf but produces chlorosis by depressing the absorption of Fe. Johnson (1917) also explained Mn-induced Fe deficiency by inhibited Fe absorption. In Fe-deficient soils, Mn-induced Fe deficiency may be related to excessive mobilization of soil Mn as a result of reductant and proton release by plant roots responding to Fe deficiency (Horst, 1988).

Other explanations to the effect of Mn in inducing Fe deficiency could be that the plant species susceptible to Mn-induced Fe deficiency assimilate Mn preferentially over Fe and are more susceptible to interference of Mn with Fe function inside the plant or that Fe is immobilized more readily within tissues of these plants (Somers and Shive, 1942).

There may also be genotypic variations in the absorption and utilization of Mn and Fe by plants (Brown et al., 1972; Graham, 1988).

2.8.1.2 The effectiveness of Fe supply

The abilities of Fe to alleviate Mn toxicity depends on whether or not the toxicity is due to Mn-induced Fe deficiency or to accumulation of excess Mn in the plant tissues (Osawa and Ikeda, 1976; Foy et al., 1978). Iron supply had a marked effect on alleviating Mn toxicity in many crops (Morris and Pierre, 1947; Sideris and Young, 1949).

Iron treatments corrected Mn toxicity in Eucalyptus (*Eucalyptus sp.* L'her) (Winterhalder, 1963), soybean (Heenan and Campbell, 1983), flax (Moraghan and Freeman, 1978), and pineapples (Sideris and Young, 1949).

Morris and Pierre (1947) reported that the beneficial action of Fe in reducing Mn toxicity appeared to be due to some mechanism that prevents the excessive absorption and subsequent accumulation of Mn in the plant rather than to an increase in Fe absorption and corrective action of Fe within the plant itself. They found that alleviation of Mn toxicity by higher Fe concentration (1 mg/liter compared to 0.2 mg/liter) was not due to an increase of total iron in the plant but to an approximate 50% reduction in the Mn content of the plant.

Limitations as to the effect of Fe in overcoming Mn toxicity are evident from the research of Morris and Pierre (1947), who showed that an increase in the concentration of Fe in culture solution up to 1 mg/liter resulted in marked reduction of the Mn toxicity from

a given concentration of Mn. A further increase in the Fe concentration from 1 to 2.5 Mg/liter produced no additional decrease in Mn absorption, and higher Fe concentration resulted in suppressed growth regardless of the Mn concentration.

2.8.1.3 The effectiveness of Mo and P supply

Millikan (1948) reported that an essential function of Mo is intimately associated with the regulation of the deleterious effects of Mn on the physiological availability of Fe to the plants. Kirch et al. (1960) found that Mn absorption in tomatoes was influenced by interactions among Fe, Mn, and Mo. Iron supply counteracted the yield depressive effects of excess Mn, but the amount of Fe needed for maximum yield was increased as the Mo supply increased. Molybdenum suppressed yields at low Fe levels and increased yields at higher Fe levels.

Foy et al. (1978) reported that low P levels in the medium reduced the effect of Mn toxicity in chickpeas (variety T-3) because low P increases Fe uptake by the plants.

2.8.1.4 Manganese toxicity induced by Fe deficiency

Manganese toxicity may develop under Fe-deficient conditions (Brown and Jones, 1977a). Iron-deficiency stress seems to enhance Mn absorption in some species (Heenan and Campbell, 1983; Sideris and Young, 1949; Somers and Shive, 1942). Brown and Jones (1977a; 1977b; 1977c) reported increased Mn absorption by soybean, cotton, or sorghum, grown under Fe-deficiency stress. The absorption of Fe and Mn was greater in 'Hawkeye' (Fe-efficient) than in 'PI' (Fe-inefficient) soybean. When these cultivars were grown under Fe-sufficient conditions, both cultivars absorbed low levels of Fe and Mn (Brown and Jones, 1962). Dicots respond to Fe deficiency by an increased potential for Fe absorption and translocation (Fleming, 1989; Brown and Ambler, 1973). When the Fe response is

initiated (roots release reductants) the absorption of Mn, as well as Fe, is enhanced in Fe efficient species such as sunflower and soybean (Fleming, 1989; Brown and Ambler, 1973). The magnitude of the enhanced Mn absorption is related to the intensity of Fe stress as well as the cultivar sensitivity to Mn.

In a study on snapbean, Fleming (1989) found that under alkaline conditions, Mn absorption by 'Bush Blue Lake (BBL) 290' snapbean was increased dramatically with Fe stress, whereas a moderate increase was found for 'BBL 274'. Fleming also found that foliar symptoms of Mn toxicity, observed on Fe stressed 'BBL 290', increased in severity at higher Mn concentrations.

2.8.1.5 Manganese-iron toxicity

Contrary to the precedent findings, susceptibility to Mn toxicity and Fe toxicity seem to coincide in 'Bragg' and 'Forrest' soybeans (Brown and Jones, 1977a), marigold (Albano and Miller, 1996), and geranium (Bachman and Miller, 1995). Brown and Jones (1977a) suggested that high Fe levels in the shoots of 'Bragg' soybean may accentuate Mn toxicity or create Fe toxicity per se. Bachman and Miller (1995) found that 1 to 60 mg/kg Fe in the medium caused Fe/Mn toxicity in 'Aurora' geranium plants. Symptomatic tissues of affected geraniums had higher concentrations of Fe and Mn than asymptomatic tissues. Iron concentration increased with increasing Fe level in the medium, in both symptomatic and asymptomatic tissues. Manganese, however, increased in symptom tissues as Fe level increased; and remained the same in asymptomatic tissue, among all Fe treatments. Vlamis and Williams (1964) also reported that the chlorotic spots of lettuce contained high concentration of Fe and Mn. Iron content of lespedeza plants increased with increased Mn

concentration in the nutrient solution (Morris and Pierre, 1947). High Mn concentration in the medium may thus cause Fe deficiency in the plants if the medium is not sufficient in Fe.

2.8.2 Interaction with Si

Abundant evidence show that a soluble source of silicon (Si) in the growth medium can protect plants against Mn toxicity (Bowen, 1972; Lewin and Reimann, 1969; Peaslee and Frink, 1969; Williams and Vlamis, 1957b). The higher absorption of Si by monocots than dicots may help explain the higher tolerance of monocots to Mn toxicity (Foy et al., 1978). Lewin and Reimann (1969) reported that low silica plants accumulate higher concentrations of Mn than high silica plants. Galvez et al. (1989) reported that acid-soil-tolerant genotypes had higher leaf Si concentrations than the acid-soil-susceptible genotypes grown at higher-acid soil stresses; Si might be the cause of the plant tolerance to high acidity and hence to Mn toxicity. Silicon reduced or prevented Mn toxicity in barley, rice, rye (*Secale sp.* L.), ryegrass (Vlamis and Williams, 1967); Sudan-grass (*Sorghum bicolor* (L.) Moench) (Bowen, 1972); and sorghum (Galvez et al., 1989).

Van Der Vorm and Van Diest (1979) attributed the effect of Si in alleviating Mn toxicity to a decreased rate of Mn absorption. Peaslee and Frink (1969) found that treating acid soils with H_2SiO_3 significantly decreased Mn concentrations in the plant tissues. Williams and Vlamis (1957b), however, reported that the effect of Si in suppressing Mn toxicity does not necessarily result from decreased Mn absorption. They found that Si prevented the formation of necrotic spots of high Mn concentrations in barley leaves although it did not significantly alter the absorption of Mn by plants.

The effect of Si in alleviating Mn toxicity also was attributed to decreased Mn transport from the roots to the shoots (Horigushi, 1988a) and to homogeneous distribution

of Mn throughout the leaves instead of concentrating in some areas more than others (Vlamis and Williams, 1967). Vlamis and Williams (1967) reported that the distribution of Mn was homogeneous in the leaves of the high silica plants whereas it was not homogeneous in those of the low silica plants and that Mn was more concentrated in the affected areas than in the green areas. Silicon enhances the tolerance of plants to Mn toxicity by increasing Mn critical toxicity concentration (Horst and Marshner, 1978b; Galvez et al., 1989). Horst and Marshner (1978b) reported that the critical toxicity concentration of Mn in beans increased from about 100 mg/kg dry weight of the leaves to about 1000 mg/kg in the presence of Si.

Galvez et al. (1989) found that Si enhanced the tolerance of rice to Mn toxicity by increasing root oxidizing conditions to decrease Mn (and Fe) reducing capacities at root surfaces, so that toxic levels of Mn (and Fe) in the reduced form would not be available to plants. Foy et al. (1978) reported that Mn oxidation was greater in rice plants supplied with Si than in Si-deprived plants and that Si decreased excessive absorption of Mn and Fe.

Other reported effects of Si in alleviating Mn toxicity are moderated respiration rate and decreased peroxidase activity (Horigushi, 1988a). Silicon in the cell, is deposited in close association with other constituents of the cell wall (Lewin and Reimann, 1969). Silicon may play a role in maintaining the compartmentalization of the cell wall and preventing peroxidase from coming into contact with the precursors of the brown substrates (Horigushi, 1988a). Silicon also was found to limit the increase of P in plants caused by excess Mn in the nutrient solution (Galvez et al., 1989).

Limitations as to the effect of Si in overcoming Mn toxicity were reported (Galvez et al., 1989). If Mn was sufficiently high to inhibit dry matter yields of sorghum by about 80% or more, added Si had no beneficial effect on overcoming Mn toxicity.

2.8.3 Interaction with Ca

Calcium deficiency is induced by Mn toxicity in dicotyledons such as cotton ("crinkle leaf") (Foy et al., 1981) and bean (*Phaseolus vulgaris* L.)(Horst and Marshner, 1978a). When the supply of Mn is excessive, the translocation of Ca to the shoot apex, especially, is inhibited (Horst and Marshner, 1990). This inhibition might be related to the fact that high Mn levels decrease the cation exchange capacity of the leaf tissue (Horst and Marshner, 1978a) and decrease the IAA levels in the areas of new growth (Morgan et al., 1966; Morgan et al., 1976). Auxin was reported to be responsible for the formation of new binding sites for the transport of Ca to the apical meristems (Horst and Marshner, 1978a).

Manganese-induced suppressions in concentrations of Ca have been reported (Clark et al., 1981; Galvez et al., 1989). The higher tolerance of strain L_6 of lespedeza (compared to strain L_{39}) to excess Mn appears to be attributed to its greater tolerance to soluble Mn and to its lower requirement for Ca (Morris and Pierre, 1949). The calcium content of peanut decreased markedly as the Mn concentration of culture solution increased (Bekker et al., 1994).

Increased Ca levels in the growth medium often decreased Mn absorption and toxicity (Heenan and Carter, 1975; Robson and Loneragan, 1970; Shuman and Anderson, 1976; Hewitt, 1945). Vose and Jones (1963) found that increasing the Ca level in the solution culture reduced the adverse effects of high Mn level on suppressing the number of nodules on white-clover (*Trifolium repens* L.) roots.

The Ca/Mn ratio was important in nodulation and Mn tolerance in soybean (Foy et al., 1978). Bekker et al. (1994) reported the importance of a Ca/Mn-ratio above 80 for a desirable Ca/Mn balance in peanut tissue. Ouellette and Dessureaux (1958) found that Mn-tolerant clones of alfalfa contained lower concentrations of Mn in their shoots and higher concentrations of Mn and Ca in their roots than the sensitive clones. They concluded that Ca absorption regulated Mn toxicity by reducing Mn transport to shoots. Contrary to these findings, Chapman (1931) and Morris and Pierre (1947) found that Mn toxicity was not alleviated by additional Ca and was even greater at high Ca concentrations.

The conflicting results between Hewitt (1945) and Chapman (1931) and Morris and Pierre (1947) may be due to the different crops used. Morris and Pierre (1947) pointed out that high Ca levels (300 mg/liter) in the nutrient solution can limit plant growth regardless of the concentration of Mn in the solution, probably because of unbalanced nutrient solution. They concluded that optimal Ca concentration (60 mg/liter) was crucial for optimal plant growth and that Ca addition to the nutrient solution may cause a nutritional unbalance to the plants.

2.8.4 Interaction with Mg

Large concentrations of Mn in the medium can induce Mg deficiency in the plant (Mn-induced Mg deficiency) (Heenan and Campbell, 1981). Excess Mn may reduce Mg uptake by up to 50% (Kazda and Znacek, 1989) due to competition (Heenan and Campbell, 1981). The competition between Mg and Mn for binding sites in the roots during absorption, inhibits Mg absorption, since Mn competes more effectively than Mg and even blocks the binding sites for Mg (Horst and Marshner, 1990).

Manganese toxicity often can be counteracted by a large Mg supply (Elamin and Wilcox, 1986a; 1986b; Lohnis, 1960). Magnesium reduced Mn uptake both by excised and intact roots of several plant species (Harrison and Bergman, 1981; Lohnis, 1960; Maas et al., 1969).

In some cases, Mg application is not a practical method for the avoidance of Mn toxicity (Davis, 1996). The ability of Mg to reduce Mn uptake depends on the concentration of Mn in the medium. Elamin and Wilcox (1986a) found that at high Mn concentration, Mg had little effect on Mn uptake and the plants were able to accumulate toxic levels of Mn at all levels of Mg supply. In addition, using Mg to prevent Mn toxicity would require large Mg applications, which could lead to serious nutritional imbalance because Mg would interfere with Ca uptake.

2.8.5 Interaction with K

In comparison, the absorption of K is only slightly affected by increasing Mn concentrations (Heenan and Campbell, 1981). Brown and Jones (1977b) speculated that high K levels in the shoots of Mn-tolerant 'Lee' soybean alleviated the harmful effects of high internal Mn concentrations.

2.8.6 Interaction with P

Bortner (1935) suggested that P may reduce the toxicity of excess Mn by rendering it inactive within the plant. Phosphorus may detoxify excess Mn in the plant roots through precipitation (Heintze, 1968). However, P does not seem to detoxify excess Mn in the stems and the leaves since no precipitation was found in those parts of the plant (Morris and Pierre, 1947).

Applications of soluble phosphates to soils or nutrient solutions containing high amounts of Mn increased crop yields and suppressed development of Mn toxicity symptoms (Bortner, 1935). Bortner (1935) attributed these effects to the additional available P and a decrease in soluble Mn in the medium by precipitation.

Contrary to these findings, Morris and Pierre (1947) found that the solubility of Mn in the nutrient solution was not affected by the additional phosphate and that P did not have any effect in preventing Mn toxicity but may even accentuate it if added at high concentrations to the medium. Le Mare (1977) reported that P application to the soil increases P content of the plants and increases Mn absorption. Phosphorus also increased Mn absorption by potato and accentuated the severity of visual symptoms of Mn toxicity at high temperature (Marsh et al., 1989).

Millikan (1948) reported an indirect negative effect of P on Mn toxicity. He found that P may precipitate Mo in the medium making it unavailable to plants and may accentuate Mn toxicity.

2.8.7 Interaction with Mo

Anderson and Thomas (1946) found general similarities between Mo deficiency and Mn toxicity. Molybdenum deficient plants showed chlorosis with a necrosis of the margins of the lower leaves, which are also symptoms of Mn toxicity.

Molybdenum availability increases with soil alkalinity whereas Mn availability increases with acidity. Thus the effect of soil reaction on Mo availability is the opposite of its effect on the availability of Mn.

Plants with a high requirement for Mo may have a low requirement for Mn. On highly acid soils, therefore, such plants likely would be injured by excess Mn in the soil, unless supplied with an adequate concentration of Mo.

Liming of Mo-responsive soils improves growth. This beneficial effect of lime has been attributed to an increase in the availability of Mo following a rise in the pH of the soil, but concomitantly with such an improvement in the Mo status in the soil there would be a decrease in the availability of Mn.

Millikan (1948) found that small applications of Mo to the medium, prevented Mn toxicity symptoms (lower leaf necrosis) of flax grown in strongly acid soil rich in $MnSO_4$.

The report of Davies (1945) that "whiptail" disease of cauliflower may be prevented by Mo is also of interest here as Mn toxicity symptoms in cauliflower appear to be similar to those of "whiptail" disease (Wallace, 1946; Wallace et al., 1945).

Anderson and Thomas (1946) and Anderson and Oertel (1946) found that Mo alleviated the symptoms of Mn toxicity in the case of clover but not in the case of grass. This difference in responsiveness to Mo is probably due to a difference in the normal Mo requirements of the two species of plants as clover has been shown to have much higher content of Mo than grass (Lewis, 1943; Millikan, 1948).

One of the essential functions of Mo is to regulate the deleterious effects of Mn on the physiological availability of Fe to plants (Millikan, 1948). The addition of 5 to 25 mg/liter Mo to the nutrient solution reduced the severity of Mn induced Fe deficiency (Millikan, 1948). With excess Mn (25 to 150 mg/liter), the addition of 5 to 25 mg/liter Mo retarded the appearance of symptoms and reduced the severity of lower leaf necrosis, which is a characteristic symptom of Mn toxicity (not Fe deficiency).

Manganese toxicity of plants may still occur with the presence of fair quantity of Mo in the medium, if the pH is low and the concentration of phosphate is high in the medium (Millikan, 1948). This is probably because of the precipitation of Mo under these conditions.

2.8.8 Other Interactions

Plant tolerance to soluble Mn may also be affected by the concentration of sulfur (S), aluminum (Al), zinc (Zn), and copper (Cu) in the medium. Additional S may lower the pH of the growth medium and increase the availability of Mn to plants. Wallace et al. (1974) reported that S significantly decreased the yields of soybeans suffering from Mn toxicity. Aluminum reduced and prevented Fe chlorosis caused by excess Mn in flax (Millikan, 1949), but also increased Mn absorption and toxicity symptoms in soybeans (Foy et al., 1978). Zinc (Hewitt, 1948) and Cu (Landi and Fagioli, 1983) were found to reduce Mn absorption and toxicity in plants.

2.9 CONCLUSIONS

Manganese toxicity in plants is often not a clearly identifiable disorder; instead, it may be the result of complex interactions of Mn with other nutrients and with some environmental factors. Several of these nutritional and environmental interactions depend on changes in growth rate. To some extent, plant tolerance to Mn toxicity depends on growth rate, cell size, and particularly the size of the vacuole, which is the site of Mn accumulation. Any factor that restricts growth might induce Mn toxicity in plants growing under high Mn concentrations.

Although excess Mn may produce some common effects on plants in general, such as development of brown spots on older leaves, there are many cases of specific

differential effects of Mn on different plant genotypes. The phytotoxic mechanisms of Mn involve different biochemical pathways in different plant species and varieties.

Plant availability of Mn in soil depends on soil adsorption strength as well as plant effectors such as root exudates for Mn chelation or reduction. Adsorption and chelation are strongly affected by soil pH. At acid pH, soil Mn is naturally much more plant available. Several other soil factors play a role in Mn binding, although these are much less important than pH. The organic matter, clay, and hydrous oxides can adsorb Mn; soils with higher Mn sorption capacity have lower potential for plant absorption of Mn.

Manganese toxicity is associated with increased Mn absorption by plant roots, rapid translocation of Mn from roots to shoots, or localized accumulation of oxidized Mn in leaves. Low Fe/Mn or Ca/Mn ratios in plant tissues may be associated with the disorder. Plants high in Fe and Ca may tolerate higher internal levels of Mn, perhaps by restricting Mn transport to the shoots or by preventing localization of Mn into toxic spots.

Excess Mn in the plant tissue interferes with plant metabolism by disturbing enzyme activities or displacing essential elements from functional sites. For example, excess Mn increases peroxidase activity and stimulates phenolic metabolism. If peroxidase comes into contact with substrates such as caffeic acid or chlorogenic acid, which are easily oxidized to form brown substances, necrotic browning of the tissue appears.

Plant species and genotypes within species differ in tolerance to Mn toxicity. Greater Mn tolerance may be attributed to restricted absorption, restricted translocation of excess Mn to the shoots, or greater tolerance to high Mn levels within plant tissue. Plants

that tolerate high levels of Mn in their tissue may oxidize excess Mn to a metabolically inactive form.

Some plants may escape Mn toxicity by lowering the Mn^{2+} concentration in the growth medium [some plants may increase the pH of the growth medium and thereby decrease the concentrations of Mn^{2+} or secrete O_2 through their roots and oxidize Mn^{2+} to Mn^{4+} (MnO_2)], retaining excess Mn in the roots, accumulating excess Mn in the woody parts of the plant, or preventing Mn translocation from the stems and roots to the leaves.

The ability of plants to escape toxicity by limiting Mn absorption is low for plants grown in wet soils rich in organic matter, for plants that are fertilized with high rates of superphosphate, acid-forming fertilizers, or NO_3^- as a source of N; plants grown under high temperature or high light intensity, or for plants that are low in Si or deficient in Fe, Ca, or Mg. The translocation rate of Mn from roots to shoots is higher in plants low in Si or deficient in Ca or P. Plants that are low in Si or deficient in Fe, Ca, Mg, or P may accumulate higher concentrations of Mn in their shoots and are more susceptible to Mn toxicity than plants adequately supplied with these elements.

Research on Mn toxicity should not be confined to a single plant species because the symptoms and the critical toxicity concentration associated with toxicity vary considerably among different genotypes within the same species. What is needed is comparative studies among different genotypes of the same species.

Identification of genetic control mechanisms of Mn tolerance may be combined with other desirable traits to produce plants that are better adapted to high Mn levels in the growth medium. Studies of biochemical mechanisms of tolerance may help select or breed plants having greatest tolerance to Mn toxicity. Such biochemical knowledge could

also lead to the development of improved agricultural practices for problem soils (fertilization, liming, organic matter, tillage, drainage).

Other approaches in studying Mn toxicity in tolerant and sensitive plant genotypes include: determining the chemical compartmentalization of Mn in various plant fractions; levels and kinds of organic matter which may act as Mn chelators and detoxifiers or toxifiers; NH_4^+ vs NO_3^- preference (in relation to pH change and Mn activity in root zone and plant fractions); physiological and biochemical effects of essential and non essential ions on excess Mn; effect of peat medium as related to moisture level; and effect of stage of plant development.

Studies on Mn toxicity require collaboration among soil scientists, plant breeders, plant physiologists, plant biochemists, and plant nutritionists. A multidisciplinary approach is needed to limit the losses in crop quality and production attributed to Mn toxicity.

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CHAPTER 3

MAGNESIUM DEFICIENCY IN MARIGOLD

3.1 ABSTRACT

Iron/manganese toxicity disorder in marigold has been related to high concentrations of Fe and Mn and low concentrations of Ca and Mg in the affected leaves. Deficiency of Mg in marigold may occur because most bedding plants are grown in peat based media and peat based media are poor in Ca and Mg unless well-limed. To investigate the effect of poor supply of Mg on marigold (*Tagetes erecta* L. 'First Lady') growth, appearance, and nutrient (Mn, Fe, Ca and Mg) content of the shoots, a solution culture study with various Mg concentrations was conducted. Concentrations of Mg up to 8mg/liter resulted in stunted plants with a significant amount of chlorotic and necrotic symptoms on the leaves. The concentration 10 mg Mg/liter resulted in plants relatively free of symptoms. Concentrations above 10 mg/l resulted in relatively healthy looking plants. In an earlier experiment, 10 mg/liter Mg resulted in reduced dry weight. Ten mg Mg/liter was thus considered incipient deficiency concentration of Mg in marigold. This concentration of Mg in solution corresponded to 1.5 % Mg in the plant shoots. In this experiment low Mg had no effect on Fe content in the shoot tissue but resulted in increased Mn and Ca contents and decreased Mg content.

3.2 INTRODUCTION

Magnesium, the central atom of chlorophyll, is vital for the process of photosynthesis and therefore for the life of plants in general. In addition to its implication in the synthesis and maintenance of chlorophyll, Mg also has a favorable influence on the synthesis of other leaf pigments such as carotene and xantophyll (Bergmann, 1992).

Although Mg deficiency leads to the rapid breakdown of chlorophyll and subsequent chlorosis, the Mg in chlorophyll accounts for only 15-20% of the total Mg contained in a healthy plant (Bergmann, 1992). So apart from its implication in photosynthesis, Mg is needed in substantial quantities for other important functions and physiological processes in the plant metabolism.

Magnesium functions in plants are related to its mobility within the cells, its capacity to interact with strongly nucleophilic ligands through ionic bonding, and its ability to act as a bridging element and form complexes of different stabilities (Bergman, 1992).

A high proportion of the total Mg in plants is involved in the regulation of cellular pH and the cation-anion balance (Sigel and Sigel, 1990).

Magnesium also acts as a cofactor and activator for many enzyme and substrate transfer reactions. According to Gunther (1981) about 300 enzyme reactions are influenced by Mg ions. First, Mg may serve to link the enzyme and substrate together, as for example in reactions involving phosphate transfer from ATP, in which Mg serves as a link binding the enzyme to its substrate (Bidwell, 1974). Second, Mg may serve to alter the equilibrium constant of a reaction by binding with a product, as for example in some kinase reactions (Bidwell, 1974). Third, Mg may act by complexing with an enzyme inhibitor (Bidwell, 1974). Magnesium is thus an activator, by one or more of these mechanisms, of many of the phosphate transfer reactions (except phosphorylases), of enzymes involved in the synthesis of nucleic acids, and also of many of the enzymes involving carbon dioxide transfer-carboxylation and decarboxylation reactions (Bidwell, 1974). Magnesium has also been found to activate Na/K-ATPase, which acts as an ion pump (efflux of sodium, influx of potassium) (Bergmann, 1992).

Magnesium has great affinity for the pyrophosphate structure of ADP and ATP and is able to establish a chelate-like link (bridge-bond) between these substrates (Bergmann, 1992). Magnesium thus is involved in virtually all phosphorylation processes serving the transfer and conversion of energy (Bergmann, 1992). Consequently, almost all energy transfers involving phosphorylation in the metabolism of the cell needs Mg ions, starting from photosynthetic phosphorylation through ATP dependent CO_2 fixation, carbohydrate, protein, fat and lipoid synthesis to glycolysis (breakdown of carbohydrates into pyruvic acid) and respiration metabolism (Bergmann, 1992). Aikawa (1981) stated that whenever there is ATP, there is an obligatory need for Mg.

Tisdale et al. (1985) reported that Mg activates the formation of polypeptide chains from amino acids. Indeed, Mg plays a role in the stabilization of ribosomes, the particles responsible for "translating" the genetic code during the synthesis of polypeptide chaines, and are therefore also necessary for protein synthesis (Bergmann, 1992 and Bidwell, 1974).

Owing to their hydrating properties, Mg ions together with Ca ions influence the volume of plasma colloids and thus have a an influence on the water budget in the plant (Bergmann, 1992).

According to Kiss (1981) Mg influences the phytohormone balance and nitrate reduction. So Mg deficiency inhibits nitrate reduction and the production of phytohormones.

As in the case of other elements, plants vary widely in their Mg needs. Tobacco and citrus species for example require especially high concentrations of Mg in the nutritive medium (Bergmann, 1992). Potatoes, beets, brassicas, clover, lucern and maize are also crops with a high Mg requirement and cotton requires even higher levels of

available Mg in the soil than maize (Bergmann, 1992). Nelson and Barber (1964) reported that legumes need two to three times as much Mg as grasses.

Magnesium deficiency leads to the breakdown of chlorophyll and affects the plant metabolism as a whole resulting in stunted chlorotic and necrotic plants. Lyon and Garcia (1944) reported that Mg deficiency led to small pith cells and chlorenshyma is densely packed with chloroplasts. Adequate supply of Mg produced maximum size and smallest numbers of chlorenchyma cells. Magnesium deficiency has also been reported to inhibits nitrate reduction and phytohormone production (Kiss, 1981). Because of its interaction with other divalent cations in the medium, Mg deficiency has been related to numerous nutritional and physiological disorders in plants (Koranski, 1988; Harrison and Bergman, 1981; Bergmann, 1992).

Iron/manganese toxicity disorder in marigold has been related to low concentrations of Mg in leaf tissue of the affected leaves (Koranski, 1988). In fact most bedding plants are grown in soilless, peat-based media that provide little Mg to the plants (Bunt, 1988; Nelson, 1991). Despite the importance of this problem, data on the effect of Mg nutrition in marigold on nutrient content in the plant tissue are meager. Available information on Mg requirement for marigold is also scant. This experiment was designed to investigate Mg requirements in marigold and the effect of Mg concentration in the medium on nutrient concentration in the plant tissue. Determination of the incipient deficiency concentration of Mg is needed for further investigation of the relationship of Fe/Mn toxicity disorder in marigold to Mg deficiency. The objectives were: (1) to determine the symptoms of Mg deficiency in marigold; (2) to determine the incipient deficiency concentrations of Mg in

marigold; and (3) to determine the effect of Mg concentration in the medium on the concentrations of Mn, Fe, Ca and Mg in the tissue of the plant shoots.

3.3 MATERIALS AND METHODS

Seeds of marigold (*Tagetes erecta* L. 'First Lady') were planted in vermiculite #3 on February 12, 1996 in the greenhouse. Three-week-old seedlings were transplanted into treatment solutions in 1.6-liter opaque plastic containers. The basal nutrient solution contained 107 mg/l Ca, 79 mg/liter K, 63 mg/liter P, 75 mg/liter NO_3-N, 75 mg/liter NH_4-N, 2.3 mg/liter Fe, 0.02 mg/liter Cu and 0.5 mg/liter of B, Mn, Zn and Mo. Magnesium treatments were 0; 2; 4; 6; 8; 10 and 12 mg Mg/liter solution. Magnesium was added to the nutrient solution as $MgSO_4$-$7H_2O$. The hydroponic cultures were aerated continuously and the nutrient solution was replaced with a fresh one every 10 days. Deionized water was added to the containers as needed to maintain volume.

Forty-two containers consisting of seven treatments and six replicates were placed on two tables in the greenhouse in a completely randomized design. The photoperiod was about 16/8. The temperature was maintained as closely as possible to 21/17 ^{o}C (day/night). The plants were harvested and divided into roots and shoots on May 26, 1996. The roots were rinsed with tap water then deionized water to discard nutrients from the root surface.

The plant parts were dried at 70 ^{o}C for 48 hours and weighed. The plant shoots were ground (20 mesh) and analyzed by atomic absorption spectroscopy (Appendix A) for Mn, Fe, Ca, and Mg contents. The data obtained were statistically analyzed using Regression Analysis and Analysis of Variance (ANOVA) to test for significance of main effects and interactions, and terms were considered significant at $p<0.05$.

3.4 RESULTS AND DISCUSSION

3.4.1 Description of Symptoms

Results reflected the close link between Mg function in the plant metabolism, its mobility and symptom manifestation of its deficiency. The function of Mg as a constituent of chlorophylls a and b naturally determined some of the most important effects caused by the deficiency of this element. Chlorosis commenced as a marked interveinal yellow green mottling that was distributed uniformly over most of the older leaves while most vascular tissue remained green. In addition to the chlorotic patterns, the margins became brilliantly tinted with yellow green color. Necrosis followed chlorosis and pigmentation. It developed in the chlorotic areas and on the margins. Plants grown in Mg deficient medium were stunted and their flowering was delayed.

Since Mg is quite soluble and readily transported around the plant, symptoms of its deficiency usually appear in older leaves first (Bidwell, 1974). The withdrawal of Mg from the older leaves leads to the gradual inhibition of the energetic enzyme reactions needed to maintain metabolism and this eventually leads to the decomposition of chlorophyll.

The visual symptoms of Mg deficiency in marigold were the outward signs of disturbed metabolic processes in which Mg is implicated. Indeed, in addition to its implication in the synthesis and maintenance of chlorophyll, Mg has a key role in many of the metabolic processes of the plant (Follet et al., 1981; Gunther, 1981; Bergmann, 1992; Aikawa, 1981). All oxidative decarboxylation reactions are catalyzed exclusively by Mg ions, which owing to their ability to activate acetyl coenzyme A, also play a key role in the citric acid cycle and fat metabolism (Bergmann, 1992). Magnesium deficiency thus results in reduced growth and delayed maturity.

The symptoms of Mg deficiency in marigold are similar to those shown in lettuce, citrus (Camp et al., 1941), tomato and pear (Hewitt, 1963). They are however different from those shown in potato, pea, broad bean, red clover, white clover, and lucerne in that their margins frequently become brilliantly tinted with orange, red or purple colors (Stewerd, 1963; Hewitt, 1944; Hewitt, 1945; Hewitt, 1946; Wallace, 1930; Wallace, 1961;). In tobacco, celery and parsnip, the margins and the leaf tips are the first to be affected (McMurtry, 1964; Garner et al., 1923; Stewerd, 1963).

3.4.2 Effect of Mg Deficiency on Plant Dry Weight

Magnesium concentration in the medium had a highly significant effect on the root, shoot and total dry weight (Appendix C). The plant dry weight increased as Mg concentration in the medium increased (Figure 3.1). Reduced plant dry weight as a response to reduced amount of Mg in the nutrient solution is due to the vital role of Mg in the biological production and conversion of matter in the metabolism of plants. Magnesium as plant nutrient is required for the formation of chlorophyll and the absence of this element leads to inadequate photosynthesis. Inadequate supply of Mg also reduces enzyme activities, nitrate reduction, phytohormone and polypeptide production and other vital metabolic processes in the plant (Gunther, 1981; Kiss, 1981; Tisdale et al., 1985; Bidwell, 1974; Bergmann, 1992).

Beringer and Forster (1981) found that the weight of Mg deficient cereal crops was below normal.

Dry weight reduction resulting from Mg shortage seems to depend on when the deficiency occurs and how acute it is. McMurtrey (1964) reported that reduction in yield

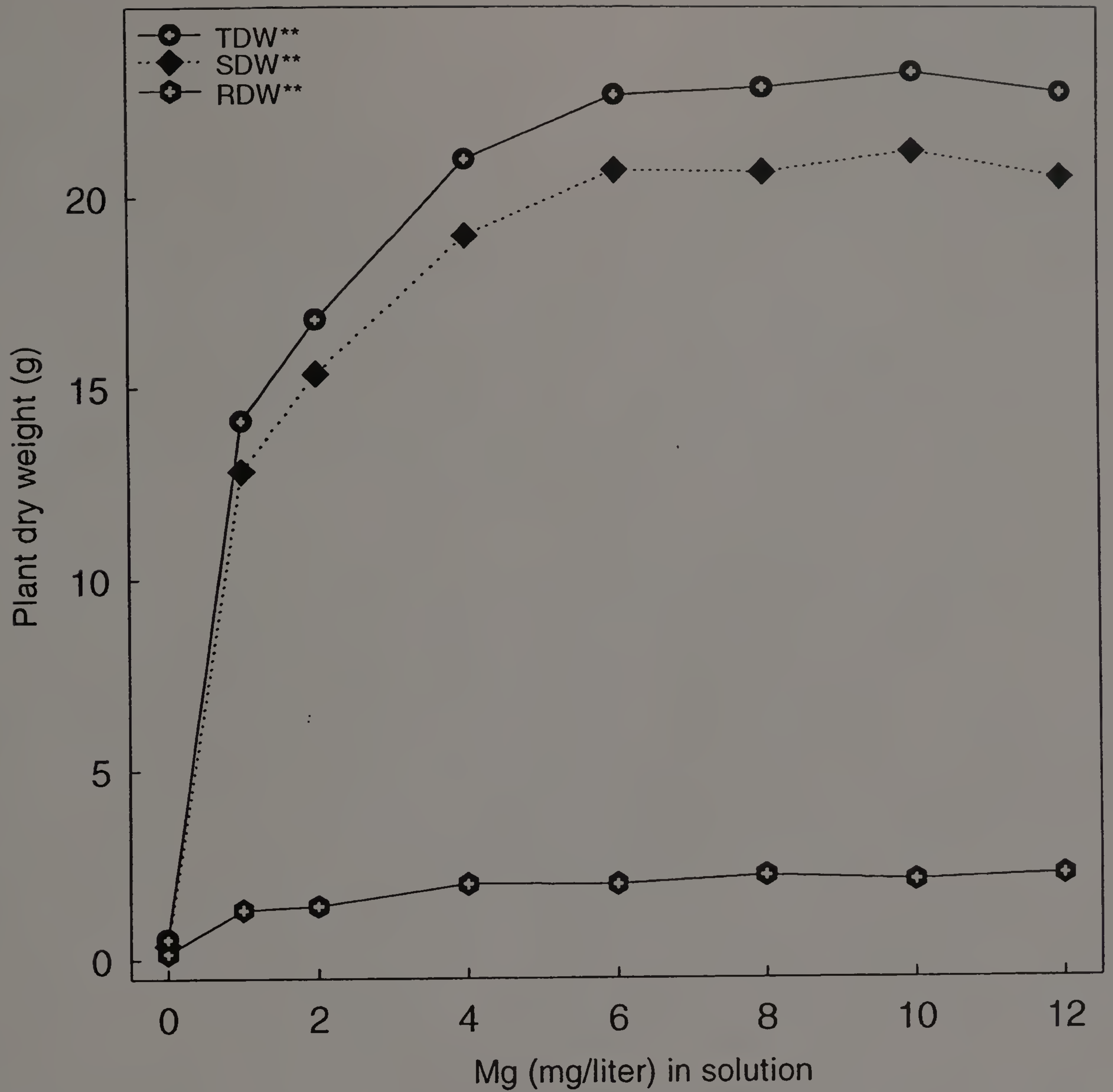

Figure 3.1: Effect of Mg level in solution on the plant dry weight (see Appendix C, Tables 1, 3, 4)

of tobacco is less severe when Mg deficiency occurs in late stages of growth rather than earlier stages.

3.4.3 Incipient Deficiency Concentration of Mg

Incipient deficiency refers to the situation where a crop needs more of a given nutrient, yet it does not show marked outward symptoms of deficiency. If not detected an incipient deficiency will continue to develop to the point that deficiency is severe enough to cause symptoms and a significant reduction in growth and yield.

Our results suggest that 10 mg Mg/liter nutrient solution was in this experiment the incipient deficiency solution concentration of Mg. Indeed, the plants grown in concentrations from 0 to 8 mg/liter Mg showed significant amount of symptoms. The plants grown in 10 mg Mg/liter and higher concentrations did not show symptoms. In previous experiment, it was noted that 10mg Mg/liter reduced the plant dry weight (data not shown). When Mg in solution was 10 mg/liter, the shoot concentration of Mg was 1.3%. This level of Mg is considered the incipient deficiency plant concentration of Mg.

Incipient deficiency of Mg may be damaging to a crop. If the Mg content drops below a certain critical level, which is not the same for all species, no net assimilation is possible (Bergmann, 1992). According to Kirkby and Mengel (1976) for barley this level is 0.012% fresh weight, but even before this level is reached major accumulation of starch are found in the leaves because of inhibition of the transformation and translocation of synthesis products. The leaves feel brittle and the protein, carbohydrate (starch, sugar) and fat contents of the seeds and fruit are reduced.

3.4.4 Effect of Mg Deficiency on the Nutrient Concentrations in the Shoots

Blasl and Mayer (1978) pointed out that more attention should be paid to the relative proportions of certain nutrients and the possibility of overabundance of plant nutrients. A deficiency of one element therefore means simultaneously that there is a relative or absolute excess of the others.

3.4.4.1 Effect of Mg in Solution on Mn in the Shoots.

Manganese concentration in the shoots was significantly high in Mg deficient plants (Appendix C). As Mg concentration in the medium increased Mn concentration in the shoots decreased (Figure 3.2). Our results agree with Bergmann (1992) when he reported that Mn uptake is inhibited by the presence of Mg.

The different ways in which Mn reacts show that it is similar, in some degree, to Mg in its mode of action. In fact both Mg and Mn are activators of many enzymes and can even replace each other to some extent (Bergmann, 1992). Manganese, Mg, Fe and zinc appear together to be very finely balanced in respect of their regulation of metabolic processes affecting plant growth in general and carbohydrate, protein and chlorophyll synthesis in particular (Bergmann, 1992).

Since Mg deficiency is most common on acid soils, it is often accompanied by Mn toxicity (Bergmann, 1992). Hu and Schmidhalter (2001) reported that when Mg is low in the growth medium, Mn actually replaces Mg in some of its metabolic functions. This may cause an increase in the concentration of Mn in the plant tissue.

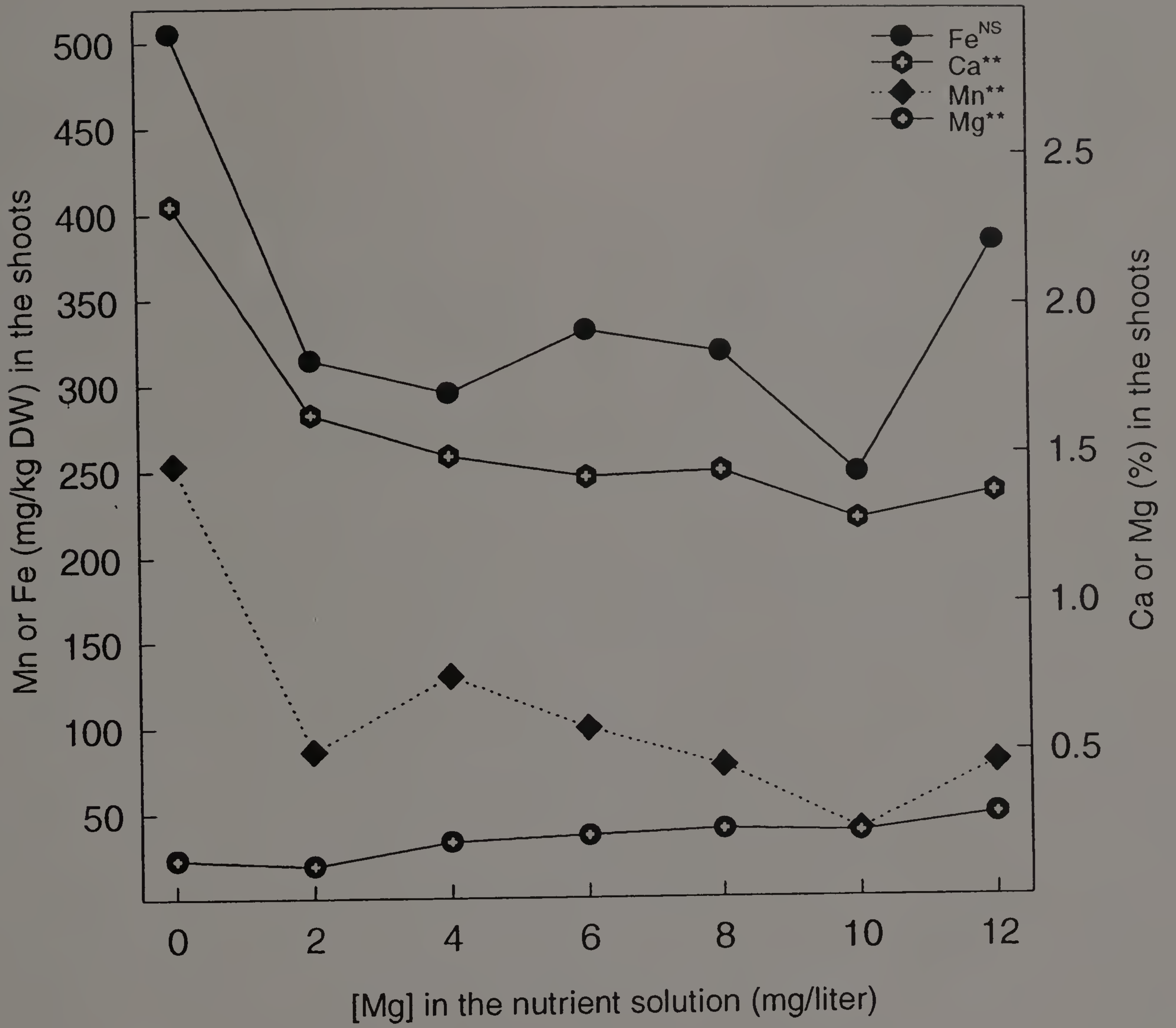

Figure 3.2: Effect of Mg in solution on nutrient concentrations in the plant shoots (see Appendix C, Tables 2, 3, 4)

3.4.4.2 Effect of Mg in Solution on Fe in the Shoots.

Magnesium concentration in the nutrient solution had no significant effect on Fe concentration in the plant shoots. Yet, there was a tendency for plants grown at low Mg to have higher concentrations of Fe in their tissue (Figure 3.2). The concentration of Fe in the shoot tissue decreased as the concentration of Mg in the medium increased (Figure 3.2). Our results differ from those reported by Straub and Wurm (1971). They found that the damaged leaves of magnesium deficient apple plants were characterized by low Fe contents. In a study on maize and sunflower plants, Scherer (1978) found that magnesium level was high in the plant tissue when the plants are deficient in Fe. Scholl (1979a, b) reported the same results. They found that magnesium content increased from 9% in normal leaves to 14% in Fe deficient leaves.

3.4.4.3 Effect of Mg in Solution on Ca in the Shoots

Magnesium concentration in the medium had a highly significant effect on Ca concentration in the plant shoots (Appendix C). Calcium concentration in the shoots was higher in the Mg deficient plants than in the Mg sufficient plants (Figure 3.2). As Mg in the nutrient solution increased, Ca in the shoots decreased (Figure 3.2).

Our results differ from those reported by Straub and Wurm (1971). They found low concentration of Ca in the leaves of apple plants deficient in Mg.

Schwartz and Bar-Yosef (1983) found that Ca concentration in tomato (*Lycopersicon esculentum* Mill.) plants decreased with increased Mg concentration in the medium. They also found that the concentration of Ca was relatively more reduced in the roots than in the shoots.

Wichmann (1976) found that the ratio of Mg to Ca content in the shoots was important when estimating whether cereal plants were adequately supplied with Mg. On the basis of his experiments he determined that the Mg:Ca ratio should be 0.30 for adequate yield of wheat, barley and oats.

Bergmann (1992) attributed the effect of high level of Mg in the medium on Ca content in the plant tissue to ion antagonism. Schwartz and Bar-yosef (1983) found that Mg interference operates by decreasing Ca binding to absorption sites or carrier sites in the root but not by reducing the capacity of these sites for Ca.

3.4.4.4 Effect of Mg in Solution on Mg in the Shoots.

Magnesium concentration in the nutrient solution had a highly significant effect on Mg in the plant shoots. As Mg in the nutrient solution increased, Mg in the shoots increased (Figure 3.2).

Csatho et al. (2000) found that the correlation between soil Mg and Mg contents in winter wheat followed a quadratic polynomial. They found that Mg in the shoots increased with Mg in the soil up to certain level, then when this level is exceeded, an increase in Mg supply caused a decrease in Mg in the shoots.

Schwartz and Bar-Yosef (1983) found that Mg in the shoots increased about threefold when increasing Mg in the medium from 0.05 to 1.35 meq/l. Mg in the roots did not change or slightly increased.

3.5 CONCLUSION

Magnesium deficiency affected the appearance and the growth of marigold. Low concentrations of Mg in the solution up to 8 mg/liter caused severe damage to the plant. The plants were stunted and they developed chlorotic and necrotic symptoms on the

leaves. The damage caused by Mg deficiency was undoubtedly due to its role in important metabolic processes in the plant such as photosynthesis; protein, carbohydrate and fat synthesis and its involvement in about 300 enzyme activities.

Plants grown in 10 mg Mg/liter nutrient solution were still relatively stunted but there were no significant symptoms affecting their appearance. The concentration 10mg/liter may thus be considered incipient deficiency solution concentration of Mg in marigold. In this experiment, this concentration was the turning point of Mg nutrition from deficiency to sufficiency. It did not fulfill the Mg requirement of marigold for adequate growth, but it was just enough to prevent chlorosis. The incipient deficiency solution concentration corresponded to a shoot tissue concentration of 1.3%.

The present data suggest that Mg in the medium had no significant effect on Fe in the shoot tissue. Magnesium in the medium did have highly significant effects on Mn, Ca and Mg concentrations in the plant shoots. Manganese and Ca concentrations in the shoot tissue were high when Mg in the medium was low. An increase in Mg in the medium caused a decrease in Mn and Ca in the plant shoots. Magnesium in the shoot tissue increased with increased Mg in the medium. The interrelationship between Mg in the medium and Mn and Ca in the plant shoots may be due to ion antagonism or to the similarities between Mg and Mn in their mode of action. Reduced Ca in the plant shoots as a response to increased Mg in the medium may be attributed to decreased Ca binding to absorption sites or carrier sites in the root.

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CHAPTER 4

CALCIUM DEFICIENCY IN MARIGOLD

4.1 ABSTRACT

Iron/manganese toxicity disorder in marigold has been related to high concentrations of Fe and Mn and low concentrations of Ca and Mg in the affected leaves. Deficiency of Ca may occur because of low medium pH and to lack of Ca in many of the fertilizers used in greenhouse crop production. To investigate the effect of poor supply of Ca on marigold (*Tagetes erecta* L. 'First Lady') growth, appearance, and nutrient (Mn, Fe, Ca and Mg) content of the shoots, a solution culture study with various Ca concentrations was conducted. Concentrations of Ca up to 15 mg/l resulted in stunted plants with significant amount of chlorotic and necrotic symptoms on the leaves. The concentration 20 mg Ca/l resulted in stunted plants relatively free of symptoms. Concentrations above 20 mg/l resulted in relatively healthy looking plants. The concentration 20 mg/l Ca may thus be considered incipient deficiency concentration of Ca in marigold. At this solution concentration, the level of Ca in the plant shoots was 0.54%. Under the experimental conditions used here, low supply of Ca had no effect on Fe concentration in the growing point but resulted in a high concentration of Fe in the old part of the shoots. Calcium deficiency did not affect the concentration of Mn in the old part of the shoots but resulted in high concentration of Mn in the growing point new leaves, buds and stems). Low Ca in the solution resulted in low Ca and high Mg in the shoots.

4.2 INTRODUCTION AND LITERATURE

Sprengel (1787-1859) was the first to show that Ca is essential as a nutrient for plants. Calcium is present in salts of organic and inorganic acids, bound by sorption to plasma colloids, and in immobilized form (Bergmann, 1992).

4.2.1 Calcium Uptake and Translocation

Calcium is absorbed by plants only in ionic form and is transported to the shoots with the transpiration flow through the xylem (Bergmann, 1992).

In contrast to potassium and Mg ions which, like nitrogen and phosphorus compounds, are easily transported in the phloem, Ca ions are transported exclusively through the vessels of the xylem, being supplied mainly to young meristematic tissues (Bergmann, 1992).

Since little or no Ca is redistributed in the plant, plants must continuously take up Ca and transport it to wherever it is needed in order to maintain metabolism and growth (Bergmann, 1992). A continuous supply of Ca to the growing plants is necessary to ensure that the needs of older tissue are met as well as those of the younger growing tissues and fruit (Shear, 1975). However, the transport of Ca at a rate that is adequate to ensure normal shoot growth, does not guarantee adequate supply of Ca to the roots or fruit (Shear, 1975). Apple fruit, for example, which accumulate about 40% of the net assimilate of the tree, contain only 2% of the total Ca uptake (Shear, 1975).

The Ca content of fruit and storage organs is often reduced by inhibited transpiration. If transpiration increases, however, it does not necessarily mean that the supply of Ca to the fruits is adequate, because under such conditions the leaves and fruit

compete for both water and Ca, and the fruit are usually less competitive than the leaves (Mix and Marschner 1976a,b).

An experiment performed by Bradfield (1976) showed that nutritional conditions leading to high organic acid concentrations (citric acid and malic acid) in the xylem, for instance after nitrogen fertilization, increases Ca mobility. Indeed, because of the chelating agents formed in the roots, over 50% of the Ca is transported through the xylem in the form of chelates (Bradfield, 1976).

4.2.2 Role of Ca in Plant Metabolism

Calcium is responsible for the structural and physiological stability of plant tissues. Together with other substances, Ca regulates influx and efflux processes in cells and tissues (Christiansen and Foy, 1979; Millaway and Wiersholm, 1979).

Calcium is necessary for regulating the actions of phytohormones. Calcium ions and indolacetic acid (IAA), for instance, influence cell elongation and differentiation. A lack of Ca leads to enlarged undifferentiated cells because of the lack of structural stability. It therefore follows that Ca ions are necessary for systematic cell division, cell wall construction and cell elongation in the meristematic tissue of the vegetative points of the shoot and root and cannot be replaced in this role by any other ion (Bergmann, 1992).

As far as its physiological effects are concerned, Ca is usually regarded as the counterpart to potassium. Because of its dehydrating properties, it opposes the plasma-expanding action of potassium ions. Calcium ions are therefore important as a factor influencing the stability of the structure of protoplasm. Its colloid-chemical functions, however, extend beyond the simple regulation of cell hydration and must be considered mainly in connection with its chelate-forming properties (Bergmann, 1992).

In the regulation of ion uptake by plants, Ca ions have both synergistic and antagonistic effects. Low Ca concentrations encourage ion uptake, probably because Ca has a membrane-stabilizing effect, and through the fact that it encourages respiratory metabolism, synthetic activity and electron transport (Bergmann, 1992). High concentrations of Ca, on the other hand, inhibit the uptake of other cations owing to ion-antagonistic effects (Mengel, 1979), and this can be a major advantage, particularly if the availability of other non-essential ions (especially heavy metals) is high. Damage due to toxic concentrations of ions is less severe in such cases (Bergmann, 1992). Wallace (1979) regarded this "protection effects" against high concentrations of trace elements as one of the most important roles of Ca in plants because the phytotoxicity of these elements (except molybdenum), especially aluminum and heavy metals, increases considerably if Ca uptake is reduced.

One of the important roles played by Ca is that of stabilizing the pectin of the middle lamella in the primary cell wall by forming Ca pectinate. Similarly, by stabilizing the micellar structure of membrane phosphatides, it is important for maintaining the integrity of membranous structures (Bergmann, 1992). The stabilization of biological membranes is important for regulating metabolic processes at the cellular and tissue level. These membranes prevent ions and molecules from permeating passively between the different intracellular compartments. Membranes also prevent ions from escaping out of the cell, thus making possible the enzymatically regulated systematic transport of substances between intracellular compartments (Marshner and Gunther, 1964; Weisenseel and Ruppert, 1977).

According to Simon (1978) this regulated transport of substances between intracellular compartment explains how Ca deficiency symptoms develop: Ca deficiency leads to permeable cell membranes. Turgor is consequently lost and cell sap spills out into the tissue, giving it its glassy appearance. The tissues eventually collapse and whither.

4.2.3 Calcium Deficiency: Cause and Impact

According to Marshner (1974) research on Ca in plants is not only of academic interest, but is also of great economic importance because of the widespread occurrence of Ca deficiency in fruit (for example, "bitter pit of apples" and "blossom-end rot" in tomato, melon and other fruit). Collier et al. (1979) estimates that losses exceeding 10 million German Marks a year are caused by Ca deficiency in England alone.

While the use of N, P and K fertilizers has increased considerably in many countries in recent decades, Ca is frequently ignored in fertilization practice (Bussler, 1979). Reliance is usually placed on the lime reserves of the soil and the ability of plants to take up sufficient amounts of Ca ions as long as the pH value is not too low. In fact, while the heavy applications of nitrogen, phosphorus and potassium fertilizers naturally increase yields, the plant's Ca demand is often ignored (Bergmann, 1992). Moreover, the increasing use of nitrogenous fertilizers with an acidifying effect is increasingly stressing the Ca potential of the soil (Bergmann, 1992).

In addition to Ca extraction by plants, leaching of Ca ions can also be considerable. At least 80-100 Kg Ca per hectare for 600-700 mm of precipitation is leached annually, and often as high as 400-500 Kg per hectare (Schffer and Schachtchabel, 1982).

It seems there is a link between the pH of growth medium and Ca deficiency. Vegetable plants grown on acid, easily leached sandy soils often show signs of Ca deficiency (Maynard, 1979).

One of the main causes for the appearance of Ca deficiency is the irregularity of water availability (Millaway and Wiersholm, 1979). Latent and acute Ca deficiencies in plants are caused mainly by insufficient transport of Ca to the apical meristem, flowers and fruits. Calcium deficiencies are obviously more common in fruits if transpiration there is low (Isermann, 1978). “Bitter pit” in apples, “blossom-end rot” in tomatoes, “black heart” in celery, “leaf tip-burn” in lettuce and cabbages, and “cavity spot” in carrots and parsnips have become increasingly common in recent decades (Shear and Faust, 1971; Forster, 1972; Shear, 1974, 1975; Carolus, 1975; Yamada, 1975; dekock et al., 1981).

Unbalanced fertilization and/or high humidity may easily induce Ca deficiency in greenhouse crops of cucumbers, lettuce, pepper, tomatoes, garden cabbage, Chinese cabbage and Kohlrabi (Bussler, 1979).

Apart from low concentration of Ca in the nutritive medium, high concentrations of other nutrients such as Mn and Mg can inhibit the uptake of Ca by plants and thus induce Ca deficiency. Antagonism between Ca and aluminum may also reduced the uptake and utilization of Ca by plants (Bergmann, 1992).

Calcium deficiency increases the permeability of the cell membrane, and this may lead to ionic inundation of the vacuoles or permit ions to escape. Ultimately, it can lead to the breakdown of intracellular compartmentalization, the escape of enzymes, and autolytic phenomena (Hecht-Buchholz, 1979).

Further consequences of Ca deficiency also include the destruction of structures within the cell nucleus and reduced chromosomal stability, and therefore disturbance of nuclear and cell division, and the destruction of mitochondria. Since mitochondria are particularly rich in Ca, Ca deficiency may impair the respiratory metabolism (Bergmann, 1992).

Calcium deficiency leads to abnormal nuclear division: both small nuclei and large polyploid nuclei are produced. The chromatin is accumulated in granules. Meristematic tissue in particular therefore needs Ca for normal differentiation (Bergmann, 1992).

Parenchymatic and phloem tissues are also at risk when Ca is deficient in the plant. According to Bussler (1962a, b, 1963), when Ca is deficient, the middle lamellae is more exposed to the effects of pectinases emerging through the membranes. The cells separate from each other, autolyze and turn brown. This is often preceded by the infiltration of cell sap into the air-filled intracellular spaces, giving the tissue a glassy appearance. The tissue of the vascular bundles is brownish, and the medullary cells disintegrate into dark brown granules, the dead zone then containing only clogged lignified vessels.

However, Pissareck (1979) thought differently regarding the autolysis of cell walls. According to his observations the darkening is induced by increased phenol synthesis when Ca is deficient. The phenols, oxidized to chinons, react with amino acids and proteins to form melanins which, finally, give the Ca deficient tissue its dark appearance.

Low concentrations of Ca in fruit accelerate ripening and reduce firmness and suitability for storage. Richardson and Al-Ani (1982), for example, found that in pears there was a negative correlation between Ca content and particularly the water-soluble Ca content, of the fruit and their development of ethylene during ripening. Elevated Ca contents also reduce respiration of the fruit. Pears containing less than 0.007% Ca (fresh weight) and with a N:Ca quotient higher than 10 ripened faster in cold storage, and this was accompanied by more intense ethylene development.

Yamada (1975) reported that Ca deficiency leads to the accumulation of nitrite (NO_2^-) because intercellular (NO_2^-) transport is reportedly disrupted. Calcium deficient plants are therefore unable to use nitrate (NO_3^-) efficiently as a source of N.

Calcium deficiency in legumes prevents the development of nodule bacteria, thus affecting nitrogen fixation. Consequently, Ca deficiency induces nitrogen deficiency in legumes (Bergmann, 1992).

Calcium deficiency also inhibits the transport of carbohydrates in plants. In the case of tomatoes, for instance, Rangnekar (1974) was able to show that under conditions of Ca deficiency the transport of photosynthetic products out of the leaves (source) was inhibited so that growth of the terminal meristem (sink) was slowed down. Gosset et al. (1977) observed the same effect in soybeans.

Because of its interaction with other divalent cations in the medium, Ca deficiency has been related to numerous nutritional and physiological disorders in plants (Koranski, 1988; Harrison and Bergman, 1981; Bergmann, 1992).

Iron/manganese toxicity disorder in marigold has been related to low Ca in leaf tissue of the affected leaves (Koranski, 1988). In fact most bedding plants are grown in

soilless peat based media that provides little Ca to the plants (Bunt, 1988; Nelson, 1991). Despite the importance of this problem, data on the effect of Ca nutrition in marigold on nutrient content in the plant tissue are meager. Available information on Ca requirement for marigold is also scant. This experiment was designed to investigate Ca requirements in marigold and the effect of Ca concentration in the medium on nutrient concentration in the plant tissue. Determination of the incipient deficiency concentration of Ca is needed for further investigation of the relationship of Fe/Mn toxicity disorder in marigold to Ca deficiency. The objectives were: (1) to determine the symptoms of Ca deficiency in marigold; (2) to determine the incipient deficiency concentrations of Ca in marigold; and (3) to determine the effect of Ca concentration in the medium on the concentrations of Mn, Fe, Ca and Mg in the tissue of the plant shoots.

4.3 MATERIALS AND METHODS

Seeds of marigold (*Tagetes erecta* L. 'First Lady') were planted in vermiculite #3 on June 1st, 1996 in the greenhouse. Three-week-old seedlings were transplanted into treatment solutions in 1.6-liter opaque plastic containers. The basal nutrient solution contained 48 mg/l Mg, 79 mg/l potassium, 63 mg/l phosphorus, 75 mg/l nitrate-nitrogen, 75 mg/l ammonium-nitrogen, 2.3 mg/l Fe, 0.02 mg/l copper and 0.5 mg/l of boron, Mn, zinc and molybdenum. Calcium treatments were 2.5; 5; 7.5; 10; 12.5; 15; 20; 40; 60; 80; and 100 mg Ca/l solution. Calcium was added to the nutrient solution as $CaCl_2$. The hydroponic cultures were aerated continuously and the nutrient solution was replaced with a fresh one every 10 days. Deionized water was added to the containers as needed to maintain volume.

Sixty-six containers consisting of 11 treatments and 6 replicates were placed on two tables in the greenhouse in a completely randomized design. The plants were grown under

ambient light conditions. The temperature was maintained as closely as possible to 21/17 °C (day/night). The plants were harvested and separated into roots, growing point and older part of the shoots on August 10, 1996. The growing point consisted of the apical meristematic parts of the terminal buds. The roots were rinsed with tap water then deionized water to discard nutrients from the root surface.

The plant parts were dried at 70 °C for 48 hours and weighed. The plant shoots were ground (20 mesh) and analyzed by atomic absorption spectroscopy (Appendix A) for Mn, Fe, Ca, and Mg contents. The data obtained were statistically analyzed using Regression Analysis and Analysis of Variance (ANOVA) to test for significance of main effects and interactions, and terms were considered significant at $p<0.05$.

4.4 RESULTS AND DISCUSSION

4.4.1 Description of Symptoms

The symptoms of Ca deficiency in marigold consisted of white necrotic spots, between the veins and close to the margins of the newly matured leaves. The new leaves showed a yellowish green color. In advanced stages of Ca deficiency, the yellowish green young leaves developed chlorotic spots between the veins and along the main vein. These chlorotic spots became brown necrotic areas. The overall look of the plant was bushy and the young leaves were smaller than normal. In severe deficiency conditions, the youngest leaves and roots died starting from the tip.

Our results suggest that there is a close link between Ca function in the plant metabolism, its mobility and symptom manifestation of its deficiency.

Since Ca deficiency is closely linked with the specific behavior (migration with the transpiration flux, accumulation in older leaves, immobility) and the functions of Ca

ions in plants, the symptoms manifested themselves first on the youngest and young tissues and organs where differentiation was still taking place. Plant growth was therefore inhibited, and the plants had a bushy appearance. The youngest leaves, which were affected first of all, were small. Chlorotic spots and blotches with necrotic lesions developed along the margins and particularly at the tips of the leaves, spread into the veinal areas and later united. Necrotic lesions appeared on the leaf blade as well. The leaves died from the tip to the base, as also did the apical meristematic tissue of the terminal buds and the roots.

The symptoms of Ca deficiency in marigold differ slightly from those in other plants. In tomato, margins of terminal and then lateral leaflets on recently expanded leaves become purple. Central interveinal areas become chlorotic then necrotic (Hewitt, 1963). Beet (*Beta vulgaris*), cauliflower (*Brassica oleracea* var. *botrtis*), tobacco (Garner et al., 1930; McMurtry, 1941), and cocoa (Maskell, et al., 1953) develop a characteristic "hooking" of the leaf tip when deficient in Ca. In moderate deficiency, the number of shoots in flax and potato plants increases producing a markedly bushy look. In severe deficiency cases, the stem apex dies, which is characteristic of Ca deficiency in many plants (Hewitt, 1963).

Bierman et all (1989) reported that the young expanding leaves of poinsettia plants affected by Ca deficiency exhibit a marginal chlorosis which rapidly progresses to necrosis, while interior portions of the leaf retain a normal green color. They stated that as marginal tissue ceases to grow continued expansion of the leaf blade causes a puckering of the leaf surface and downward curling of the leaf tip and margins.

4.4.2 Effect of Ca Deficiency on the Plant Dry Weight

Calcium concentration in the solution had a highly significant effect on the root, shoot and total dry weight (Appendix D). Results suggested that Ca deficiency caused a reduction in the plant dry weight (Figure 4.1).

Plants that received no Ca were completely unviable and did not survive beyond the seedling stage. Root growth was reduced when Ca was deficient and was extremely limited without Ca (Figure 4.1). For the reasons already explained the roots remained short, became slimy, turned brown and died, starting at the tips. The formation of new root tissue always depends on a continuous Ca uptake. Root growth is controlled by Ca, which regulates cell growth, increases the elasticity and stability of the cell walls, encourages metabolic activity and binds the CO_2 from respiration in the nutritive medium.

Shoot growth was also reduced when Ca was deficient in the nutritive medium (Figure 4.1). Because of inefficient root function and because of the crucial role of Ca as a phytohormone and cell growth regulator, Ca deficiency resulted in reduced shoot growth as well.

4.4.3 Incipient Deficiency Concentration of Ca

The plants grown in concentrations up to 10 mg/l Ca showed symptoms. Those grown in 12.5 and 15 mg/l Ca treatments did not show significant symptoms but their new leaves were pale green. The plants grown in concentrations above 15 mg/l Ca had no significant symptoms.

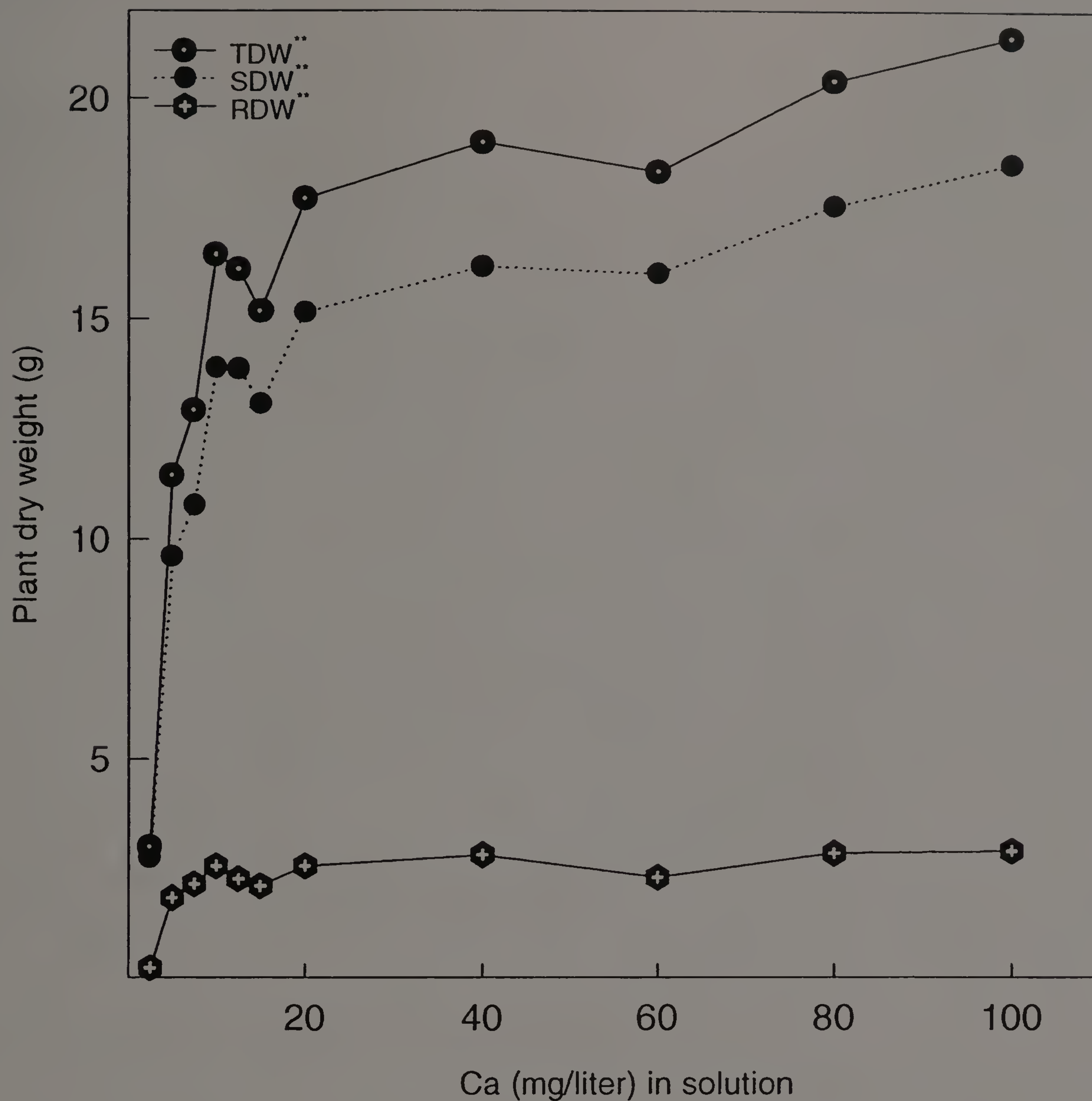

Figure 4.1: Effect of Ca level in solution on the plant dry weight (see Appendix D, Tables 1, 3)

The plants grown in 20 mg/l Ca did not show any significant symptoms but their dry weight was reduced (Figure 4.1). The concentration 20 mg/l Ca might thus be considered the incipient deficiency concentration of Ca in marigold in this experiment.

Despite its many important roles in the metabolism of plants, many plants require only a little Ca as long as the supply is not interrupted (Wallace at al., 1966). According to Millaway and Wiersholm (1979) plants show Ca deficiency symptoms when the momentary uptake rate falls below the functional demand of the tissue. Wallace and Soufi (1975) reported that Ca demand in plants is low particularly if heavy metal concentrations are low. In their opinion Ca concentrations of 0.3-2.0% DW in the leaves and 0.20-3.00% DW in the fruit should be completely sufficient for most plants. In the authors' view, Ca deficiency problems are induced by "heavy metal overstressing", and "critical Ca concentrations" depends on the heavy metal concentration in the plants concerned.

Plant species and their varieties differ greatly in their Ca needs and also in their Ca uptake and translocation ability. According to Yamada (1975) a Ca content of 0.5% in the tissues is generally considered adequate. The Ca uptake of cereals and other grasses is much lower than that of dicotyledones, particularly herbaceous plants and legumes, whose Ca needs are five times as high as those of grasses (Bergmann, 1992). Tomato, for instance, take 10-15 times as much Ca from standard nutritive solutions as wheat (Bergmann, 1992). Lupins have about the same Ca requirement as cereals and grasses (Kamprath and Foy, 1971). In vegetables, Ca concentrations of less than 0.8% in the leaf should be regarded as critical, although the Ca levels at which signs of deficiency occur vary considerably (Bergmann, 1992).

It is important to prevent incipient deficiency and deficiency symptoms rather than to correct them. Soil testing is an important tool and plant tissue analysis in combination with a good knowledge of plant nutrient requirement, help better fertilizer program.

4.4.4 Nutrient Distribution

Manganese was distributed evenly between the growing point and the older parts of the shoots (Figure 4.2). Iron and Mg was higher in the older parts of the shoots than in the growing point, and Ca was higher in the growing point than in the older parts of the shoots (Figure 4.2).

Nutrient distribution may be due to the role of these elements in the plant metabolism. Calcium is more needed in the growing point where it supports new growth. Iron and Mg, on the other hand are more involved in the "production of respiratory energy" (Bergmann, 1992) and photosynthesis respectively. These metabolic functions are more important in the older parts of the shoots than in the growing point. The involvement of manganese in many enzymatic activities makes it needed uniformly throughout the shoots.

4.4.5 Effect of Ca Deficiency on Nutrient Concentrations in the Shoots

Blasl and Mayer (1978) emphasized the importance of a balanced nutrition for adequate plant growth. They suggested that that more attention should be paid to the relative proportions of certain nutrients and the possibility of overabundance of plant nutrients. A deficiency of one element may cause a relative or absolute excess of the others.

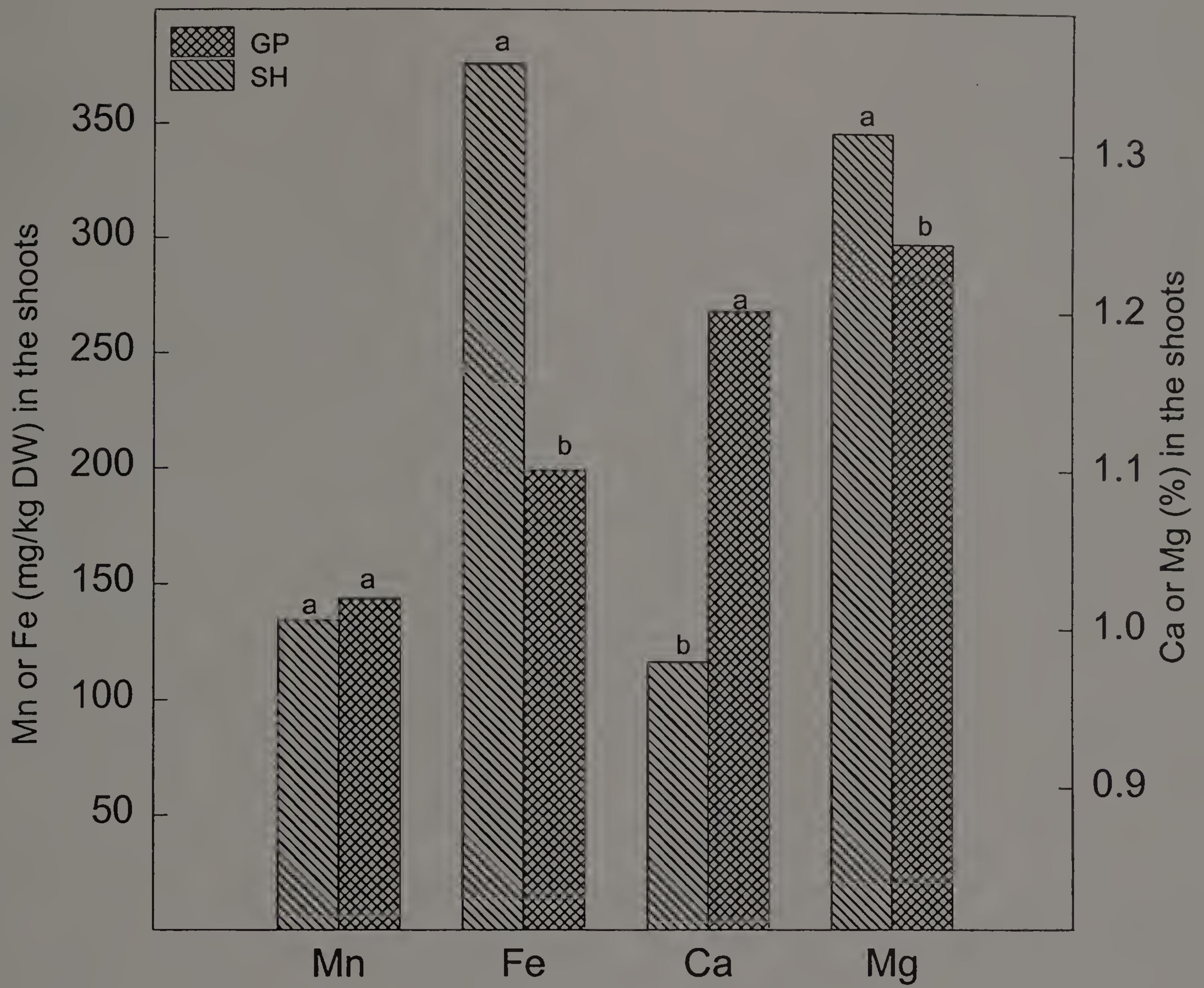

Figure 4.2: Nutrient distribution in the shoots

4.4.5.1 Effect of Ca in solution on Mn in the shoots

Calcium deficiency had no effect on Mn concentration in the older parts of the shoots but caused a highly significant increase of Mn in the growing point (Figure 4.3).

Different results were found in strawberry cultivars. Makus (1998) reported that Mn concentration increased in the plant tissue when the plants were treated with gypsum. They added that paired Ca and sulfate ions from gypsum have no effect on soil pH in most soils, and should not influence Mn availability.

4.4.5.2 Effect of Ca in solution on Fe in the shoots

Iron was higher in the older parts of the shoots than in the growing point (Figure 4.4). Calcium deficiency caused a highly significant increase of Fe in the older parts of the shoots but had no effect on Fe in the growing point (Figure 4.4).

This negative correlation between Ca in the nutritive medium and Fe concentration in the plant growing point is supported by Bergmann (1992). They reported that one of the known symptoms of excessive Ca were the “lime chlorosis”. “Lime chlorosis” is caused by a shortage of “activated Fe^{2+} ions” resulting from excessive liming or high levels of lime in the soil.

4.4.5.3 Effect of Ca in solution on Ca in the shoots

Calcium was higher in the growing point than in the older parts of the shoots (Figure 4.5). This may be because of the role of Ca in meristematic growth. Calcium deficiency resulted in a highly significant decrease of Ca in both the growing point and the old part of the shoots (Figure 4.5).

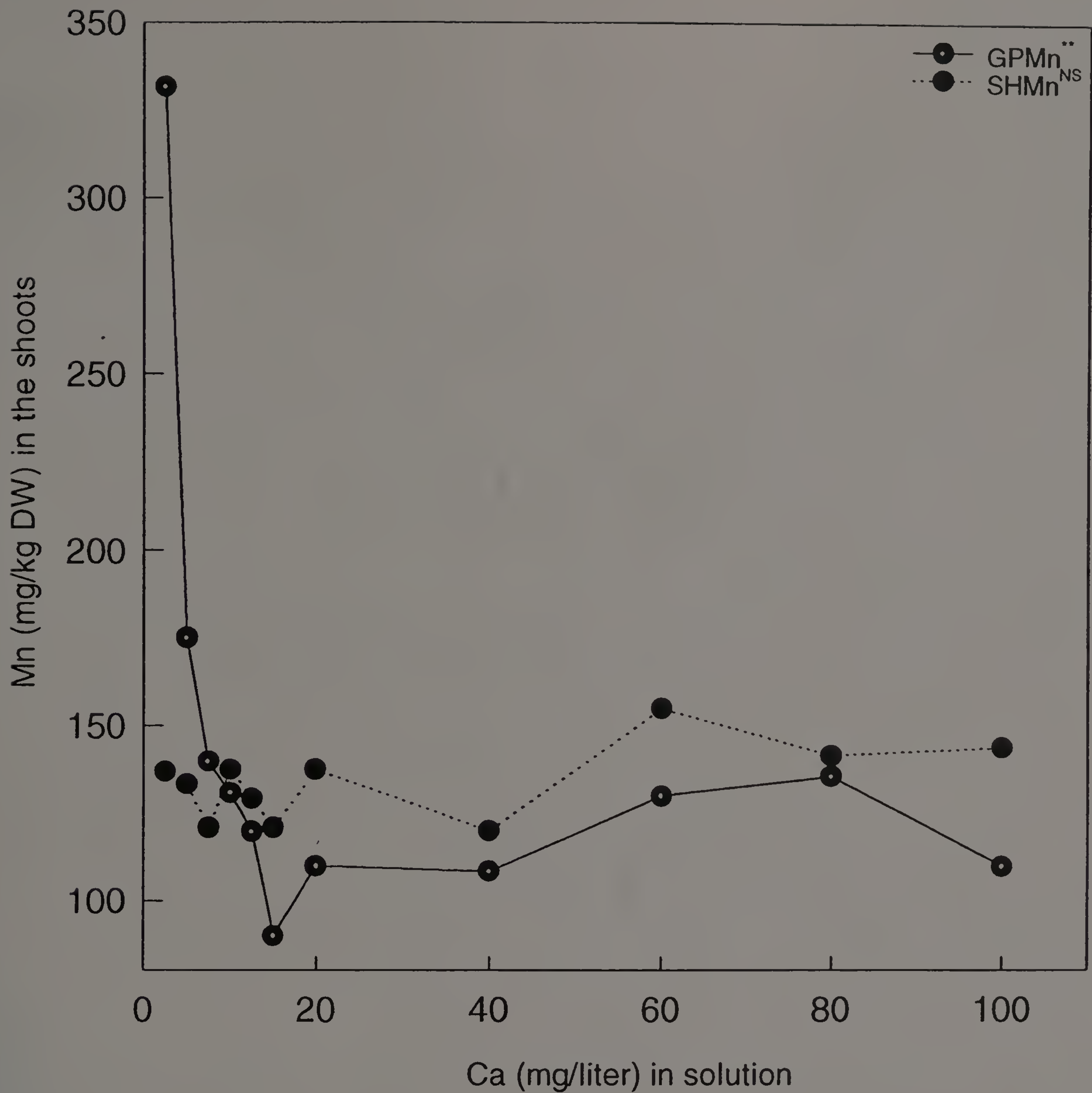

Figure 4.3: Effect of Ca level in solution on the level of Mn in the shoots (see Appendix D, Tables 2, 3)

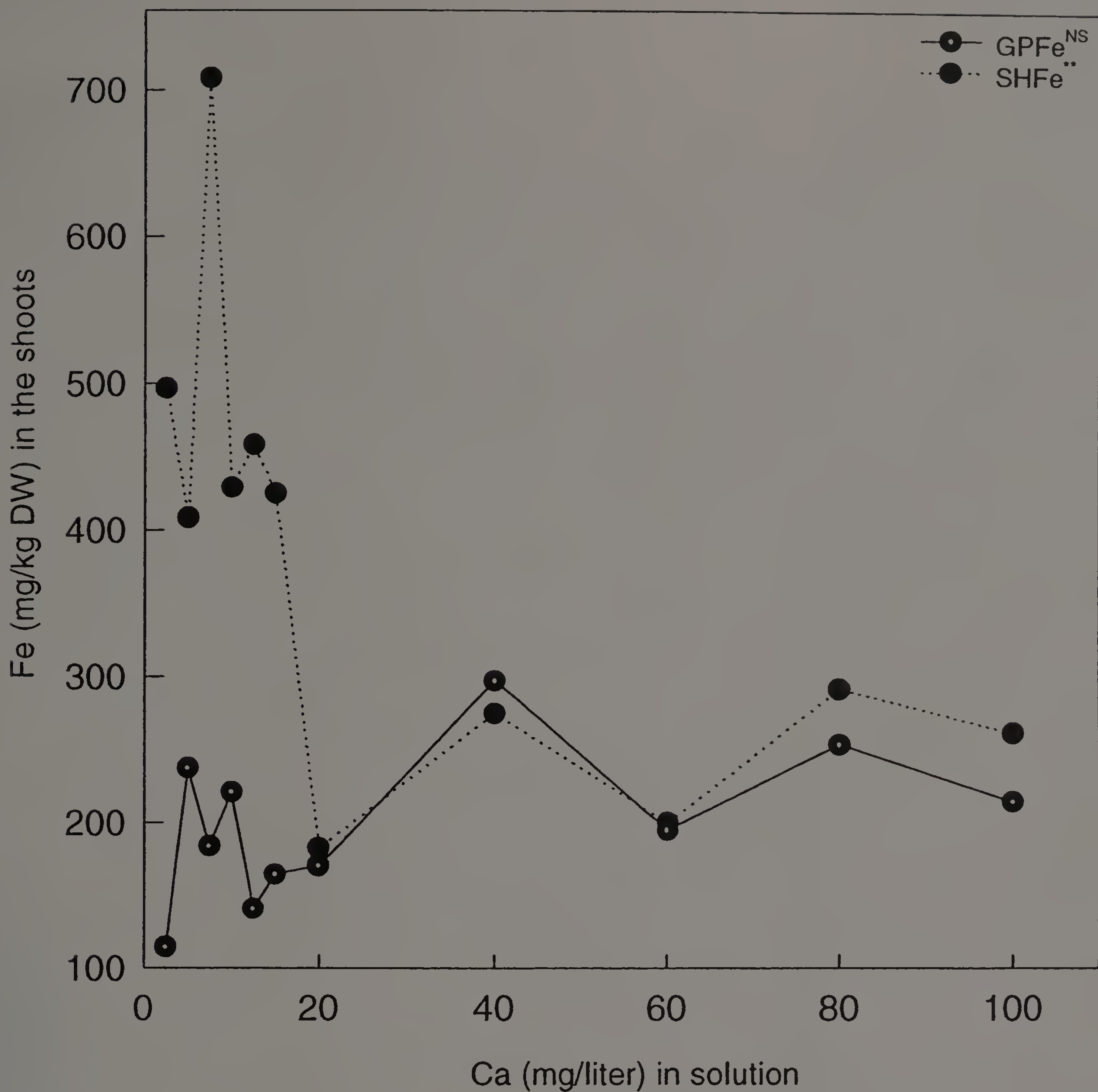

Figure 4.4: Effect of Ca level in solution on the`level of Fe in the shoots (see Appendix D, Tables 2, 3)

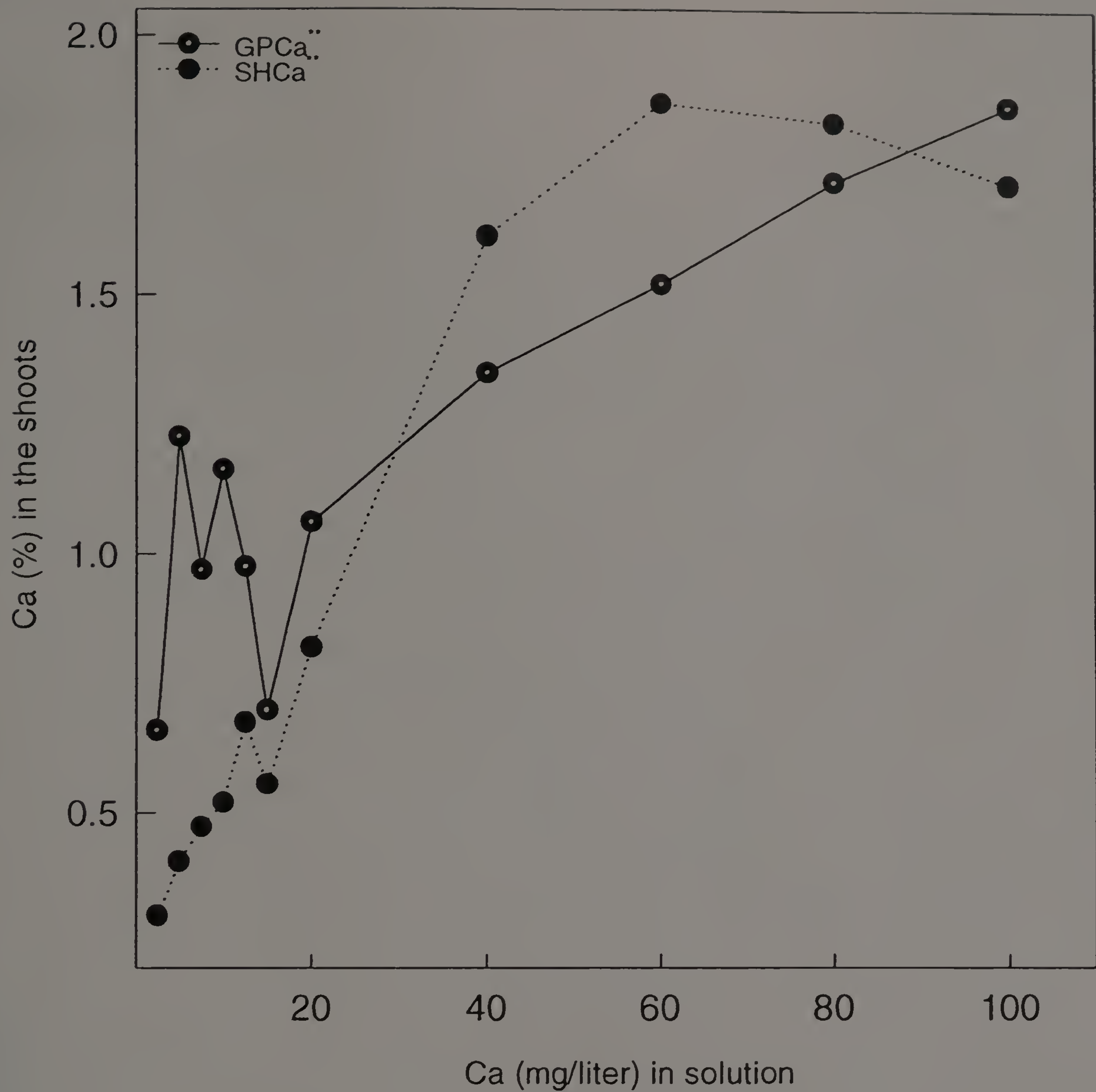

Figure 4.5: Effect of Ca level in solution on the level of Ca in the shoots (see Appendix D, Tables 2, 3)

Our results are similar to those of Gunter et al. (1996) who found that increased Ca in the medium resulted in increased Ca in potato tuber. Makus (1998) also found that $Ca(NO_3)_2$ treatment significantly improved Ca content of strawberry fruit.

4.4.5.4 Effect of Ca in solution on Mg in the shoots

Magnesium was higher in the older parts of the shoots than in the growing point (Figure 4.6). Calcium deficiency caused a highly significant increase of Mg in both the growing point and older parts of the shoots (Figure 4.6). As Ca increased in the nutrient solution, Mg decreased in the plant tissue.

These results may be explained by Moore et al. (1961) and Maas and Ogata (1971). They reported that excess Ca in the medium had an inhibitory effect on Mg influx.

Bergmann (1992) reported that usually Mg uptake is initially encouraged by liming. However, when liming becomes excessive or more frequent, the disproportion between Ca and Mg ions causes Mg deficiency symptoms to develop on the plants.

The uptake of Mg by plants and the interaction between the absorption of Mg and the absorption of Ca have been investigated by Spear et al. (1978), McLean et al. (1972), and Christenson et al. (1973). Their studies showed that Mg contents in plant tissue were suppressed by Ca, but the effect depended on the concentration of the ions in the medium.

Moor et al. (1961) and Maas and Ogata (1971) studied the influence of Ca concentration in solution on Mg uptake by excised barley (*Hordeum vulgare* L.) and corn (*Zea mays* L.) roots, respectively. In both reports it was concluded that enhanced

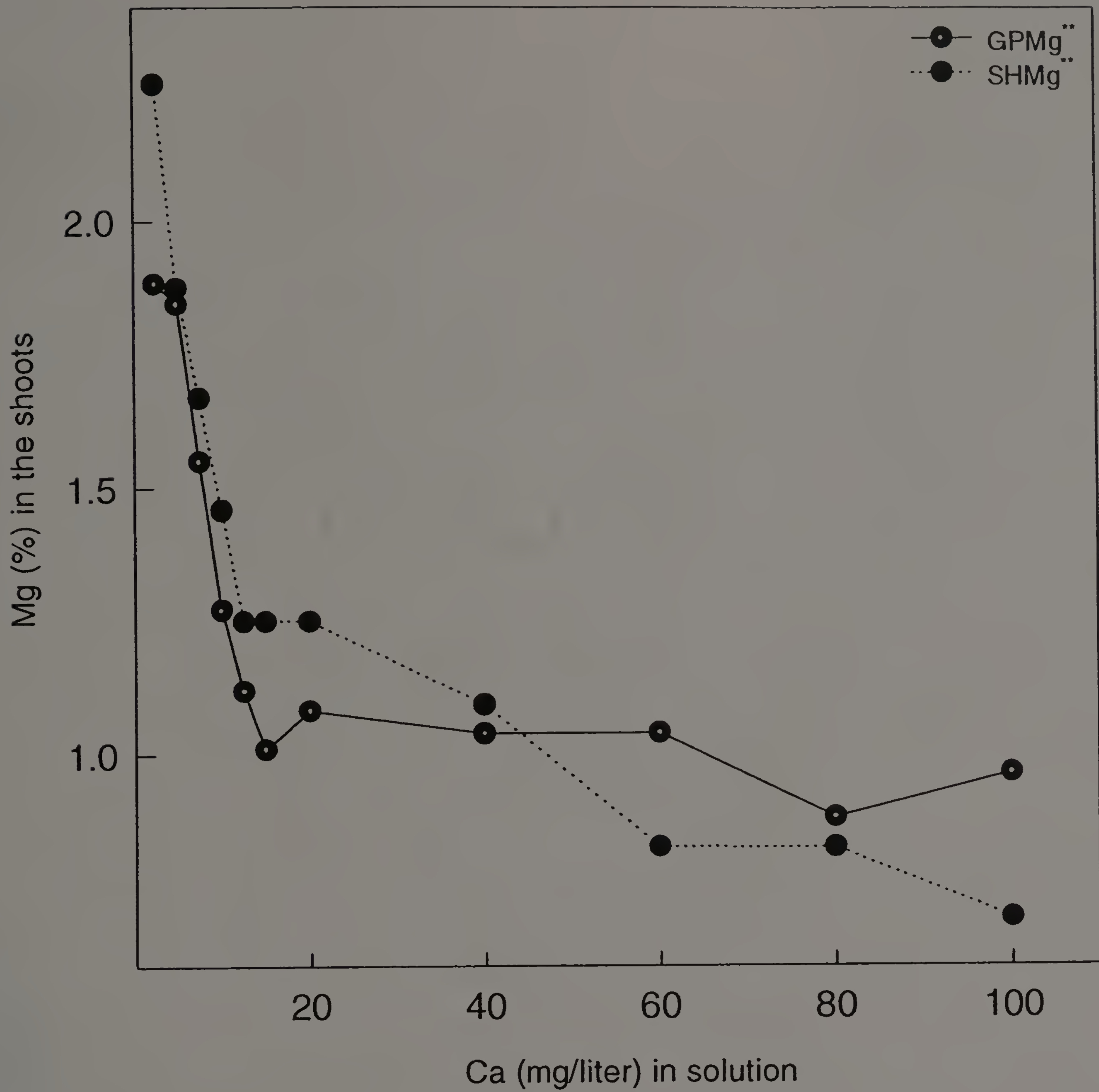

Figure 4.6: Effect of Ca level in solution on the level of Mg in the shoots (see Appendix D, Tables 2, 3)

solution Ca concentrations reduced Mg uptake rate by suppressing the Mg transport capacity of the roots rather than competing with Mg for absorption sites.

According to Shear and Faust (1971) and Shear (1975), excessive levels of Mg in the medium may increase the occurrence of bitter pit in plants.

In experiments with Mg and Ca, Schimansky and Wienecke (1976) were able to show that in fruit-bearing plants such as strawberry, tomato and grapevines, Mg and Ca ions are distributed in the same general way. Both ions are present mainly in the vegetative parts and accumulate only to a small extent in the fruit. However, the value of the distribution coefficient Mg/Ca increases with increasing distance from the point of application (root, stem, fruit stalk) because of the greater distance covered by the more mobile Mg ions.

Similarly, inside the fruit the values of the quotient Mg/Ca increase from the stalk to the flower end of the fruit. According to Wienecke (1969), this increase of the Mg/Ca quotient from the stalk to the flower end of the fruit of apples occurs because Ca ions are constantly transferred laterally into adjacent tissue causing a dilution effect as the supply of these ions diminishes.

To provide optimal nutrition for plants it is important to achieve a harmonious balance between Ca and Mg (lime-Mg law or Loew's lime factor) (Bergmann, 1958). Hao and Papadopoulos (2000) recommend 300/50-80 ppm Ca/Mg for best yield and quality of tomato.

4.5 CONCLUSION

Calcium deficiency affected the appearance and the weight of marigold. Low Ca in the nutrient solution up to 15 mg/l caused severe damage to the plant. The plants were stunted and bushy and their leaves were severely damaged by chlorotic and necrotic

stunted and bushy and their leaves were severely damaged by chlorotic and necrotic symptoms. The damage caused by Ca deficiency probably due to the role of Ca in important metabolic processes in the plant such as maintaining the integrity of cell membrane; cell division, and phytohormone regulation.

The plants grown in 20mg Mg/l nutrient solution were still relatively stunted but there were no significant symptoms affecting their appearance. The concentration 20mg/l may thus be considered incipient deficiency concentration of Ca in marigold. This concentration was the turning point of Ca nutrition from deficiency to sufficiency. It still does not fulfill the requirement of marigold in Ca for adequate growth, but it was just enough to reach meristematic tissues and support their metabolism. At 20mg/liter Ca in solution, the concentration of Ca in the shoot tissue was 0.54%.

The present data showed that Mn was evenly distributed in the plant shoots. There was no significant difference between the concentration of Mn in the growing point and the concentration of Mn in the older part of the shoots.

Calcium concentration in the medium had no significant effect on Mn concentration in the old part of the shoots but had a highly significant effect on Mn concentration in the growing point. Calcium deficiency caused a highly significant increase of Mn in the growing point. Manganese concentration in the growing point decreased as Ca concentration in the medium increased.

Calcium deficiency had no significant effect on Fe in the growing point but resulted in a significant increase of Fe in the older part of the shoots. As Ca concentration in the medium increased, Fe concentration in the older part of the shoots decreased.

Calcium was significantly higher in the growing point than in the older parts of the shoots. Calcium in the nutrient solution had a highly significant effect on Ca in the growing point as well as the older parts of the shoots. Calcium deficiency caused a decrease in Ca in the shoots. As Ca concentration in the medium increased, Ca concentration in the shoots increased.

Magnesium was significantly higher in the older parts of the shoots than in the growing point. Calcium in the medium had a highly significant effect on Mg in the older parts of the shoots as well as the growing point. Calcium deficiency in the medium caused an increase of Mg in the shoots. As Ca in the nutrient solution increased, Mg in the shoot tissues decreased. Reduced Ca in the plant shoots as a response to increased Mg in the nutrient solution may be attributed to decreased Ca binding to absorption sites or carrier sites in the root.

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CHAPTER 5

MANGANESE TOXICIY IN MARIGOLD

5.1 ABSTRACT

Iron/manganese toxicity disorder in marigold has been related to high concentrations of Mn in the affected leaves. In greenhouse crop production, preplant addition of micronutrients in the media combined with constant feed program and low medium pH, create favorable conditions for the development of Mn toxicity. To investigate the effect of excess supply of Mn on marigold (Tagetes erecta L. 'First Lady') growth, appearance, and nutrient (Mn, Fe, Ca and Mg) concentrations, a solution culture study with various Mn concentrations was conducted. Concentrations of Mn equal or above 6.5mg/l resulted in stunted plants with significant number of small brown spots, interveinal chlorotic patches, and necrotic symptoms on the tips and margins of the leaves. The concentration 4.5mg/l resulted in stunted plants relatively free of symptoms. Concentrations below 4.5mg/l Mn resulted in relatively healthy looking plants. The concentration 4.5mg/l Mn may thus be considered critical toxicity concentration of Mn in marigold. Internal concentrations close to 270mg/kg in the new leaves were associated with normal growth. Plants with 1000mg/kg Mn suffered from Mn toxicity. Using the methods of this experiment, excessive supply of Mn increased the concentration of Mn in the roots and leaves, increased the concentration of Fe in the roots, and slightly increased the overall concentration of Ca and Mg in the plant.

5.2 INTRODUCTION AND LITERATURE

In many higher plants excessive amounts of Mn disrupt various aspects of plant metabolism, leading to serious physiological and morphological disorders. Manganese

toxicity has been associated with reduced enzyme activity (Heenan and Campbell, 1981; Sirkar and Amin, 1974), reduced hormone activity (Morgan et al., 1966), inhibition of ATP synthesis, and reduced respiration rates (Sirkar and Amin, 1974).

Morgan et al. (1976) and Morgan et al. (1966) found that Mn toxicity in cotton was associated with an increased activity of indoleacetic acid oxidase, peroxidase, and polyphenol oxidase; lower activities of catalase, ascorbic acid oxidase and glutatione oxidase; lower ATP contents; and lower respiration rates. In sugar beets, excess Mn restricted leaf cell number and volume (Terry et al., 1975). Manganese toxicity has been associated with an increase in indoleacetic acid oxidase activity, which results in a destruction of auxin (IAA) (Morgan et al., 1976; Morgan et al., 1966).

Factors that affect the toxicity of Mn in plants include the concentration of Mn in the growth medium; growth medium moisture, organic matter, and pH (Foy et al., 1978; Goh and Haynes, 1978); concentration of salts, particularly those of Ca, Mg, Fe and P (Bachman and Miller, 1995; Harrison and Bergman, 1981; Le Mare, 1977); nitrogen source (Sirkar and Amin, 1974) and plant developmental stage (Hood et al., 1993). Small changes in these factors can determine the degree of Mn toxicity in a given crop.

5.2.1 High Level of Mn in the Medium

In some cases, the accumulation of Fe and Mn in the leaf tissue is the direct result of applying excessive levels of micronutrients. The continuous use of high levels of peat-lite fertilizers may cause problems if salt levels are not monitored carefully (Sheely, 1990). The preplant addition of micronutrients in the media, combined with a constant feed program, create the toxicity problem (Sheely, 1990).

5.2.2 Low Level of Ca in the Medium

The toxicity effects of high concentrations of Mn in the plant tissue are considerably modified by Ca status. They are much more severe when the Ca level in the plant tissue is low (Clark et al., 1981; Galvez et al., 1989; Foy et al., 1981; Horst and Marshner, 1978, Keisling and Fuqua, 1979; Wallace et al., 1945). Le Mare (1972, 1977) found that large concentrations of Ca in the growth medium could alleviate the harmful effects of Mn toxicity. The plants grown in a soil that supplied little Ca were very sensitive to Mn toxicity (Le Mare, 1972; Le Mare 1977).

Foy et al. (1978) reported the importance of Ca/Mn ratios in the tolerance of plants to Mn toxicity. Ratios above 80 were found desirable for a balanced nutrition in peanut (Bekker et al., 1994).

Although increasing the concentration of Ca in the medium can alleviate the detrimental effects of high concentrations of Mn, optimal concentration (60 mg/kg) of Ca is crucial for optimal plant growth (Morris and Pierre, 1947). Morris and Pierre (1947) pointed out that high Ca levels (300 mg/kg) in the medium can reduce plant growth regardless of the concentration of Mn in the medium, probably because of unbalanced nutrition.

5.2.3 Low Level of Mg in the Medium

A large concentration of Mn in the medium can induce Mg deficiency in the plant (Mn-induced Mg deficiency) (Heenan and Campbell, 1981). Kazda and Znacek (1989) reported that excess Mn in the medium reduced Mg uptake by 50%.

Manganese toxicity can often be counteracted by large Mg supply (Lohnis, 1960; Elamin and Wilcox, 1986). It was reported that Mg decreased Mn uptake both by

excised and intact roots of several plant species (Harrison and Bergmann, 1981; Lohni, 1960; Maas et al., 1969).

In some cases, Mg application is not a practical method for the avoidance of Mn toxicity (Davis, 1996). The ability of Mg to reduce Mn uptake depends on the concentration of Mn in the medium. Elamin and Wilcox (1986) found that at high Mn concentration, Mg had little effect on Mn uptake and the plants were able to accumulate toxic levels of Mn at all levels of Mg supply. In addition, using Mg to prevent Mn toxicity would require large Mg applications, which could lead to serious nutritional imbalance because Mg would interfere with Ca uptake.

In summary, Fe/Mn toxicity disorder in marigold has been related to high concentrations of Mn in the tissue of the affected leaves (Koranski, 1988). In fact, the agricultural practices such as preplant addition of micronutrients in the media and constant feed program and the low pH of the growth media create favorable conditions for the development of Mn toxicity in greenhouse crops (Sheely, 1990; Biernbaum et al., 1988; Vetanovetz and Knauss, 1989).

Despite the importance of this problem, data on the effect of Mn nutrition in marigold on nutrient content in the plant tissue are meager. Available information on Mn requirement for marigold is also scant. This experiment was designed to investigate Mn requirements in marigold and the effect of Mn concentration in the medium on nutrient content in the plant tissue. Determination of the critical toxicity concentration of Mn is needed for further investigation of the relationship of Fe/Mn toxicity disorder in marigold to Mn toxicity. The objectives were: (1) to determine the symptoms of Mn toxicity in marigold; (2) to determine the critical toxicity concentration of Mn in marigold; and (3)

to determine the effect of Mn concentration in the medium on the content of Mn, Fe, Ca and Mg in the plant tissue.

5.3 MATERIALS AND METHODS

Seeds of marigold (*Tagetes erecta* L. 'First Lady') were planted in vermiculite #3 on September 17, 1996 in the greenhouse. Three-week-old seedlings were transplanted into 800 ml half strength nutrient solution in 800 ml opaque plastic containers. The basal nutrient solution contained 100 mg/l Ca, 48 mg/l Mg, 79 mg/l potassium, 63 mg/l P, 75 mg/l nitrate-nitrogen, 75 mg/l ammonium-nitrogen, 2.3 mg/l Fe, 0.02 mg/l Cu and 0.5 mg/l of Mn, B, Zn and Mo. After 10 days of growth in the half strength basal nutrient solution, the seedlings were transferred to treatment solutions. Manganese treatments were 0.5; 2.5; 4.5; 6.5; 8.5; 10.5; 12.5; 14.5; 16.5 and 18.5 mg Mn/l solution. Manganese was added to the nutrient solution as $MnCl_2$. The hydroponic cultures were aerated continuously and the nutrient solution was replaced with a fresh one every week. Deionized water was added to the containers as needed to maintain volume.

Sixty containers consisting of 10 treatments and 6 replicates were placed on two tables in the greenhouse in a completely randomized design. The photoperiod was about 16/8. The temperature was maintained as closely as possible to 21/17 °C (day/night). The plants were harvested on November 14, 1996 into roots, stems, old leaves and young leaves separately. The roots were rinsed with tap water then deionized water to discard nutrients from the root surface.

The plant parts were dried at 70 °C for 48 hours and weighed. The different plant parts were ground (20 mesh) and analyzed by atomic absorption spectroscopy (Appendix A) for Mn, Fe, Ca, and Mg contents. The data obtained were statistically analyzed using

Regression Analysis and Analysis of Variance (ANOVA) to test for significance of main effects and interactions, and terms were considered significant at $p<0.05$.

5.4 RESULTS AND DISCUSSION

5.4.1 Description of Symptoms

The symptoms of Mn toxicity in marigold consisted of small, clearly defined blackish-brown spots on old leaves. Initially the brown spots were difficult to see unless the leaves were held against the light. In advanced toxicity the spots appeared on the new leaves as well and became surrounded by chlorotic spots. Sometimes the chlorosis was so accentuated that the leaves looked patchy and even bleached. In addition to that, necrosis developed at the edges and tips of the leaves. The roots of the plants grown in excessive concentrations of Mn were brown and brittle.

According to Bergmann (1992), the brown spots on the leaves consist of enzymatically-controlled precipitations of Mn as Mn oxide (MnO_2) in the old epidermal cells. Bussler (1958) regards such depositions as a sort of Mn detoxification by the plant. In many plants accumulations of this kind are formed before the first chlorotic symptoms appear. As the excess Mn increases, the regions around the spots marking the accumulations become chlorotic and the leaves take on a patchy appearance (Bussler, 1958).

The chlorotic patches and the bleached look of the leaves may be associated with reduced chlorophyll synthesis and to Mn-induced Fe deficiency. Indeed, excess Mn interferes with the synthesis of chlorophyll, and in some cases, it reduces Fe uptake by the plant causing Fe deficiency symptoms to appear (Bergmann, 1992).

Osawa and Ikeda (1976) found that Mn toxicity in lettuce, celery, and cabbage was characterized by marginal chlorosis of the leaves with brown necrotic spots. They found that increasing the Fe supply prevented or reduced the severity of interveinal chlorosis, but had little or no effect on reducing the marginal chlorosis and brown spots. The leaf analysis indicated that the Mn-induced interveinal chlorosis was due to Fe deficiency whereas the marginal chlorosis and brown necrotic spots were due to accumulation of excess Mn.

The brown color of the roots may be attributed to oxidation of Mn with concomitant deposition of oxidized Mn in the roots. Foy et al. (1978) reported that deposition of oxidized Mn in the roots reduces Mn translocation to the shoots and enhances the tolerance of the plant to excessive amounts of Mn. Horiguchi (1987) observed deposition of oxidized Mn on the roots of rice, which displayed a dark brown color and accumulated a large amount of Mn. They also reported that a peroxidase-malate dehydrogenase system in the root cell walls oxidized Mn.

5.4.2 Effect of Mn Toxicity on the Plant Dry Weight

High concentrations of Mn in the growth medium resulted in reduced root and shoot growth (Figure 5.1). The general plant growth associated with excess Mn concentration in the medium may be due to the depression of the phytohormone metabolism by high Mn concentrations (Morgan et al., 1966). Reduced growth may, as well, be linked to reduced enzyme activity (Heenan and Campbell, 1981; Sirkar and Amin, 1974), inhibition of ATP synthesis, and reduced respiration rates (Sirkar and Amin, 1974).

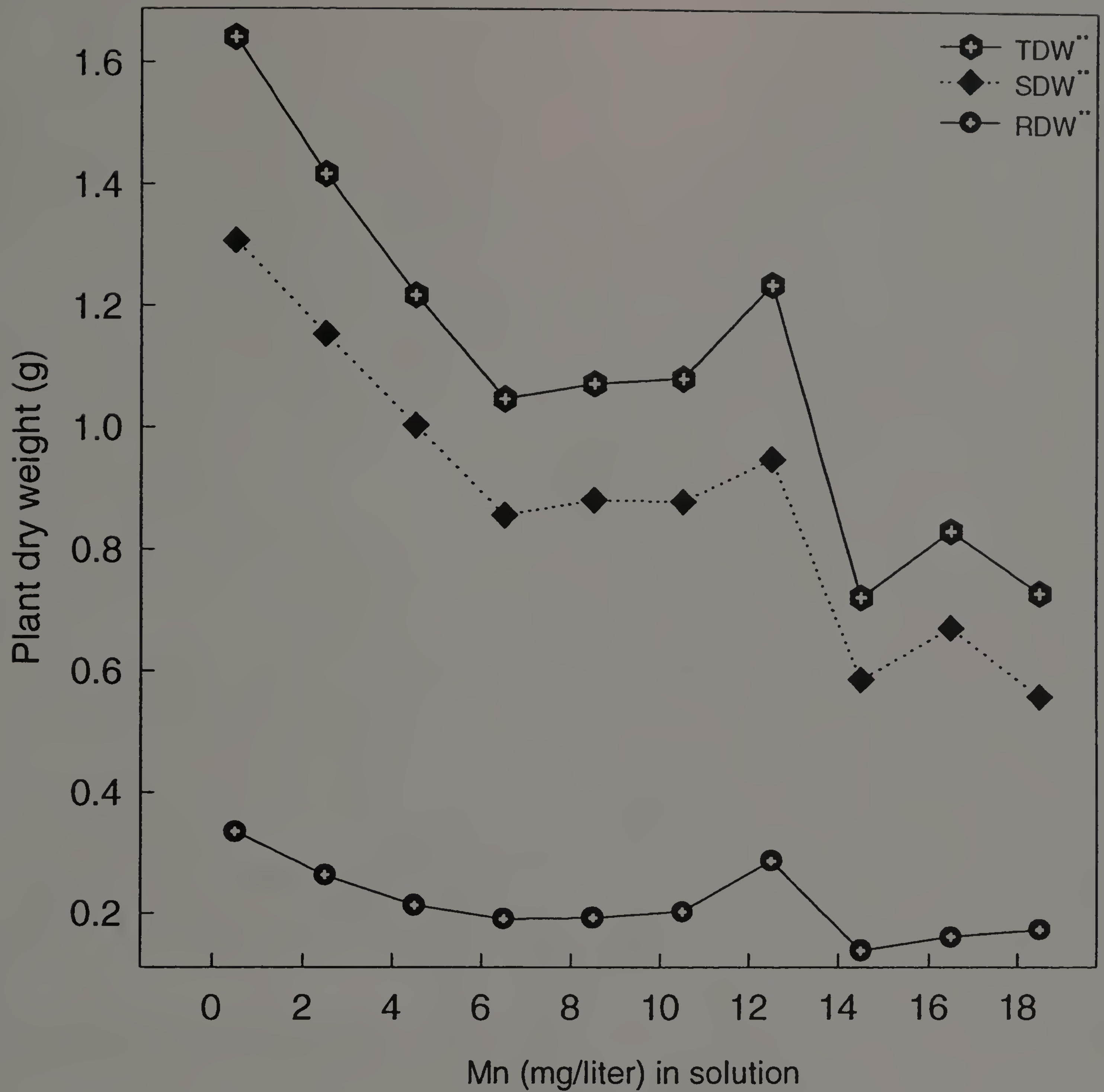

Figure 5.1: Effect of Mn level in solution on the plant dry weight (see Appendix E, Tables 1, 3)

5.4.3 Critical Toxicity Concentration of Mn

Plants grown at Mn concentrations of 0.5 and 2.5 mg/liter nutrient solution did not show any significant symptoms of Mn toxicity. Plants grown in 4.5 mg/l did not show significant symptoms but their dry weight was reduced in comparison to those grown in 0.5 and 2.5 mg Mn/liter. The plants grown at concentrations of Mn greater than 4.5 were stunted and showed symptoms. The intensity of dry weight reduction and symptom appearance depended on the concentration of Mn in the solution. These results suggest that 4.5mg/liter Mn may be considered critical toxicity concentration of Mn in marigold in this experiment. This concentration was the turning point of Mn nutrition from sufficiency to toxicity. It seems that 4.5mg/l Mn was not high enough to cause localized precipitation of Mn on the leaves or to induce Fe deficiency, but it was high enough to disturb the plant metabolism and reduce growth.

The tissue analysis suggested that the internal concentration of Mn associated with normal growth was about 270 mg/kg in the new leaves. The concentration 1000 mg/kg was associated with toxicity symptoms and stunting. The level of Mn in the plants that were stunted, but free of symptoms, was about 750 mg/kg. This internal concentration of Mn in the new leaves may be considered the internal critical toxicity concentration of Mn in marigold.

Critical toxicity concentration of Mn in plants depends on the degree of tolerance of these plants to excess Mn. Chrysanthemums, for example, are reported to be very sensitive to excess Mn in the medium; they show symptoms of Mn toxicity at very low concentration of Mn in the medium (Bergmann, 1992). It has even been suggested to use chrysanthemums as indicators of excess Mn in the medium (Foy, 1976). Carnations,

poinsettia and roses are able to tolerate higher concentrations of Mn than chrysanthemums (Foy, 1976). Some sensitive plants such as lettuce, beans and roses show toxic effects at 200-400 mg Mn/kg dry weight (Sonneveld and Voogt, 1975). Carnations show toxic effects at Mn levels 600-800 mg/Kg dry weight (Sonneveld and Voogt, 1975). Other so called tolerant plants, often show no toxic effects even at Mn levels of 1500-2000 mg/Kg dry weight (Sonneveld and Voogt, 1975).

Based on the Mn concentration in the medium associated with injury, the critical Mn concentration was found to be 1 mg/kg for tobacco (Jacobson and Swanback, 1932); 3.5 mg/kg for peas, and 10 mg/kg for soybean, peanut (Morris and Pierre, 1949), and barley (Aso, 1902).

5.4.4 Effect of Mn Toxicity on the Nutrient levels in the plant

5.4.4.1 Effect of Mn in solution on Mn level in the plant

An increase in Mn level in the nutrient solution caused an increase in the Mn concentration in the plant tissue (Figure 5.2). This increase was highly significant in the roots, old leaves and new leaves but it is not significant in the stems (Figure 5.2). Manganese seemed to accumulate much more in the roots than in any other organ of the plant (Figure 5.2). This high accumulation of Mn in the roots might be related to an attempt by the plant to prevent translocation of excess concentrations of Mn to the shoots. The brown color of the roots supports this hypothesis. It appeared that a large amount of Mn absorbed by the plants was precipitated in the roots to prevent translocation to the shoots.

Manganese accumulation and translocation is closely associated with the degree of tolerance of the plant to excessive Mn. Kamprath and Foy (1971) reported that Mn

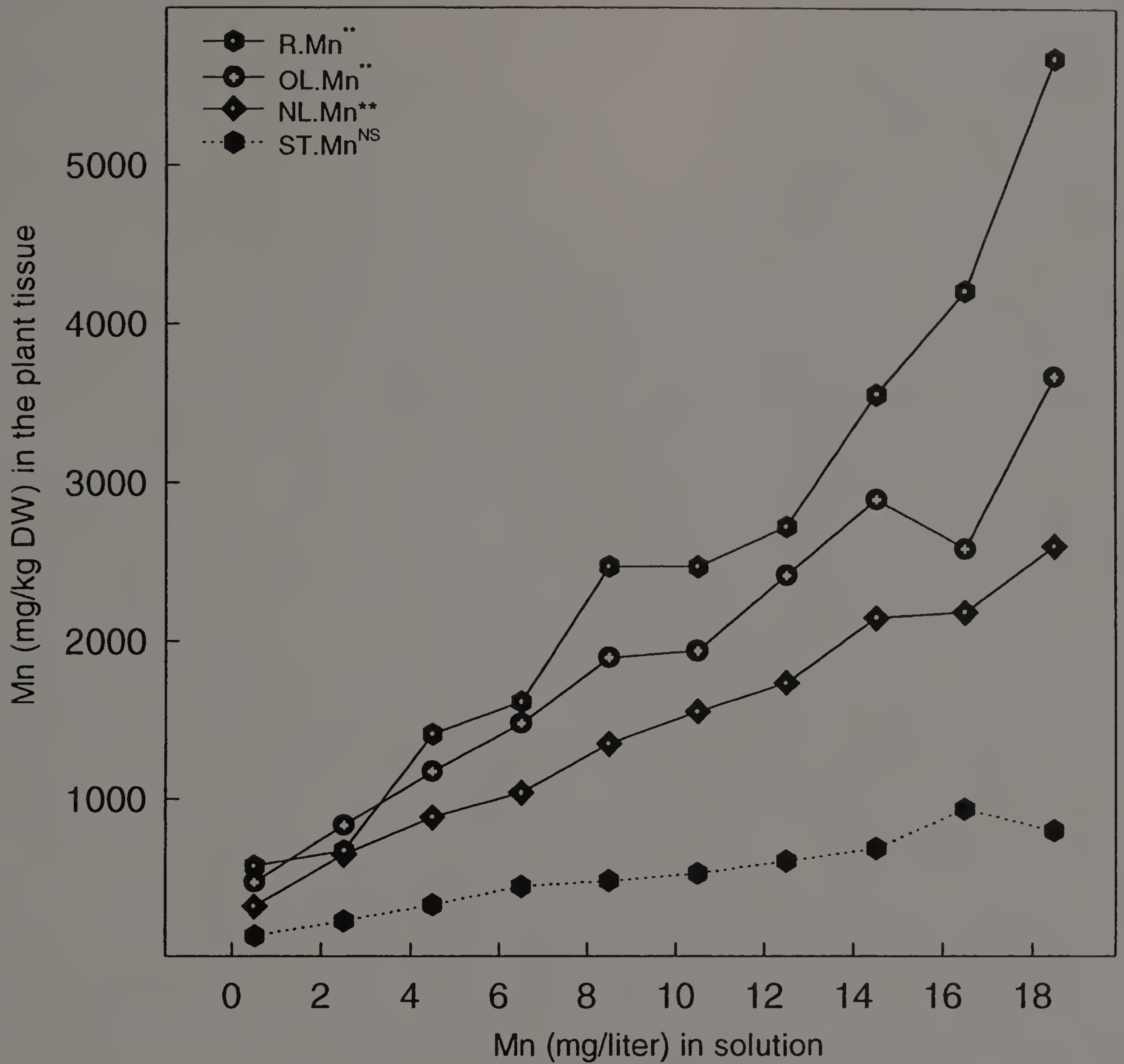

Figure 5.2: Effect of Mn level in solution on the level of Mn in the plant tissue (see Appendix E, Tables, 2, 3)

may be detoxified by accumulating in the woody portions of the azalea plants. High Mn sensitivity in peanut was associated with the accumulation of Mn in its leaves but little or no accumulation in roots, stem, or petioles (Morris and Pierre, 1949).

High retention of excess Mn in the roots may be associated with the relative tolerance of marigold to excess Mn. Andrew and Hegarty (1969) reported that the relative tolerance of either tropical or temperate legume species depends in part on the retention of excess amounts of Mn within the root system. The superior Mn tolerance of corn (compared to peanut) was associated with reduced translocation of Mn from the roots and stem to the leaves (Benac, 1976). Ouelette and Dessureaux (1958) reported that the tolerant clones of alfalfa contained lower concentrations of Mn in their shoots and higher concentrations of Mn in their roots than did the more sensitive clones.

Although Mn retention in the roots may contribute to the Mn tolerance of some species, this mechanism does not seem to reflect the Mn tolerance of marigold. Although large concentrations of Mn were retained in the roots, symptoms related to excess concentrations of Mn were expressed in the leaves.

5.4.4.2 Effect of Mn in solution on Fe level in the plant

The statistical analysis suggested that Mn level in the nutrient solution had no significant effect on Fe concentration in the stem, old leaves or young leaves, but had a highly significant effect on Fe concentration in the roots (Figure 5.3;). An increase in Mn in the nutrient solution resulted in an increase in Fe concentration in the roots (Figure 5.3).

This finding does not support our interpretation of the interveinal chlorosis of the leaves as a consequence of Mn-induced Fe deficiency. Some researchers, however,

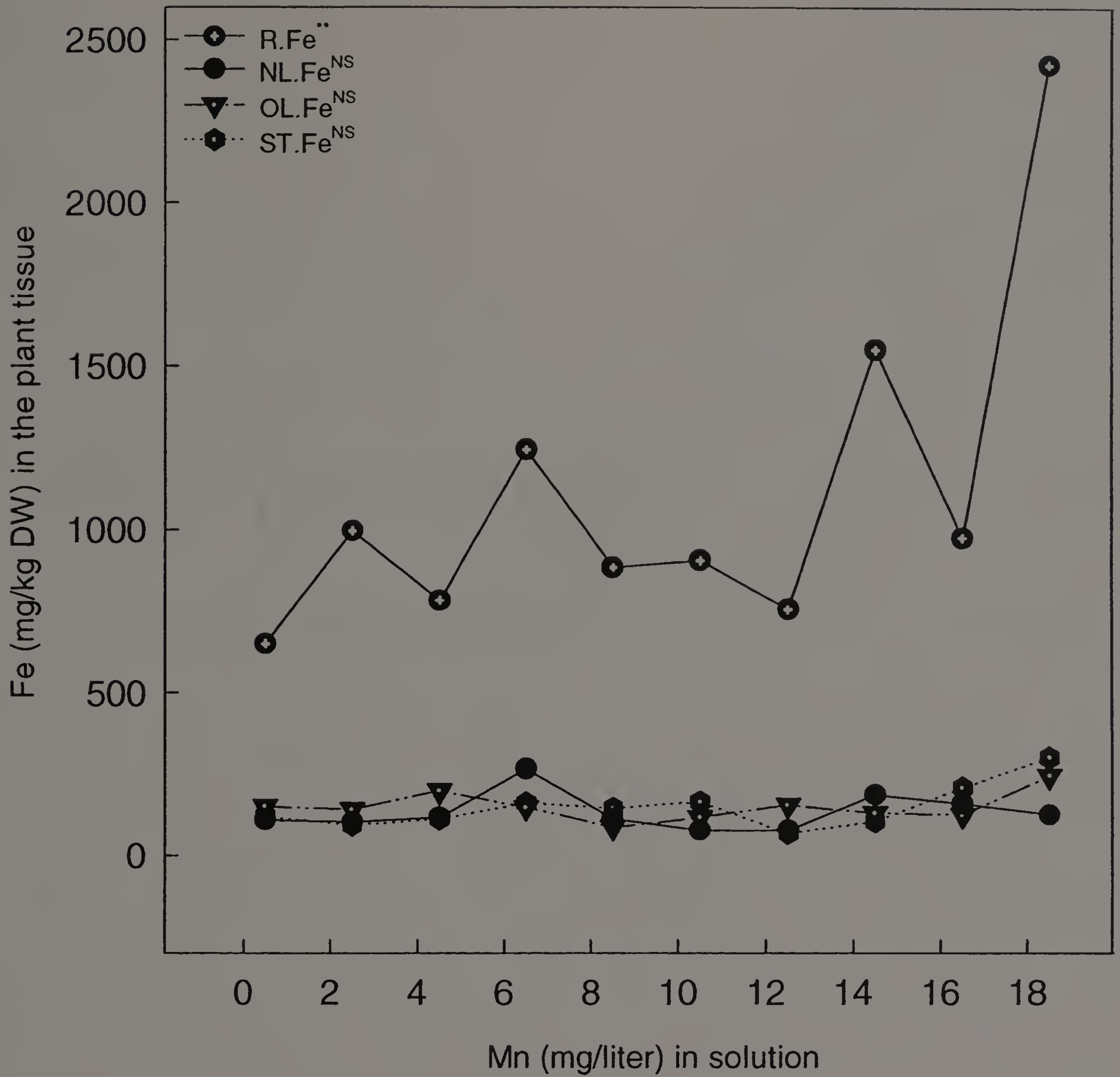

Figure 5.3. Effect of Mn level in solution on the level of Fe in the plant tissue (see Appendix E, Tables 2, 3)

found that the Fe/Mn ratio in the culture solution, rather than the total amounts of these elements, controlled the growth of plants (Somers and Shive, 1942).

Somers and Shive (1942) found that the optimum ratio was around 2.0 and any appreciable variation in this ratio would result in appearance of Fe or Mn toxicity symptoms. Morris and Pierre (1947) found that 0.5mg/liter Fe and 1mg/liter Mn made excellent growth and that the plants grown with the same ratio (2.0) but with concentrations five times higher than the former treatment resulted in poor growth and severe Mn toxicity symptoms.

Leach and Taper (1954) found that the optimum Fe/Mn ratios in plants ranged from 1.5 to 3 for kidney beans and from 0.5 to 5 for tomato. Iron deficiency developed at lower ratios.

Osawa and Ikeda (1976) reported that in spinach the iron/Mn ratio in nutrient solution was related closely to plant growth. Decreasing this ratio gave Mn-induced chlorosis (Mn-induced Fe deficiency) and decreased growth. They also reported that in tomato, pepper, bean, and eggplant both Fe/Mn ratio and Mn concentration in nutrient solution affected plant growth and occurrence of Mn-induced chlorosis.

The actual means by which Mn induces Fe deficiency in marigold is to be explained by the statistical analysis which suggests that excess Mn in the solution had no effect on Fe concentration in the shoots and it even caused an increase in Fe concentration in the roots. These results suggested that it is not Fe absorption but its action in the tissue that is unfavorably affected by excess Mn as shown in other studies (Rippel, 1923; Chapman, 1931; Millikan, 1949).

Somers and Shive (1942) suggested that this effect was probably due to the Mn catalysis of the oxidation of the physiologically active form of Fe (Fe^{2+}) to the inactive form (Fe^{3+}). They also suggested the probable formation of an insoluble ferric-phosphate-organic complex.

5.4.4.3 Effect of Mn in solution on Ca level in the plant

The statistical analysis suggested that an increase in Mn level in the nutrient solution caused a small increase in the overall Ca concentration in the plant tissue (Figure 5.4). Even though this increase was highly significant from the statistical point of view, it is not as important an increase as that of Mn (R^2 = 20% for Ca compared to 90% for Mn). This slight increase in the overall Ca concentration in the plant may be due to reduced growth, which results in increased concentration, rather to increased Ca uptake. It has been reported that excess Mn does not result in increased Ca uptake, but rather it inhibits Ca uptake by plants (Keil et al., 1980; Smith, 1979).

As to the repartition of Ca in the plant, it seems that most Ca was localized in the stem and the leaves. Calcium was lower in the new leaves and very low in the roots (Figure 5.5).

Low Ca in the new leaves may be due to the effect of high Mn level in the nutrient solution. Indeed, when the supply of Mn is excessive, the translocation of Ca into the shoot apex is inhibited (Horst and Marschner, 1990). This inhibition might be related to the fact that high Mn levels decrease the cation exchange capacity of the leaf tissue (Horst and Marschner, 1978a) and decrease the IAA levels in the areas of new growth (Morgan et al., 1966; Morgan et al., 1976). Auxin was reported to be responsible for the

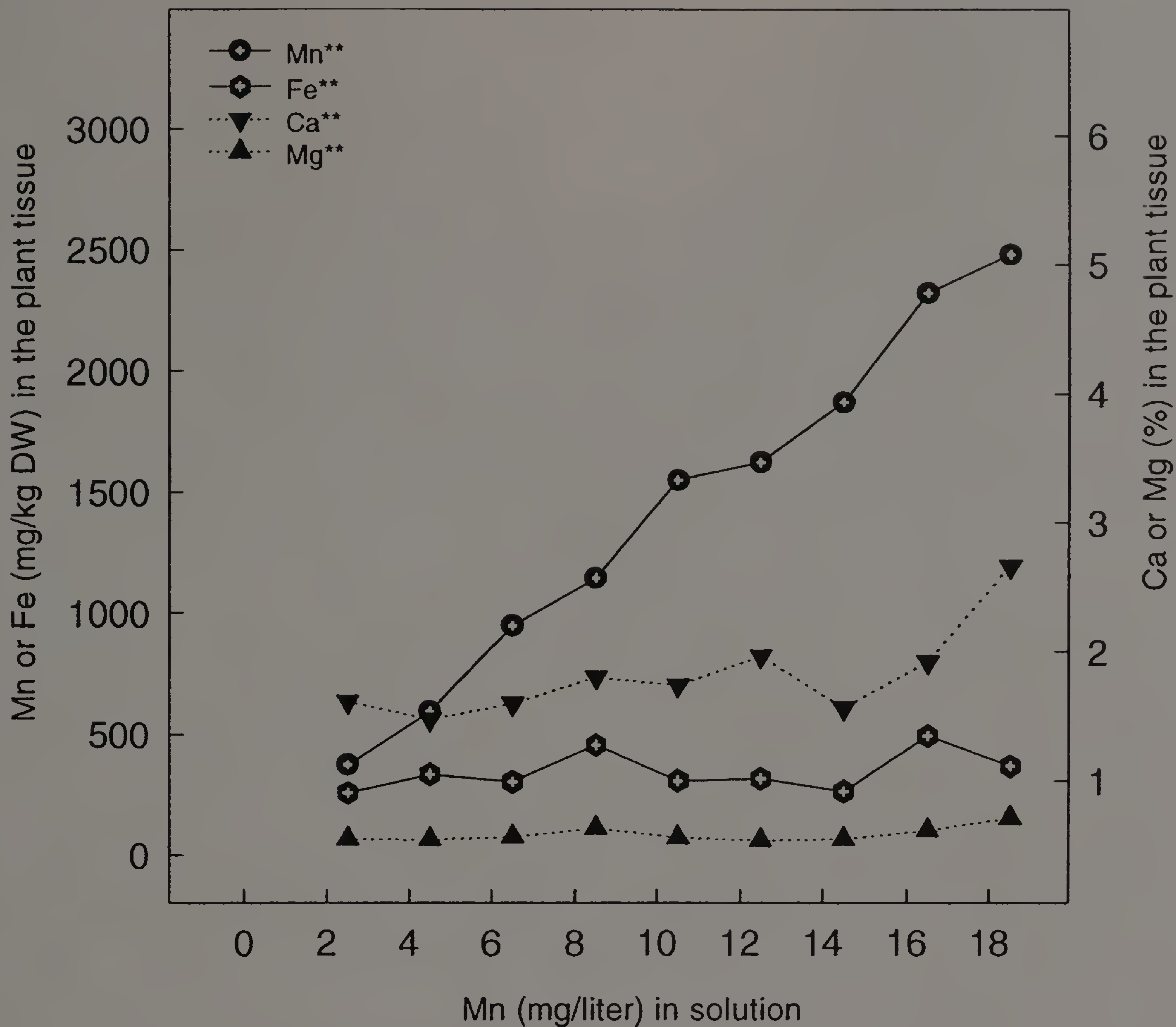

Figure 5.4: Effect of Mn level in solution on the level of nutrients in the plant tissue (see Appendix E, Tables 2, 3)

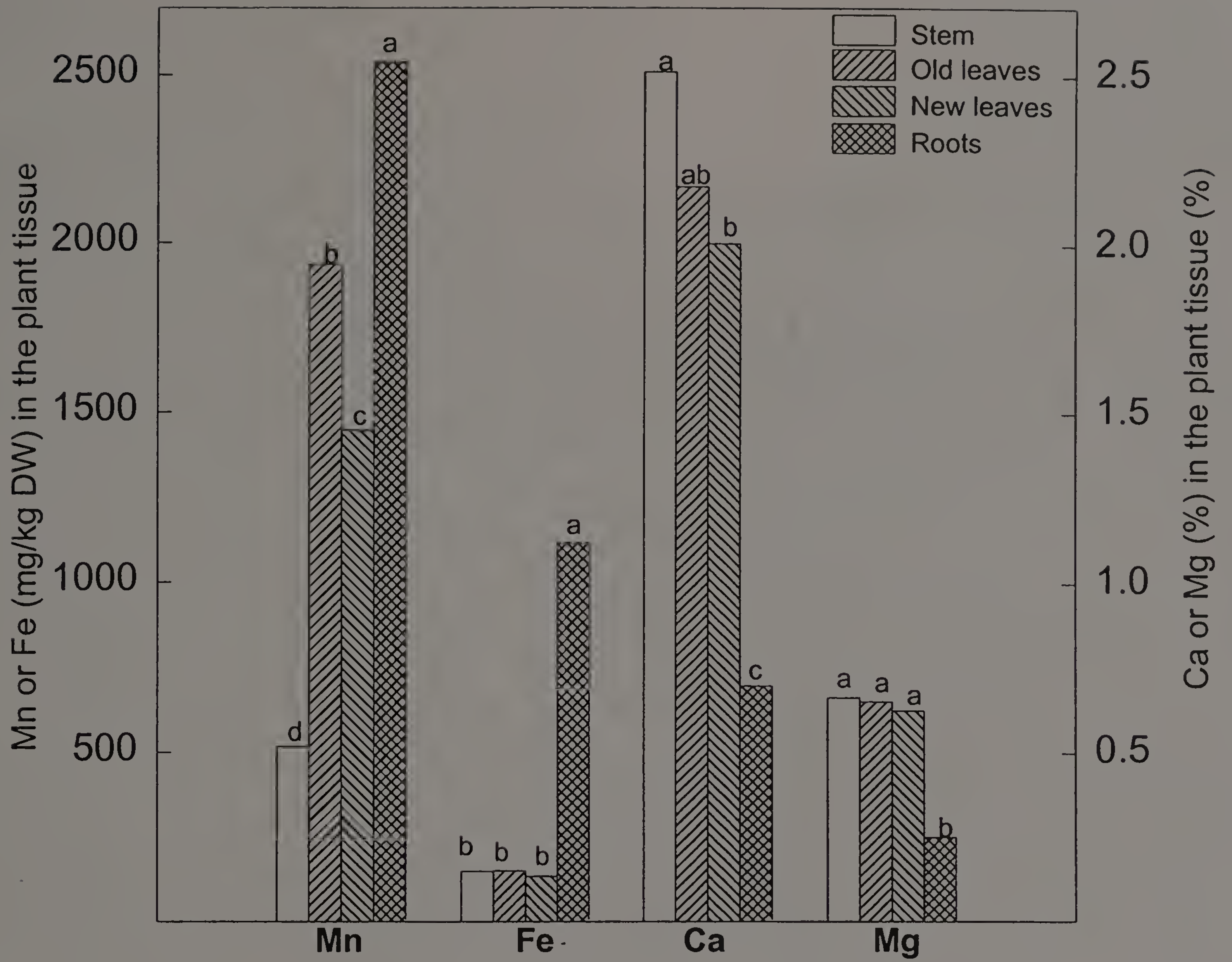

Figure 5.5: Nutrient distribution in the plant

formation of new binding sites for the transport of Ca to the apical meristems (Horst and Marschner, 1978a).

Horst and Marschner (1978c) found that the translocation of Ca to the leaves of bean plants was reduced when Mn levels were high. The reduced auxin levels caused by the increased IAA-oxidase activity associated with an excess of Mn is believed to inhibit cell enlargement and the creation of negative binding sites, thus reducing the translocation of Ca ions (Bergmann, 1992).

5.4.4.4 Effect of Mn in solution on Mg level in the plant

An increase in Mn level in the nutrient solution caused an increase in the overall Mg concentration in the plant (Figure 5.4). Magnesium was higher in the stem and leaves than in the roots (Figure 5.5). Even though the increase in Mg concentration was highly significant from the statistical point of view, it was not as an important increase as that of Mn (R^2 = 12% for Mg compared to 90% for Mn). Our findings were different from those of Heenan and Cambell (1981). The authors found that high concentrations of Mn in the medium actually induced Mg deficiency in the plant. Competition between Mg and Mn for binding sites in the roots during absorption inhibits Mg absorption, since Mn competes more effectively than Mg, and even blocks the binding sites for Mg (Horst and marschner, 1990). Keil et al. (1980) found that excess Mn in the nutritive medium inhibits the uptake of Mg.

The slight increase in the overall Mg in the plant associated with increased Mn is probably due to reduced growth, but it might also be related to the concentrations of Mg and Mn used in this study. Elamin and Wilcox (1986a, 1986b) and Lohnis (1960) found that a large supply of Mg can actually counteract the effects of Mn toxicity. Magnesium

was found to reduce Mn uptake both by excised and intact roots of several plant species (Harrison and Bergmann, 1981; Lohni, 1960; Maas et al., 1969).

5.5 CONCLUSION

Manganese toxicity affected the appearance and reduced the growth of marigold. Concentrations of Mn equal or above 6.5mg/liter nutrient solution caused severe damage to the plant. The plants were stunted and their leaves were severely damaged by the appearance of small brown spots and/or chlorotic patches and necrotic margins. The damage caused by excessive amounts of Mn may be due the detrimental effects of high Mn content in the plant on the metabolism, such as reduced enzyme activity, reduced hormone activity, inhibition of ATP synthesis, and reduced respiration rates. The symptoms related to high Mn level in the nutrient solution may as well be related to localized accumulations of Mn oxide and to Fe deficiency caused by Mn toxicity.

Plants grown in 4.5 mg/l Mn in the nutrient solution were still relatively stunted but there were no significant symptoms affecting their appearance. The concentration 4.5 mg/l may thus be considered critical toxicity concentration of Mn in marigold. This concentration was the turning point of Mn nutrition from sufficiency to toxicity. It seems that 4.5 mg/l Mn was not high enough to cause localized precipitation of Mn on the leaves or to induce Fe deficiency, but it was high enough to disturb the plant metabolism and reduce growth.

The data show that high Mn in the nutrient solution caused an increase of Mn in the roots and the leaves. Manganese accumulated to high concentrations in the roots perhaps as a way reducing translocation to the shoots. High Mn concentration in the nutrient solution also resulted in an increase of Fe in the roots and a slight increase in the

overall Ca and Mg concentration of the plant. Increased Ca and Mg in the overall plant tissue as a response to excess Mn in the nutrient solution may have resulted from a reduced dry weight rather than an increase in Ca and Mg uptake by the plants.

Increased Fe in the roots as result of high Mn in the nutrient solution may have been caused by reduced translocation of Fe from the roots to the shoots. Excess Mn catalyses the oxidation of the physiologically active form of Fe (Fe^{2+}) to the inactive form (Fe^{3+}). In addition to that, the formation of insoluble ferric-phosphate-organic complex in the roots due to high Mn concentration in the roots may have prevented Fe translocation to the shoots.

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CHAPTER 6

MANGANESE TOXICIY IN MARIGOLD AS AFFECTED BY CALCIUM

6.1 ABSTRACT

Iron/Manganese toxicity disorder in marigold has been related to high concentrations of Mn and low concentrations of Ca in the affected leaves. Preplant addition of micronutrients in the media combined with constant feed program and low medium pH create favorable conditions for the development of Mn toxicity in greenhouse crops. Deficiency of Ca is due in part to low medium pH and to lack of Ca in many of the fertilizers used in greenhouse production. To investigate the effect of Ca on Mn toxicity effects (growth, appearance, and nutrient concentrations) in marigold (*Tagetes erecta* L. 'First Lady'), a solution culture study with various Ca and Mn concentrations was conducted.

Treatments 20/6.5, 20/4.5, and 20/2.5 mg/l Ca/Mn concentrations resulted in stunted plants with significant number of small brown spots, interveinal chlorotic patches and necrotic symptoms on the tips and margins of the leaves, deformed leaves, and necrotic margins and tips of the leaves. The treatments 20/0.5, 100/2.5, and 100/4.5 resulted in stunted plants relatively free of symptoms. Treatment 100/6.5 resulted in stunted plants with blackish brown spots, chlorotic patches, and necrotic tips and margins on the leaves. The treatment 100/0.5 resulted in fully-grown and healthy looking plants.

Concentrations of Ca as high as the incipient deficiency concentration reduced the critical toxicity concentration of Mn in marigold from 4.5 to 2.5mg/l nutrient solution. Low concentrations of Ca in the nutrient solution accentuated the effect of excessive Mn

on Mn and Fe in the plant tissue, but had no effect on Ca and Mg. Low Ca in the medium accentuated the increase of Mn in the leaves and stem. It also accentuated the increase of Fe in the roots resulting from excessive Mn concentration in the medium.

6.2 INTRODUCTION AND LITERATURE

High Mn supply combined with low Ca supply disrupts various aspects of plant metabolism, leading to serious physiological and morphological disorders. Manganese toxicity has been associated with reduced enzyme activity (Heenan and Cambell, 1981; Sircar and Amin, 1974), reduced hormone activity (Morgan et al., 1966), inhibition of ATP synthesis, and reduced respiration rates (Sircar and Amin, 1974).

Calcium deficiency has been associated with increased permeability of the cell membrane (Hecht-Buchholz, 1979), destruction of structures within the cell nucleus, reduced chromosomal stability, disturbance of nuclear and cell division, destruction of mitochondria, and impaired respiratory metabolism (Bergmann, 1992).

Calcium deficiency has also been associated with abnormal nuclear division, accelerated fruit ripening and reduced firmness and suitability for storage (Richardson and Al-Ani, 1982), accumulation of nitrite (NO_2^-) (Yamada, 1975), and inhibited transport of carbohydrates in plants (Rangnekar, 1974; Gosset et al., 1977).

Factors that affect the toxicity of Mn and deficiency of Ca include the concentration of Mn in the growth medium; growth medium moisture, organic matter and pH (Foy et al., 1978; Goh and Haynes, 1978); concentrations of salts, particularly those of Mg, Fe and phosphorus (Bachman and Miller, 1995; Harrison and Bergman, 1981; Le Mare, 1977); nitrogen source (Reddy and Mills, 1991) and plant developmental stage

(Hood et al., 1993). Small changes in these factors can determine the degree of Mn toxicity and Ca deficiency in a given crop.

6.2.1 High Level of Mn in the Medium

The accumulation of Mn in the leaf tissue may result directly from applying excessive amounts of micronutrients. The continuous use of high levels of peat-lite fertilizers may cause problems if salt levels are not monitored carefully (Sheely, 1990). Preplant addition of micronutrients in the growth medium, combined with constant feed program, creates toxicity problems (Sheely, 1990).

High concentrations of Mn in the growth medium can also inhibit the uptake of Ca by plants and thus induce a Ca deficiency (Bergmann, 1992).

6.2.2 Interaction with Ca

The second part of Fe/Mn toxicity disorder is deficiency of Ca due in part to low medium pH and to lack of Ca in many of the fertilizers used in greenhouse crop production (Koranski, 1988). The toxicity effects of high concentrations of Mn in the plant tissue are considerably modified by Ca status. The effects are much more severe when the Ca level in the plant tissue is low (Clark et al., 1981; Galvez et al., 1989; Foy et al., 1981; Horst and Marshner, 1978; Keisling and Fuqua, 1979; Wallace et al., 1945). Le Mare (1972; 1977) found that large concentrations of Ca in the medium could alleviate the harmful effects of Mn toxicity. The plants grown in a medium that supplied little Ca were very sensitive to Mn toxicity (Le Mare, 1972; Le Mare, 1977).

Foy et al. (Foy et al., 1978) reported the importance of Ca/Mn ratios in the tolerance of plants to Mn toxicity. Ratios above 80 were found desirable for a balanced nutrition in peanut (Bekker et al., 1994).

Although increasing the concentration of Ca in the medium can alleviate the detrimental effects of high concentrations of Mn, optimal concentration (60 mg/kg) of Ca is crucial for optimal plant growth (Morris and Pierre, 1947). Morris and Pierre (1947) pointed out that high Ca levels (300 mg/kg) in the medium can reduce plant growth regardless of the concentration of Mn in the medium, probably because of unbalanced nutrition.

6.2.3 Interaction with Mg

A large concentration of Mn in the medium can induce Mg deficiency in the plant (Heenan and Cambell, 1981). Kazda and Znacek (1989) reported that excess Mn in the medium reduced Mg uptake by 50%.

Manganese toxicity can often be counteracted by a large Mg supply (Lohnis, 1960; Elamin and Wilcox, 1986). It was reported that Mg decreased Mn uptake both by excised and intact roots of several plant species (Harrison and Bergman, 1981; Lohnis, 1960; Maas et al., 1969).

In some cases, Mg application is not a practical method for the preventionof Mn toxicity (Davis, 1996). The ability of Mg to reduce Mn uptake depends on the concentration of Mn in the medium. Elamin and Wilcox (Elamin and Wilcox, 1986b) found that at high Mn concentration, Mg had little effect on Mn uptake and the plants were able to accumulate toxic levels of Mn at all levels of Mg supply. In addition, using Mg to prevent Mn toxicity would require large Mg applications, which could lead to serious nutritional imbalance because Mg would interfere with Ca uptake.

In summary, Fe/Mn toxicity disorder in marigold may be caused by Mn toxicity since the affected plants contain high concentrations of Mn in their tissue. Basic factors

affecting Mn toxicity in plants include Ca deficiency. The relationship of Ca deficiency to the incidence of Fe/Mn toxicity in marigold needs to be investigated.

Despite the importance of this problem and its relation to high concentrations of Ca in the affected leaves, no research has been done on the effect of Ca nutrition on manganese toxicity in marigold. Available information on the interaction between ca and Mn are meager. This experiment was designed to investigate the effect of Ca on Mn toxicity in marigold.

The objectives were: (1) to determine the effect of Ca in the nutrient solution on the sensitivity of marigold to high concentrations of Mn; (2) to determine the effect of Ca in the nutrient solution on the critical toxicity concentration of Mn; and (3) to determine the effect of the interaction between Ca and Mn in the nutrient solution on the concentration of Mn, Fe, Ca and Mg in the plant tissue.

6.3 MATERIALS AND METHODS

Seeds of marigold (*Tagetes erecta* L. 'First Lady') were planted in vermiculite #3 on September 8, 1997 in the greenhouse. Three-week-old seedlings were transplanted into 800 ml half strength nutrient solution in 1.6 liter opaque plastic containers. The basal nutrient solution contained 48 mg/l Mg, 79 mg/l potassium, 63 mg/l phosphorus, 75 mg/l nitrate-nitrogen, 75 mg/l ammonium-nitrogen, 2.3 mg/l Fe, 0.02 mg/l copper and 0.5 mg/l of Mn, boron, zinc and molybdenum. After 10 days of growth in the half strength basal nutrient solution, the seedlings were transferred to treatment solutions. Mn treatments were 0.5; 2.5; 4.5 and 6.5mg/l nutrient solution. Mn was added to the nutrient solution as $MnCl_2$. Calcium treatments were 100 and 20 mg/l. The concentration 100 mg/l is considered optimal concentration of Ca for marigold growth (Chapter 4). The concentration 20mg/l is

considered incipient deficiency concentration of Ca in marigold (Chapter 4). This concentration is enough to fulfill the metabolic needs of the plant but not enough for optimal growth. The hydroponic cultures were aerated continuously and the nutrient solution was replaced with a fresh one every week. Deionized water was added to the containers as needed to maintain volume.

Forty-eight containers consisting of 4 Mn treatments, 2 Ca treatments and 6 replicates of treatment combinations were placed on two benches in the greenhouse in a completely randomized design. Plants were grown under ambient light conditions. The temperature was maintained as closely as possible to 21/17 °C (day/night). The plants were harvested on October 24, 1997 into roots, stems, old leaves and young leaves separately. The roots were rinsed with tap water then deionized water to wash residual nutrients from the root surface.

The plant parts were dried at 70 °C for 48 hours and weighed. The different plant parts were ground (20 mesh) and analyzed by inductive coupled plasma (ICP) (Appendix B) for Mn, Fe, Ca, and Mg contents. The data obtained were statistically analyzed using Regression Analysis, Analysis of Variance (ANOVA) and procedure mixed to test for significance of main effects and interactions. Terms were considered significant at $p<0.05$.

6.4 RESULTS AND DISCUSSION

6.4.1 Symptoms

The nature of the symptoms and their severity depended on the treatment. The plants grown in 100 mg/l Ca-0.5 mg/l Mn did not show any symptoms. The plants grown in 100mg/l Ca-2.5 mg/l Mn and 100 mg/l-4.5 mg/l Mn did not show significant symptoms but the plants looked smaller relative to 100mg Ca/0.5 Mn. The plants grown

in 100 mg/l Ca-6.5 mg/l Mn were also small and showed typical symptoms of Mn toxicity as observed in earlier experiments (Chapter 5). The Mn toxicity symptoms appeared in the old leaves first then spread toward the rest of the plant. The symptoms started as small blackish brown spots on the leaves. Patches of chlorosis appeared on the tips and margins of the leaves afterward. When the plants were exposed to the same treatment for a longer time the chlorotic areas became necrotic.

The plants treated with 20/6.5, 20/4.5, and 100/6.5 mg/l Ca-Mn showed symptoms. The symptoms however were most severe in the plants treated with 20 mg/l Ca-6.5 mg/l Mn. The plants were also very small per comparison to the plants grown with the other treatments. The symptoms included small brown spots on the surface area of the leaves, deformation of the leaves, interveinal and marginal chlorosis, rolling and crinkling of necrotic leaf margins and brown brittle roots. After being exposed to the treatment for a longer time, necrosis developed on the margins and at the tips of the leaves.

The plants treated with 20/0.5 or 20/2.5 mg/l Ca/Mn did not show significant symptoms but the plants were smaller than those treated with 100/0.5 mg/l Ca/Mn.

The small brown spots shown in the affected leaves the brown color of the roots may have been associated with accumulation of Mn as precipitated Mn oxide (MnO_2) in the leaves and roots (Horst and Marshner, 1978a, b, c).

Interveinal chlorotic patches on the leaves may have been associated with Mn-induced Fe deficiency. Local accumulations of Mn may interfere with Fe dependent metabolic functions and thus lead to Fe deficiency (Horst and Marshner, 1978a).

The deformation and curling of the leaves may be due to deficiency of plant hormones. Indeed, toxic concentrations of Mn increase the activities of polyphenoloxidase, peroxidase and IAA oxidase, which result in decomposition of auxins (Morgan et al., 1966; Cheng and Ouelette, 1971; Kamprath and Foy, 1971; Foy, 1973).

Horst and Marshner (1978c) reported that high concentrations of Mn reduced Ca translocation in the plant leaves. They believed that this reduced translocation of Ca in the leaves was responsible for the deformation and crinkling of the leaves.

According to their findings, excess Mn increases IAA-oxidase activity, which reduces auxin level in the leaves. Reduced auxin level inhibits cell enlargement and the creation of negative binding sites, thus reducing the translocation of Ca ions.

6.4.2 Dry Weight

Manganese concentration in the solution had no significant effect on root dry weight, but did have a significant effect on shoot and total dry weight. When Mn in the solution increased, the dry weight of the shoots decreased (Figure 6.1). The concentrations of Mn used in this study were probably not high enough to affect the growth of roots but, were high enough to affect shoot growth and development. In Chapter 5 high Mn in the nutrient solution reduced root and shoot dry weight. The reduced plant growth associated with high Mn may have resulted from the depression of phytohormone metabolism by excess Mn (Marshner, 1978a).

Low concentration of Ca in the nutrient solution resulted in a decrease in the root and shoot dry weight (Figure 6.2). This decrease in plant growth is due to the crucial role of Ca in plant metabolism.

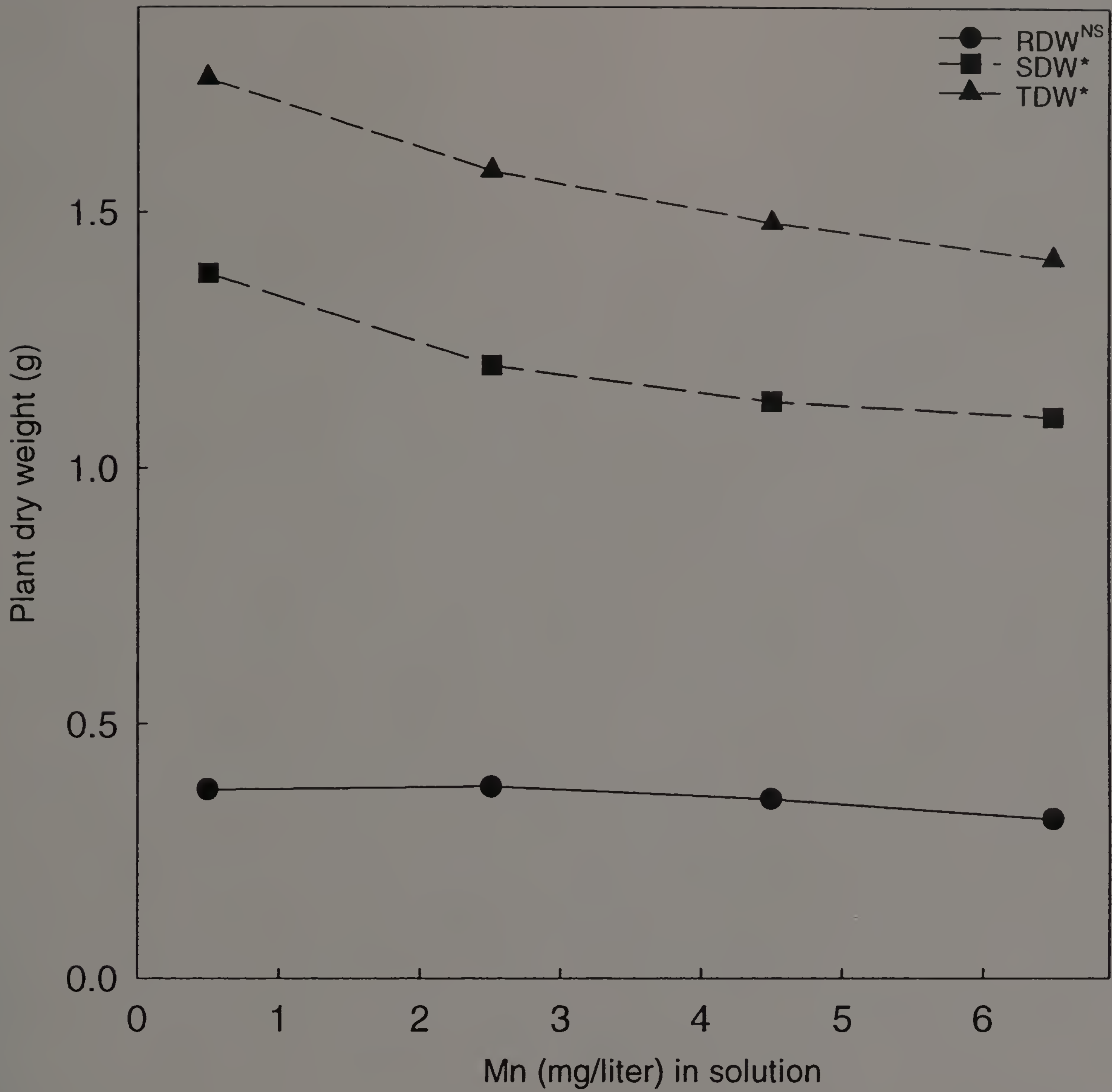

Figure 6.1: Effect of Mn level in solution on the plant dry weight (see Appendix F, Table1)

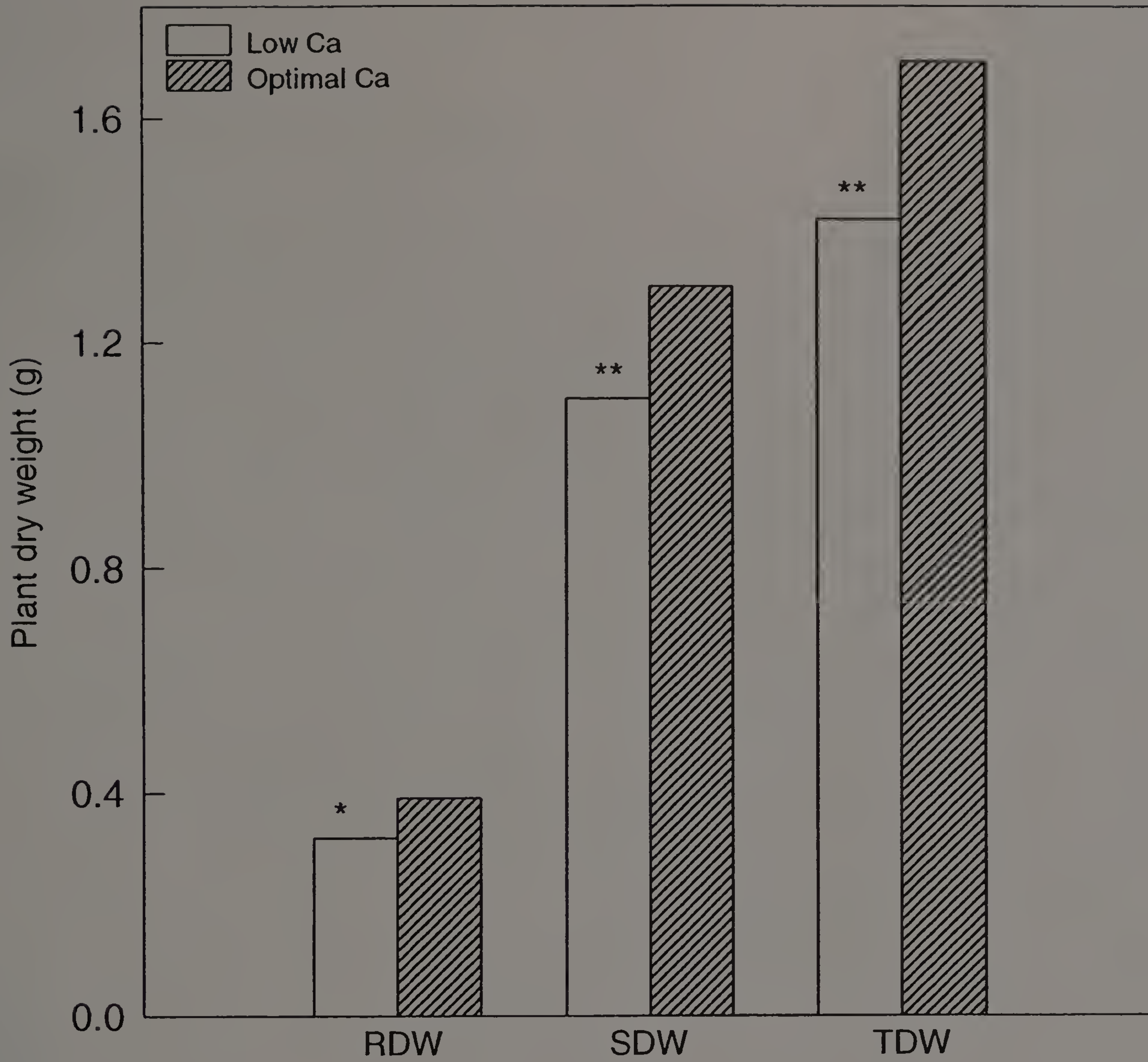

Figure 6.2: Effect of Ca level in solution on the plant dry weight (see Appendix F, Table 1)

The interaction between Ca and Mn in the medium had no significant effect on the plant dry weight (Appendix F).

6.4.3 Critical Toxicity Concentration

Plant appearance suggested that low concentrations of Ca in the medium decreased the critical toxicity solution concentration of Mn in marigold from 4.5 to 2.5mg/l. At optimal concentration of Ca in the medium, 2.5 and even 4.5mg/l Mn did not cause any significant symptoms. However, when Ca level in the medium was low (incipient deficiency concentration), the low concentration of Mn (2.5 mg/l) resulted in significant symptom development.

6.4.4 Nutrient concentrations in the plant tissues

6.4.4.1 Manganese

The Mn level in the nutrient solution had a highly significant effect on Mn in the roots, stems, and old leaves; and a significant effect on Mn in the young leaves (Appendix F). Increased Mn level in the solution resulted in increased Mn in all the plant parts (Figure 6.3). The increase in Mn in the stem and leaves as a result of increased Mn in the nutrient solution was greater when Ca was low (Figure 6.4). The increase in the overall Mn in the plant as a result of increased Mn in the nutrient solution also was greater when Ca was low (Figure 6.4a). Calcium in the solution had no significant effect on Mn in the roots (Figure 6.4).

The effect of Ca in solution on Mn increase in the stem and leaves may have been due to the effect of Ca on Mn absorption by plants. Increased Ca level in the growth medium often decreases Mn absorption (Heenan and Carter, 1975; Robson and Loneragan, 1970; Shuman and Anderson, 1976; Hewitt, 1945). Ouellette and

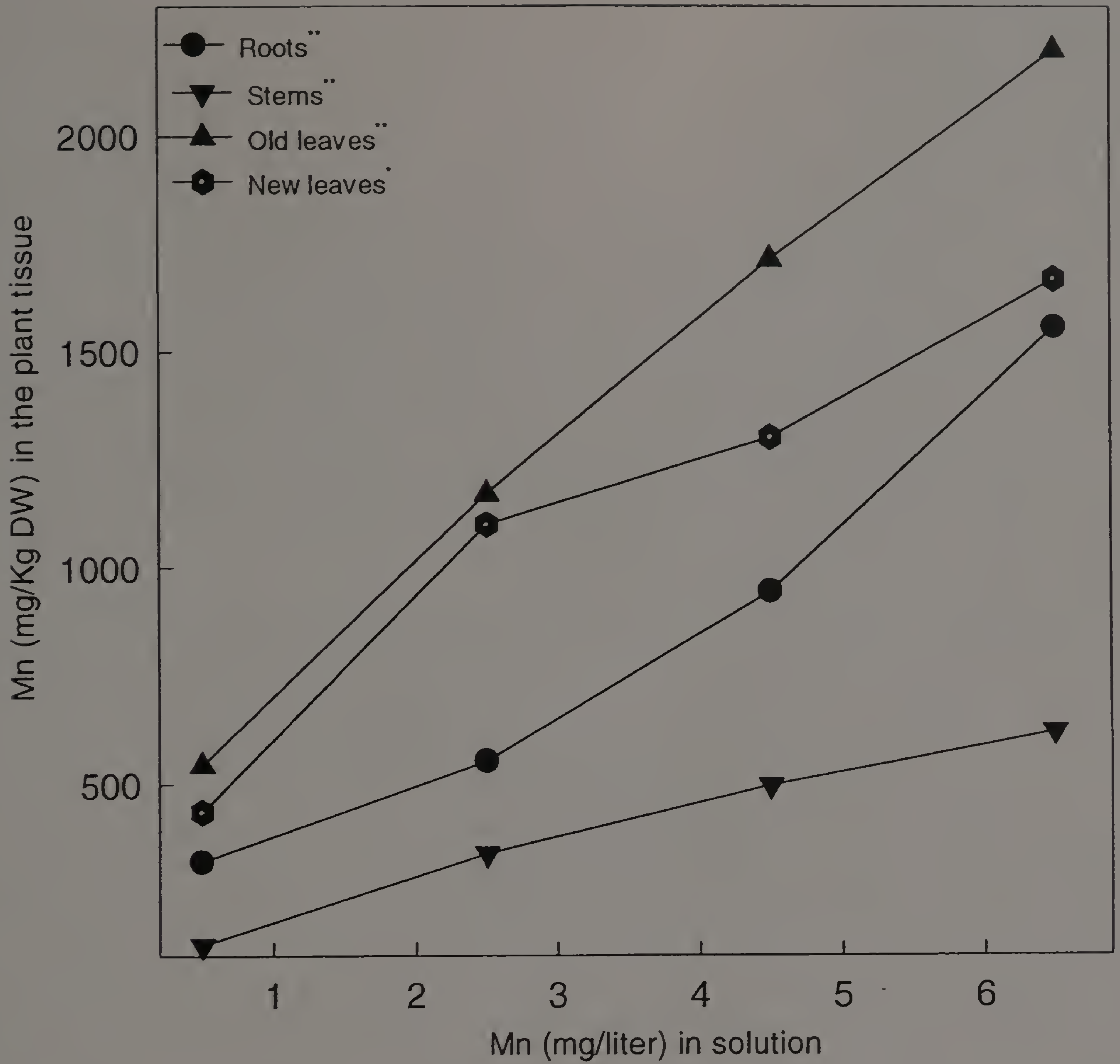

Figure 6.3: Effect of Mn level in solution on the level of Mn in the plant tissue (see Appendix F, Table 2)

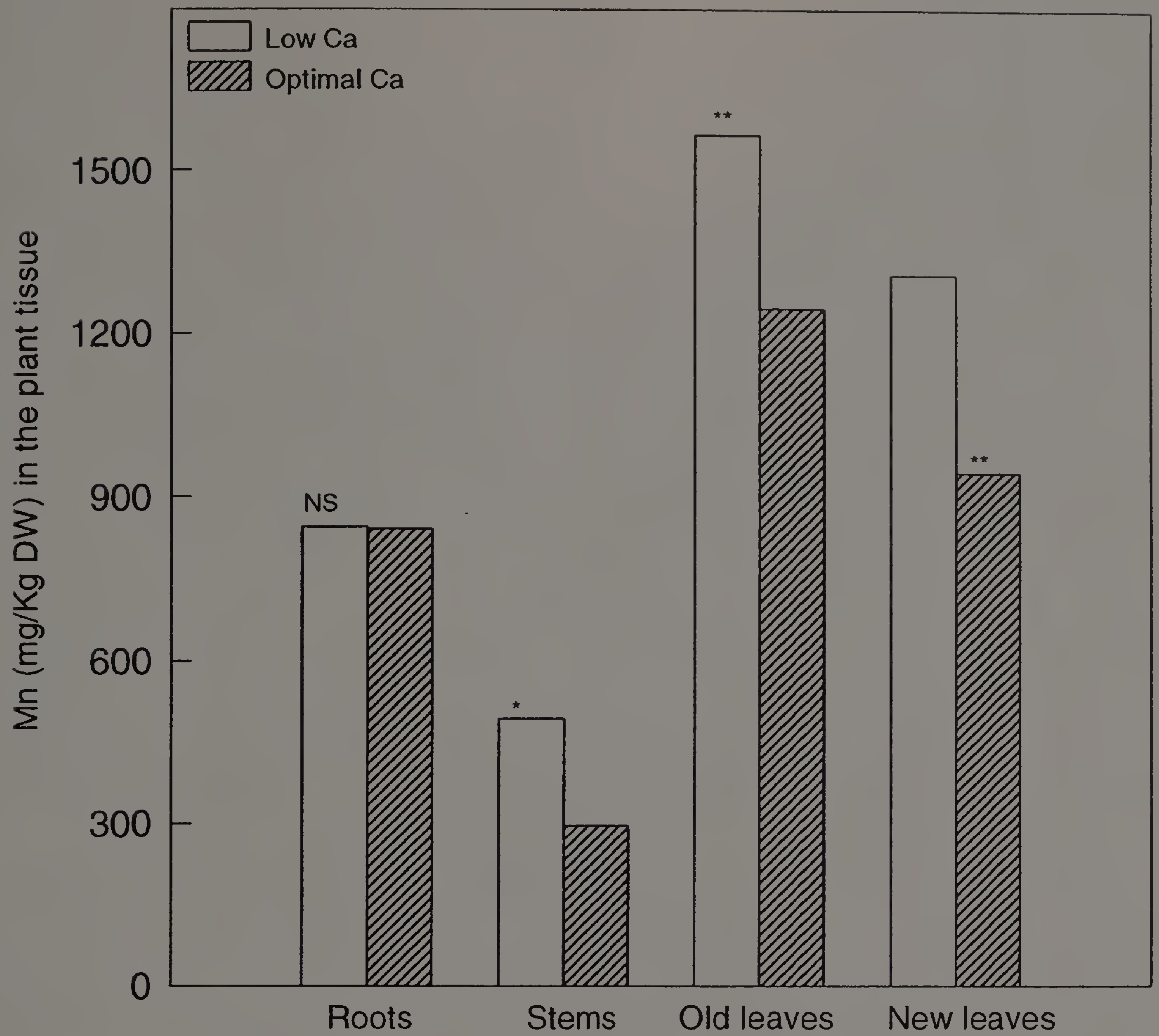

Figure 6.4: Effect of Ca in solution on the level of Mn in the plant tissue (see Appendix F, Table 2)

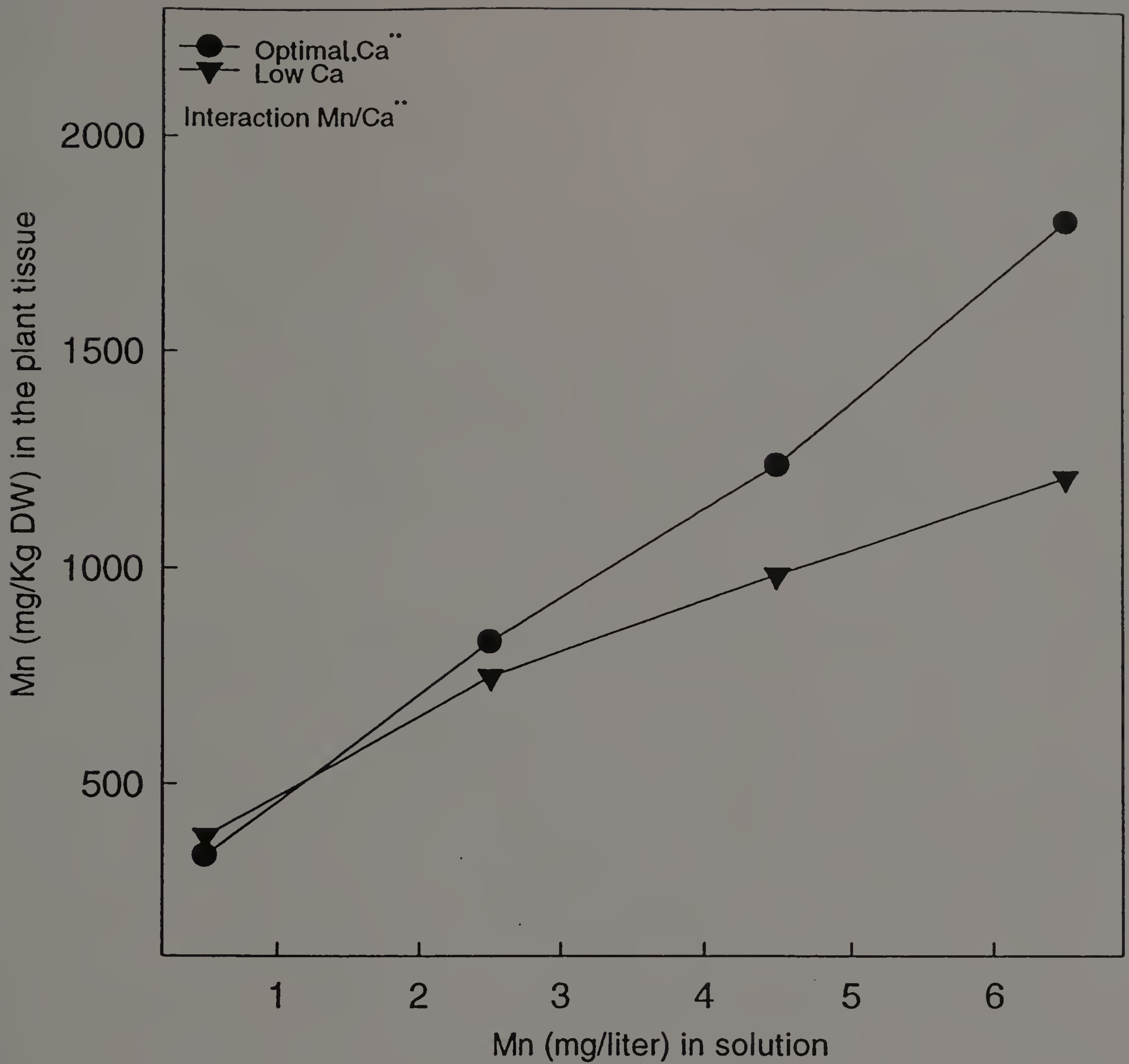

Figure 6.4a: Effect of Mn level in solution on the level of Mn in the plant tissue under low and optimal levels of Ca in solution (see Appendix F, Table 2)

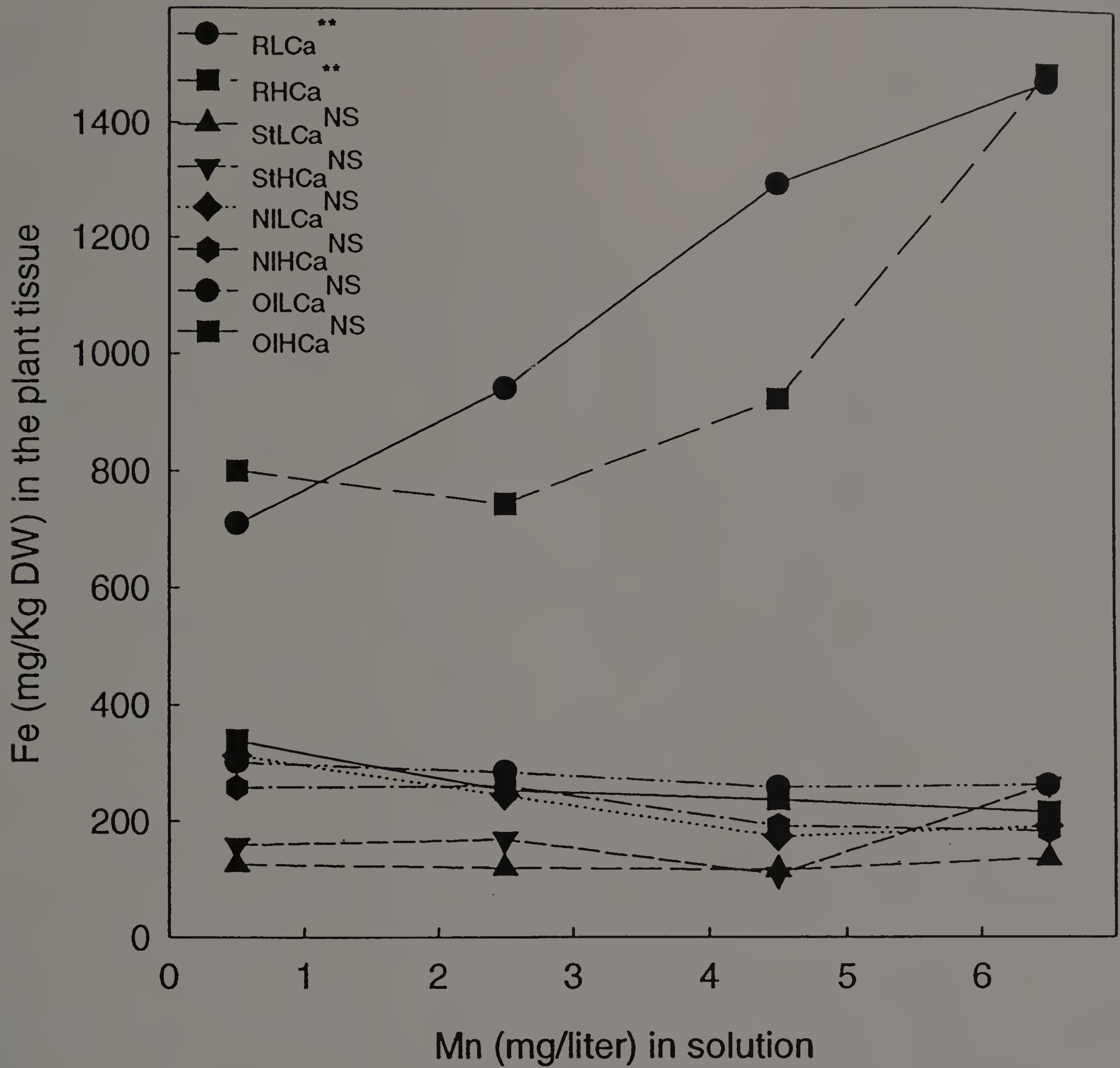

Figure 6.5: Effect of Mn level in solution on the Fe level in the plant tissue under low (L) and optimal (H) levels of Ca (see Appendix F, Table 2)

Somers and Shive (1942) suggested that the unfavorable effect of excess Mn on Fe may be due to the Mn catalysis of the oxidation of the physiologically active form of Fe (Fe^{2+}) to the inactive form (Fe^{3+}). They also suggested the probable formation of an insoluble ferric-phosphate-organic complex.

6.4.4.3 Calcium

Manganese in the solution had no significant effect on Ca in the roots (Appendix F). It however had a significant effect on Ca in the stems and old leaves and a highly significant effect on Ca concentration in the young leaves (Appendix F). As Mn in the solution increased, Ca concentration decreased in the stem, old and young leaves (Figure 6.6).

These results are similar to those of Bekker et al. (1994), Foy et al. (1981) and Horst and Marshner (1978a). They found that the Ca content of cotton, bean and peanut decreased markedly as the Mn concentration of the medium increased. Clark et al. (1981) and Galvez et al. (1989) also found that excess Mn concentration in the nutritive medium suppressed Ca concentration in the plant tissue.

Excess Mn inhibits the translocation of Ca into the shoot apex (Horst and Marshner, 1990). This inhibition might be related to the fact that high Mn levels decrease the cation exchange capacity of the leaf tissue (Horst and Marshner, 1978a) and decrease the IAA levels in the areas of new growth (Morgan et al., 1966; Morgan et al., 1976). Auxin was reported to be responsible for the formation of new binding sites for the transport of Ca to the apical meristems (Horst and Marshner, 1978a).

Calcium level in the nutrient solution had a highly significant effect on Ca concentration in the roots, stems, old leaves and young leaves (Appendix F). Calcium

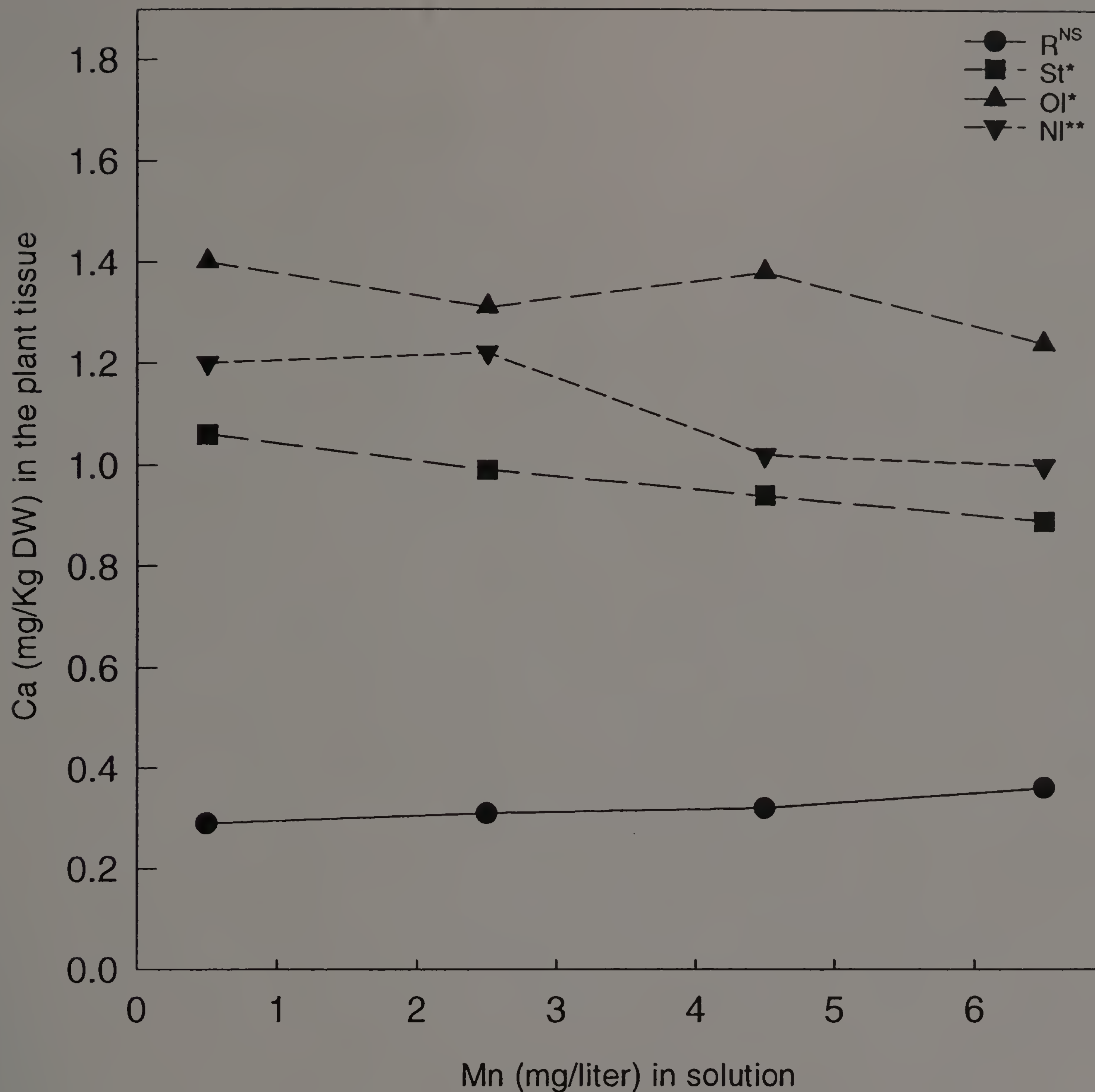

Figure 6.6: Effect of Mn level in solution on the level of Ca in the plant tissue (see Appendix F, Table 2)

was much higher in the roots, stem, old leaves and young leaves when Ca in the nutrient solution was optimal rather than low (Figure 6.7). Our results are similar to those of Gunter et al. (1996) and Makus (1998). They found that increased Ca in the medium resulted in increased Ca in potato and strawberries.

Under the conditions of this experiment, the interaction between Mn and Ca in the nutrient solution had no effect on Ca in the tissue of the plant parts (Appendix F).

6.4.4.4 Magnesium

Manganese in the solution had no significant effect on Mg in the roots, stems, and old leaves, but did have a significant effect on Mg in the young leaves (Appendix F). Magnesium in the young leaves decreased as Mn in the solution increased (Figure 6.8).

This decrease in Mg in the young leaves may have been due to reduced Mg uptake by marigold. Heenan and Campbell (1981) found that large concentrations of Mn in the medium can induce Mg deficiency in the plant. Kazda and Znacek (1989) and Heenan and Campbell (1981) reported that excess Mn may reduce Mg uptake by up to 50% due to competition. The competition between Mg and Mn for binding sites in the roots during absorption, inhibits Mg absorption, since Mn competes more effectively than Mg and even blocks the binding sites for Mg (Horst and Marshner, 1990).

Calcium level in the nutrient solution had a highly significant effect on Mg in the roots, stems, old leaves and young leaves (Appendix F). Magnesium in the plant was greater when Ca in the medium was low (Figure 6.9).

High Ca in the medium inhibits Mg influx. Moore et al. (1961) and Maas and Ogata (1971) found that high Ca in the medium reduced Mg uptake rate by suppressing the Mg transport capacity of the roots rather than competing with Mg for absorption sites.

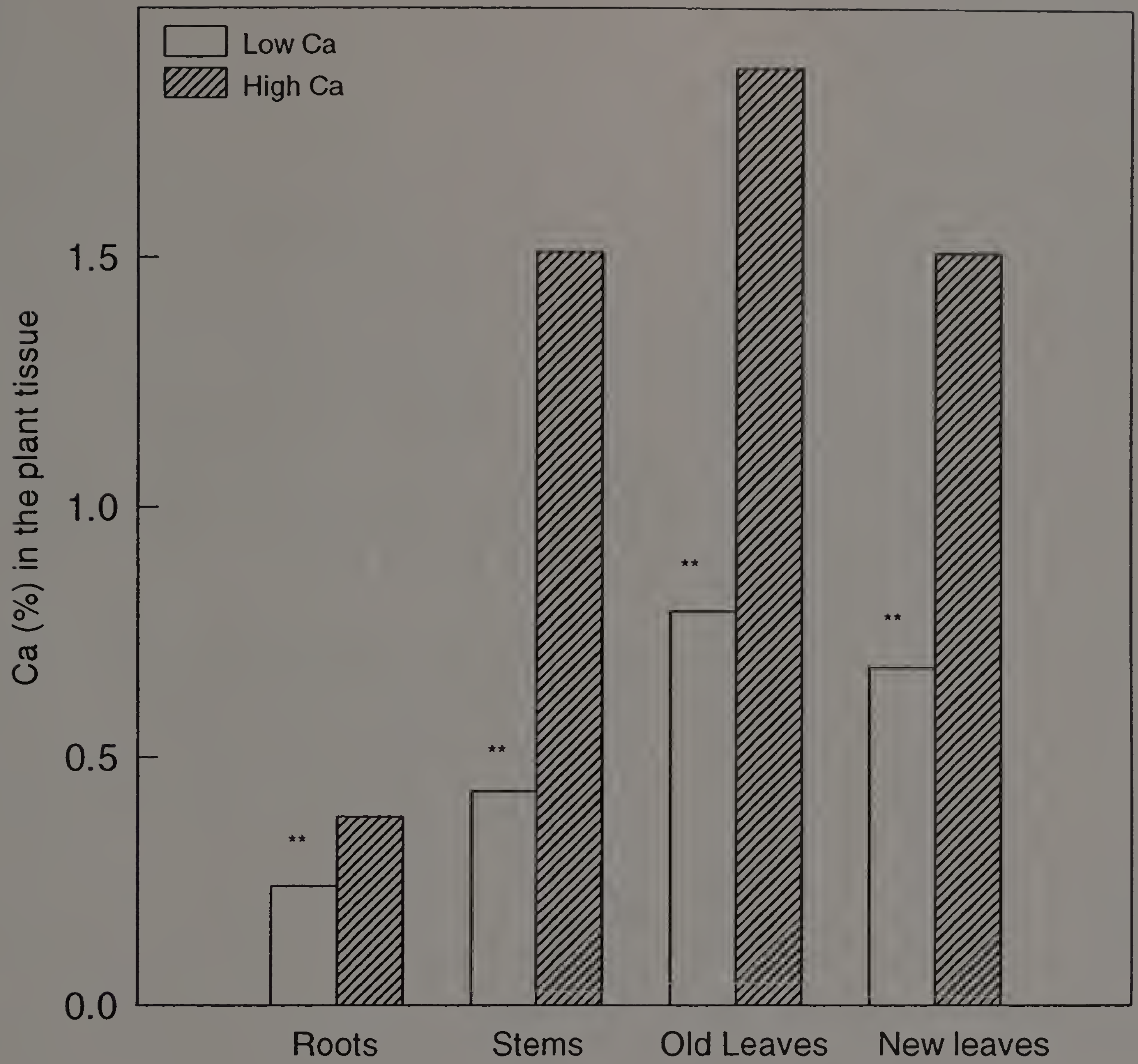

Figure 6.7: Effect of Ca level in solution on the level of Ca in the plant tissue (see Appendix F, Table 2)

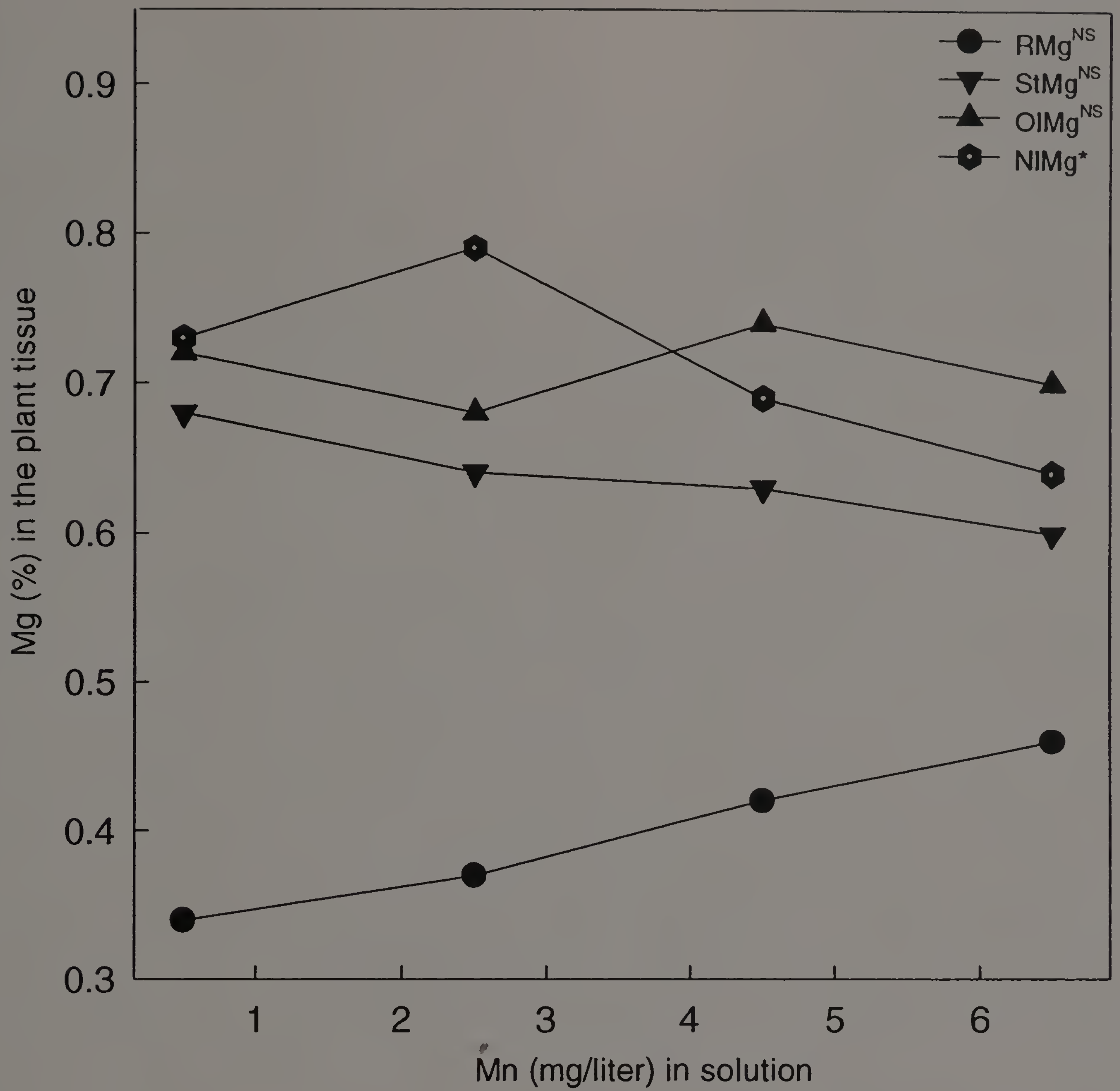

Figure 6.8: Effect of Mn level in solution on the level of Mg in the plant tissue (see Appendix F, Table 2)

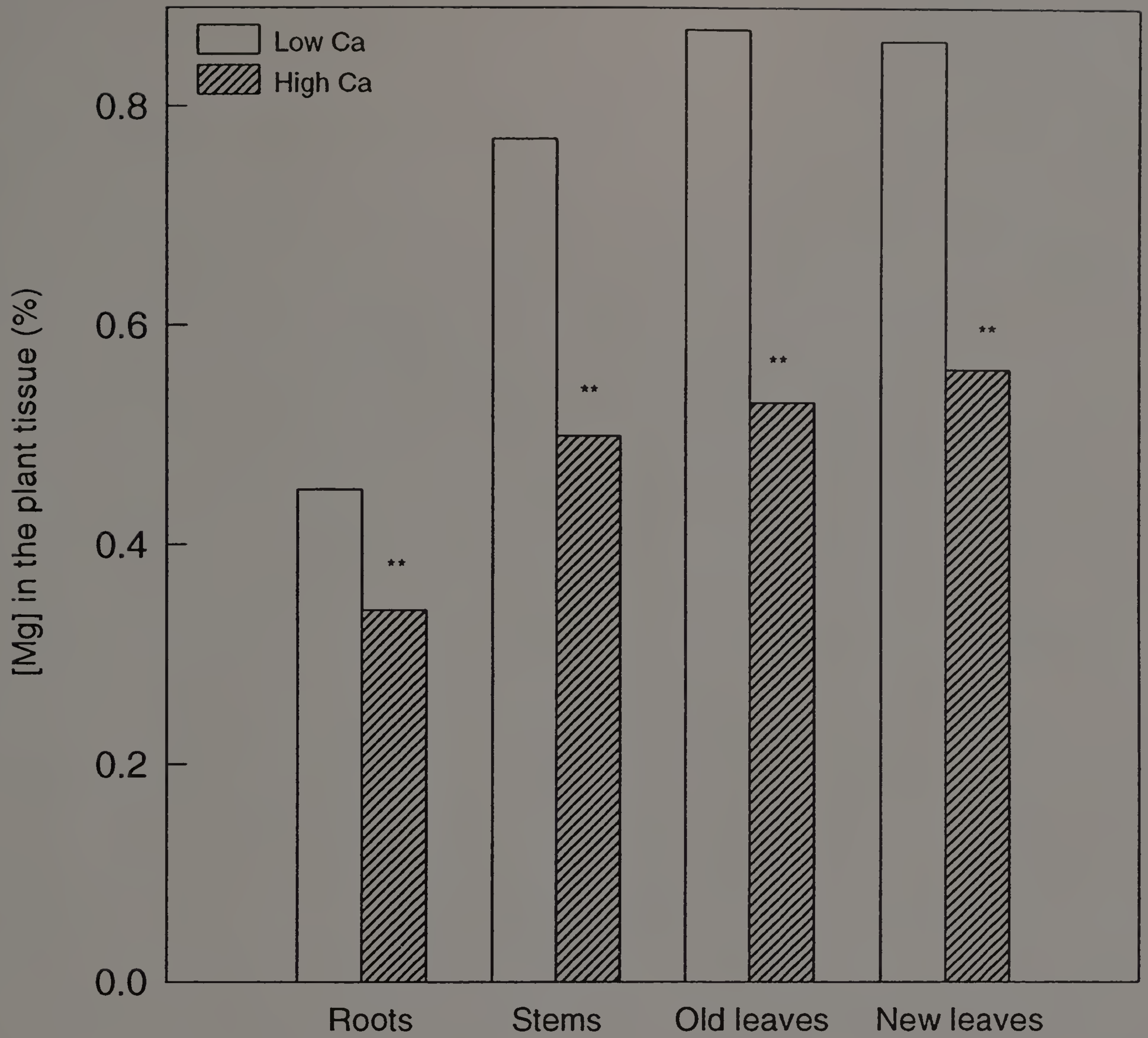

Figure 6.9: Effect of Ca level in solution on the level of Mg in the plant tissue (see Appendix F, Table 2)

6.5 CONCLUSION

Low Ca level in the nutrient solution reduced the critical toxicity solution concentration of Mn in marigold from 4.5 to 2.5mg/l. High concentrations of Mn in the nutrient solution combined with a low concentration of Ca resulted in stunted plants with curled deformed leaves. The leaves were covered with small brown spots and chlorotic almost bleached patches. The tips of the leaves and edges were necrotic and the roots were brittle and brown.

The small brown spots and the brown color of the leaves may have resulted from localized precipitations of Mn as Mn oxide (MnO_2). The chlorotic bleached patches on the leaves may have been due to Mn-induced Fe deficiency. The deformation and crinkling of the leaves and the necrosis on the tips and edges may be due to Mn-induced Ca deficiency.

Results suggest that the interaction between Mn and Ca in the solution had a highly significant effect on Mn and Fe in the plant tissue but had no significant effect on Ca and Mg. High Mn in the solution resulted in increased Mn in all the plant parts. This increase was greater when Ca in the solution was low rather than optimal. Calcium probably regulates Mn toxicity by reducing Mn absorption and transport to the shoot.

High Mn in the solution resulted in increased Fe in the roots. This increase was greater when Ca in the solution was low.

High Mn in the solution resulted in decreased Ca in the stem, old leaves and young leaves; and decreased Mg in the young leaves. This decrease in Ca and Mg as a result of excess Mn was not affected by the level of Ca in the solution.

Decreased Mg in the young leaves may have been due to reduced Mg uptake by marigold. Decreased Ca may have been due to inhibited translocation of Ca into the shoot apex. Manganese decreases the cation exchange capacity of the leaf tissue and decreased the IAA levels in the areas of new growth. Decreased auxin results in limited formation of new binding sites for the transport of Ca to the apical meristems.

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CHAPTER 7

MANGANESE TOXICIY IN MARIGOLD AS AFFECTED BY MAGNESIUM

7.1 ABSTRACT

Iron/manganese toxicity disorder in marigold has been related to high concentrations of Mn and low concentrations of Mg in the affected leaves. Preplant addition of micronutrients in the media combined with constant feed program and low medium pH create favorable conditions for the development of Mn toxicity in greenhouse crops. Deficiency of Mg is due in part to low medium pH and to lack of Mg in most of the fertilizers used in greenhouse production. To investigate the effect of Mg on Mn toxicity effects (growth, appearance, and nutrient concentrations), a solution culture study with various Mg and Mn concentrations was conducted.

In this experiment, the treatments 10/6.5, 10/4.5, 5/6.5, and 5/4.5mg/l Mg-Mn solution concentrations resulted in stunted plants with significant number of small brown spots, interveinal chlorosis and necrotic symptoms on the tips and margins of the leaves. The treatments 10/0.5, 15/4.5, and 20/4.5, resulted in stunted plants relatively free of symptoms. Treatment 20/6.5, 15/6.5 resulted in stunted plants with blackish brown spots, chlorotic patches and necrotic tips and margins on the leaves. The treatment 20/0.5 and 15/0.5 resulted in fully-grown and healthy looking plants.

Concentrations of Mg as high as the incipient deficiency concentration reduced the critical toxicity solution concentration of Mn in marigold from 4.5 to 2.5mg/l.

High Mn in the nutrient solution affected the plant appearance and reduced the dry weight of the plants. High Mn also increased the concentration of Mn in the roots

and leaves, increased Fe in the roots, reduced the concentration of Fe in the stem and old leaves, reduced Ca in the leaves, and reduced Mg in the plant.

Magnesium concentration in the nutrient solution did not affect the effect of excessive Mn in the medium on Mn, Fe, Ca or Mg in the plant.

7.2 INTRODUCTION AND LITERATURE

High Mn supply combined with low Mg supply disrupts various aspects of plant metabolism, leading to serious physiological and morphological disorders (Davis, 1996; Elamin and Wilcox, 1986b, c). Manganese toxicity has been associated with reduced enzyme and hormone activities, inhibition of ATP synthesis, and reduced respiration rates (Heenan and Campbell, 1981; SirKar and Amin, 1974; Morgan et al., 1966).

Magnesium deficiency has been associated with the breakdown of chlorophyll, large number of small chlorenchyma cells, inhibition of nitrate reduction, inhibition of phytohormone production, reduced stability of cell membrane, reduced regulation of the intra- and extra-cellular balance of cations, and numerous nutritional and physiological disorders in plants (Koranski, 1988; Harrison and Bergman, 1981; Bergmann, 1992; Lyon and Garcia, 1944; Kiss, 1981).

Factors that affect the toxicity of Mn and deficiency of Mg include the concentrations of Mn and Mg in the growth medium; growth medium and moisture content, nitrogen source, plant developmental stage, organic matter and pH, and concentrations of salts, particularly those of Ca, Fe and phosphorus (Foy et al., 1978; Goh and Haynes, 1978; Bachman and Miller, 1995; Harrison and Bergman, 1981; Le Mare, 1977; Reddy and Mills, 1991; Hood et al., 1993). Small changes in these factors can determine the degree of Mn toxicity and Mg deficiency in a given crop.

7.2.1 Concentration of Mn in the Growth Medium

The accumulation of Mn in the leaf tissue may result directly from applying excessive amounts of micronutrients. The continuous use of high levels of peat-lite fertilizers may cause problems if salt levels are not monitored carefully (Sheely, 1990). Preplant addition of micronutrients in the growth medium, combined with constant feed program, creates toxicity problem (Sheely, 1990).

Large concentrations of Mn in the medium can induce Mg deficiency in the plant (Heenan and Campbell, 1981). Excess Mn may reduce Mg uptake by up to 50% (Kazda and Znacek, 1989) due to competition (Heenan and Campbell, 1981). The competition between Mg and Mn for binding sites in the roots during absorption, inhibits absorption of Mg, since Mn competes more effectively than Mg and even blocks the binding sites for Mg (Horst and Marshner, 1990).

7.2.2 Concentration of Mg in the Growth Medium

A second aspect of Fe/Mn toxicity disorder may be deficiency of Mg due in part to low medium pH and to lack of Mg in most of the fertilizers used in greenhouse crop production (Koranski, 1988). Manganese toxicity often can be counteracted by a large supply of Mg (Elamin and Wilcox, 1986a; 1986b; Lohnis, 1960). Magnesium reduced Mn uptake both by excised and intact roots of several plant species (Harrison and Bergman, 1981; Lohnis, 1960; Maas et al., 1969).

The ability of Mg to reduce Mn uptake depends on the concentration of Mn in the medium. Elamin and Wilcox (1986a) found that at high Mn concentration, Mg had little effect on Mn uptake and the plants were able to accumulate toxic levels of Mn at all levels of Mg supply.

Although increasing the concentration of Mg in the medium can alleviate the detrimental effects of high concentrations of Mn, Mg application is not a practical method for the avoidance of Mn toxicity (Davis, 1996). Indeed, using Mg to prevent Mn toxicity would require large applications of Mg, which could lead to serious nutritional imbalance because Mg would interfere with Ca uptake.

In summary, Fe/Mn toxicity disorder in marigold may be caused by Mn toxicity since the affected plants contain high concentrations of Mn in their tissue. Basic factors affecting Mn toxicity in plants include Mg deficiency. The relationship of Mg deficiency to the incidence of Fe/Mn toxicity in marigold needs to be investigated.

In spite of the fact that Fe/Mn toxicity has been related to high concentrations of Mn and low concentrations of Mg in the affected leaves, no research has been done on the effect of Mg nutrition on manganese toxicity in marigold. Available information on the interaction between Ca and Mn are meager. This experiment was designed to investigate the effect of Mg on Mn toxicity in marigold.

The objectives were: (1) to determine the effect of Mg on the sensitivity of marigold to high levels of Mn in the nutrient solution; (2) to determine the effect of Mg on the critical toxicity solution concentration of Mn; and (3) to determine the effect of the interaction between Mg and Mn in the nutrient solution on Mn, Fe, Ca and Mg in the plant tissue.

7.3 MATERIALS AND METHODS

Seeds of marigold (*Tagetes erecta* L. ‘First lady’) were planted in vermiculite #3 on January 13, 1998 in the greenhouse. Three-week-old seedlings were transplanted into 800ml half strength nutrient solution in 1 liter opaque plastic containers. The basal

nutrient solution contained 48 mg/l Mg, 100 mg/l Ca, 79 mg/l potassium, 63 mg/l phosphorus, 75 mg/l nitrate-nitrogen, 75 mg/l ammonium-nitrogen, 2.3 mg/l Fe, 0.02 mg/l copper and 0.5 mg/l of Mn, boron, zinc and molybdenum. After 10 days of growth in the half strength basal nutrient solution, the seedlings were transferred to treatment solutions. Manganese treatments were 0.5; 2.5; 4.5 and 6.5mg/l nutrient solution. Manganese was added to the nutrient solution as $MnCl_2$. Magnesium treatments were 5; 10; 15; and 20mg/l. Magnesium was added as $MgSO_4$-$7H_2O$. Based on the results of Chapter 3, the concentration 20mg/l is considered optimal concentration of Mg for marigold growth. Magnesium at 10mg/l is considered incipient deficiency solution concentration of Mg in marigold. This concentration is enough to fulfill the metabolic needs of the plant but not enough for optimal growth. The hydroponic cultures were aerated continuously and the nutrient solution was replaced with a fresh one every week. Deionized water was added to the containers as needed to maintain volume.

Eighty containers consisting of 4 Mn treatments, 4 Mg treatments and 5 replicates were placed on two benches in the greenhouse in a completely randomized design. Plants were grown under ambient light conditions. The temperature was maintained as closely as possible to 21/17 °C (day/night). The plants were harvested on March 29, 1998 into roots, stems, old leaves and young leaves separately. The roots were rinsed with tap water then deionized water to discard nutrients from the root surface.

The plant parts were dried at 70 °C for 48 hours and weighed. The different plant parts were ground (20 mesh) and analyzed by inductive coupled plasma (ICP) (Appendix B) for Mn, Fe, Ca, and Mg contents. The data obtained were statistically analyzed using

Analysis of Variance (ANOVA) and procedure Mixed to test for significance of main effects and interactions. Terms were considered significant at $p<0.05$.

7.4 RESULTS AND DISCUSSION

7.4.1 Symptoms

The nature of the symptoms and their severity depended on the treatment. The plants grown in 20/0.5 and 15/0.5 mg/l Mg-Mn did not show any symptoms and they looked big and healthy, while the plants grown in 15/2.5 and 15/4.5 mg/l Mg-Mn did not show significant symptoms but the plants were smaller. The plants grown in 20/6.5 and 15/6.5 mg/l Mg-Mn were small and showed typical symptoms of Mn toxicity as described in chapter 5: the symptoms appeared in the old leaves first then spread toward the rest of the plant. The symptoms started as small blackish brown spots on the leaves. Patches of chlorosis appeared on the tips and margins of the leaves afterward. When the plants were exposed to the same treatment for a longer time the chlorotic areas became necrotic.

The plants treated with 10/6.5, 5/6.5, 10/4.5, and 5/4.5 mg/l Mg-Mn showed symptoms. The symptoms, however, were much more severe in the plants treated with 5/6.5 and 5/4.5 Mg-Mn. The plants were also very small per comparison to the plants grown with the other treatments. The symptoms included small brown spots on the surface area of the leaves, deformation of the leaves, interveinal and marginal chlorosis, rolling and crinkling of necrotic leaf margins and brown brittle roots. After being exposed to the treatment for a longer time, necrosis developed on the margins and at the tips of the leaves.

The plants treated with 10 mg/l Mg-0.5 mg/l Mn or 10 mg/l Mg-2.5 mg/l Mn did not show significant symptoms but the plants were smaller than those treated with 10 mg/l Mg-0.5 mg/l Mn.

The small brown spots shown in the affected leaves and the brown color of the roots may have been associated with accumulation of Mn as precipitated Mn oxide (MnO_2) in the leaves and roots (Horst and Marshner, 1978a, b, c).

Interveinal chlorotic patches of the leaves may have been associated with Mn-induced Fe deficiency (Horst and Marshner, 1978a). Local accumulations of Mn may interfere with Fe dependent metabolic functions and thus lead to Fe deficiency (Horst and Marshner, 1978a).

The deformation and curling of the leaves may be due to deficiency of plant hormones. Toxic concentrations of Mn increase the activities of polyphenoloxidase, peroxidase and IAA oxidase, which result in decomposition of auxins (Morgan et al., 1966; Cheng and Ouelette, 1971; Kamprath and Foy, 1971; Foy, 1973).

7.4.2 Dry Weight

Manganese concentration in the nutrient solution had a highly significant effect on root dry weight, shoot dry weight and total dry weight. When Mn in the solution increased the dry weight of the roots and shoots decreased (Figure 7.1).

Magnesium concentration in the solution had a significant effect on the root dry weight, but it had no significant effect on the shoot and total dry weight. As Mg concentration decreased in the solution, the root dry weight decreased (Figure 7.2).

Under the conditions of this experiment, there was no significant effect of the interaction between Mg and Mn in the solution on the plant dry weight (Appendix G).

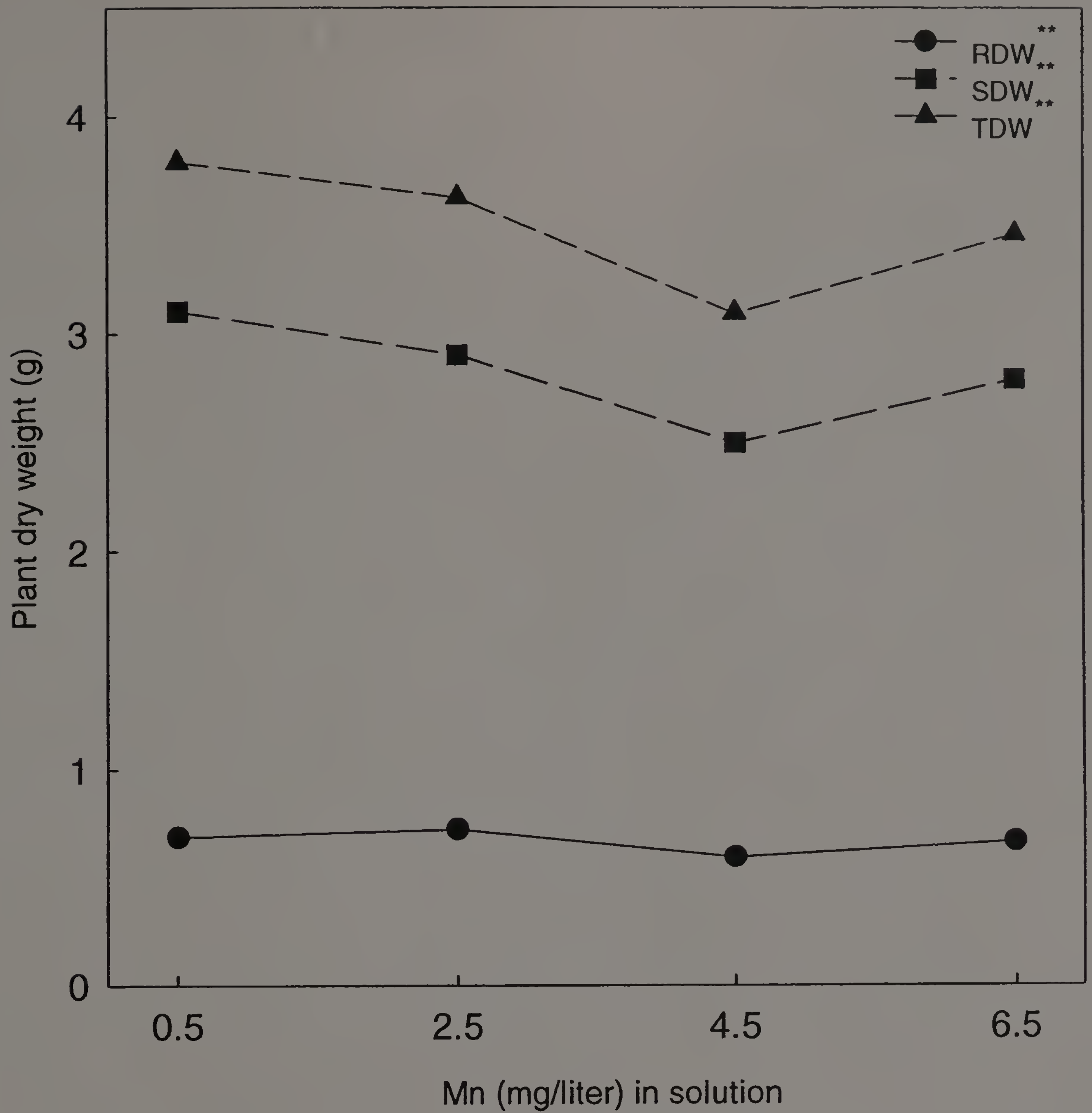

Figure 7.1: Effect of Mn level in solution on the plant dry weight (see Appendix G, Table 1)

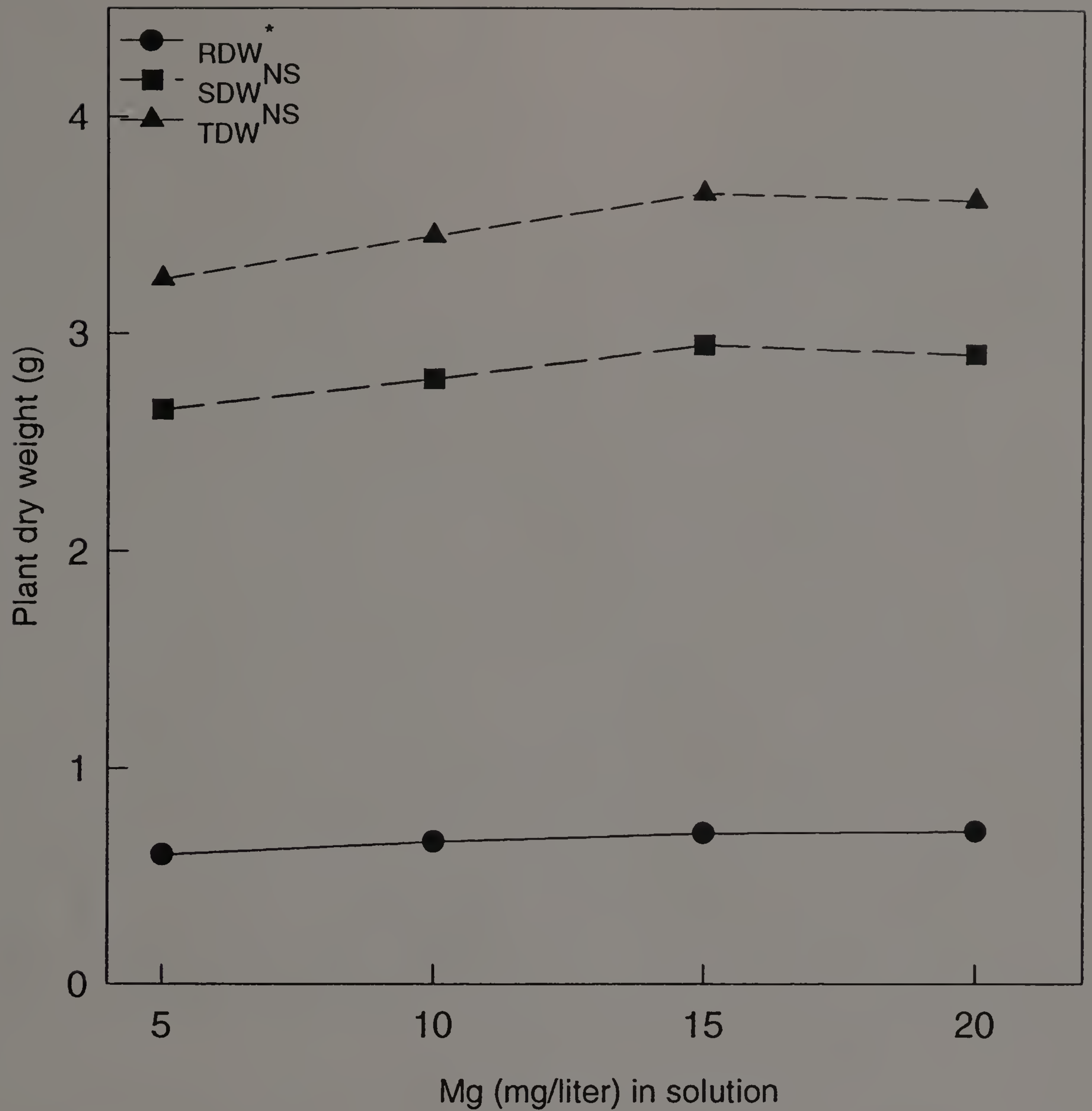

Figure 7.2: Effect of Mg level in solution on the plant dry weight (see Appendix G, Table 1)

The reduced plant growth associated with high concentrations of Mn in the medium may have resulted from the depression of phytohormone metabolism by excess Mn (Marshner, 1978a).

Low Mg in the nutrient solution resulted in a decrease in the root dry weight and did not affect the shoot dry weight (Figure 7.2). These results are different from what was expected considering the crucial role of Mg in plant metabolism (Chapter 3). The results obtained in this experiment may have been due to the concentrations of Mg used in this experiment combined with the conditions of growth.

7.4.3 Critical Toxicity Concentration of Mn

In this experiment, plant appearance suggested that low concentrations of Mg in the nutrient solution decreased the critical toxicity solution concentration of Mn in marigold from 6.5 to 2.5mg/l. At optimal concentration of Mg (20 mg/l), in the medium, 2.5 and even 4.5mg Mn/l did not cause any significant symptoms to appear. However, when Mg level in the medium was at the incipient deficiency concentration (10mg/l), concentrations of Mn as low as 4.5 resulted in significant symptom development that affected the plant appearance. These results suggest that low concentrations of Mg in the medium decrease the critical toxicity concentration of Mn. Some researchers actually found that Mg reduces Mn uptake by plants (Harrison and Bergmann, 1981; Lohnis, 1960; Maas et al., 1969).

7.4.4 Nutrient Concentrations in the Plant Tissues

7.4.4.1 Manganese

Manganese in the solution had a highly significant effect on Mn in the roots, young leaves and old leaves and no significant effect on Mn in the stem (Appendix G).

Increased Mn level in the medium resulted in increased Mn in the roots, old leaves and young leaves (Figure 7.3). The increase of Mn in the roots and leaves as a result of increased Mn in the nutrient solution was not affected by Mg in the nutrient solution (Figure 7. 3a). In other words the interaction between Mn and Mg in the nutrient solution was not significant (Appendix G).

7.4.4.2 Iron

Manganese in the nutrient solution had no significant effect on Fe in the new leaves. Manganese level, however had a highly significant effect on Fe in the roots, stem and old leaves (Appendix G). As Mn level in the nutrient solution increased, Fe concentration increased in the roots and decreased in the stem and old leaves (Figure 7.4). This effect of Mn in the nutrient solution on Fe level in the plant tissue was not affected by Mg concentration in the nutrient solution. In other words, the interaction between Mn and Mg was not significant (Appendix G).

Some of the symptoms of Mn toxicity in marigold were bleached patches on the leaves, which are typical symptoms of Fe deficiency. Perhaps excess Mn in the medium caused a Mn-induced Fe deficiency. Yet, the concentration of Fe in the roots increased as the concentration of Mn increased in the medium. This suggests that high Mn concentration in the medium does not inhibit Fe absorption, but rather transport or accumulation in the shoots. Others have found that excess Mn in the medium does not affect Fe absorption, but it unfavorably affects Fe action in the plant tissues (Rippel, 1923; Chapman, 1931; Millikan, 1949).

Somers and Shive (1942) suggested that the unfavorable effect of excess Mn on Fe may be due to the Mn catalysis of the oxidation of the physiologically active form of

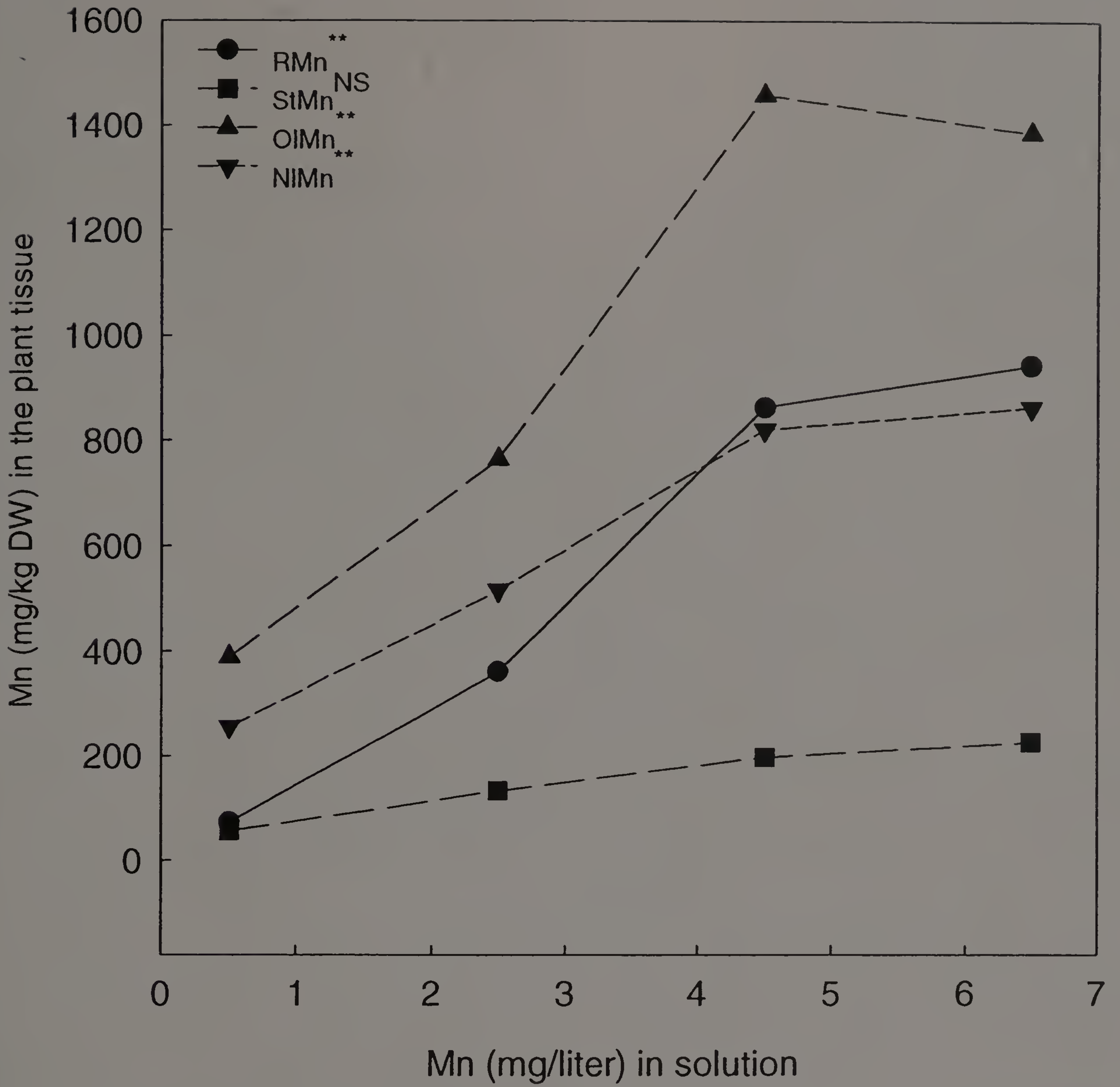

Figure 7.3: Effect of Mn level in solution on the level of Mn in the plant tissue (see Appendix G, Table 2)

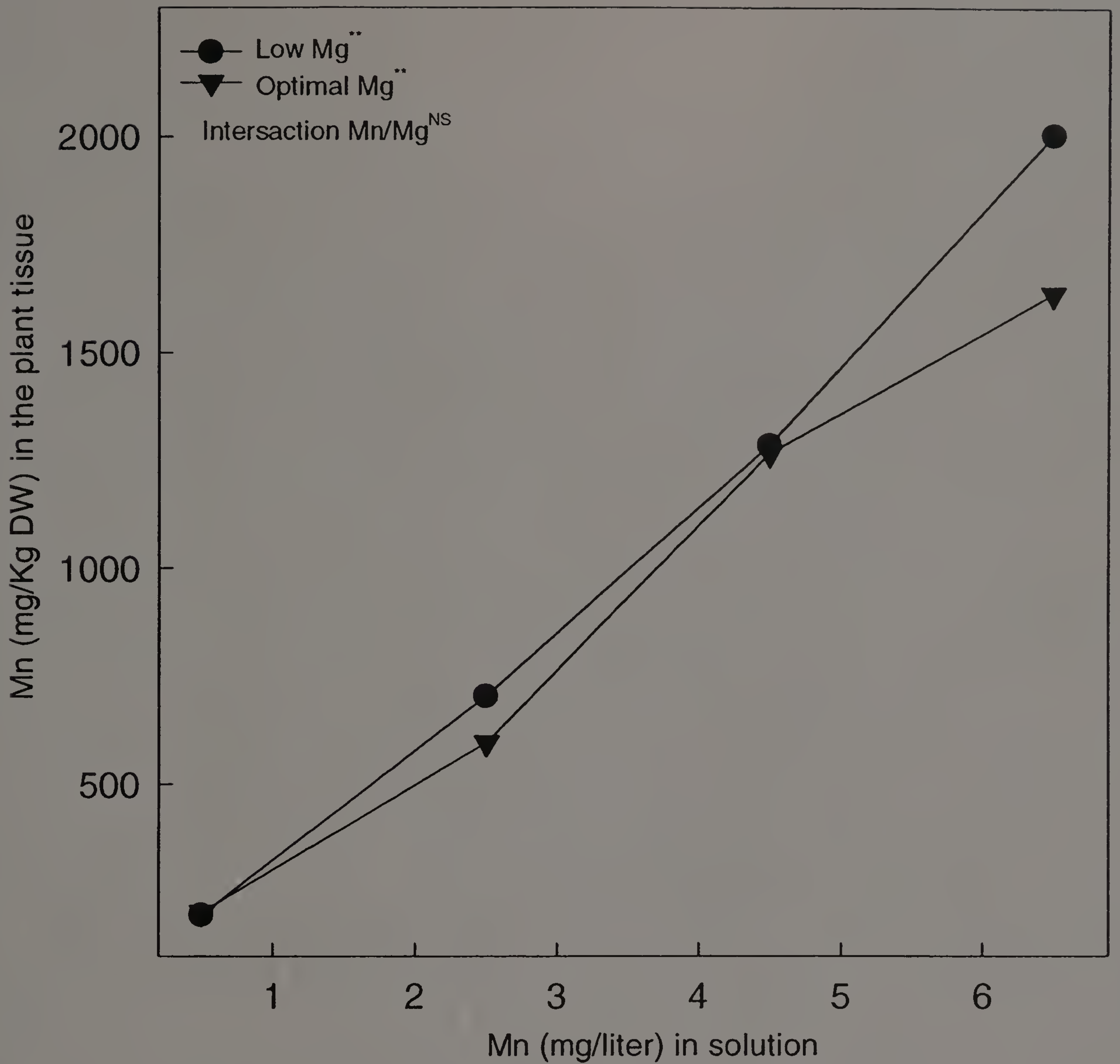

Figure 7.3a: Effect of Mn level in solution on the level of Mn in the plant tissue under low and optimal levels of Mg in solution (see Appendix G, Table 2)

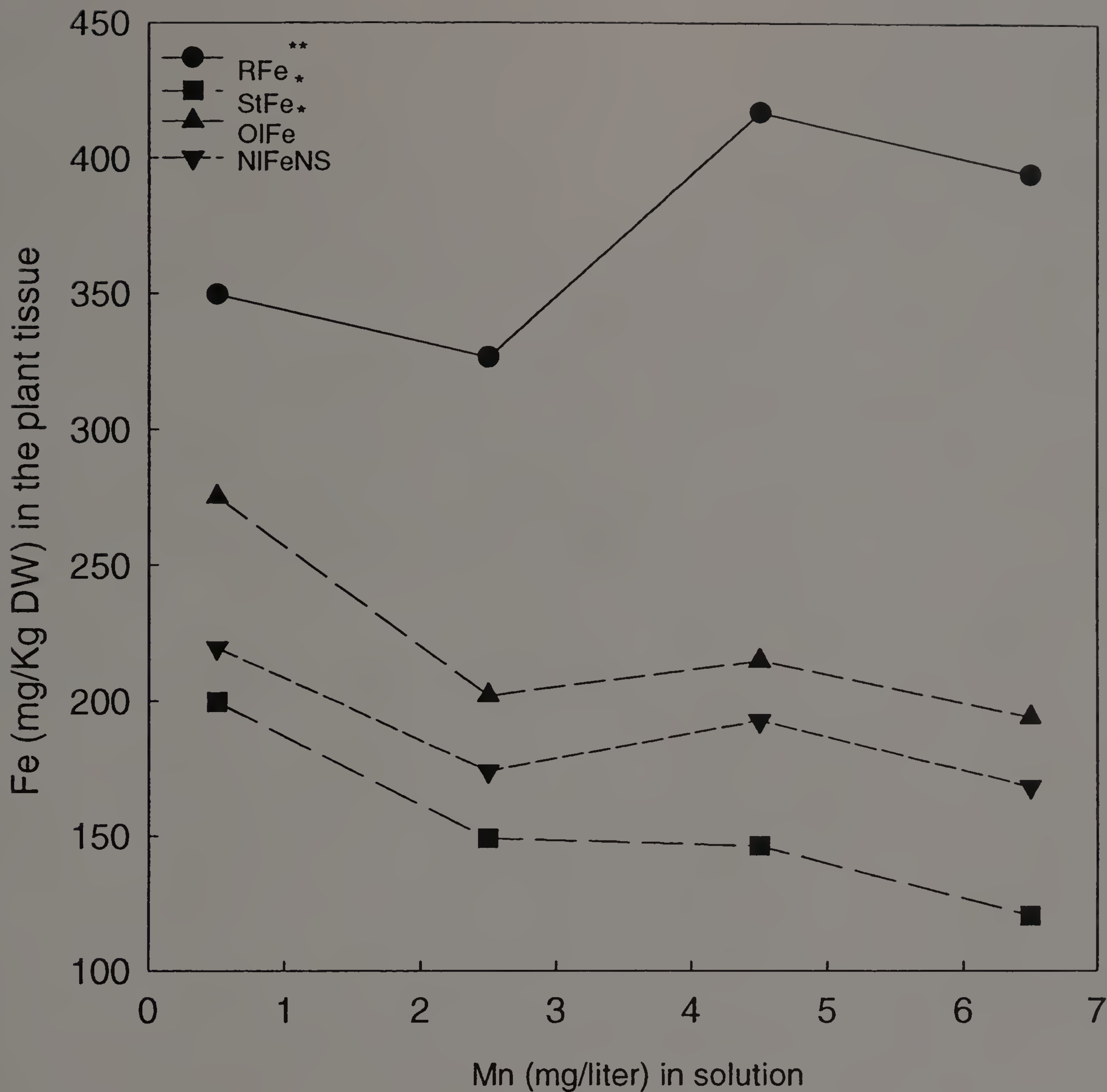

Figure 7.4: Effect of Mn level in solution on the level of Fe in the plant tissue (see Appendix G, Table 2)

Fe (Fe^{2+}) to the inactive form (Fe^{3+}). They also suggested the probable formation of an insoluble ferric-phosphate-organic complex.

Magnesium concentration in the medium had no significant effect on Fe in the shoots, but had a highly significant effect on Fe in the roots (Appendix G). As the Mg in the solution decreased below 15mg/l, Fe in the roots increased (Figure 7.5).

Our results differ from those reported by Straub and Wurm (1971). They found that Mg concentration in the medium affected Fe concentration in the leaves. They also found that the leaves of Mg deficient apple plants had low Fe contents. In a study on maize and sunflower plants, Scherer (1978) found that Mg level was high in the plant tissue when the plants were deficient in Fe. Scholl (1979a, b) reported the same results. They found that Mg content increased from 9% in normal leaves to 14% in Fe deficient leaves.

7.4.4.3 Calcium

Manganese in the solution had no significant effect on Ca concentration in the roots and stem (Appendix G). It however had a significant effect on Ca concentration in the new leaves and a highly significant effect on Ca concentration in the old leaves (Appendix G). As Mn in the solution increased, Ca decreased in the leaves (Figure 7.6). This decrease of Ca in the leaves due to excess Mn was not affected by Mg level in the medium. In other words, the interaction between Mn and Mg in the medium was not significant (Appendix G).

These results are similar to those of Bekker et al. (1994), Foy et al. (1981) and Horst and Marshner (1978a). They found that Ca content of cotton, been and peanut decreased markedly as the Mn concentration in the medium increased. Clark et al. (1981)

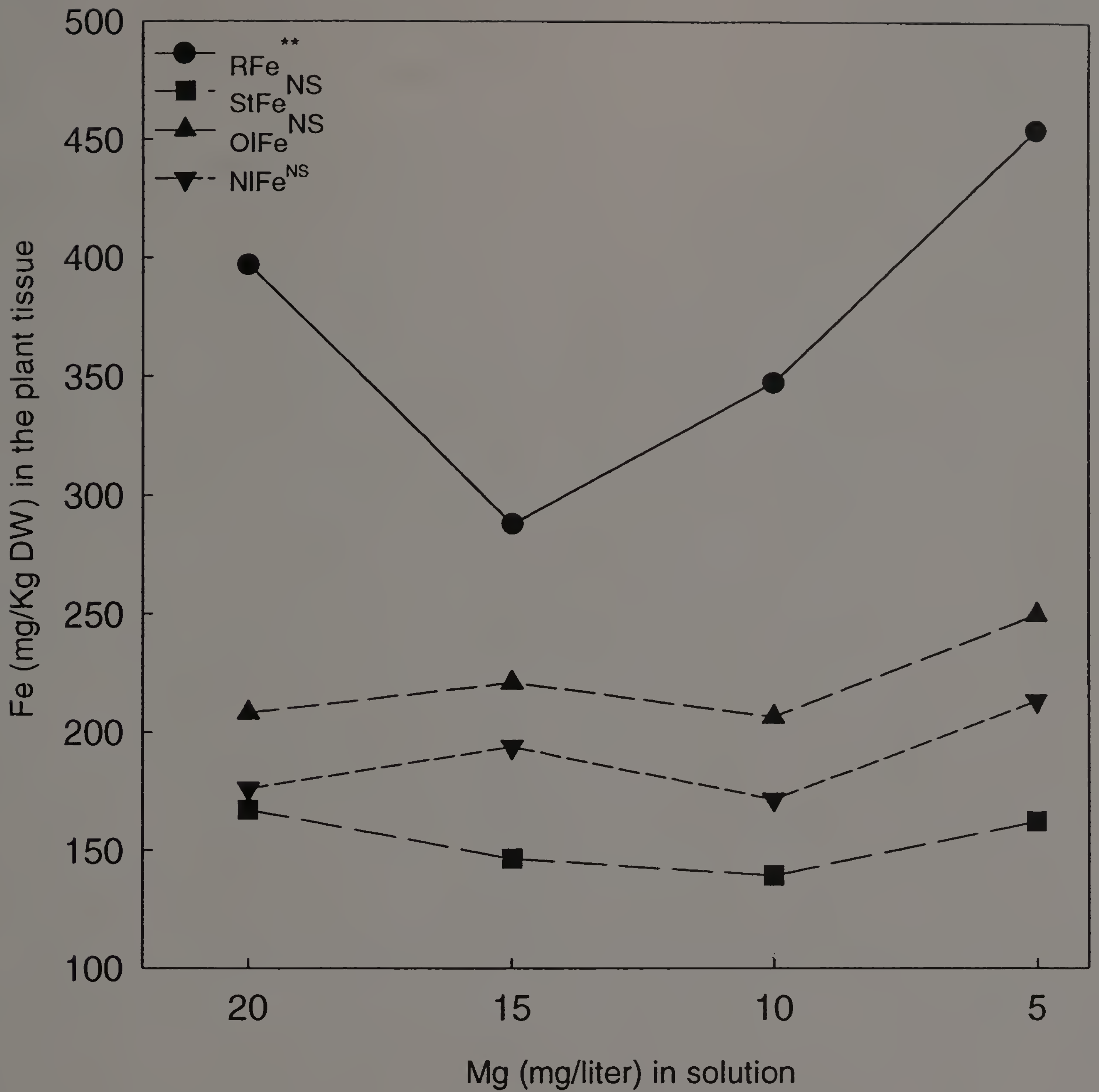

Figure 7.5: Effect of Mg level in solution on the level of Fe in the plant tissue (see Appendix G, Table 2)

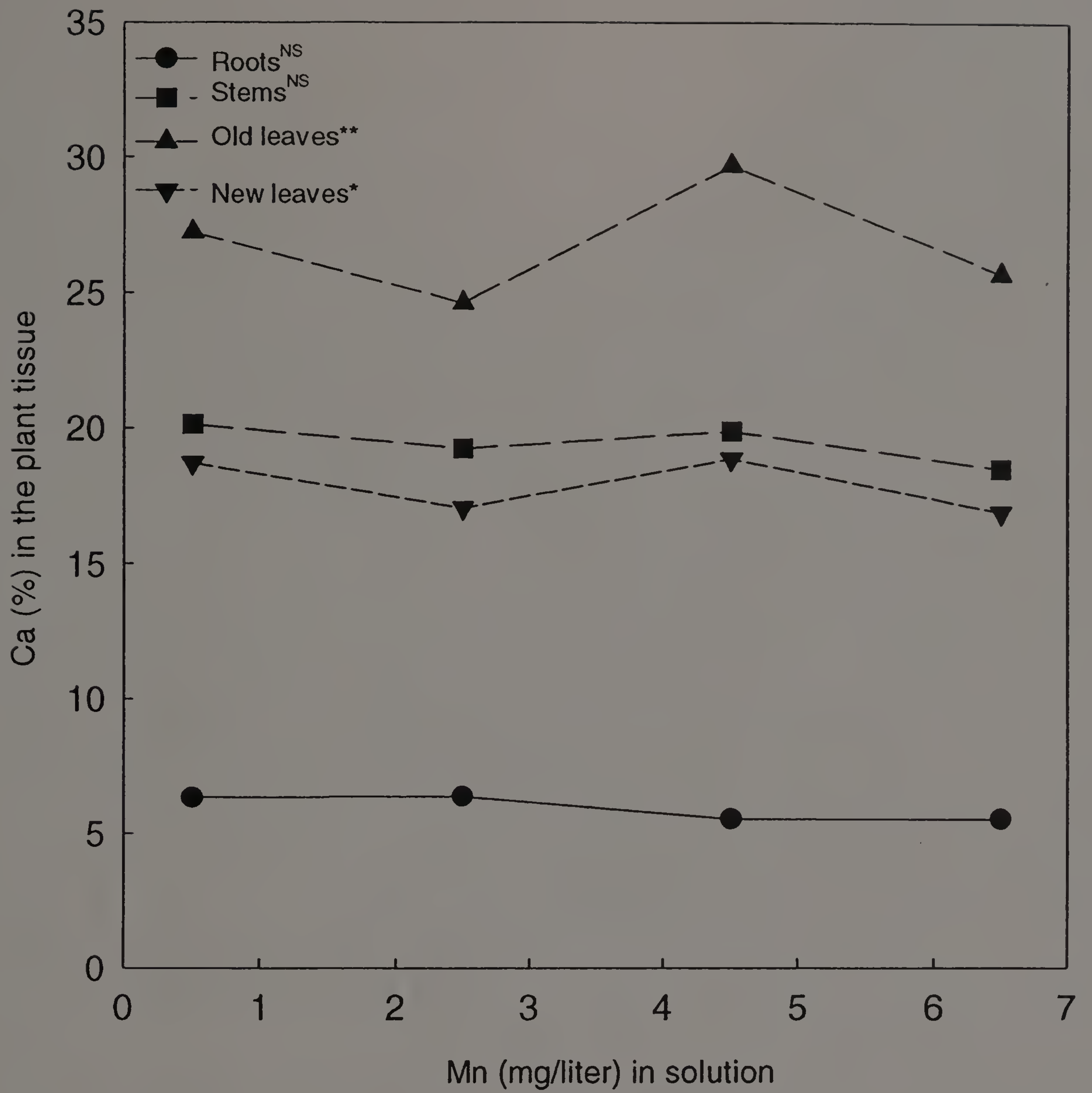

Figure 7.6: Effect of Mn level in solution on the level of Ca in the plant tissue (see Appendix G, Table 2)

and Galvez et al. (1989) also found that excess Mn concentration in the nutritive medium suppressed Ca in the plant tissue.

Excess Mn inhibits the translocation of Ca into the shoot apex (Horst and Marshner, 1990). This inhibition might be related to the fact that high Mn levels decrease the cation exchange capacity of the leaf tissue (Horst and Marshner, 1978a) and decrease the IAA levels in the areas of new growth (Morgan et al., 1966; Morgan et al., 1976). Auxin was reported to be responsible for the formation of new binding sites for the transport of Ca to the apical meristems (Horst and Marshner, 1978a).

Under the conditions of this experiment, Mg in the solution had no significant effect on Ca concentration in the plant tissue (Appendix G).

7.4.4.4 Magnesium

Manganese concentration in the solution had a highly significant effect on the overall concentration of Mg in the plant (Appendix G). The effect of Mn in the medium on Mg in the plant tissue was the same regardless of the plant part. In other words the interaction between Mn level in the medium and plant part was not significant (Appendix G). Results show that the overall Mg concentration in the plant tissue decreased as Mn in the nutrient solution increased (Figure 7.7). This decrease was not affected by Mg concentration in the solution. Indeed, the interaction between Mn and Mg in the nutrient solution was not significant (Appendix G).

The decrease in Mg in the plant due to high concentration of Mn in the solution may be due to reduced Mg uptake by marigold. Heenan and Campbell (1981) found that large concentrations of Mn in the medium can induce Mg deficiency in the plant (Mn-induced Mg deficiency). Kazda and Znacek (1989) and Heenan and Campbell (1981)

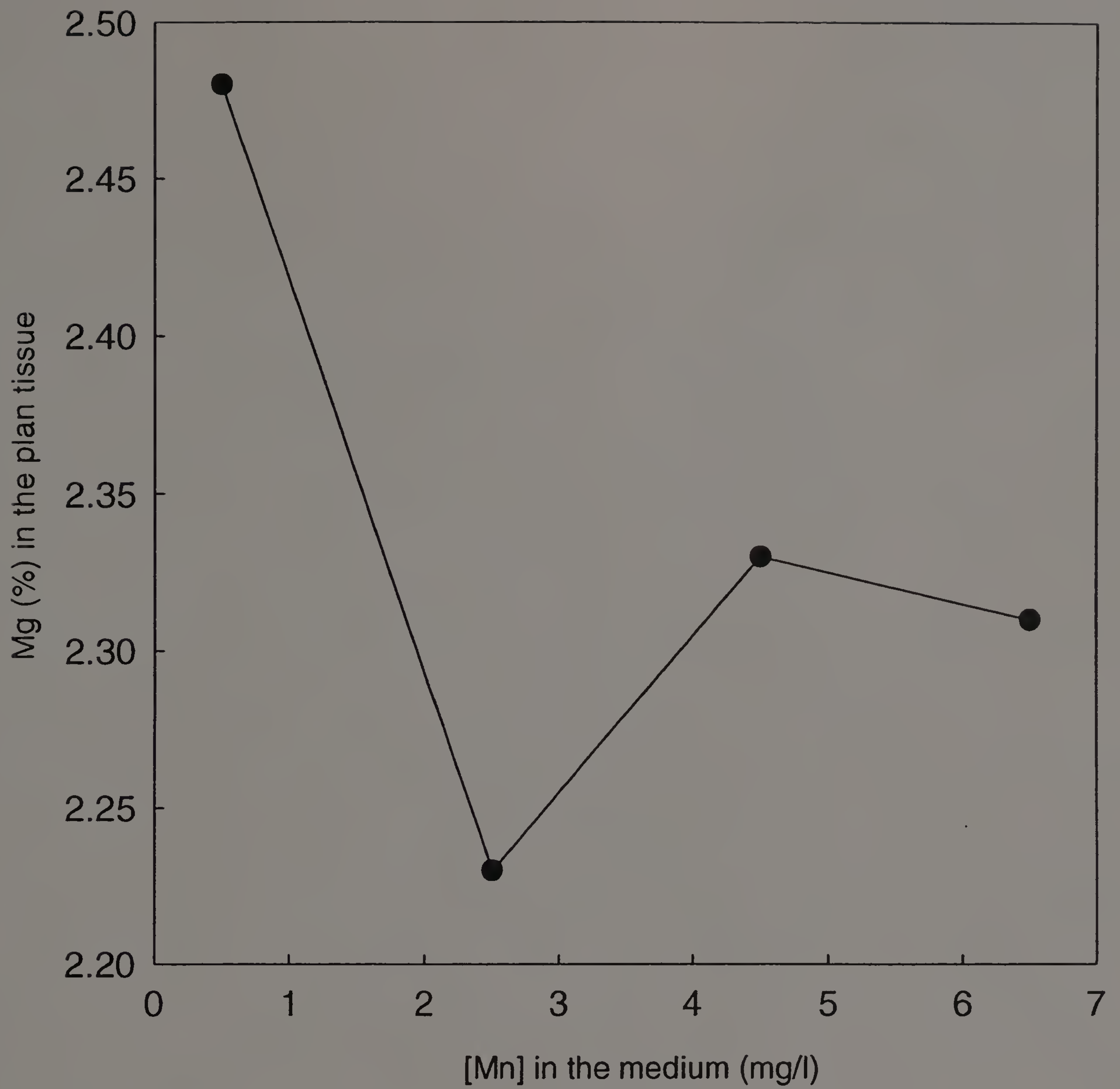

Figure 7.7: Effect of Mn level in solution on the level of Mg in the plant tissue (see Appendix G, Table 2)

reported that excess Mn may reduce Mg uptake by up to 50% due to competition. The competition between Mg and Mn for binding sites in the roots during absorption, inhibits Mg absorption, since Mn competes more effectively than Mg and even blocks the binding sites for Mg (Horst and Marshner, 1990).

Magnesium concentration in the solution had a highly significant effect on Mg in the roots, stems, old leaves and young leaves (Appendix G). As Mg in the nutrient solution increased, Mg in the plant tissue increased as well (Figure 7.8).

7.5 CONCLUSION

When Mg was low in the solution, the critical toxicity solution concentration of Mn in marigold decreased from 4.5 to 2.5 mg/l. High levels of Mn in the nutrient solution combined with low concentration of Mg (10 mg/l) (incipient deficiency concentration) resulted in relatively stunted plants with curled deformed leaves. The leaves showed chlorotic areas as well as small brown spots. The tips of the leaves and edges were necrotic and the roots were brownish.

The small brown spots and the brown color of the leaves may have resulted from localized precipitations of Mn as Mn oxide (MnO_2). The chlorosis on the leaves may have been due to Mn-induced Fe deficiency.

The reduced critical toxicity concentration of Mn at low levels of Mg in the solution does not seems to be due to increased Mn in the plant tissue as a results of high Mn and low Mg in the solution. Magnesium level in the solution did not affect the effect of high Mn in the solution on Mn in the plant tissue. The reduced critical toxicity concentration of Mn may be due just to the conditions of this experiment.

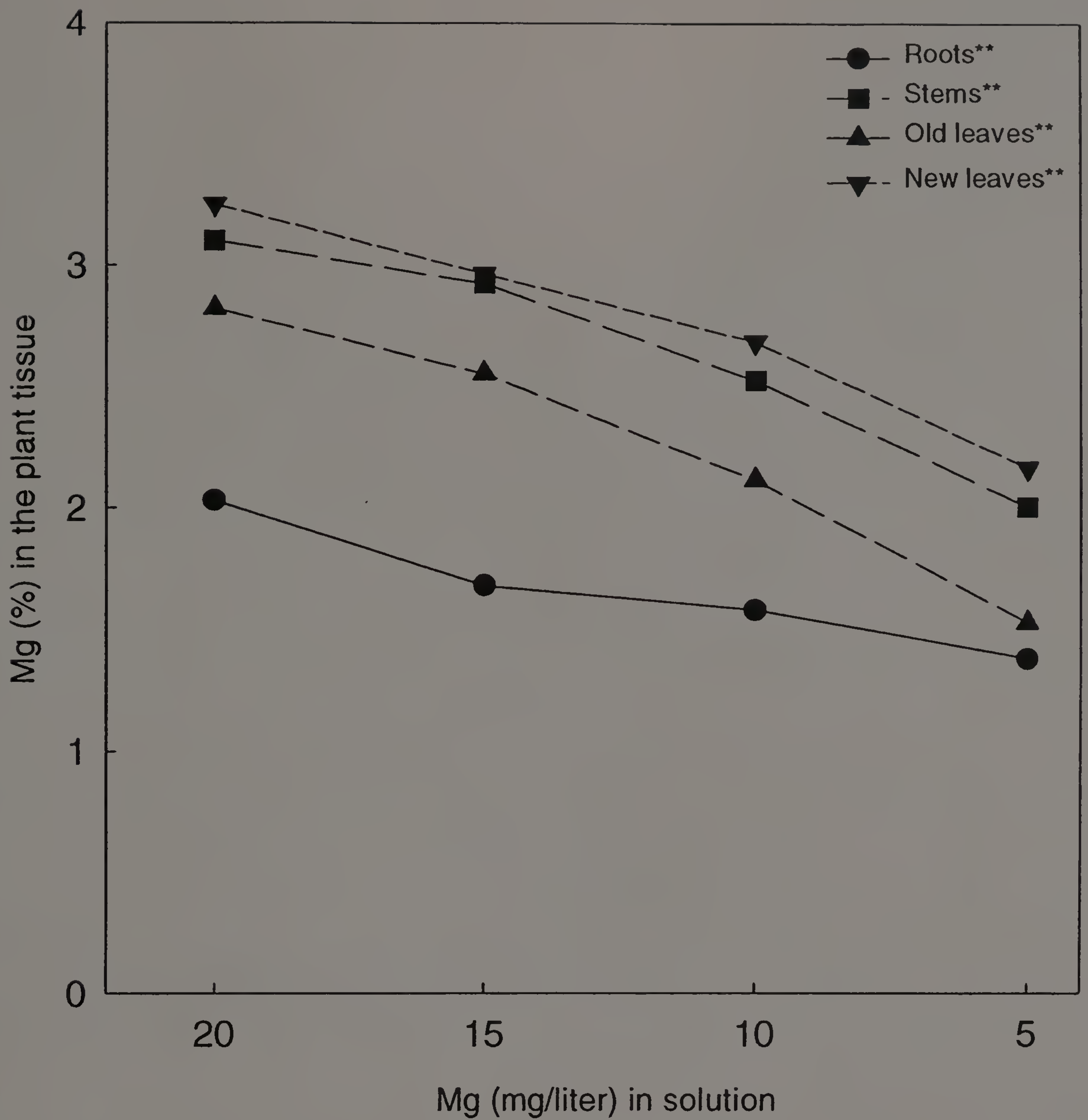

Figure 7.8: Effect of Mg level in solution on the level of Mg in the plant tissue (see Appendix G, Table 2)

Results of this experiment suggest that the interaction between Mn and Mg in the nutrient solution had no significant effect on Mn, Ca or Mg concentrations in the plant tissue. Large concentrations of Mn in the nutrient solution resulted in increased Mn in all the plant parts. This increase was not affected by Mg in the nutrient solution.

High Mn in the nutrient solution resulted in increased Fe in the roots and decreased Fe in the shoots. This effect of Mn in the nutrient solution on Fe in the plant tissue was not affected by Mg in the nutrient solution.

High Mn in the nutrient solution resulted in decreased Ca in the leaves and decreased overall concentration of Mg in the plant tissue. Magnesium did not affect the decrease of Ca and Mg in the plant tissue due to excess Mn in solution.

Decreased Mg in the plant tissue due to excess Mn in the solution may have resulted from reduced Mg uptake by marigold. Manganese competes with Mg for the binding sites in the roots during absorption.

Decreased Fe in the shoots may have been due to the oxidation of the physiologically active form of Fe (Fe^{2+}) to the inactive form (Fe^{3+}) or to the formation of insoluble ferric-phosphate-organic complex.

Decreased Ca may have been due to inhibited translocation of Ca into the shoot apex. Manganese decreases the cation exchange capacity of the leaf tissue and decreased the IAA levels in the areas of new growth. Decreased auxin results in limited formation of new binding sites for the transport of Ca to the apical meristems.

In conclusion, even though symptoms of manganese toxicity appeared at 4.5 instead of 6.5 mg/l Mn in the solution, Mg does not seem to have an effect in preventing

or reducing the occurrence of manganese toxicity in marigold because it does not affect Mn level in the plant tissue.

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CHAPTER 8

MANGANESE TOXICIY IN MARIGOLD AS AFFECTED BY CALCIUM AND MAGNESIUM

8.1 ABSTRACT

Iron/manganese toxicity disorder in marigold has been related to high concentrations of Mn and low concentrations of Ca and Mg in the affected leaves. Preplant addition of micronutrients in the media combined with constant feed program and low medium pH create favorable conditions for the development of Mn toxicity in greenhouse crops. Deficiency of Ca or Mg is due in part to low medium pH and to a lack of Mg and Ca in most of the fertilizers used in greenhouse production. To investigate the effect of Ca and Mg on Mn toxicity effects (growth, appearance, and nutrient concentrations), a solution culture study with various Ca, Mg and Mn concentrations was conducted.

The treatments 0.5/20/10 mg/l Mn-Ca-Mg concentrations resulted in stunted plants with curled leaves, a significant number of small brown spots, interveinal chlorosis, and necrotic areas on the tips and margins of the leaves. The treatments 0.5/20/20, 0.5/100/20, and 4.5/100/20 resulted in stunted plants relatively free of symptoms.

Low concentrations of Mg and Ca (incipient deficiency concentration of Mg and Ca) reduced the critical toxicity concentration of Mn in marigold from 4.5 to 0.5 mg/l.

Large concentrations of Mn in the medium affected plant appearance, reduced the dry weight of the plants, and increased the concentration of Mn in all the plant parts.

This increase was greater when Ca level in the medium was low, but was not as much affected by the concentration of Mg.

High Mn in the solution also resulted in increased Fe in the roots. This increase of Fe concentration in the roots was not affected by Mg in the solution, but was greater when Ca level in the solution was low.

High concentrations of Mn in the nutrient solution resulted in increased Ca in the roots, stems, and new leaves. This increase in Ca was not affected by Mg but was greater when Ca level was high. High Mn in the nutrient solution resulted in increased Ca in the old leaves when Mg level was low in solution. Calcium in the old leaves was reduced when Mg in solution was high.

High Mn in solution resulted in increased Mg in the roots when Ca level in solution was low. Excess Mn in solution also resulted in increased Mg in the stem when Ca level in solution was low and reduced Mg when Ca level in solution was high. High Mn had no effect on Mg in the old leaves, but resulted in increased Mg in the new leaves when Ca level in solution was low.

8.2 INTRODUCTION AND LITERATURE

High Mn supply disrupts various aspects of plant metabolism, leading to serious physiological and morphological disorders. Manganese toxicity has been associated with reduced enzyme and hormone activities, inhibition of ATP synthesis, and reduced respiration rates (Heenan and Cambell, 1981; Sircar and Amin, 1974; Morgan et al., 1966).

Factors that affect the toxicity of Mn, deficiency of Ca, and deficiency of Mg include the concentrations of Mn, Ca and Mg in the growth medium; growth medium and

moisture content, nitrogen source, plant developmental stage, organic matter and pH, and concentrations of salts, particularly those of Ca, Fe and phosphorus (Foy et al., 1978; Goh and Haynes, 1978; Bachman and Miller, 1995; Harrison and Bergman, 1981; Le Mare, 1977; Reddy and Mills, 1991; Hood et al., 1993). Small changes in these factors can determine the degree of Mn toxicity, Mg deficiency, and Ca deficiency in a given crop.

8.2.1 Concentration of Mn in the Growth Medium

The accumulation of Mn in the leaf tissue may result directly from applying excessive amounts of micronutrients. The continuous use of high levels of peat-lite fertilizers may cause problems if salt levels are not monitored carefully (Sheely, 1990). Preplant addition of micronutrients in the growth medium, combined with a constant feed program, creates toxicity problem (Sheely, 1990).

High concentrations of Mn in the growth medium can also inhibit the uptake of Ca by plants and thus induce Ca deficiency (Bergmann, 1992).

High concentrations of Mn in the medium can induce Mg deficiency in the plant (Mn-induced Mg deficiency) (Heenan and Campbell, 1981). Excess Mn may reduce Mg uptake by up to 50% (Kazda and Znacek, 1989) due to competition (Heenan and Campbell, 1981). The competition between Mg and Mn for binding sites on the roots during absorption, inhibits absorption of Mg, since Mn competes more effectively than Mg and even blocks the binding sites for Mg (Horst and Marshner, 1990).

Manganese toxicity can induce Ca deficiency "crinkle leaf" in dicotyledons such as cotton (Foy et al., 1981) and bean (*Phaseolus vulgaris* L.)(Horst and Marshner, 1978a). When the supply of Mn is excessive, the translocation of Ca into the shoot apex, especially, is inhibited (Horst and Marshner, 1990). This inhibition might be related to the fact that

high Mn levels decrease the cation exchange capacity of the leaf tissue (Horst and Marshner, 1978a) and decrease the IAA levels in the areas of new growth (Morgan et al., 1966; Morgan et al., 1976). Auxin was reported to be responsible for the formation of new binding sites for the transport of Ca to the apical meristems (Horst and Marshner, 1978a).

Manganese-induced suppressions in concentrations of Ca have been reported (Clark et al., 1981; Galvez et al., 1989). Bekker et al. (1994) found that Ca content of peanut decreased markedly as the Mn concentration of culture solution increased.

8.2.2 Concentration of Ca in the Growth Medium

Anoter aspect of Fe/Mn toxicity disorder may be a deficiency of Ca due, in part, to low medium pH and to a lack of Ca in many of the fertilizers used in the greenhouse crop production (Koranski, 1988). The toxicity effects of high concentrations of Mn in the plant tissue are considerably modified by Ca status. Manganese toxicity is much more severe when the Ca level in the plant tissue is low (Clark et al., 1981; Galvez et al., 1989; Foy et al., 1981; Horst and Marshner, 1978; Keisling and Fuqua, 1979; Wallace et al., 1945). Le Mare (1972, 1977) found that large concentrations of Ca in the medium could alleviate the harmful effects of Mn toxicity. The plants grown in a medium that supplied little Ca were very sensitive to Mn toxicity (Le Mare, 1972; Le Mare, 1977).

Foy et al. (Foy et al., 1978) reported the importance of Ca/Mn ratios in the tolerance of plants to Mn toxicity. Ratios above 80 were found desirable for a balanced nutrition in peanut (Bekker et al., 1994).

Although increasing the concentration of Ca in the medium can alleviate the detrimental effects of high concentrations of Mn, optimal concentration (60 mg/kg) of Ca is crucial for optimal plant growth (Morris and Pierre, 1947). Morris and Pierre (1947)

pointed out that high Ca levels (300 mg/kg) in the medium can reduce plant growth regardless of the concentration of Mn in the medium, probably because of unbalanced nutrition.

Increased Ca levels in the growth medium often decreased Mn absorption and toxicity (Heenan and Carter, 1975; Robson and Loneragan, 1970; Shuman and Anderson, 1976; Hewitt, 1945). Vose and Jones (1963) found that increasing the Ca level in the solution culture reduced the adverse effects of high Mn level on suppressing the number of nodules on white-clover (*Trifolium repens* L.) roots.

The Ca/Mn ratio was important in nodulation and Mn tolerance in soybean (Foy et al., 1978). Bekker et al. (1994) reported the importance of a Ca/Mn ratio above 80 for a desirable Ca/Mn balance in peanut tissue. Ouellette and Dessureaux (1958) found that Mn tolerant clones of alfalfa contained lower concentrations of Mn in their shoots and higher concentrations of Mn and Ca in their roots than the sensitive clones. In this study, Ca absorption seemed to regulate Mn toxicity by reducing Mn transport to shoots. Contrary to these findings, Chapman (1931) and Morris and Pierre (1947) found that Mn toxicity was not alleviated by additional Ca and was even greater at high Ca concentrations.

The conflicting results between Hewitt (1945) and Chapman (1931) and Morris and Pierre (1947) may be due to the different crops used. Morris and Pierre (1947) pointed out that high Ca levels (300 mg/liter) in the nutrient solution can limit plant growth regardless of the concentration of Mn in the solution, probably because of unbalanced nutrient solution. Optimal Ca concentration (60 mg/liter) was crucial for best plant growth and Ca addition to the nutrient solution may have caused a nutritional imbalance to the plants.

Bergmann (1992) reported that Mg uptake is usually increased by liming. However, when liming become excessive or more frequent, the disproportion between the Ca and Mg ion concentrations causes Mg deficiency symptoms to develop on the plants.

The uptake of Mg by plants and the interaction between the absorption of Mg and the absorption of Ca was investigated by Spear et al. (1978), McLean et al. (1972), and Christenson et al. (1973). Their studies showed that the Mg content in plant tissue was suppressed by Ca, but the effect depended on the concentration of the ions in the medium.

Moor et al. (1961) and Maas and Ogata (1971) studied the influence of Ca concentration in solution on Mg uptake by excised barley and corn roots, respectively. In both reports it was concluded that enhanced solution Ca concentrations reduced Mg uptake rate by suppressing the Mg transport capacity of the roots rather than competing with Mg for absorption sites.

To provide optimal nutrition for plants it is important to achieve a harmonious balance between Ca and Mg. Wichmann (1976) found that the ratio of Mg to Ca content in the shoots was important when estimating whether cereal plants were adequately supplied with Mg. On the basis of their experiment Mg:Ca ratio should be 0.30 for adequate yield of wheat, barley and oats. Hao and Papadopoulos (2000) recommend 300/50-80 mg/kg Ca/Mg for best yield and quality of tomato.

8.2.3 Concentration of Mg in the Growth Medium

Another factor affecting Fe/Mn toxicity disorder may be deficiency of Mg due in part to low medium pH and to lack of Mg in many of the fertilizers used in greenhouse

crop production (Koranski, 1988). Manganese toxicity often can be counteracted by a large supply of Mg (Elamin and Wilcox, 1986a; 1986b; Lohnis, 1960). Magnesium reduced Mn uptake both by excised and intact roots of several plant species (Harrison and Bergman, 1981; Lohnis, 1960; Maas et al., 1969).

The ability of Mg to reduce Mn uptake depends on the concentration of Mn in the medium. Elamin and Wilcox (1986a) found that at high concentrations of Mn, Mg had little effect on Mn uptake and the plants were able to accumulate toxic levels of Mn at all levels of Mg supply.

Although increasing the concentration of Mg in the medium can alleviate the detrimental effects of high concentrations of Mn, Mg application is not a practical method for the avoidance of Mn toxicity according to Davis (1996). Using Mg to prevent Mn toxicity would require large applications of Mg, which could lead to serious nutritional imbalance because Mg would interfere with Ca uptake.

Although Fe/Mn toxicity disorder in marigold has been related to high concentrations of Mn and low concentrations of Mg and Ca in the affected leaves, no research has been done on the effect of Ca and Mg nutrition on Mn toxicity in marigold. Available information on the interaction among Ca, Mg and Mn are meager. This experiment was designed to investigate the effect of Mg and Ca on Mn toxicity in marigold.

The objectives were: (1) to determine the effects of Mg and Ca on the sensitivity of marigold to high levels of Mn in the nutrient solution; (2) to determine the effects of Ca and Mg on the critical toxicity solution concentration of Mn; and (3) to determine the

effect of the interaction among Ca, Mg and Mn in solution on Mn, Fe, Ca and Mg in the plant tissue.

8.3 MATERIALS AND METHODS

Seeds of marigold (*Tagetes erecta* L. 'First Lady') were planted in vermiculite #3 on March 4, 1998 in the greenhouse. Three-week-old seedlings were transplanted into 800ml half strength nutrient solution in 16 oz, opaque plastic containers. The basal nutrient solution contained 48 mg/l Mg, 100 mg/l Ca, 79 mg/l potassium, 63 mg/l phosphorus, 75 mg/l nitrate-nitrogen, 75 mg/l ammonium-nitrogen, 2.3 mg/l Fe, 0.02 mg/l copper and 0.5 mg/l of Mn, boron, zinc and molybdenum. After 10 days of growth in the half strength basal nutrient solution, the seedlings were transferred to treatment solutions. Manganese treatments were 0.5; 2.5; 4.5 and 6.5mg/l nutrient solution. Calcium treatments were 20 and 100 mg/l and Mg treatments were 10 and 20mg/l nutrient solution. Manganese was added as $MnCl_2$ to the basal nutrient solution. Calcium was added as $CaCl_2$-$6H_2O$ and Mg was added as $MgSO_4$-$7H_2O$. The concentration 100 mg/l is considered optimal concentration of Ca and 20 mg/l is considered the incipient deficiency concentration of Ca in marigold based on the results discussed in Chapter 4. The concentration 20 mg/l is considered optimal concentration of Mg and 10mg/l is considered the incipient deficiency concentration of Mg in marigold (Chapter 3). Incipient deficiency concentrations of Ca and Mg are considered enough to fulfill the metabolic needs of the plant in Ca and Mg but they are not enough for optimal growth. The hydroponic cultures were aerated continuously and the nutrient solution was replaced with a fresh one every week. Deionized water was added to the containers as needed to maintain volume.

Eighty containers consisting of 4 Mn treatments, 2 Ca treatments, 2 Mg treatments, and 5 replicates were placed on two benches in the greenhouse in a completely randomized design. The temperature was maintained as closely as possible to 21/17 °C (day/night). The plants were harvested on April 21, 1998 and separated into roots, stems, old leaves and young leaves. The young leaves are the leaves closest to the apical buds. The old leaves are the leaves located far from the apical buds. The roots were rinsed with tap water then deionized water to discard nutrients from the root surface.

The plant parts were dried at 70 °C for 48 hours and weighed. The different plant parts were ground (20 mesh) and analyzed by inductive coupled plasma (ICP) (Appendix B) for Mn, Fe, Ca, and Mg contents. The data obtained were statistically analyzed using Analysis of Variance (ANOVA) and procedure Mixed (tests of Fixed Effects and tests of effect Slices) to test for significance of main effects and interactions. Terms were considered significant at $p<0.05$.

8.4 RESULTS AND DISCUSSION

8.4.1 Symptoms

The nature of the symptoms and their severity depended on the treatment. The treatments 0.5/100/20 mg/l (Mn/Ca/Mg) did not show any significant symptoms. 0.5/20/20; 2.5/100/20; 4.5/100/20 did not show any significant symptoms but were smaller and bushier. The plants treated with 0.5/100/10; 0.5/20/10; 2.5/100/10; 2.5/20/10; 4.5/100/10; 4.5/20/10 and 4.5/20/20 showed symptoms similar to those described for manganese toxicity in Chapter 5. All the plants grown in 6.5 mg/l Mn showed significant symptoms and decrease in dry weight regardless of the concentrations of Ca and Mg.

The symptoms were severe on the plants treated with 0.6/20/10 and the plants were very small in comparison to the plants grown under the other treatments. Symptoms appeared in the old leaves first then spread toward the rest of the plant. They included small blackish, brown spots on the leaves, curled leaves, brown roots and chlorosis on the tips and margins of the leaves afterward. When the plants were exposed to the same treatment for a longer time the chlorotic areas became necrotic.

The brown color of the roots and the curling of the leaves occurred mostly when high concentration of Mn was combined with low concentration of Ca in the nutrient solution.

The small brown spots shown in the affected leaves and the brown color of the roots may be associated with accumulation of Mn as precipitated Mn oxide (MnO_2) in the leaves and roots (Horst and Marshner, 1978a, b, c).

Interveinal chlorosis of the leaves may be associated with Mn-induced Fe deficiency. Local accumulations of Mn may interfere with Fe dependent metabolic functions and thus lead to Fe deficiency (Horst and Marshner, 1978a).

The deformation and curling of the leaves may be due to deficiency of plant hormones. Indeed, toxic concentrations of Mn increase the activities of polyphenoloxidase, peroxidase and IAA oxidase, which result in decomposition of auxins (Morgan et al., 1966; Cheng and Ouelette, 1971; Kamprath and Foy, 1971; Foy, 1973).

Horst and Marshner (1978c) reported that high concentrations of Mn reduced Ca translocation in the plant leaves. They believed that this reduced translocation of Ca in the leaves was responsible for the deformation and crinkling of the leaves. According to their findings, excess Mn increases IAA-oxidase activity, which reduces auxin level in

the leaves. Reduced auxin level inhibits cell enlargement and the creation of negative binding sited, thus reducing the translocation of Ca ions.

8.4.2 Dry Weight

Manganese concentration in solution had a highly significant effect on root, shoot and total dry weight. As Mn in solution increased, the root, shoot and total dry weight decreases (Figure 8.1). This decrease was more pronounced when the Ca level in the medium was low (Figure 8.2).

Calcium concentration in the solution had a highly significant effect on the root and shoot dry weight. Low levels of Ca in the solution caused a decrease in the root and shoot dry weight (Figure 8.3).

Magnesium concentration in solution had a significant effect on the root and total dry weight. Low level of Mg in solution caused a decrease in the root and total dry weight (Figure 8.4).

Under the conditions of this experiment, there was no significant effect of the interaction between Mg and Mn in the medium on plant dry weight. The interaction, however, between Ca and Mn was significant (Appendix H).

The reduced plant growth associated with high Mn in solution may have resulted from the depression of phytohormone metabolism by excess Mn (Marshner, 1978a).

Low Ca and Mg in solution resulted in a decrease in the root and shoot dry weight (Figure 8.3; 8.4). This decrease in plant growth was probably due to the crucial roles of these elements in plant metabolism.

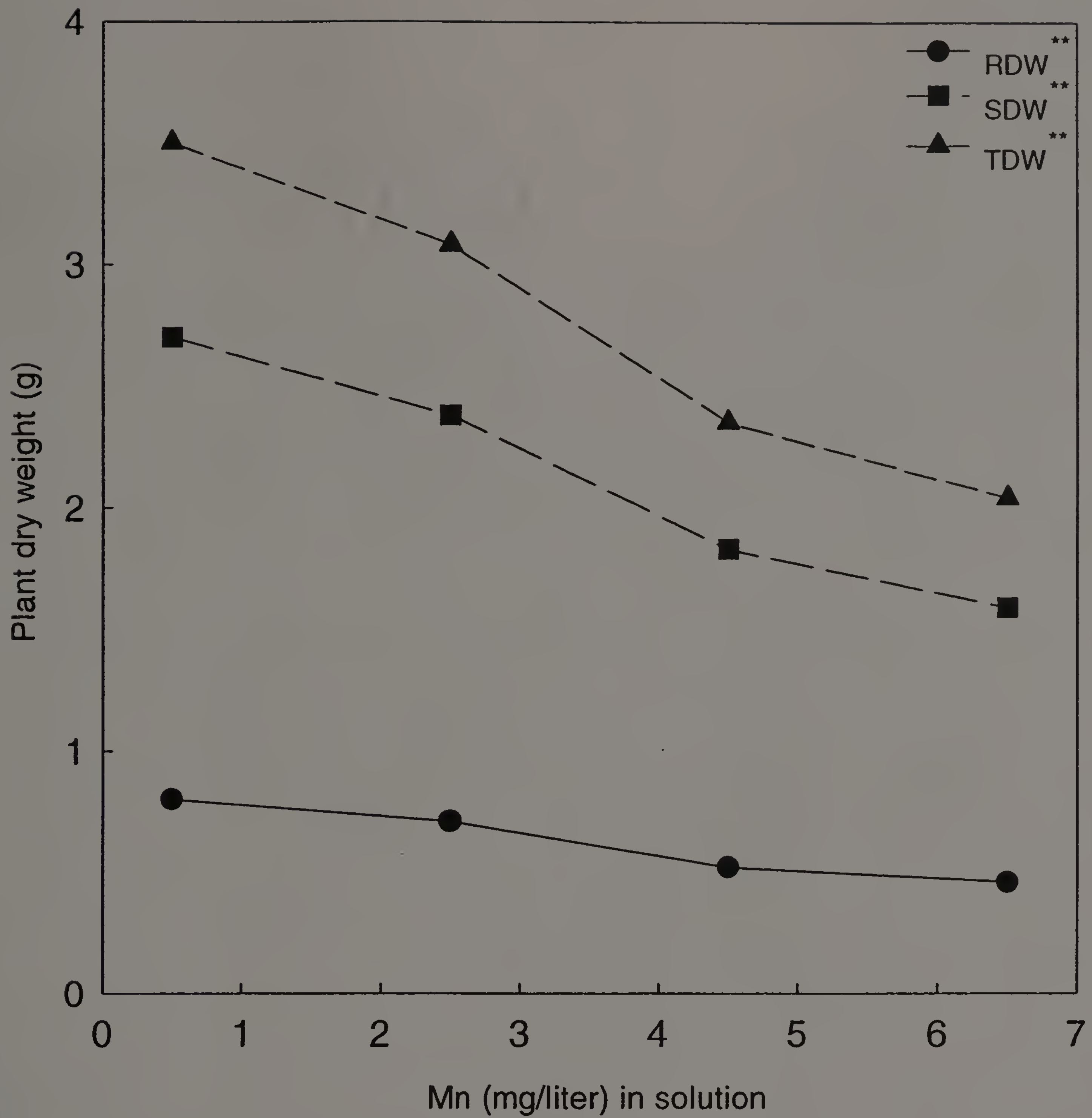

Figure 8.1: Effect of Mn level in solution on the plant dry weight (see Appendix H, Table 1)

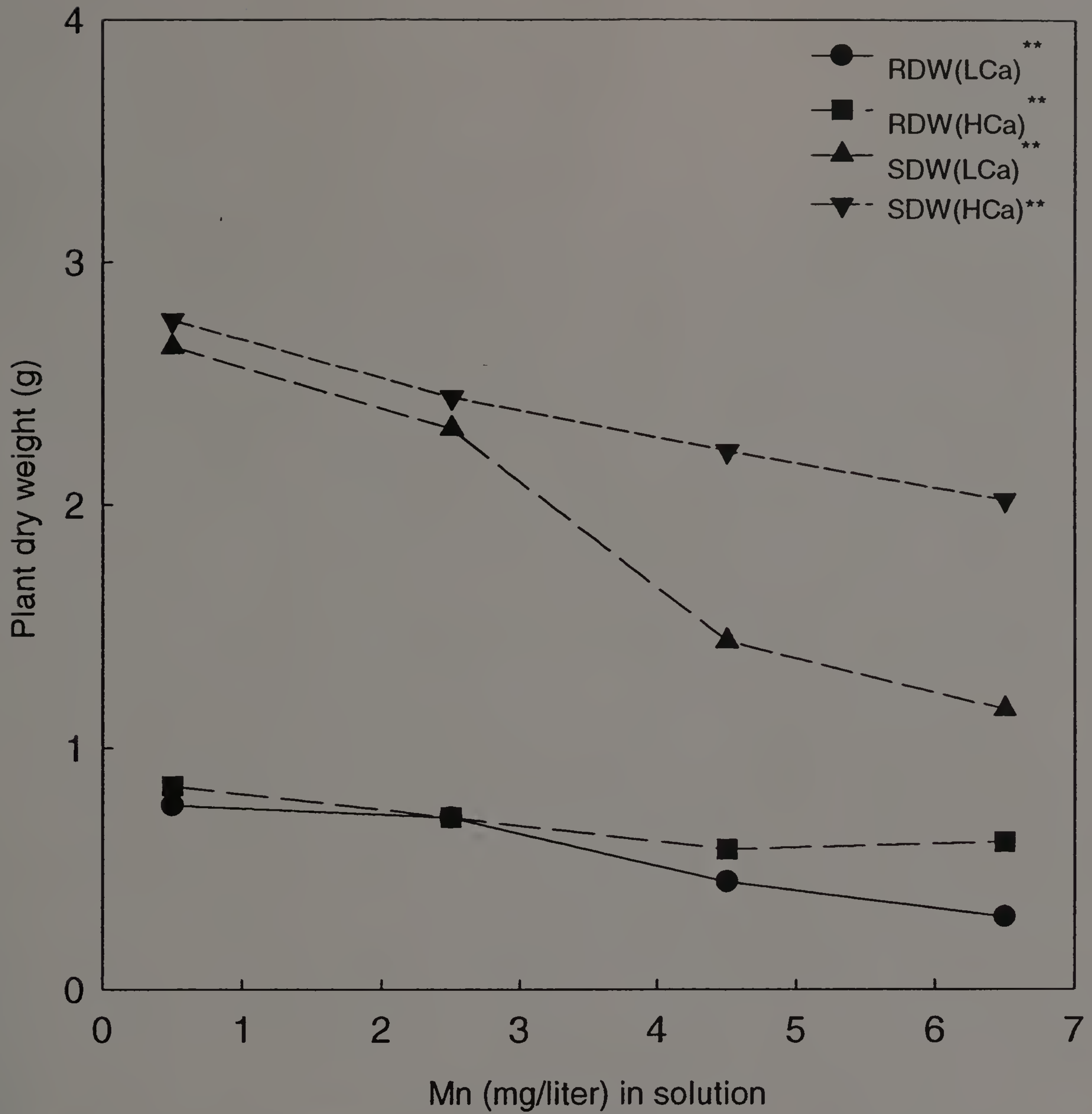

Figure 8.2: Effect of Mn in solution on the plant dry weight under low and optimal Ca in solution (see Appendix H, Table 1)

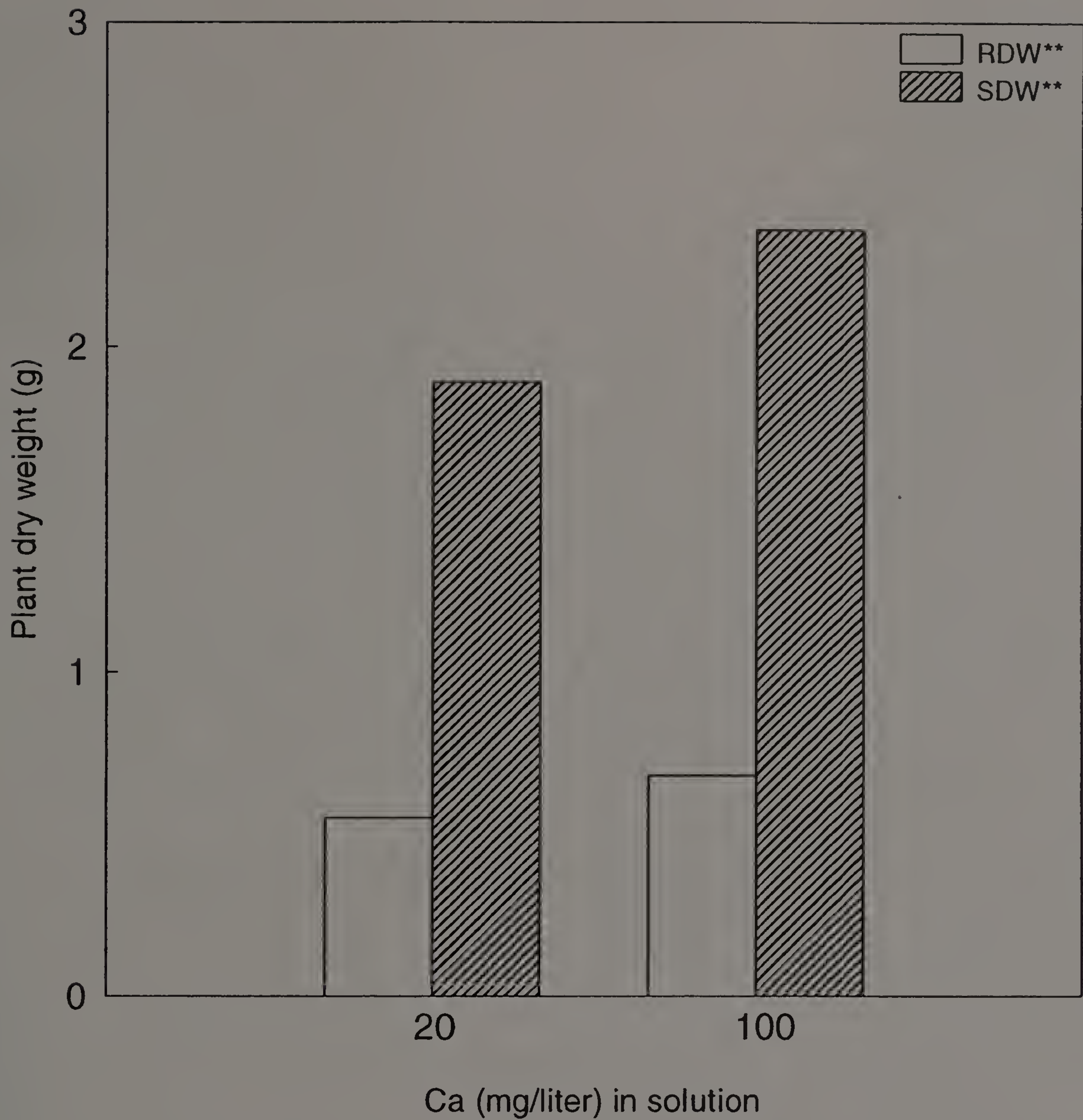

Figure 8.3: Effect of Ca level in solution on the plant dry weight (see Appendix H, Table 1)

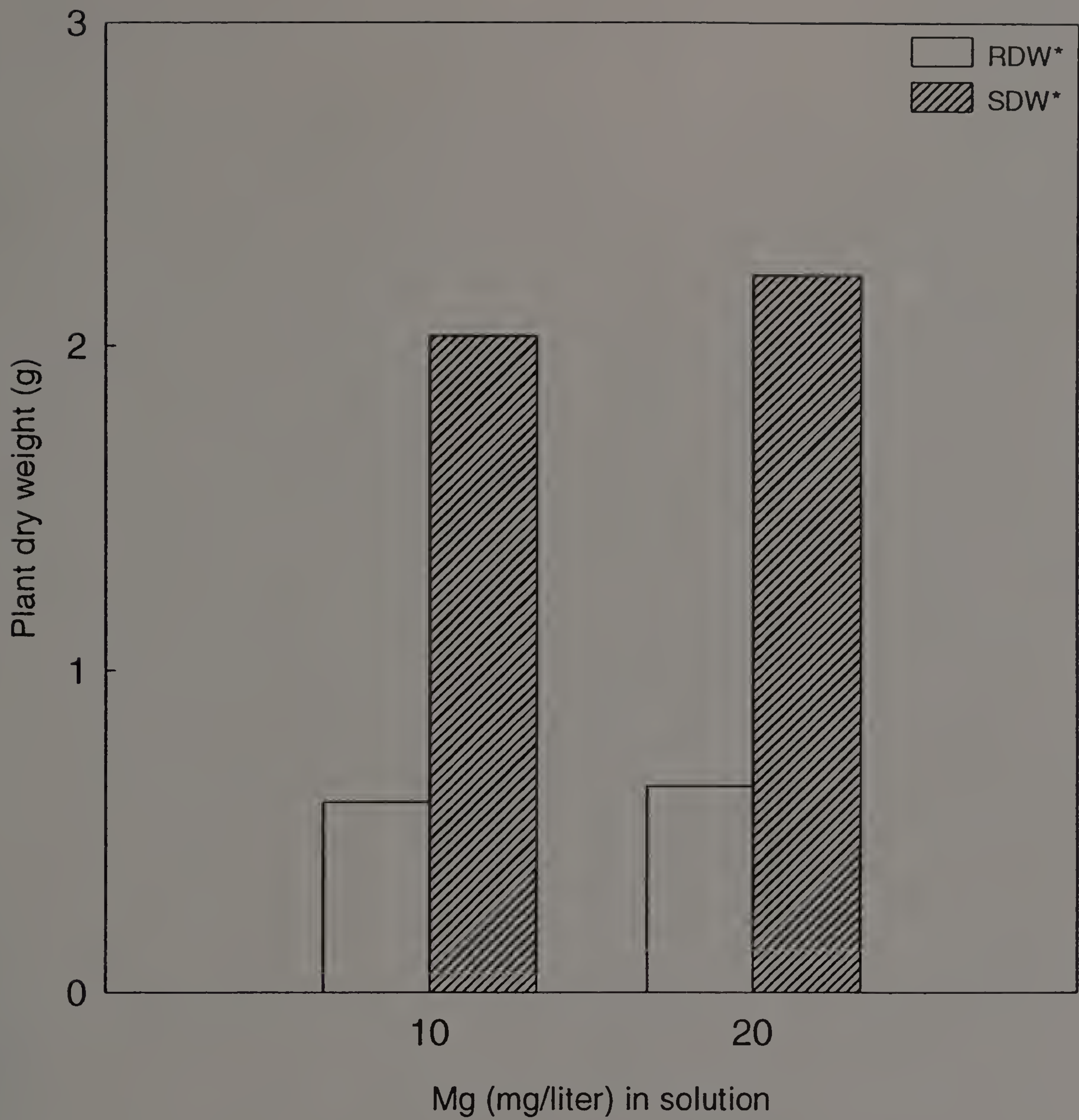

Figure 8.4: Effect of Mg level in solution on the plant dry weight (see Appendix H, Table 1)

8.4.3 Critical Toxicity Concentration of Mn

Plant appearance suggested that low Ca and/or Mg in solution decreased the critical toxicity concentration of Mn in marigold from 6.5 to 0.5 mg/l. At optimal concentration of Mg in solution, 2.5 and even 4.5 mg Mn/l did not cause any significant symptoms to appear. However, when Mg level in the medium was low (incipient deficiency concentration), and/or Ca level in solution was low, Mn as low as 2.5 resulted in the appearance of symptoms in the affected plants. These results suggest that low Mg and/or Ca in solution can decrease the critical toxicity solution concentration of Mn. Some researchers have found that Mg reduces Mn uptake by plants (Harrison and bergmann, 1981; Lohnis, 1960); Maas et al., 1969). Other researchers have found that high levels of Ca in the medium reduce Mn absorption and toxicity (Heenan and Carter, 1975; Robson and Loneragan, 1970; Shuman and Anderson, 1976; Hewitt, 1945).

8.4.4 Nutrients in the Plant Tissue

8.4.4.1 Manganese

Manganese in solution had a highly significant effect on Mn in the roots, stem, old leaves and young leaves (Appendix H). As Mn in solution increased, Mn concentration in the roots, stems, old leaves and young leaves also increased (Figure 8.5). This increase was greater in all plant parts when Ca level in solution was low (Figure 8.6, 8.7, 8.8, 8.9). The increase in Mn in the different plant parts as a response to Mn increase in solution was slightly greater when the level of Mg in solution was low (Figure 8.10, 8.11, 8.12, 8.13).

The greater increase of Mn in plant parts when Ca level in solution was low was probably due to the interaction between Mn and Ca solution. High levels of Ca in the

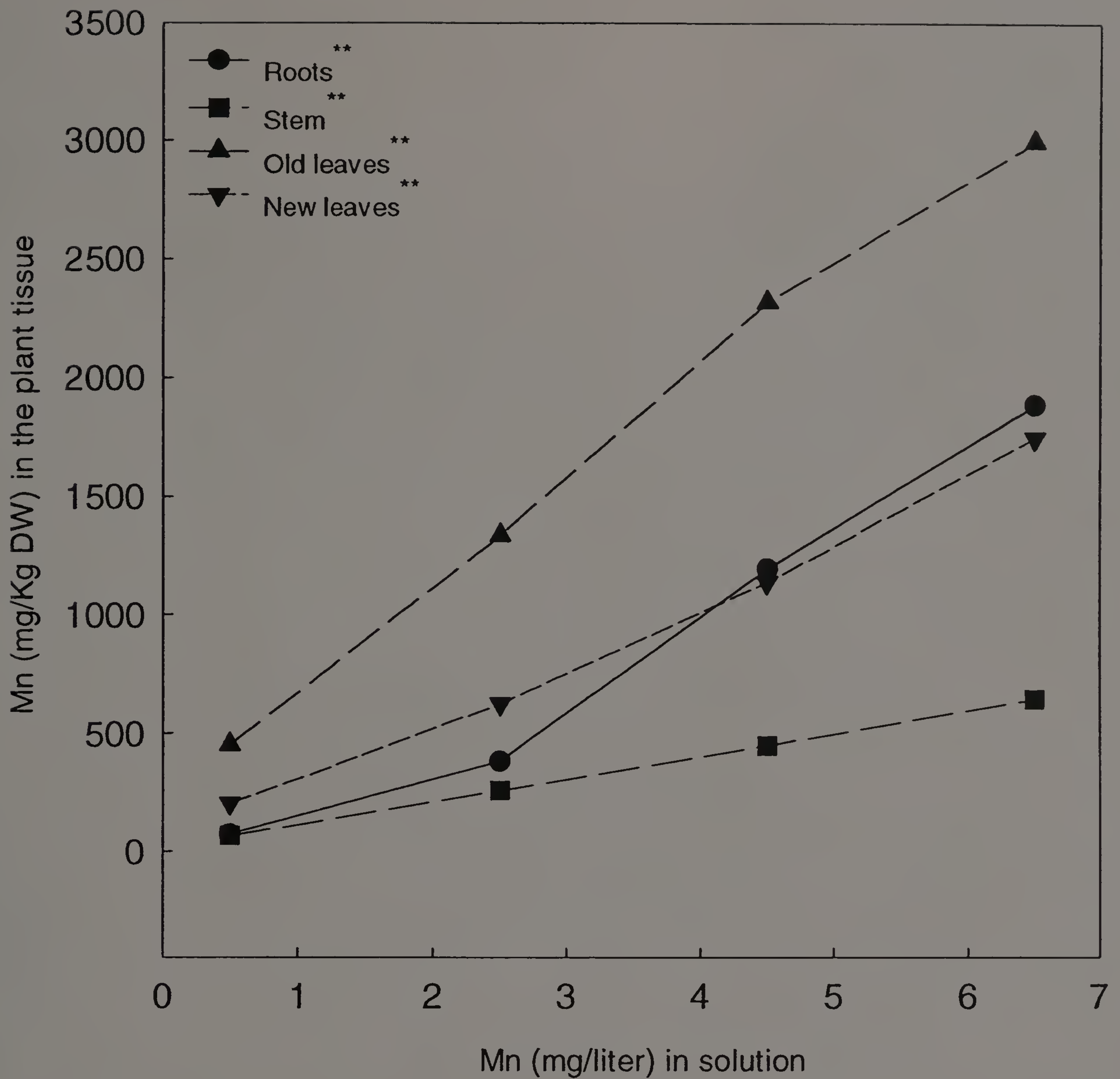

Figure 8.5: Effect of Mn level in solution on the level of Mn in plant tissue (see Appendix H, Table 2)

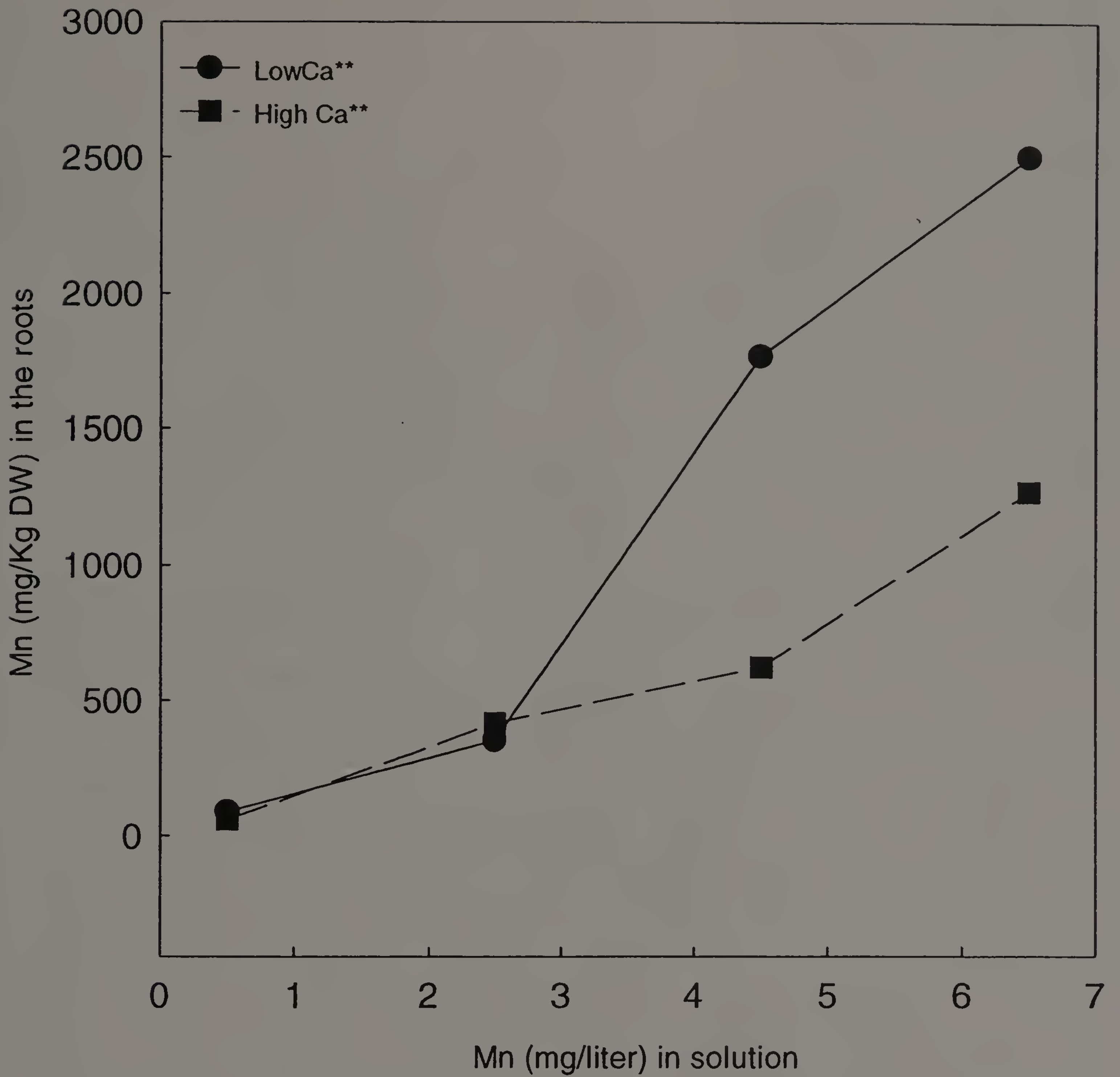

Figure 8.6: Effect of Mn level in solution on the Mn level in the roots under low and optimal levels of Ca (see Appendix H, Table 2)

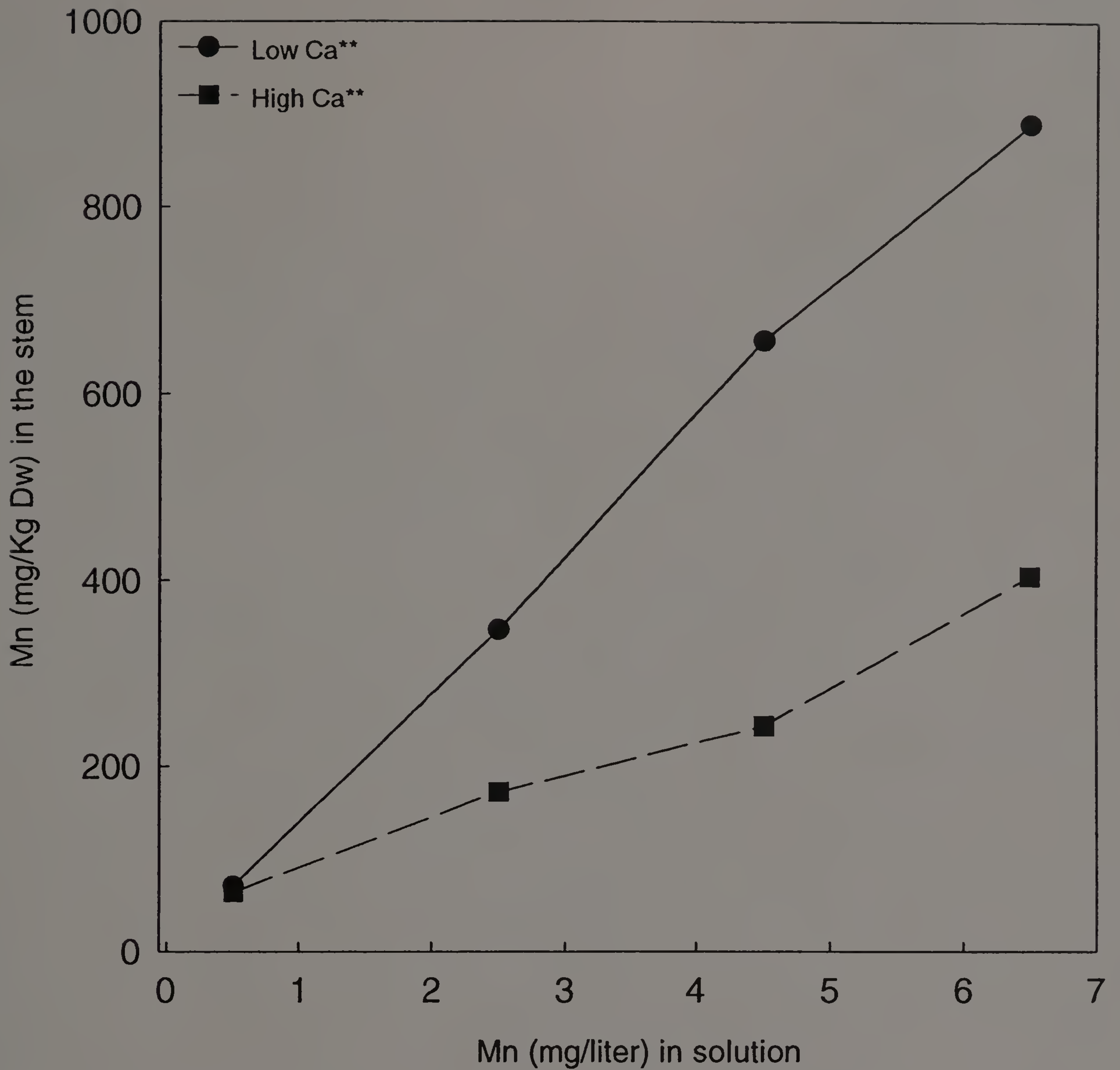

Figure 8.7: Effect of Mn level in solution on the Mn level in the stem under low and optimal levels of Ca
(see Appendix H, Table 2)

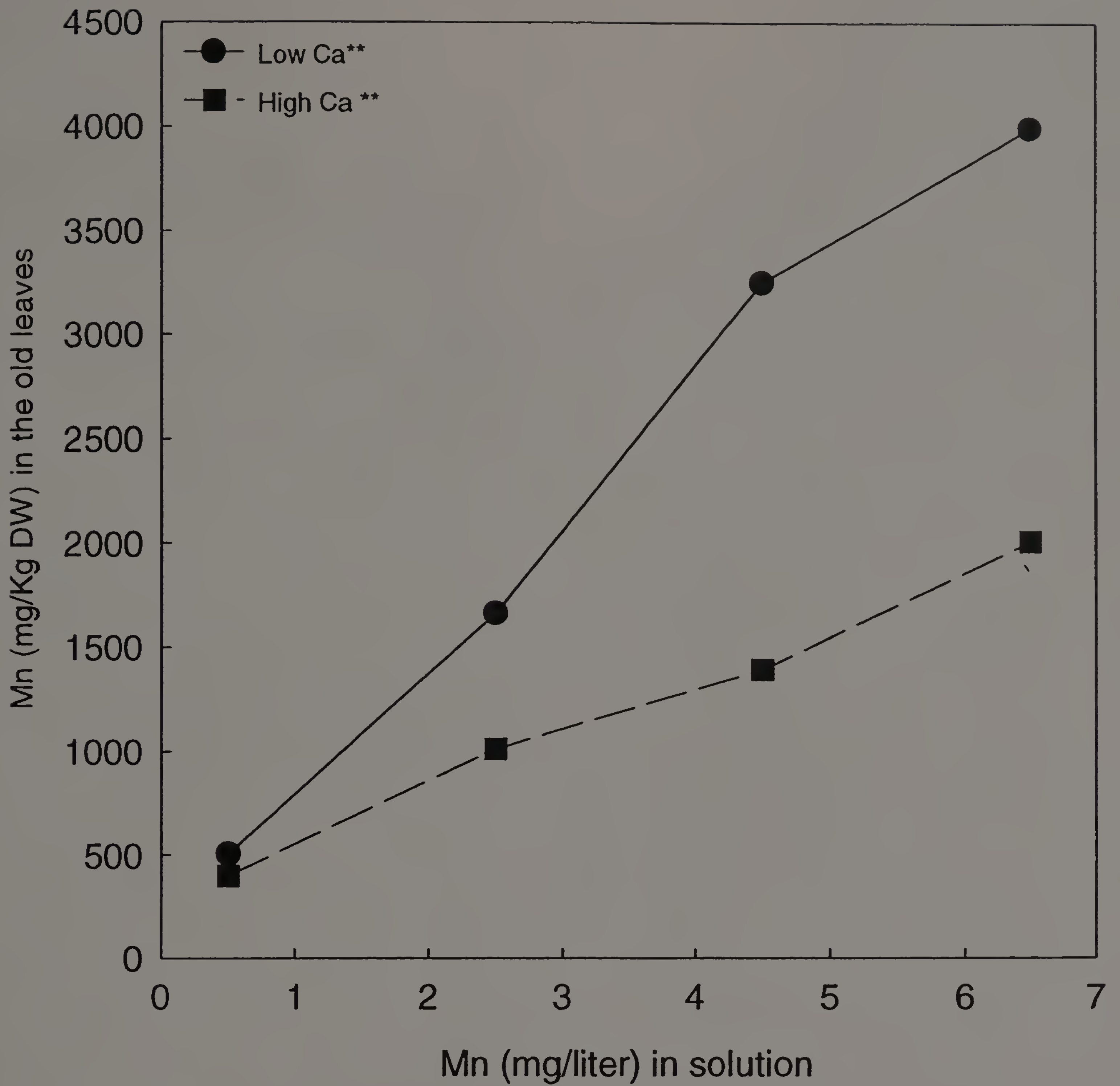

Figure 8.8: Effect of Mn level in solution on the Mn level in the old leaves under low and optimal levels of Ca
(see Appendix H, Table 2)

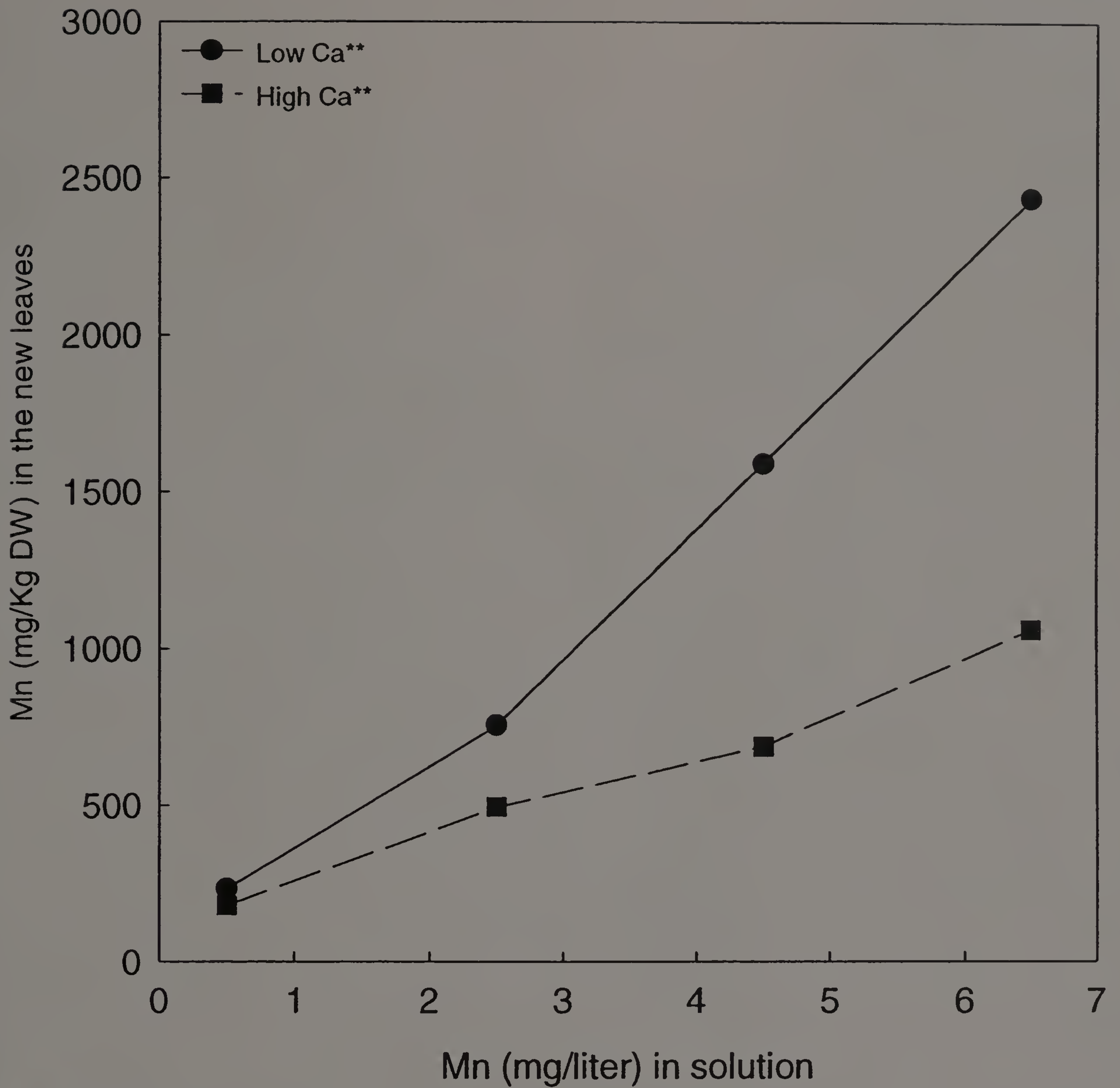

Figure 8.9: Effect of Mn level in solution on Mn level in the new leaves under low and optimal levels of Ca (see Appendix H, Table 2)

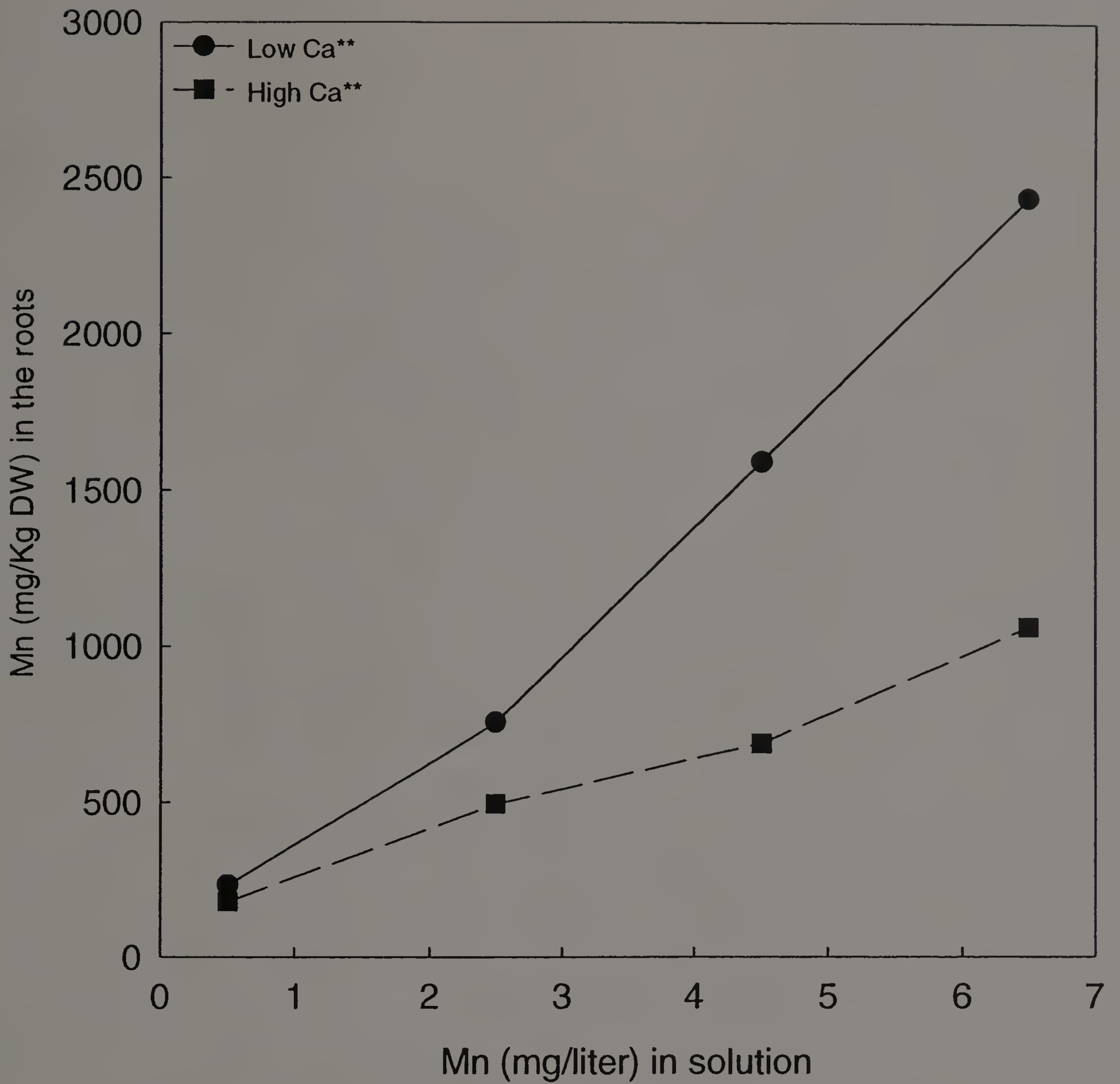

Figure 8.10: Effect of Mn level in solution on the Mn level in the roots under low and optimal levels of Mg
(see Appendix H, Table 2)

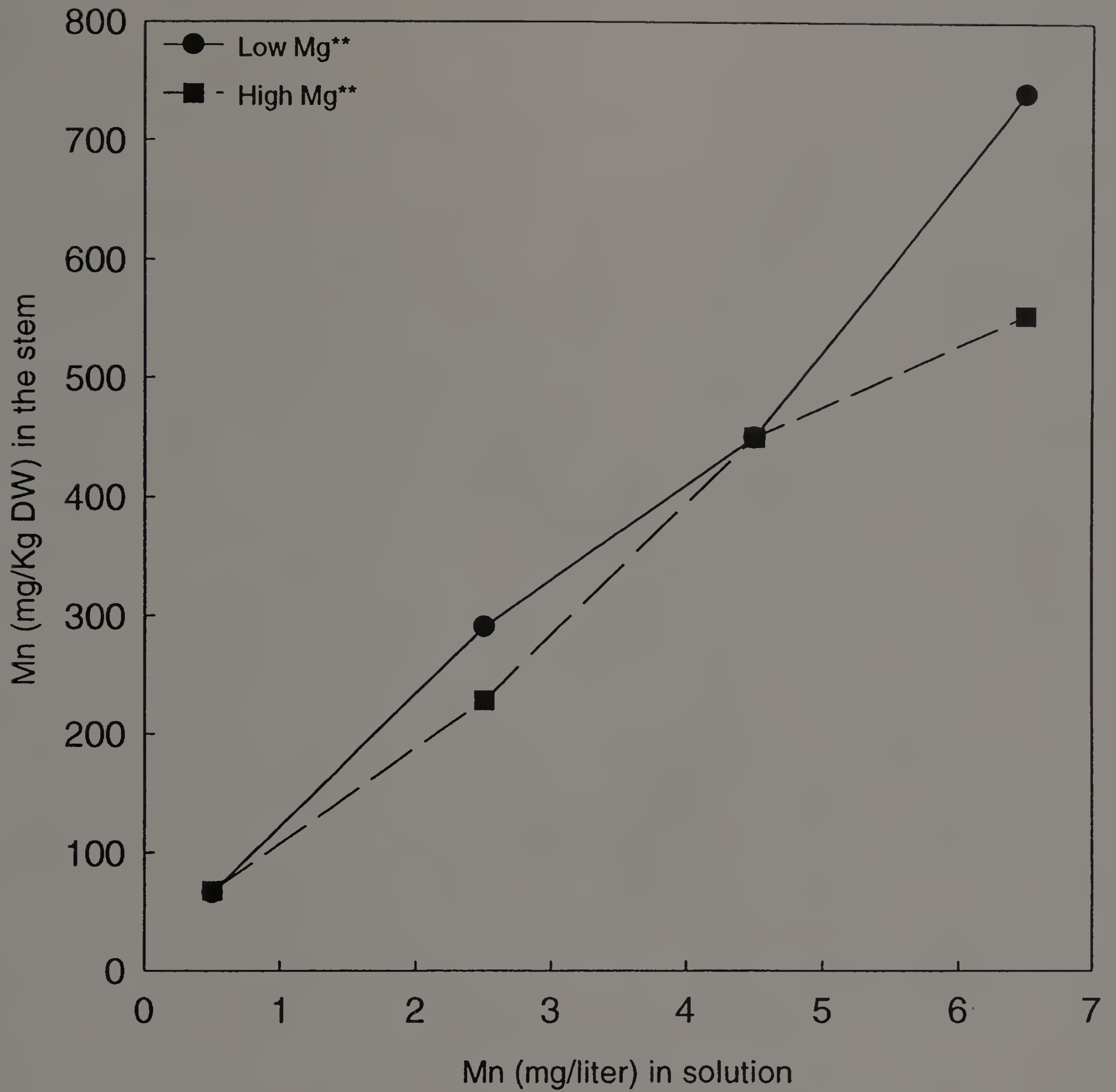

Figure 8.11: Effect of Mn level in solution on Mn level in the stem under low and optimal levels of Mg
(see Appendix H, Table 2)

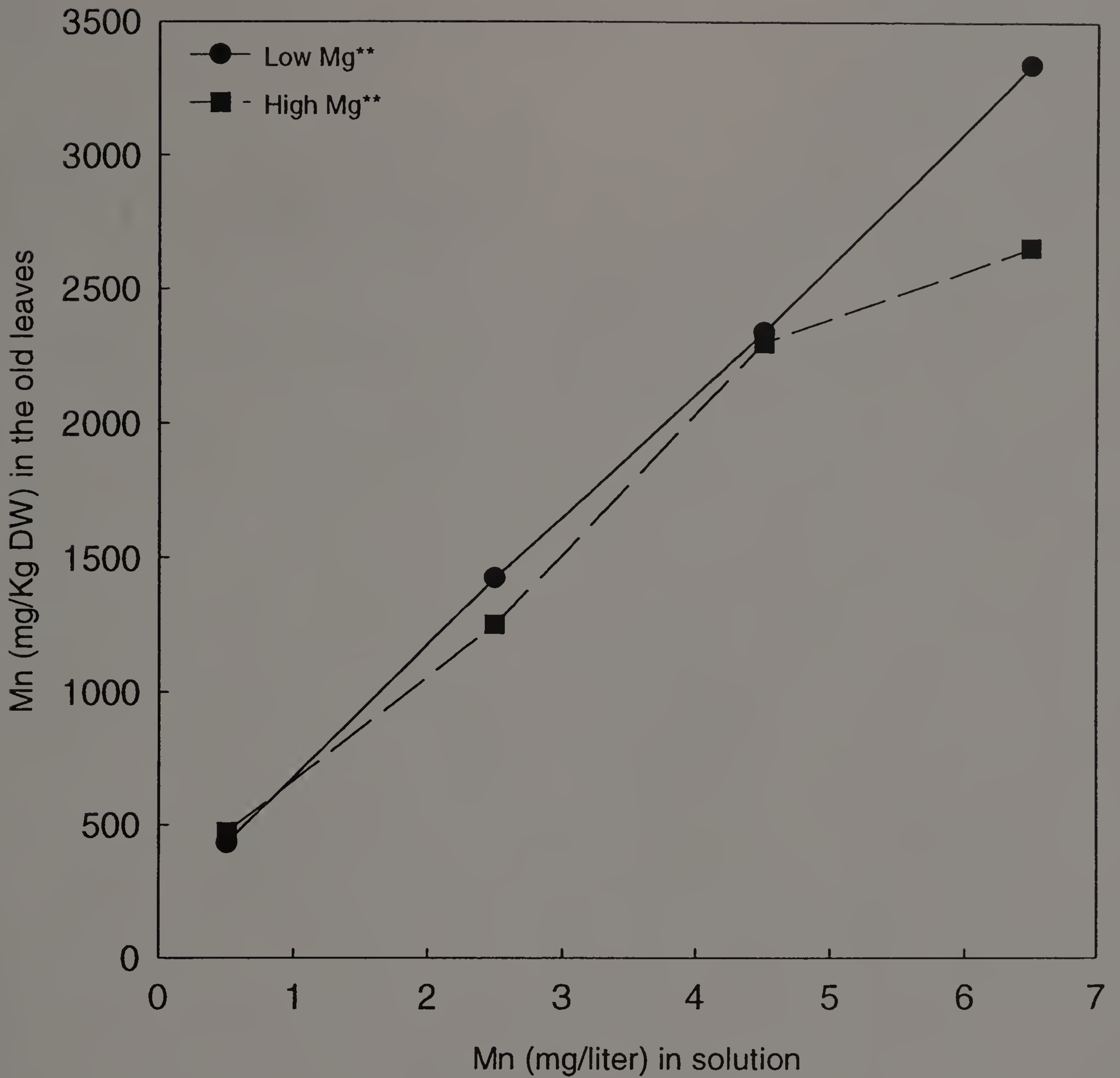

Figure 8.12: Effect of Mn level in solution on Mn level in the old leaves under low and optimal levels of Mg
(see Appendix H, Table 2)

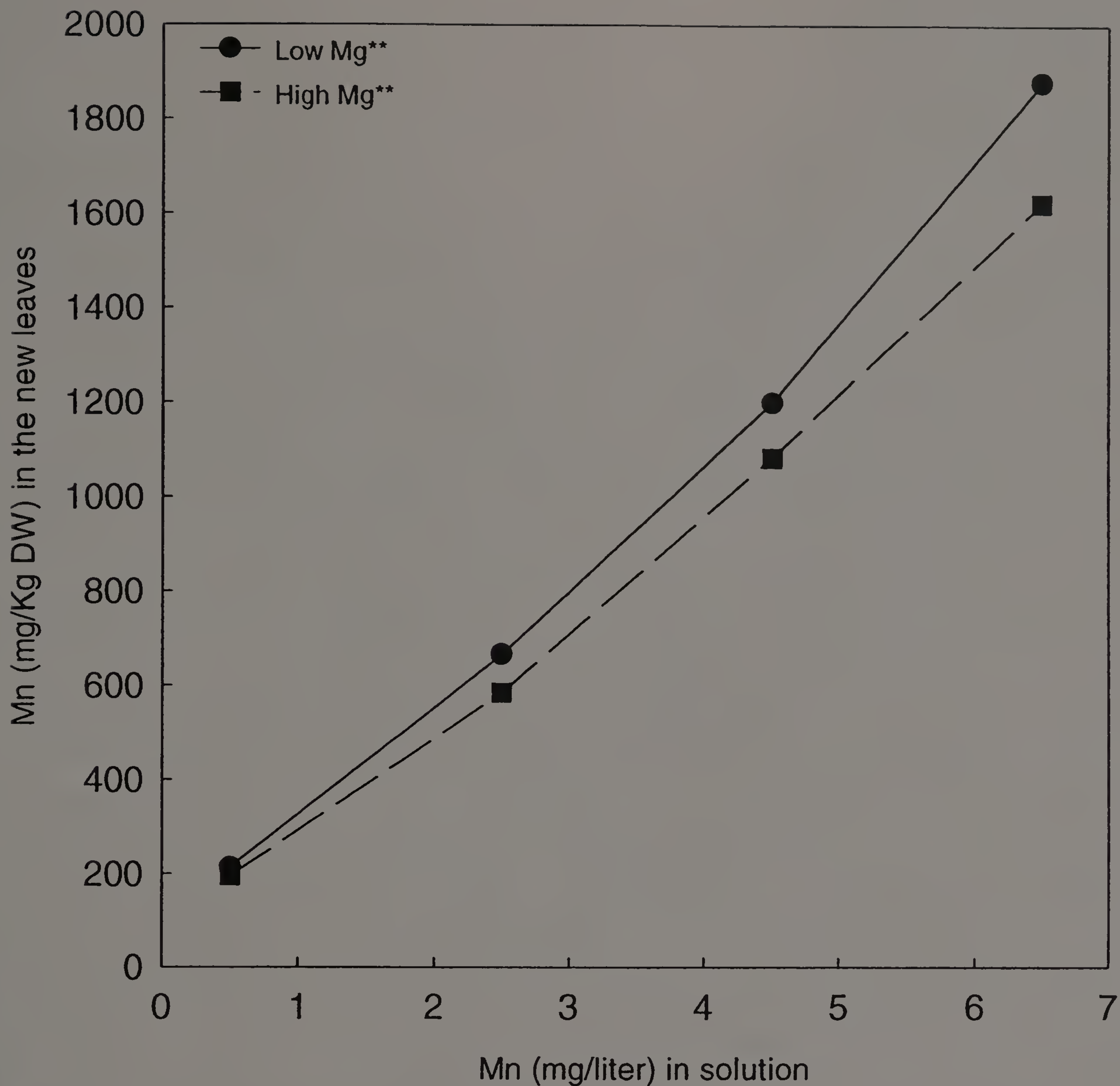

Figure 8.13: Effect of Mn level in solution on Mn level in the new leaves under low and optimal levels of Mg
(see Appendix H, Table 2)

growth medium reduce Mn absorption and toxicity in plants (Heenan and Carter, 1975; Robson and Loneragan, 1970; Shuman and Anderson, 1976; Hewitt, 1945). This decrease in Mn absorption is thus reduced when Ca level in the medium is low.

The slight accentuation of Mn increase in plant parts when Mg in solution was low was probably due to the interaction between Mn and Mg in solution. High levels of Mg in the growth medium can reduce Mn uptake by plants (Harrison and Bergman, 1981; Lohnis, 1960; Maas et al., 1969). This decrease in Mn uptake is thus reduced when Mg level in the medium is low.

Calcium concentration in solution had a highly significant effect on Mn in the roots, stem, old leaves and young leaves (Appendix H). When Ca in the solution was low, Mn was high in the roots, stem, old leaves and young leaves (Figure 8.14).

The effect of Ca in solution on Mn concentration in the different plant parts was greater when Mg level in the medium was low (Figure 8.15, 8.16, 8.17, 8.18, 8.18a).

The finding that high level of Ca in solution resulted in lower concentration of Mn in the plant tissues agree with those of Shuman and Anderson (1976), who found that high levels of Ca in the nutritive medium decreased Mn absorption in plants. Other researchers also found similar results (Heenan and Carter, 1975; Robson and Loneragan, 1970; Hewitt, 1945).

The accentuation of the effect of Ca in solution on Mn in the different plant parts when Mg in solution was low was probably due to the interaction between Mn and Mg in solution. In fact, Harrison and Bergman (1981) and other researchers found that high levels of Mg in the growth medium can reduce Mn uptake by plants (Lohnis, 1960; Maas et al., 1969).

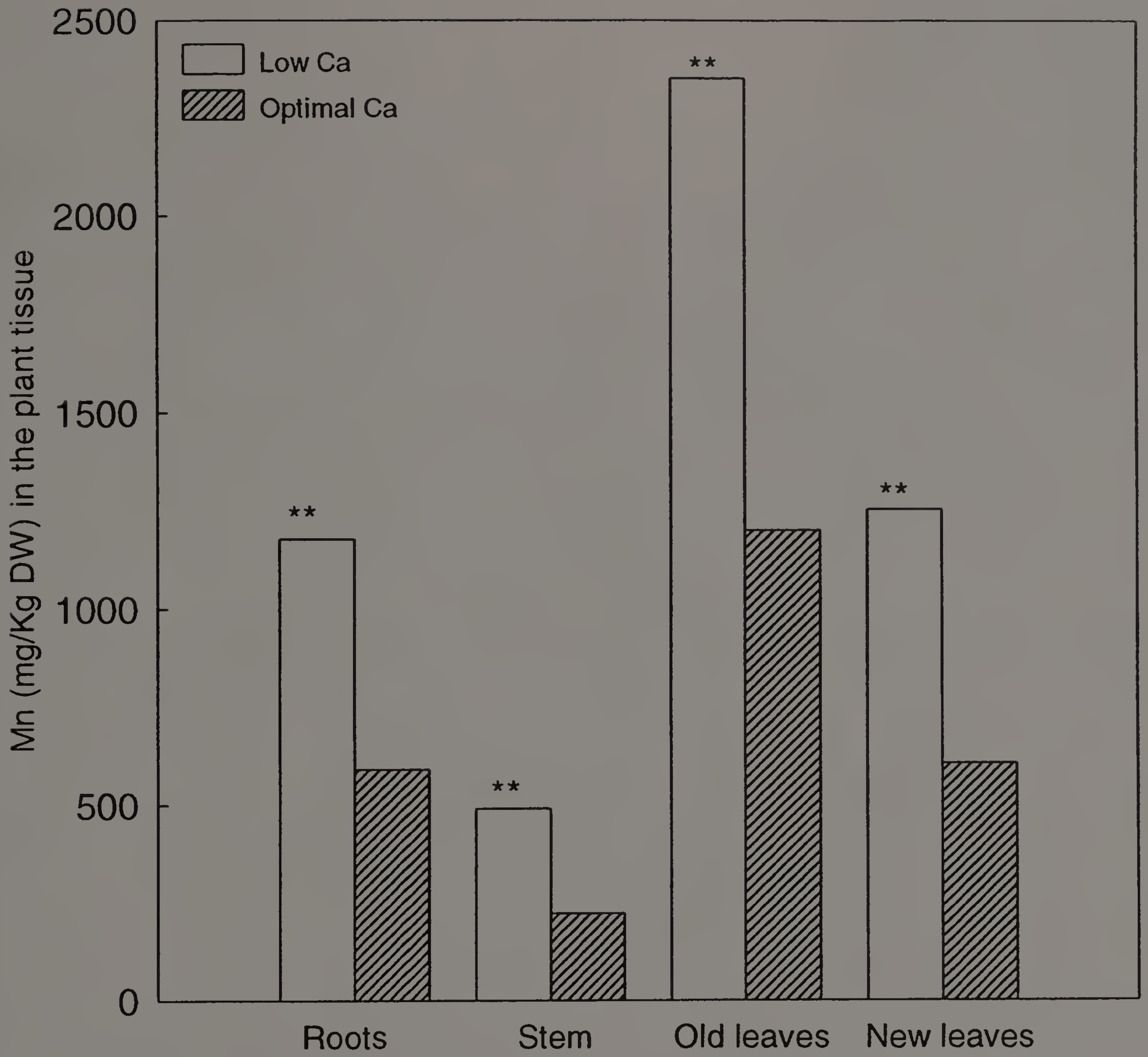

Figure 8.14: Effect of Ca level in solution on Mn level in the plant tissue (see Appendix H, Table 2)

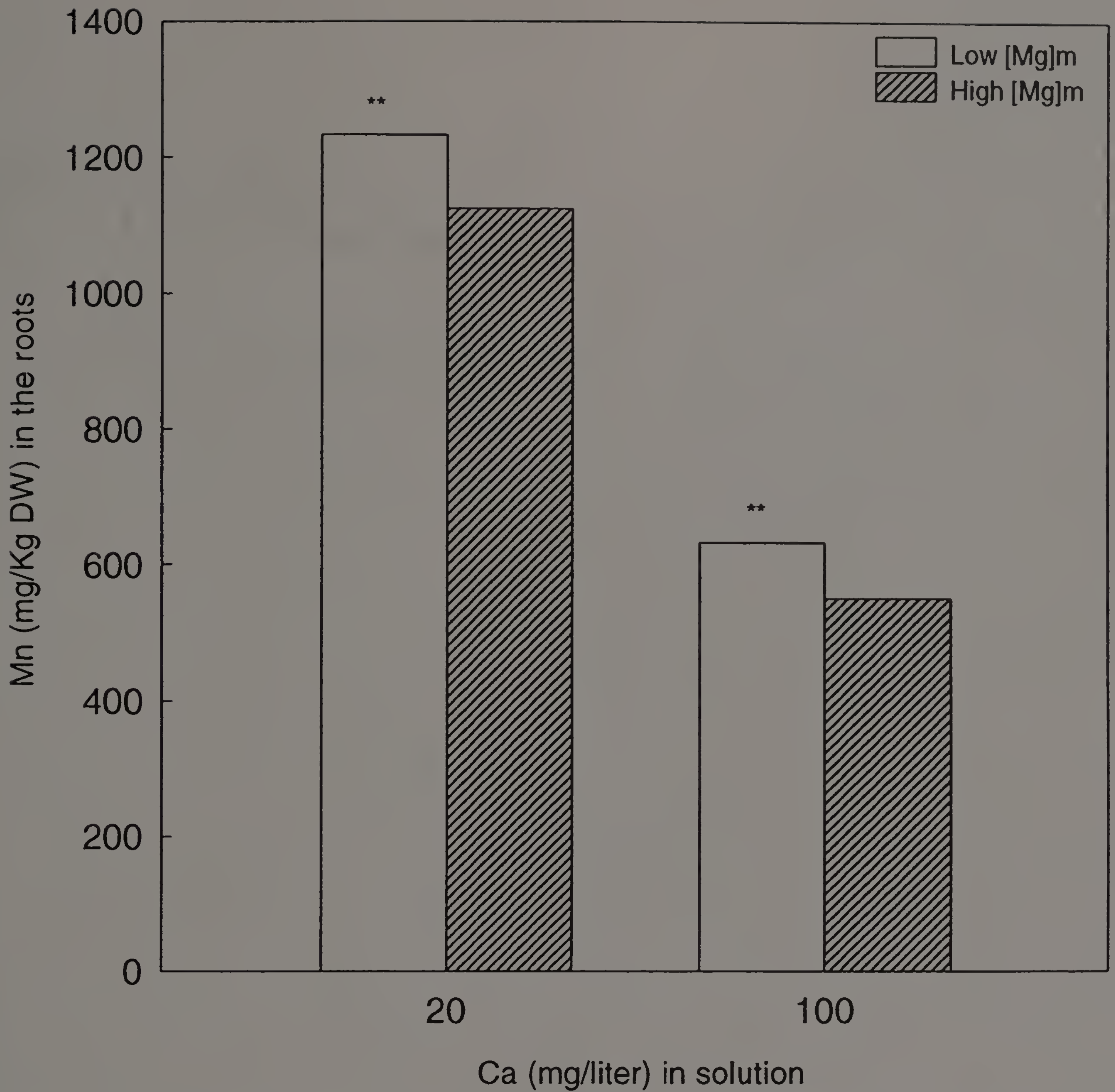

Figure 8.15: Effect of Ca level in solution on Mn level in the roots under low and optimal levels of Mg
(see Appendix H, Table 2)

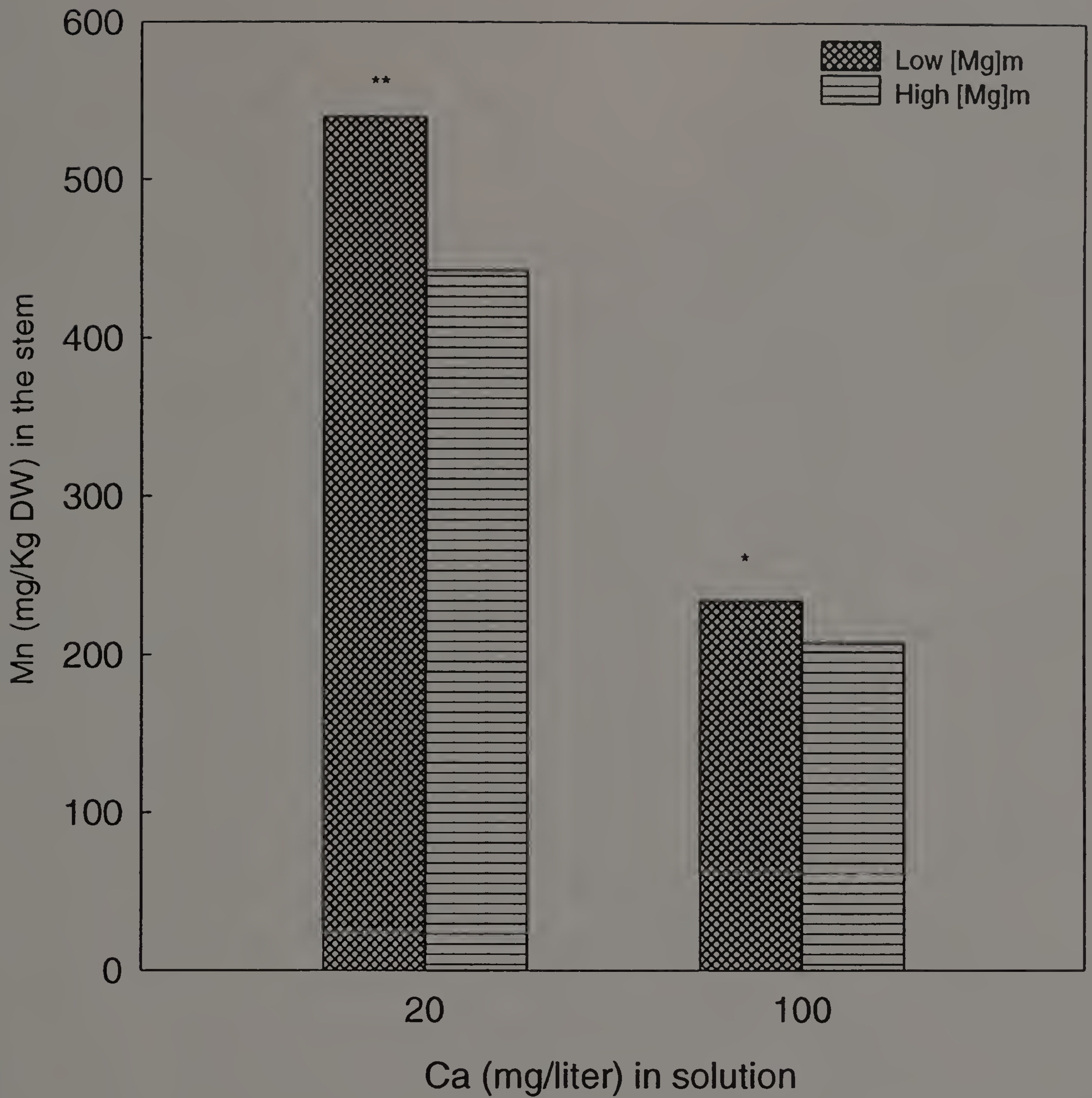

Figure 8.16: Effect of Ca level in solution on Mn level in the stem under low and optimal levels of Mg

(see Appendix H, Table 2)

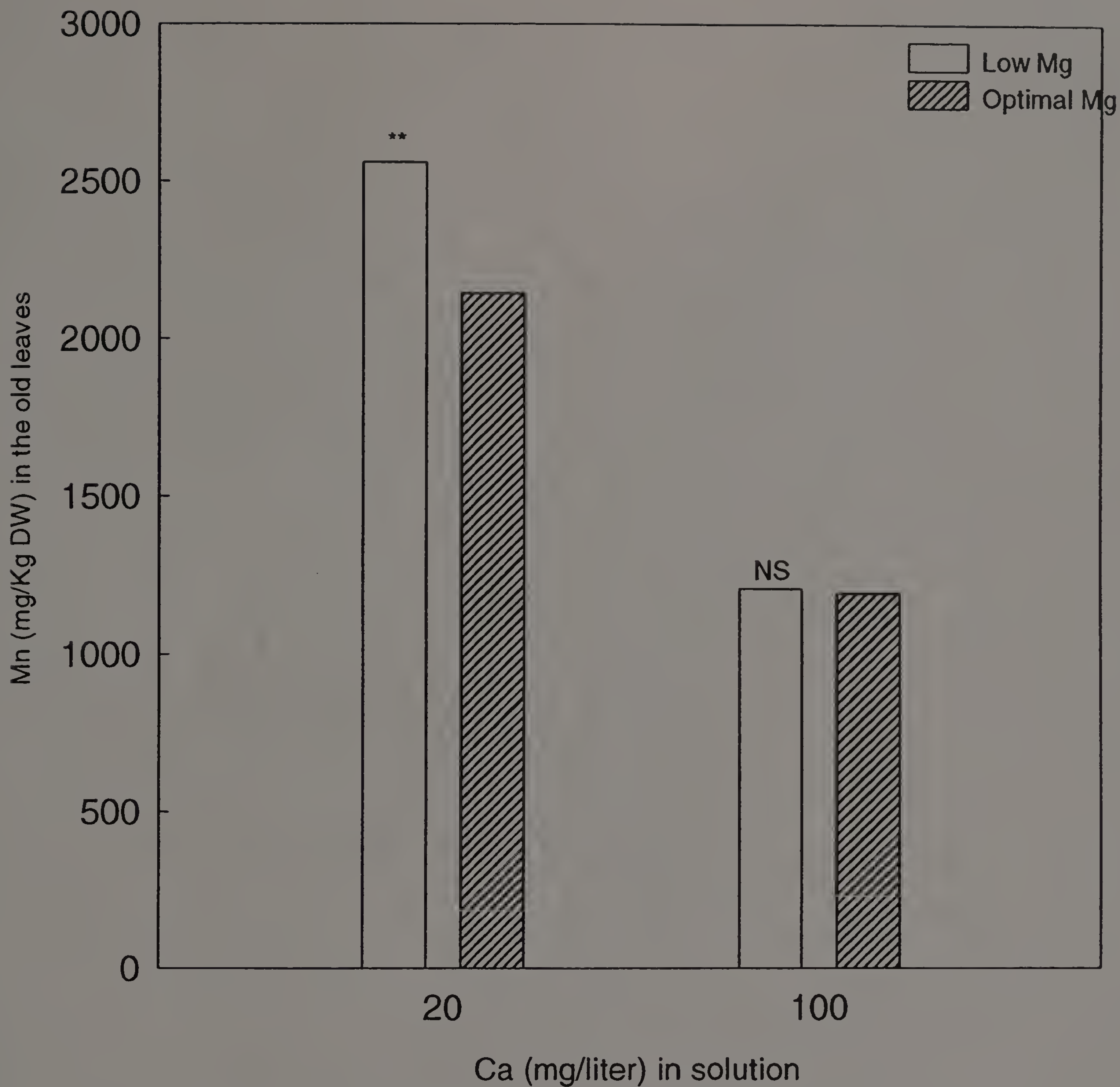

Figure 8.17: Effect of Ca level in solution on Mn level in the old leaves under low and optimal levels of Mg
(see Appendix H, Table 2)

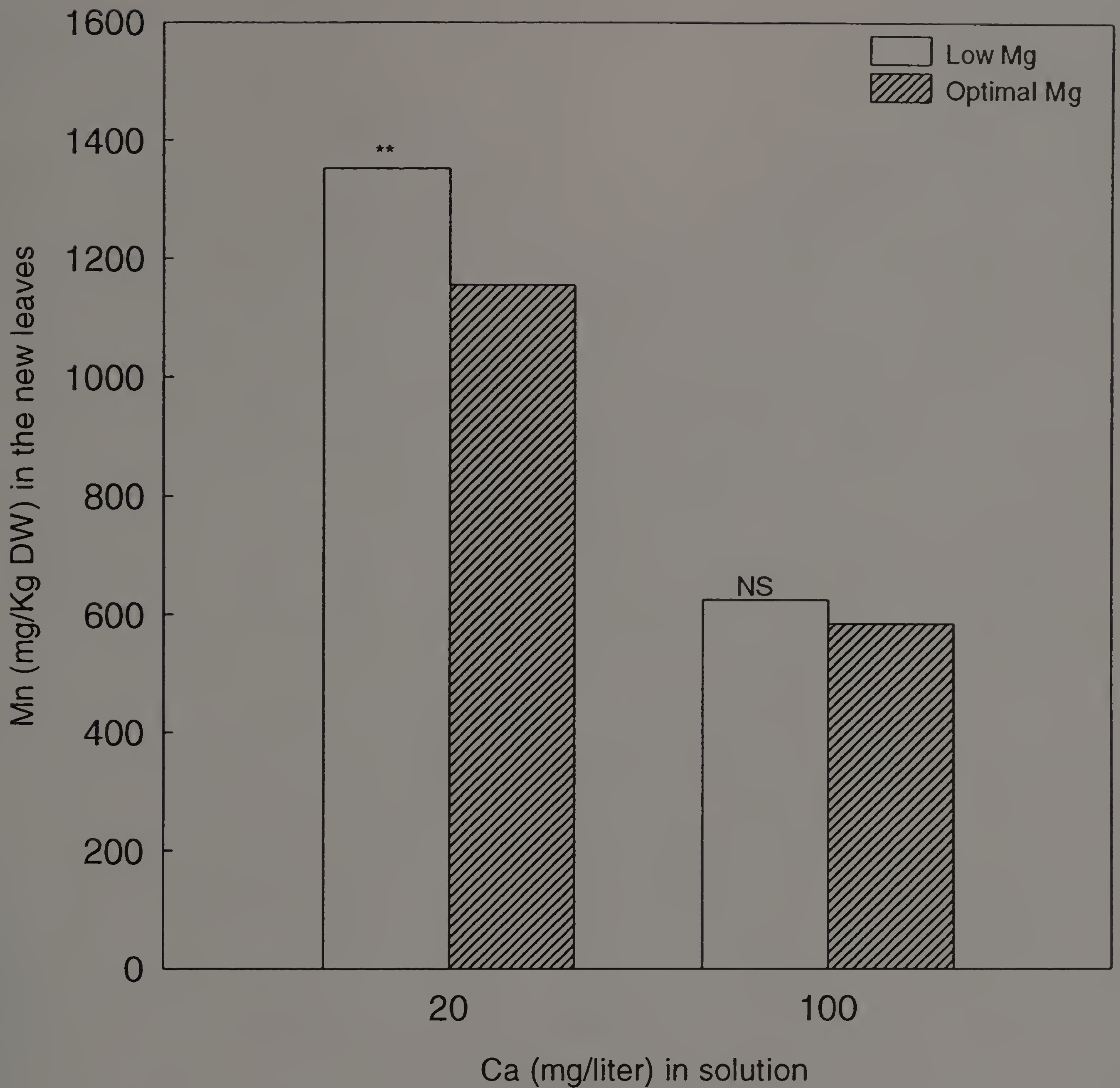

Figure 8.18: Effect of Ca level in solution on Mn level in the new leaves under low and optimal levels of Mg

(see Appendix H, Table 2)

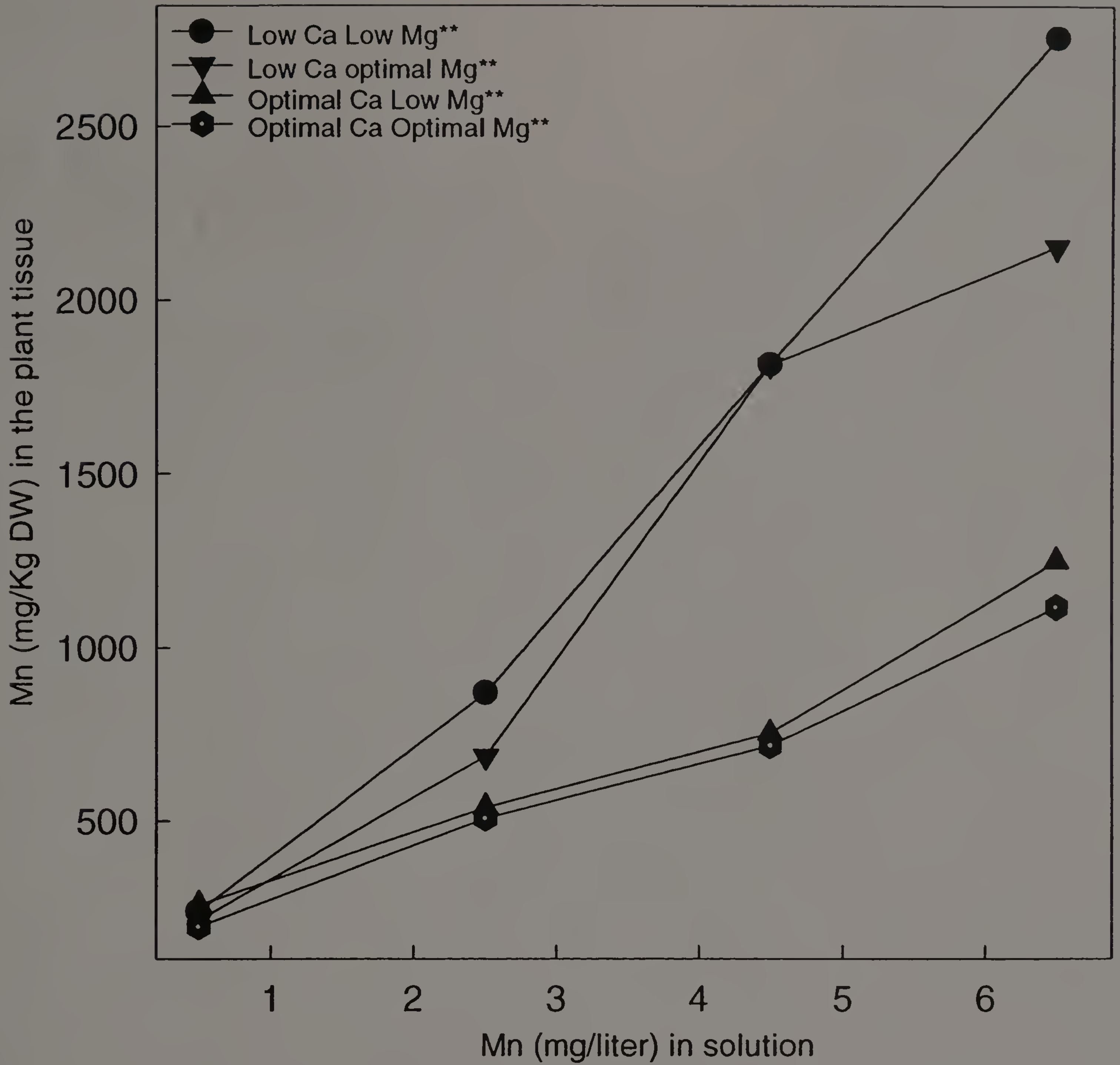

Figure 8.18a: Effect of Mn level in solution on the level of Mn in the plant tissue under different levels of Ca and Mg in solution (see Appendix H, Table 2)

Magnesium concentration in solution had a highly significant effect on the overall concentration of Mn in the plant tissue but there was no significant interaction between Mg in solution and the individual plant parts (Appendix H). Manganese concentration was high in the plant tissue when Mg level was low in solution (Figure 8.19). These results may be due to the effect of Mg in solution on Mn absorption by plants. Indeed, when Mg level in the medium is high, Mn absorption is reduced (Harrison and Bergman, 1981; Lohnis, 1960; Maas et al., 1969). When Mg level in the medium is low, Mn absorption is not reduced and Mn in the plant tissue can reach high concentrations.

8.4.4.2 Iron

Manganese concentration in solution had no significant effect on Fe in the stem and leaves, but did have a highly significant effect on Fe in the roots (Appendix H). As Mn level in solution increased, Fe in the roots also increases (Figure 8.20). This increase in Fe was much greater when Ca level solution was low (Figure 8.21). Magnesium in solution had no effect on the Fe increase in the roots (Appendix H).

Some of the symptoms of Mn toxicity in marigold were bleached or chlorotic patches on the leaves, which are similar to the typical symptoms of Fe deficiency. These findings may indicate that excess Mn in the solution causes a Mn-induced Fe deficiency. Yet, Fe increased in the roots as Mn increased in the solution. This suggests that high Mn concentration in the medium does not inhibit Fe absorption. Other researchers have found that excess Mn in the medium does not affect Fe absorption by plants but it unfavorably affects Fe action in the plant tissues (Rippel, 1923; Chapman, 1931; Millikan, 1949).

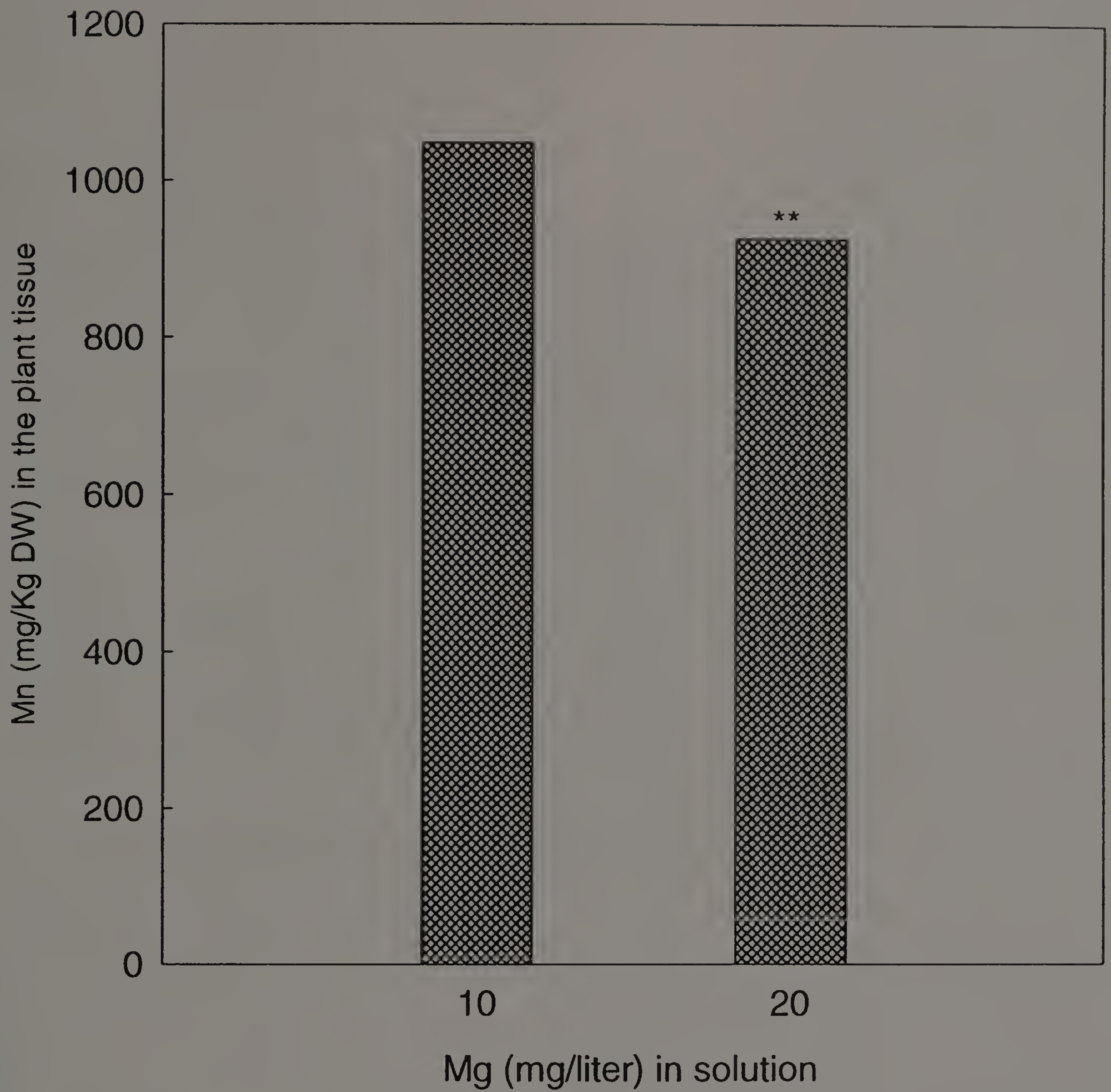

Figure 8.19: Effect of Mg level in solution on the level of Mn in the plant tissue (see Appendix H, Table 2)

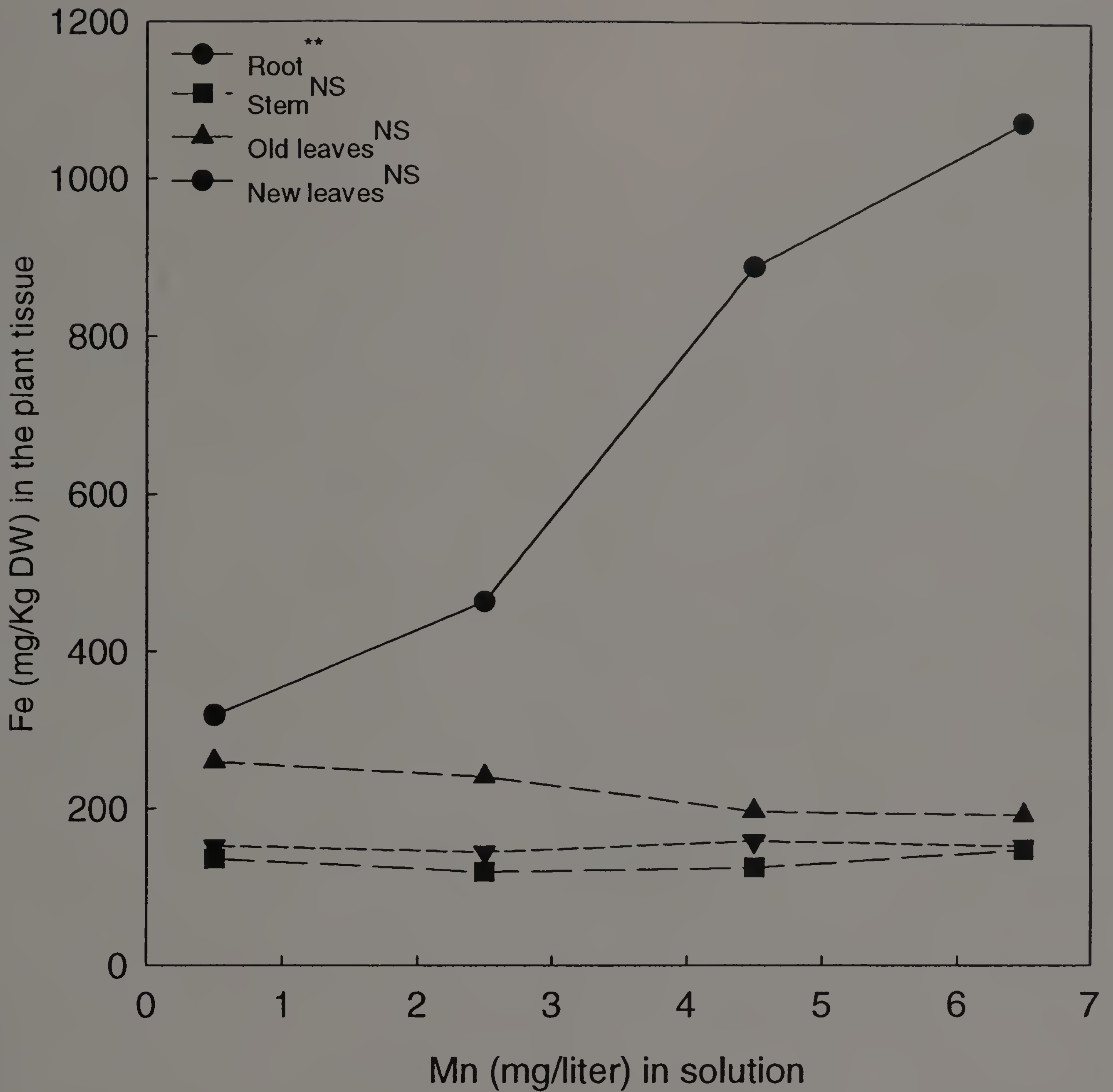

Figure 8.20: Effect of Mn level in solution on the level of Fe in the plant tissue (see Appendix H, Table 2)

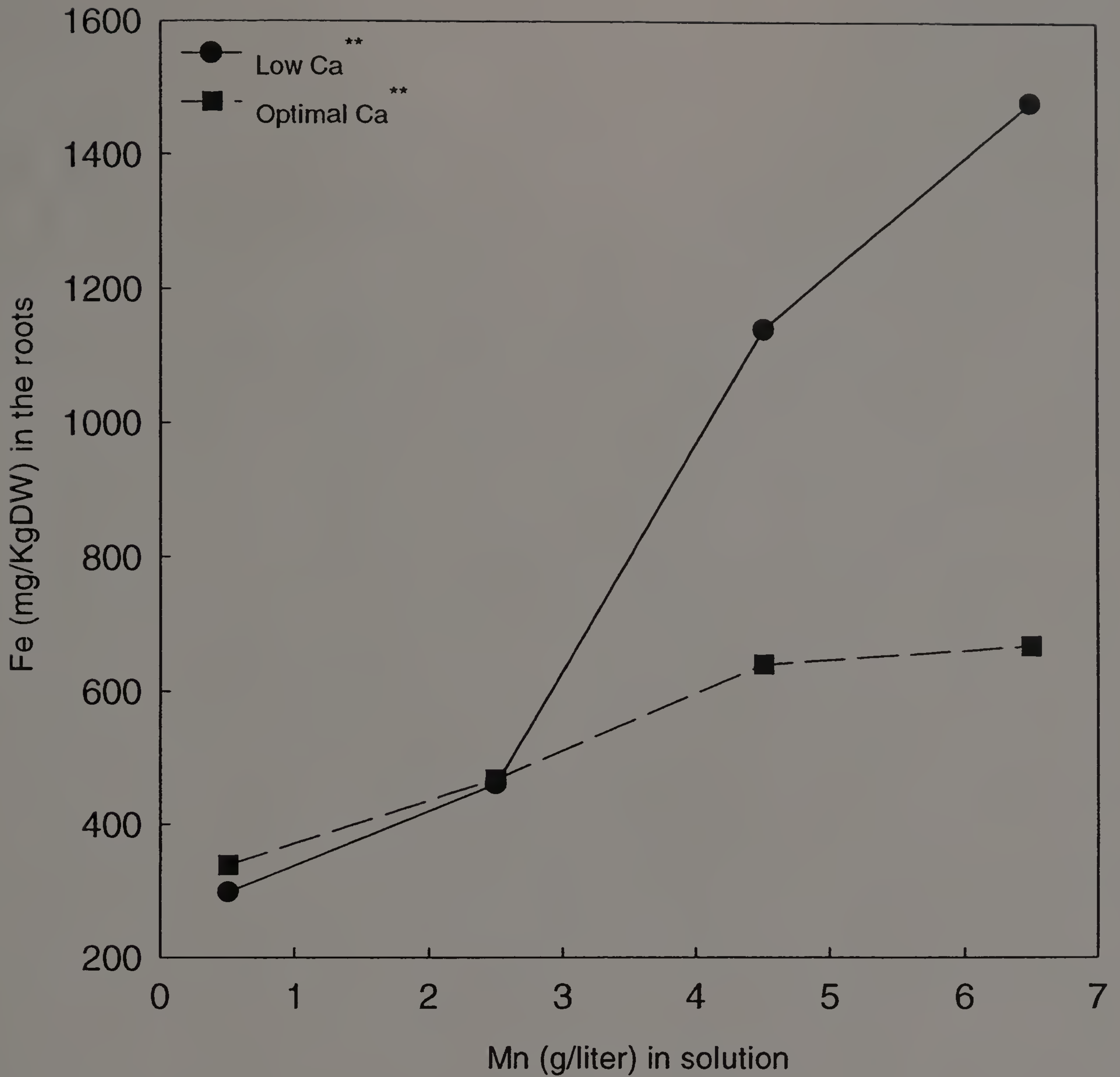

Figure 8.21: Effect of Mn level in solution on Fe level in the roots under low and optimal levels of Ca
(see Appendix H, Table 2)

Somers and Shive (1942) reported that the unfavorable effect of excess Mn on Fe may be due to the Mn catalysis of the oxidation of the physiologically active form of Fe (Fe^{2+}) to the inactive form (Fe^{3+}). They also reported the probable formation of an insoluble ferric-phosphate-organic complex.

The greater increase in Mn in the plant tissue when Mn was high and Ca was low in the solution may be due to the interaction between Mn and Ca. Indeed, high a concentration of Ca in the medium reduces Mn absorption by plants (Shuman and Anderson, 1976; Heenan and Carter, 1975; Robson and Loneragan, 1970; Hewitt, 1945). Low levels of Ca do not have this unfavorable effect on Mn absorption.

Calcium concentration in solution had no effect on Fe concentration in the stems and leaves but had a highly significant effect on Fe concentration in the roots (Appendix H). Iron level in the roots was high when Ca in solution was low and the opposite was also true (Figure 8.22). Our results agree with Rutland (1971), who found that Ca ions actually inhibit Fe uptake and translocation by precipitating Fe^{3+} compounds both outside and inside the plant. They also found that Fe accumulated in the nerves of chlorotic azalea leaves under the influence of $Ca(HCO_3)_2$.

Under the conditions of the present experiment, Mg in the solution had no significant effect on Fe concentration in any part of the plant (Appendix H). These results differ from those reported by Straub and Wurm (1971). They found that Mg concentration in the medium affected Fe concentration of the leaves. They also found that the leaves of Mg deficient apple plants had low Fe contents. In a study on maize and sunflower plants, Scherer (1978) found that Mg level was high in the plant tissue when

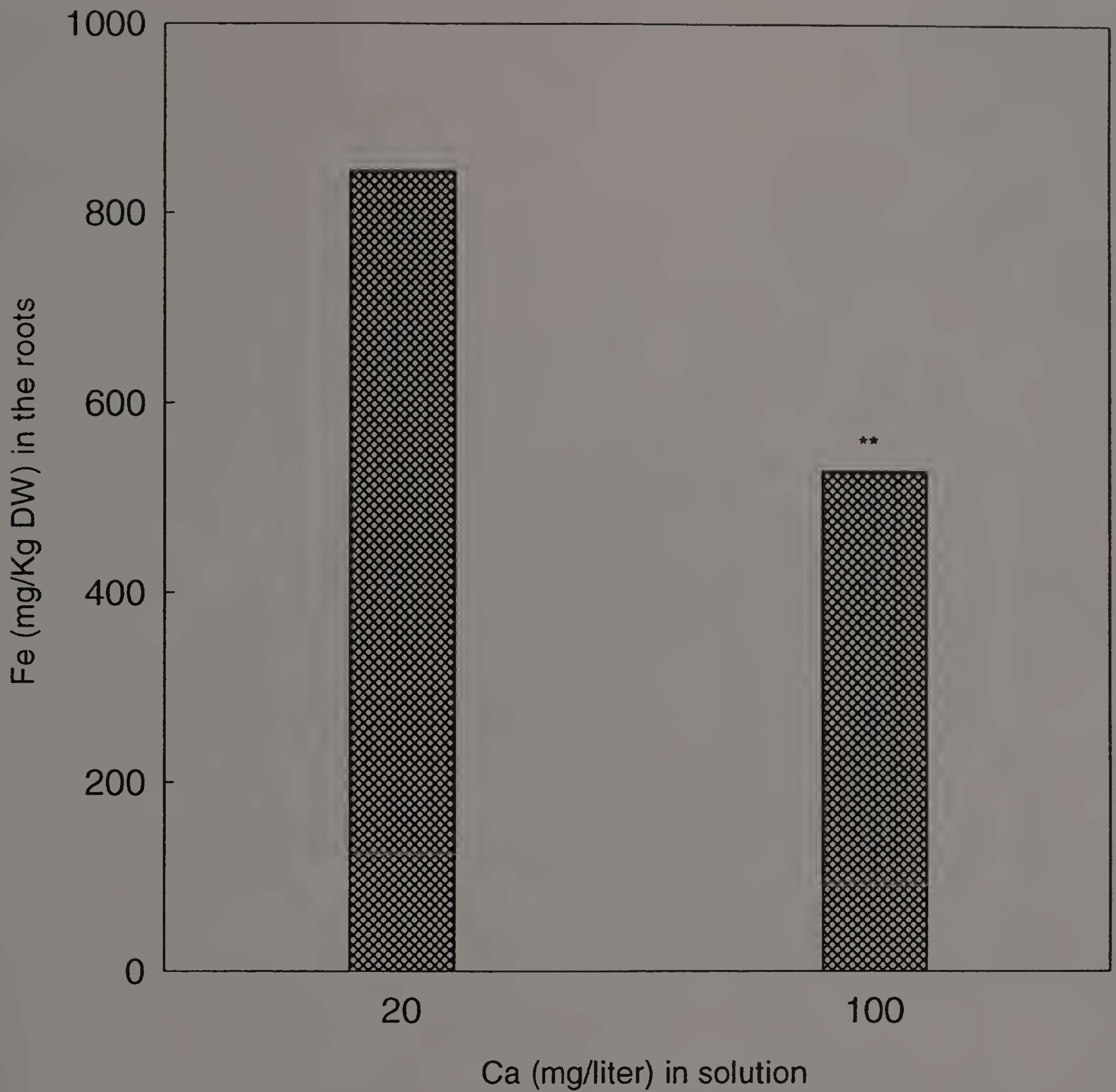

Figure 8.22: Effect of Ca level in solution on the level of Fe in the roots (see Appendix H, Table 2)

the plants were deficient in Fe. Scholl (1979a, b) reported the same results finding that Mg content increased from 9% in normal leaves to 14% in Fe deficient leaves.

8.4.4.3 Calcium

Manganese in solution had a highly significant effect on the overall Ca in the plant tissue (Appendix H). As Mn in the solution increased, the overall concentration of Ca in the plant tissue also rose (Figure 8.23). This increase was greater when Ca in solution was high (Figure 8.24) and the Mg level was low (Figure 8.25).

Manganese effect on Ca in the plant tissue was dependent on plant part and on Mg in the solution. High Mn in the solution caused an increase in Ca concentration in the roots, stems, and new leaves regardless of the Mg level in the solution. This increase of Ca was grater when the Mg level in solution was low (Figures 8.26; 8.27; 8.28). In the old leaves, excess Mn in the solution caused an increase of Ca when Mg level was low in the solution and caused a decrease in Ca when Mg was high (Figure 8.29).

Our results are different from those of Bekker et al. (1994), Foy et al. (1981) and Horst and Marshner (1978a), who found that Ca content of cotton, bean and peanut decreased markedly as the Mn in the medium increased. Clark et al. (1981) and Galvez et al. (1989) also found that excess Mn in the nutritive medium suppressed Ca in the plant tissue.

Excess Mn inhibits the translocation of Ca into the shoot apex (Horst and Marshner, 1990). This inhibition might be related to the fact that high Mn levels decrease the cation exchange capacity of the leaf tissue (Horst and Marshner, 1978a) and decrease the IAA levels in the areas of new growth (Morgan et al., 1966; Morgan et al.,

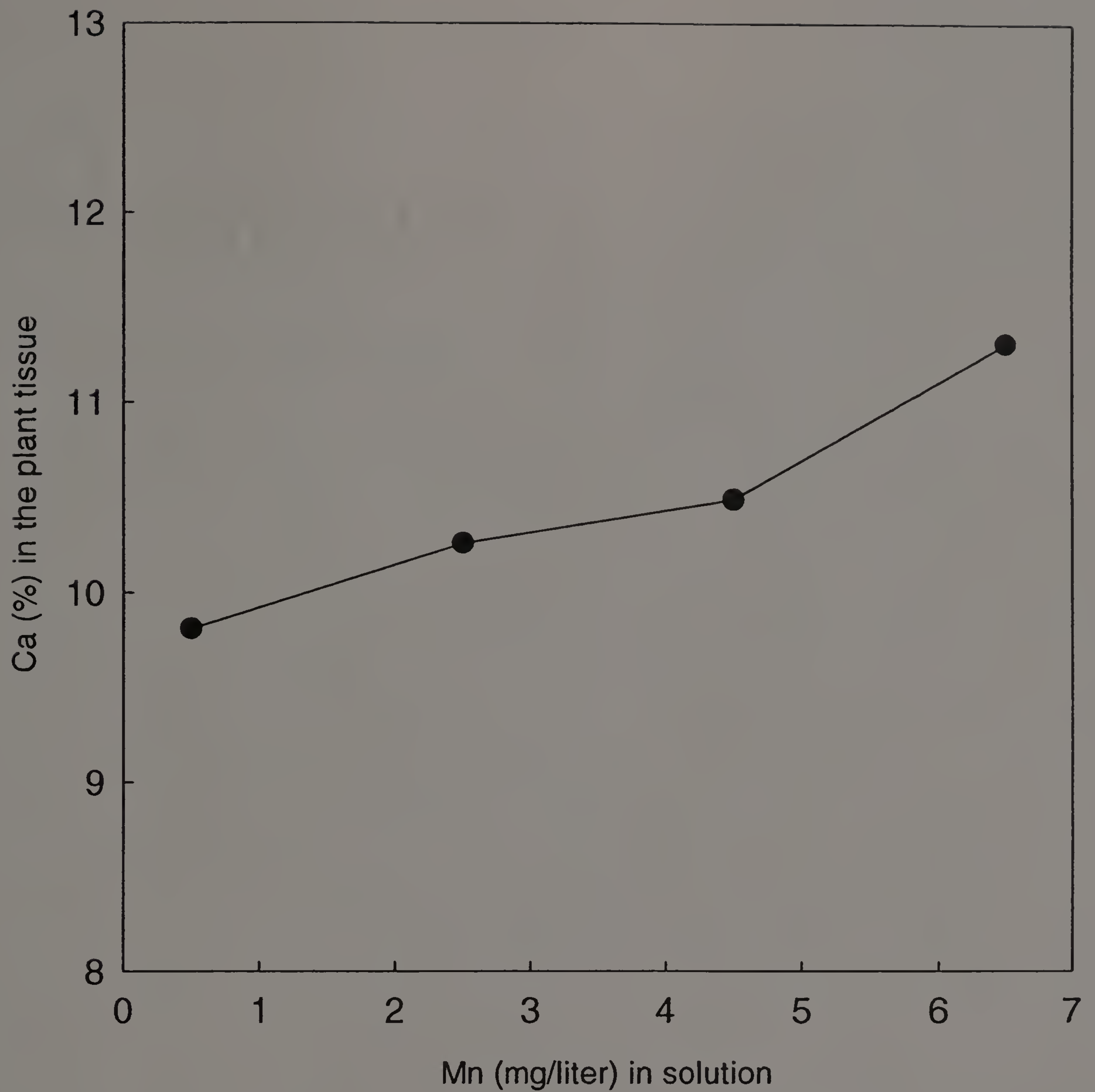

Figure 8.23: Effect of Mn level in solution on the level of Ca in the plant tissue (see Appendix H, Table 2)

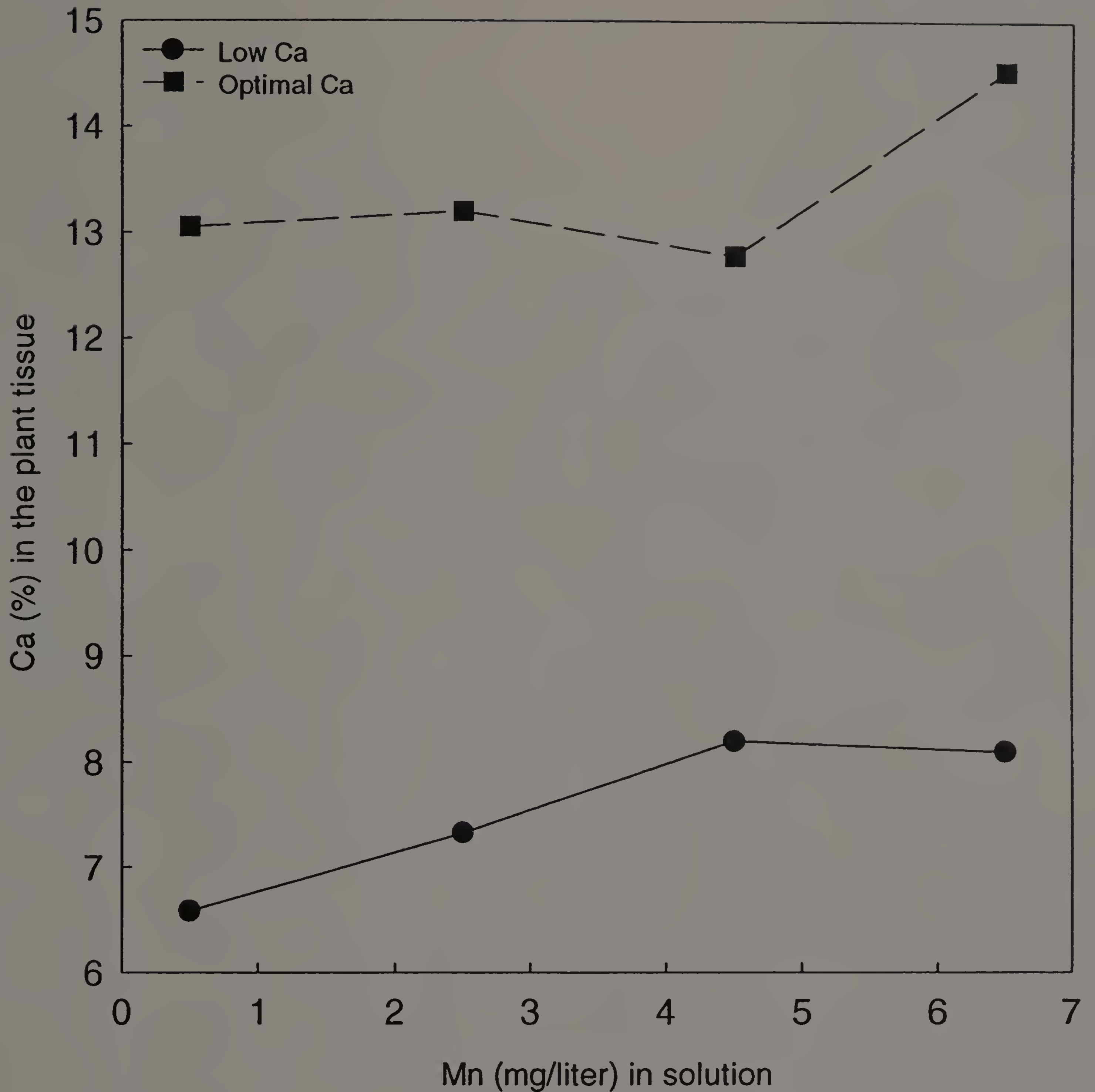

Figure 8.24: Effect of Mn level in solution on Ca level in the plant tissue under low and optimal levels of Ca
(see Appendix H, Table 2)

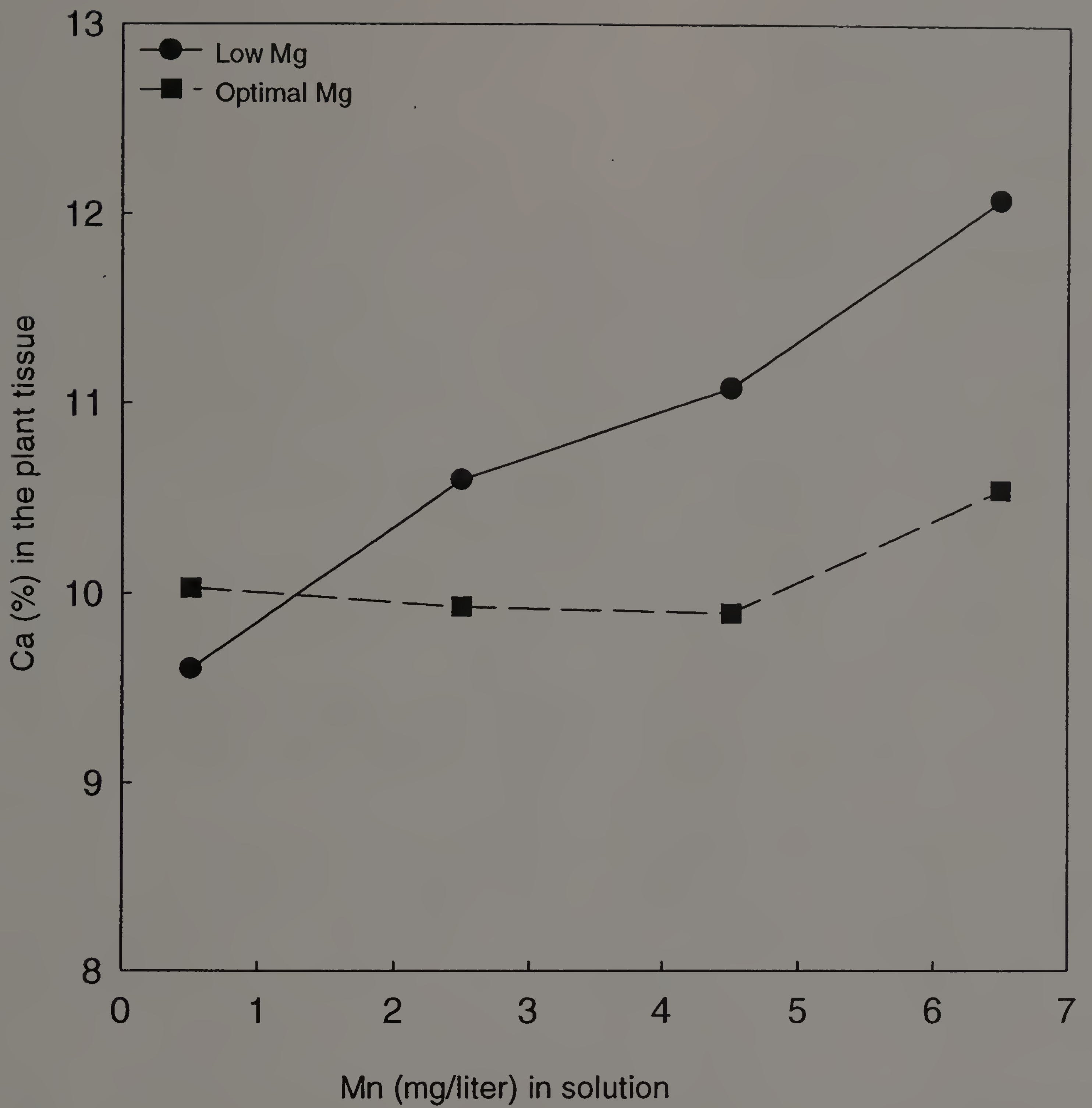

Figure 8.25: Effect of Mn level in solution on Ca level in the plant tissue under low ans optimal levels of Mg
(see Appendix H, Table 2)

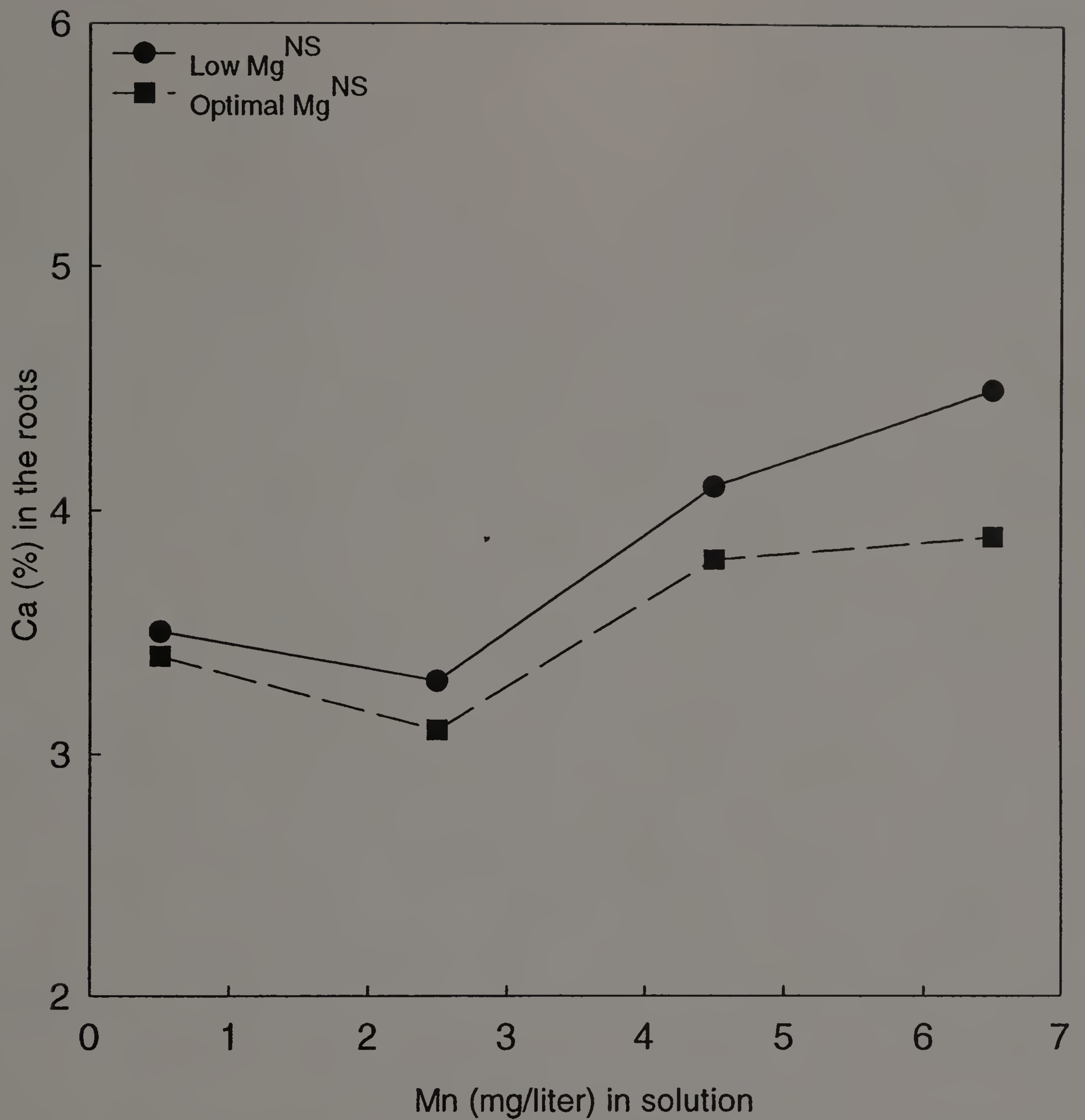

Figure 8.26: Effect of Mn level in solution on Ca level in the roots under low and optimal levels of Mg
(see Appendix H, Table 2)

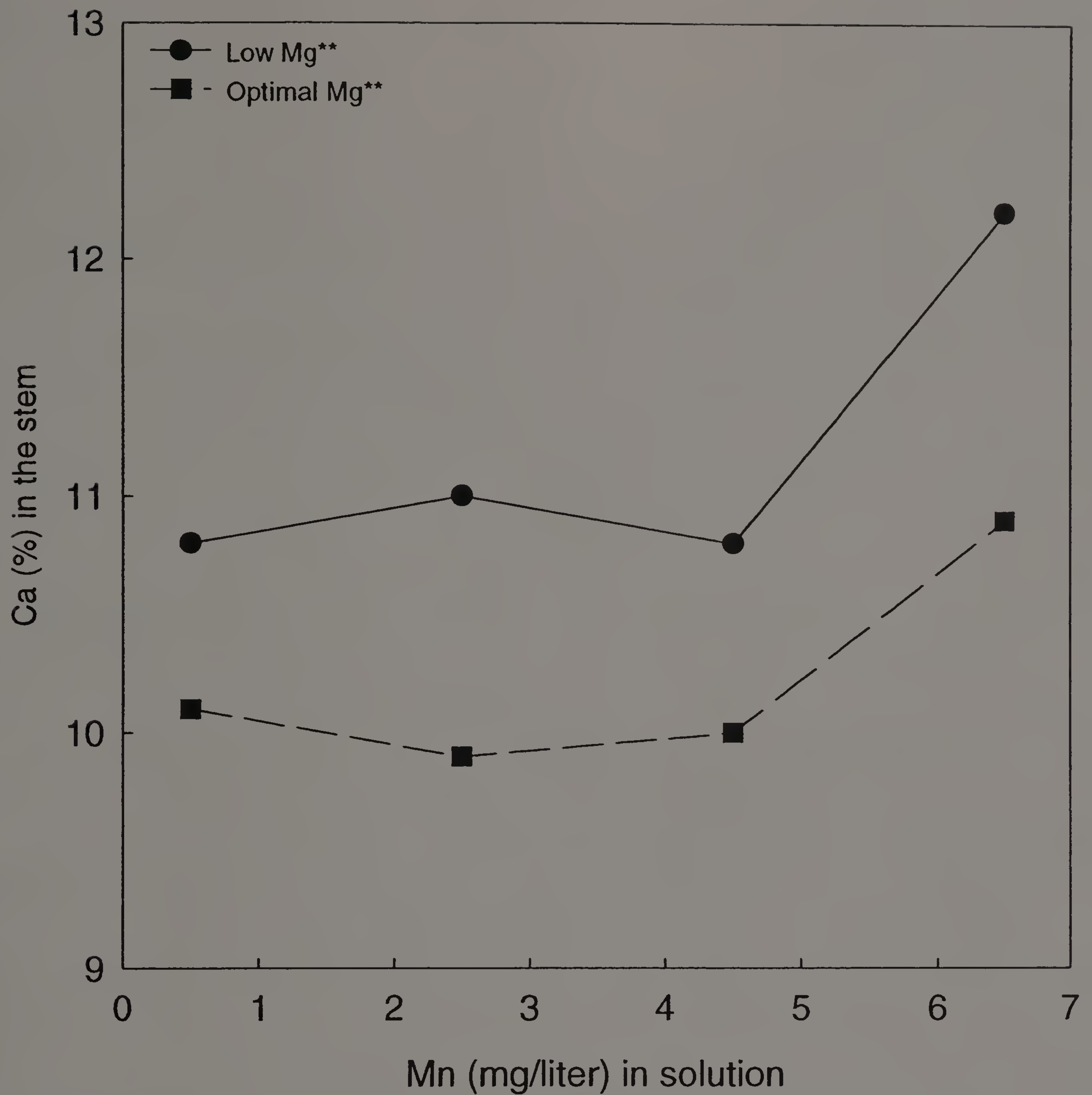

Figure 8.27: Effect of Mn in solution on Ca level in the stem under low and optimal levels of Mg

(see Appendix H, Table 2)

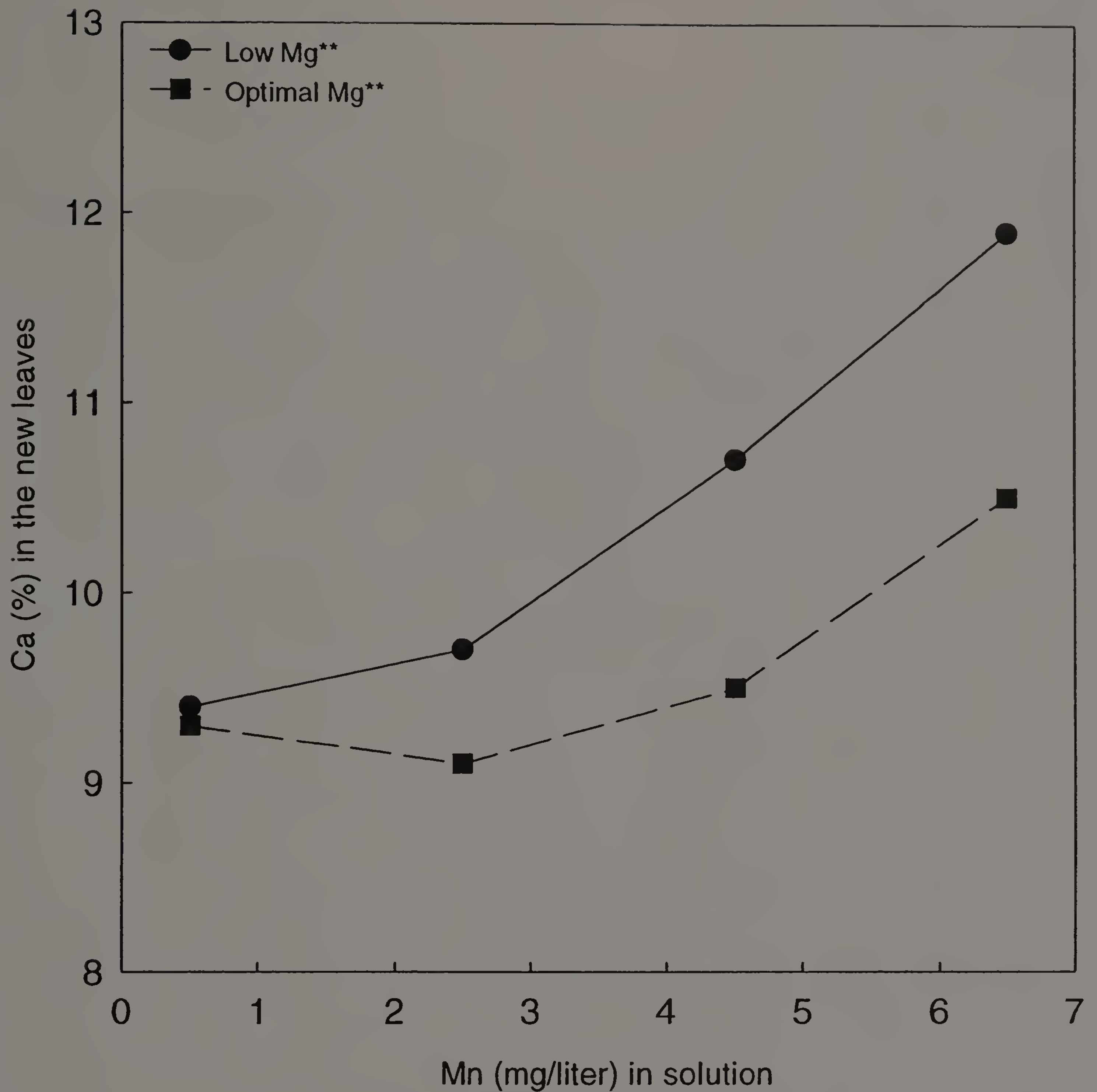

Figure 8.28: Effect of Mn level in solution on Ca level in the new leaves under low and optimal levels of Mg
(see Appendix H, Table 2)

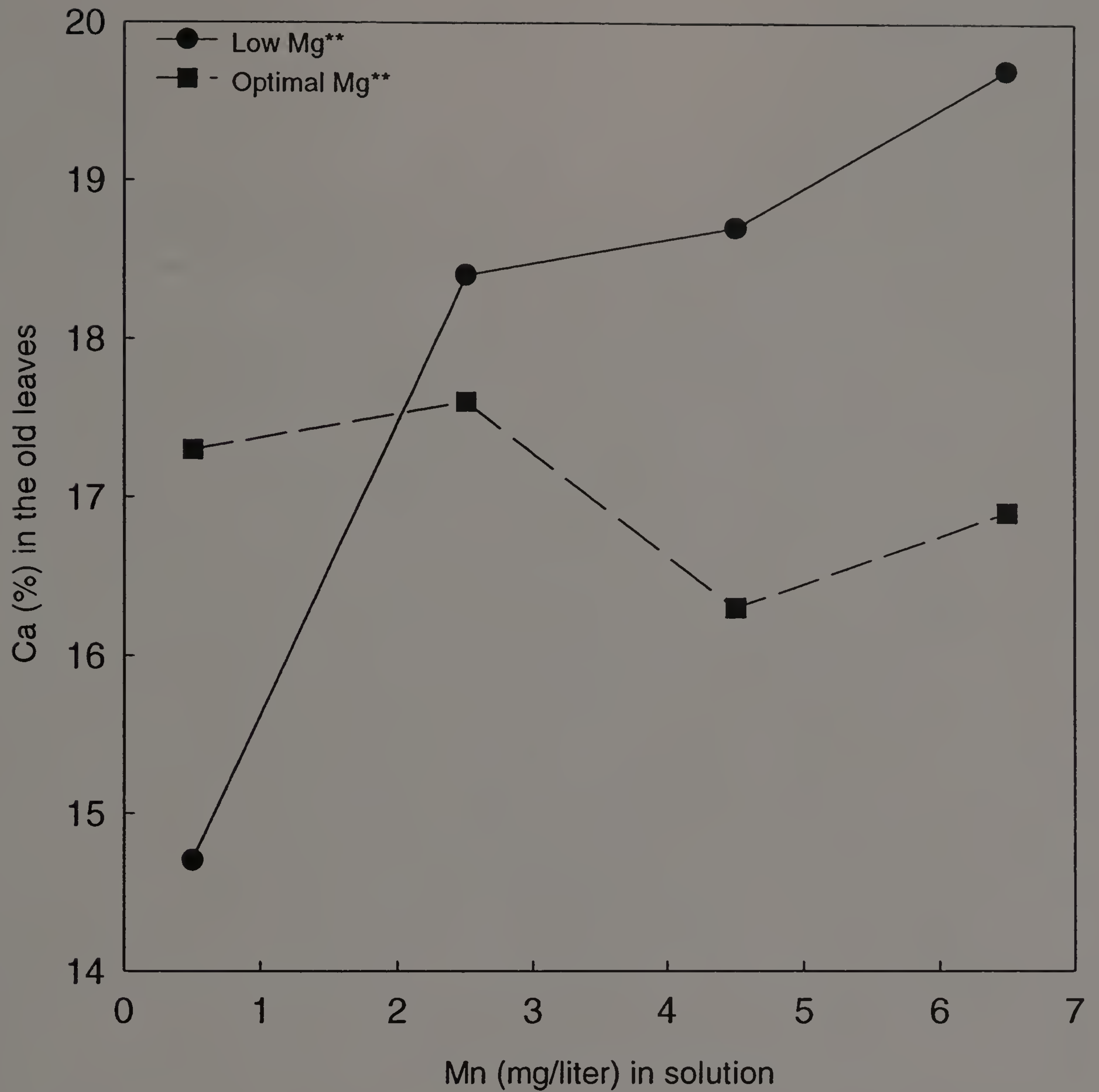

Figure 8.29: Effect of Mn level in solution on Ca level in the old leaves under low and optimal levels of Mg
(see Appendix H, Table 2)

1976). Auxin was reported to be responsible for the formation of new binding sites for the transport of Ca to the apical meristems (Horst and Marshner, 1978a).

Calcium in the solution had a highly significant effect on Ca in the roots, stems, old leaves and young leaves. Calcium concentration was much greater in the different plant parts when Ca supply was high (Figure 8.30).

Magnesium in solution had a highly significant effect on the overall Ca in the plant tissue (Appendix H). Calcium in the plant tissue was greater when Mg in solution was low (Figure 8.31). This effect of Mg in the solution on Ca in the plant tissue was probably attributed to ion antagonism between Ca and Mg. Bergmann (1992) attributed reduced Ca content in the plant tissue as a result to high level of Mg in the medium to ion antagonism.

Schwartz and Bar-yosef (1983) found that Mg interference operates by decreasing Ca binding to absorption sites or carrier sites in the root but not by reducing the capacity of these sites for Ca. Our results differ from those reported by Straub and Wurm (1971). They found that Mg deficient apples had low Ca in their leaves. Our results, however, agree with those of Schwartz and Bar-Yosef (1983). They found that Ca in tomato decreased with increased Mg in the medium.

8.4.4.4 Magnesium

Manganese in the solution had a highly significant effect on the overall Mg in the plants (Appendix H). As Mn in the solution increased, the concentration of Mg in the plant tissue also increased (Figure 8.32).

The effect of Mn in the solution on Mg in the plant tissue depended on the level of Mg in the solution. When Mg level was high in the solution, an increase in Mn

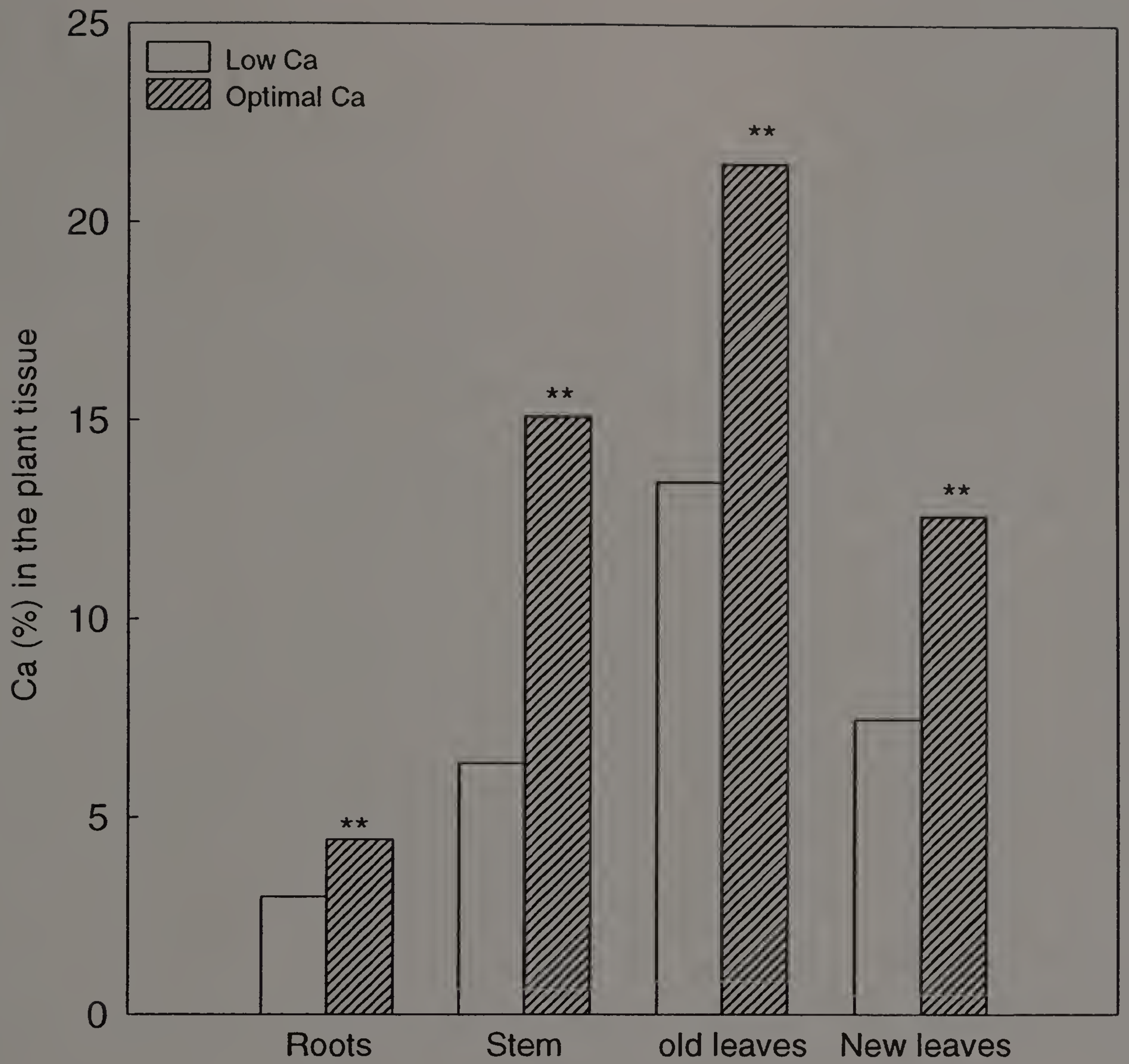

Figure 8.30: Effect of Ca level in solution on the level of Ca in the plant tissue (see Appendix H, Table 2)

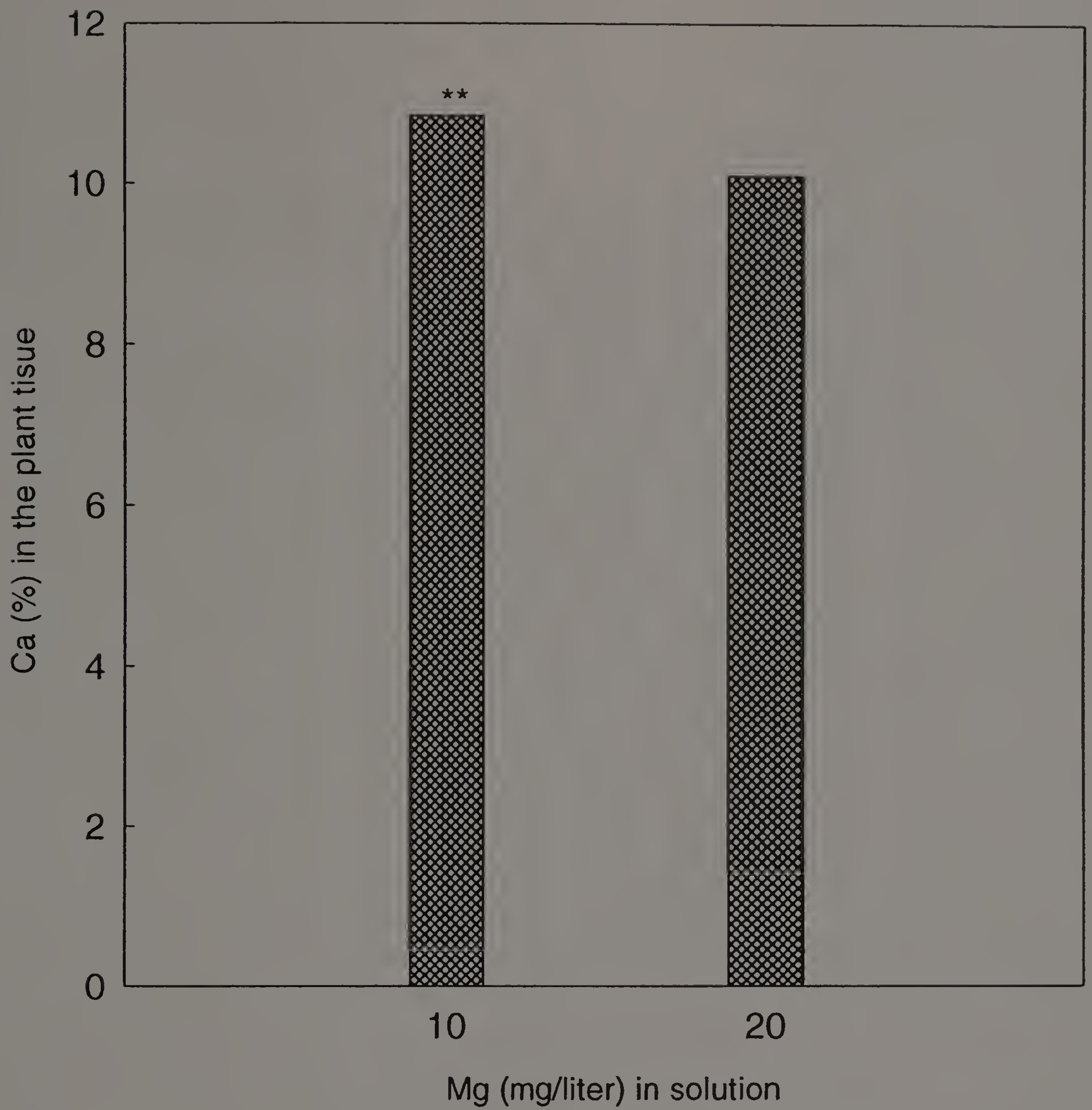

Figure 8.31: Effect of Mg level in solution on Ca level in the plant tissue (see Appendix H, Table 2)

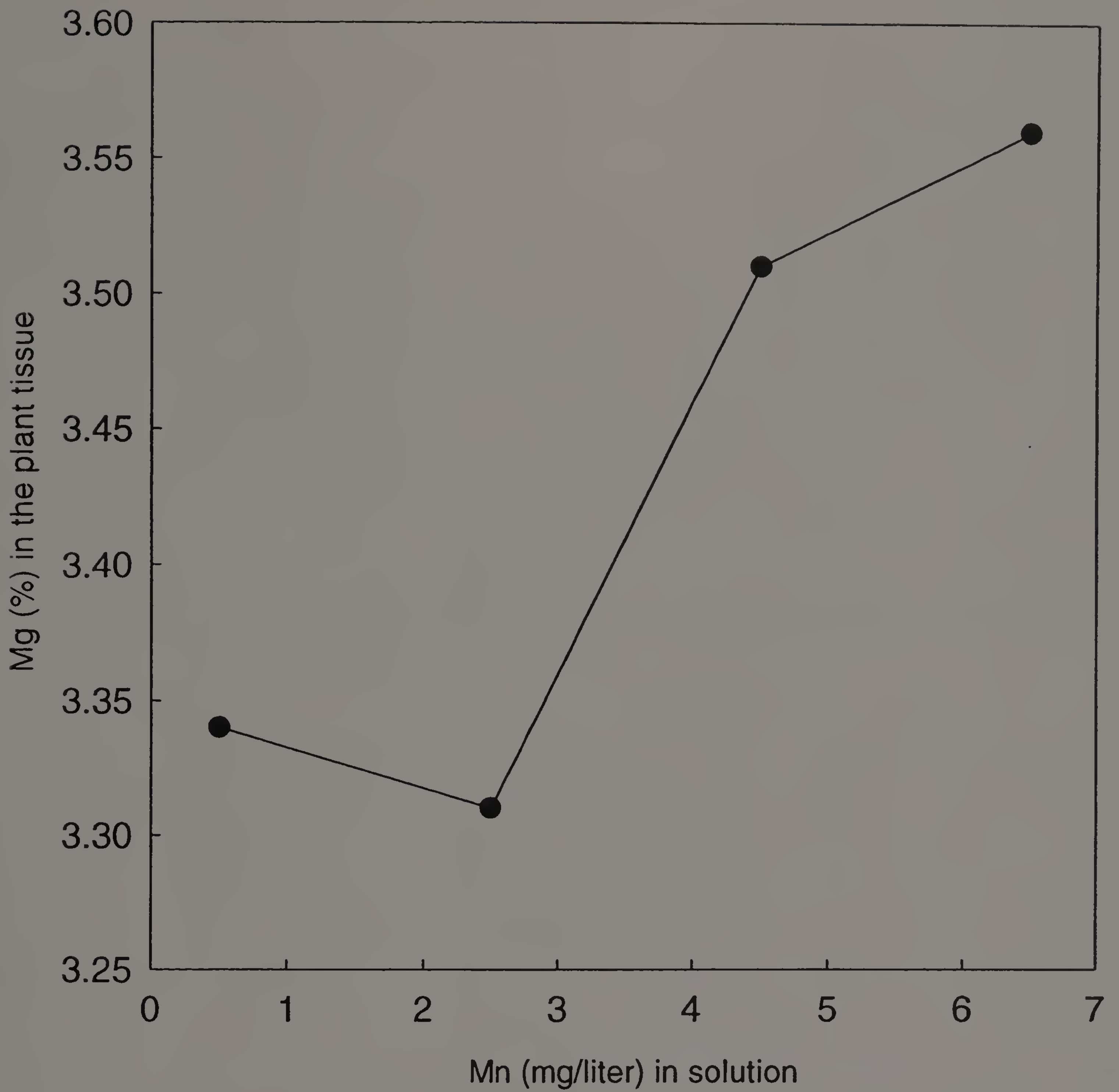

Figure 8.32: Effect of Mn level in solution on the level of Mg in the plant tissue (see Appendix H, Table 2)

concentration in the solution had no effect on Mg concentration in the plants. But when Mg was low in the solution, an increase in Mn in the solution caused a significant increase in Mg concentration in the plant tissue (Figure 8.33).

The effect of Mn in solution on Mg in the plant, also depended on Ca level in solution. When Ca level in the solution was high, there was no significant effect of Mn in the solution Mg in the plants. But when Ca was low in the solution, an increase in Mn in the solution caused a significant increase in Mg in the plants (Figure 8.34).

High Mn in the solution resulted in an increase in Mg in the roots (Figure 8.35). This increase was greater when Ca in the solution was low.

In the stem the interaction between Ca and Mn in the solution was significant. Under low Ca level in the solution, an increase in Mn in the solution caused an increase in Mg in the stem. When Ca in the solution was high, an increase in Mn in the solution caused a decrease in Mg in the stem (Figure 8.36).

The interaction between Mn and Ca in solution was not significant in the old leaves, but it was significant in the new leaves (Appendix H). Indeed, when Ca was low in the solution, an increase in Mn in the solution caused an increase in Mg in the new leaves (Figure 8.37). When Ca in the solution was high, increased Mn in the solution did have an effect on Mg in the new leaves (Figure 8.37).

These results differed from those in Chapters 6 and 7, possibly due to the interaction between Mn, Mg and Ca in the solution, and also from the findings of Heenan and Campbell (1981). They found that large concentrations of Mn in the medium can induce Mg deficiency. Kazda and Znacek (1989) and Heenan and Campbell (1981) reported that excess Mn may reduce Mg uptake by up to 50% due to competition. The

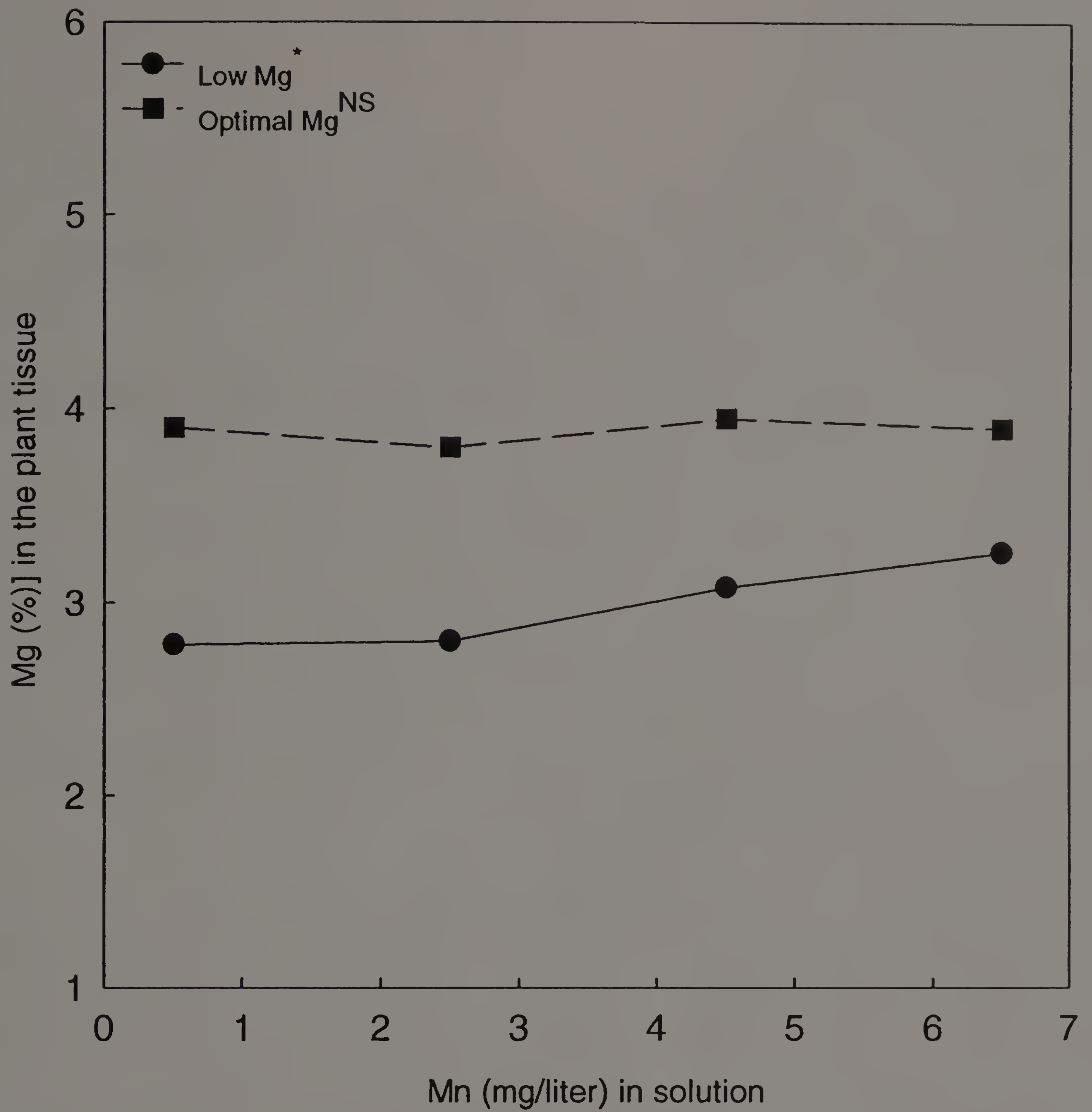

Figure 8.33: Effect of Mn level in solution on Mg level in the plant tissue under low and optimal levels of Mg
(see Appendix H, Table 2)

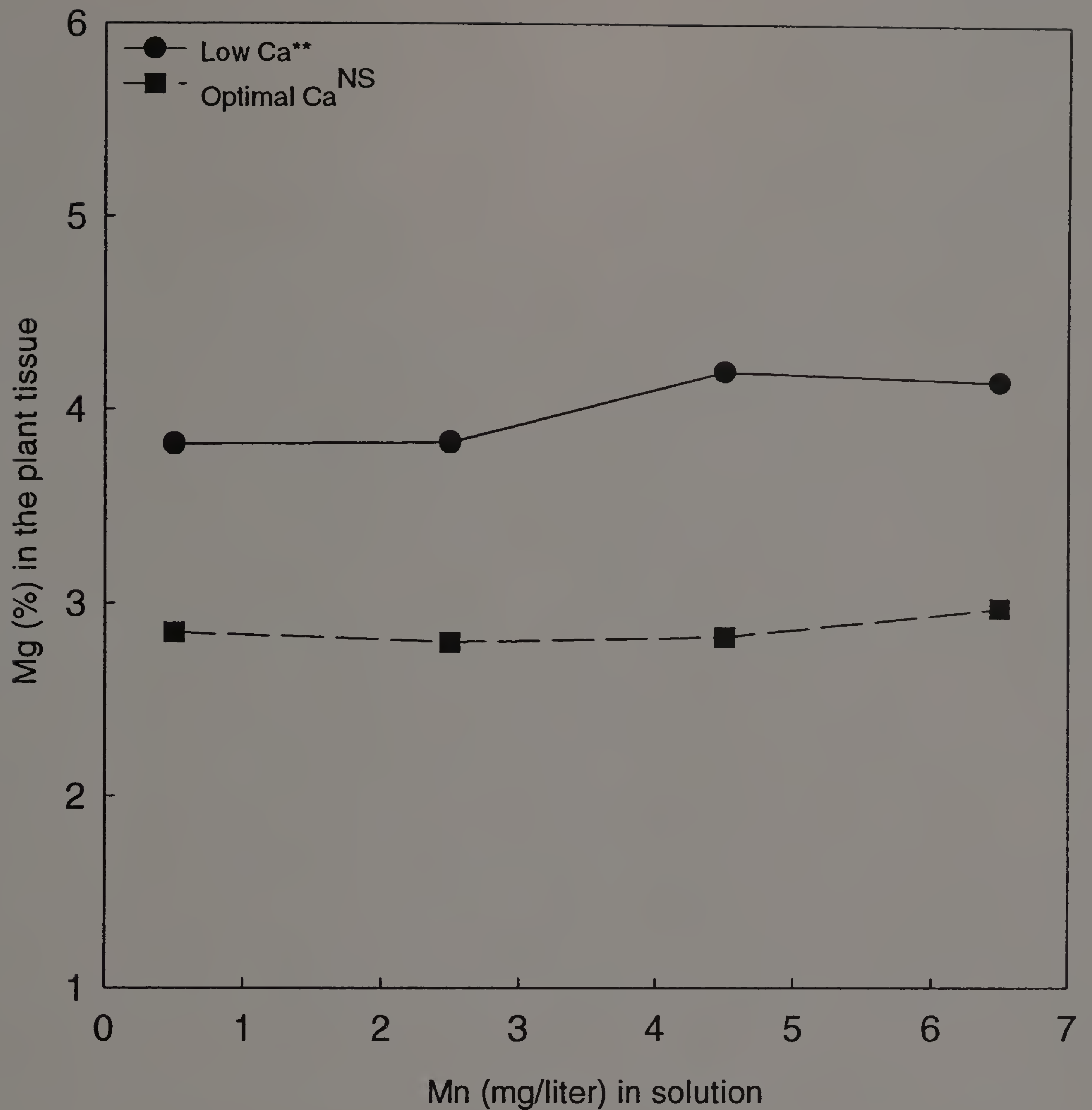

Figure 8.34: Effect of Mn level in solution on Mg level in the plant tissue under low and optimal levels of Ca
(see Appendix H, Table 2)

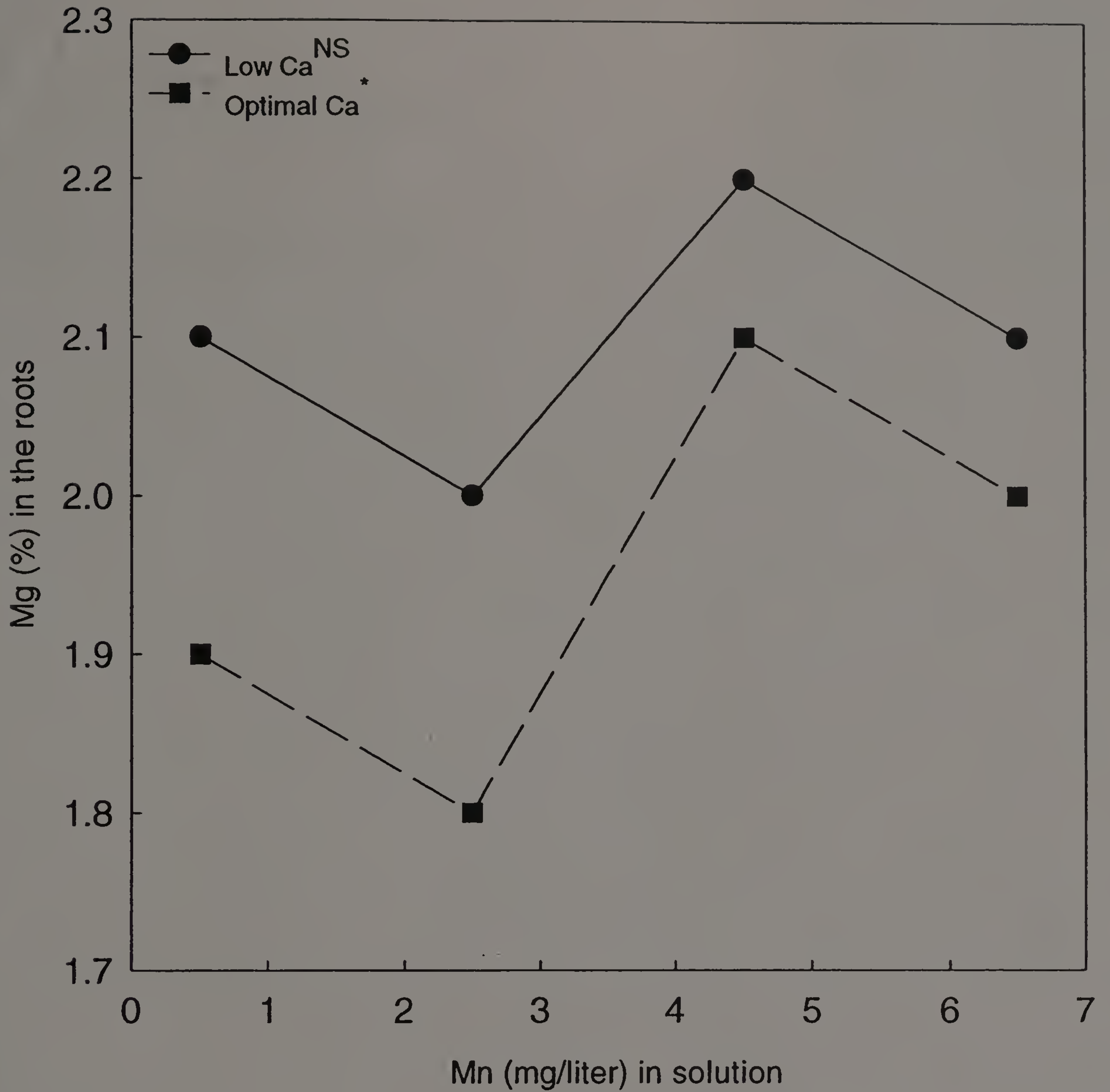

Figure 8.35: Effect of Mn level in solution on Mg level in the roots under low and optimal levels of Ca
(see Appendix H, Table 2)

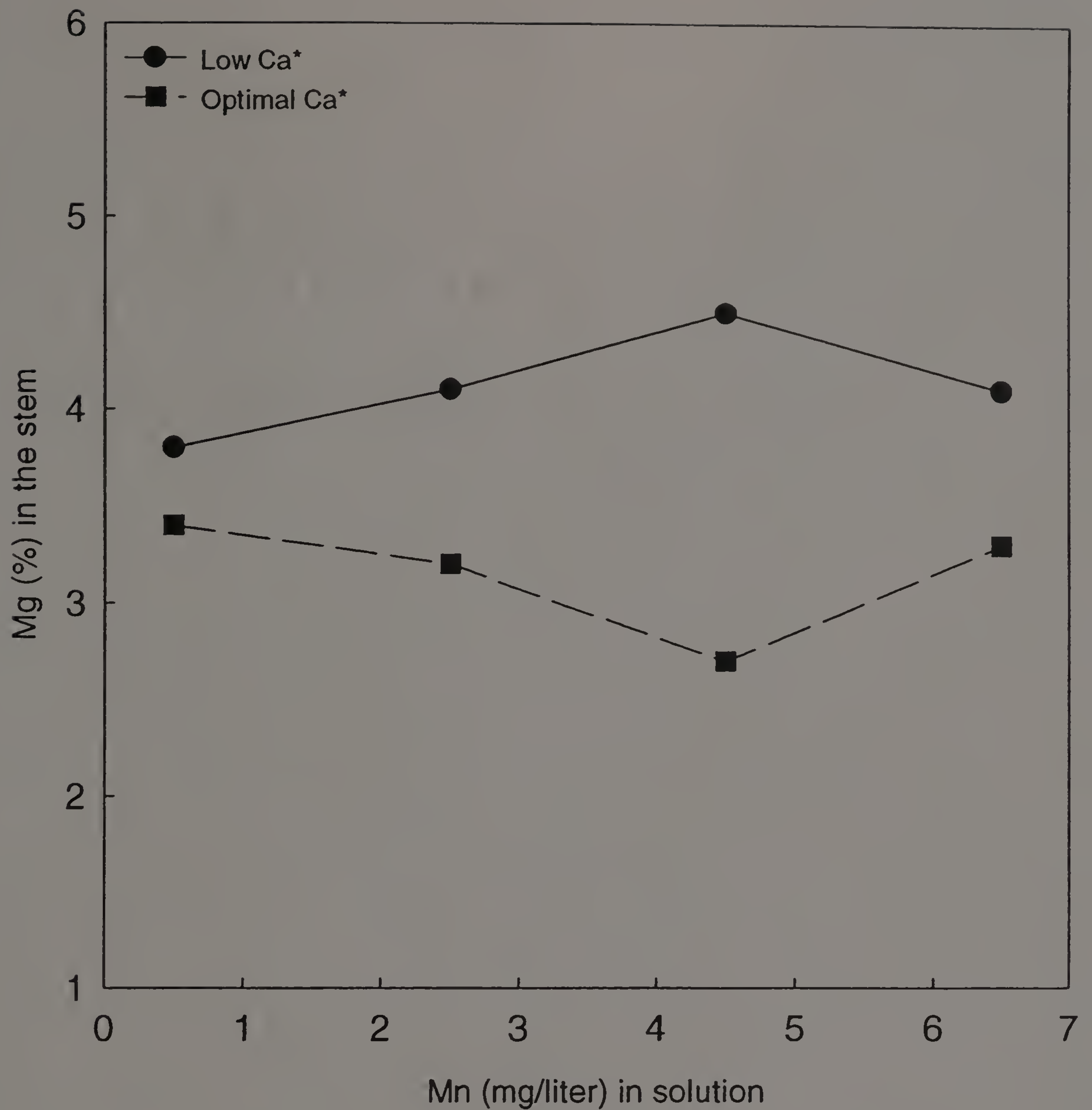

Figure 8.36: Effect of Mn level in solution on Mg level in the stem under low and optimal levels of Ca
(see Appendix H, Table 2)

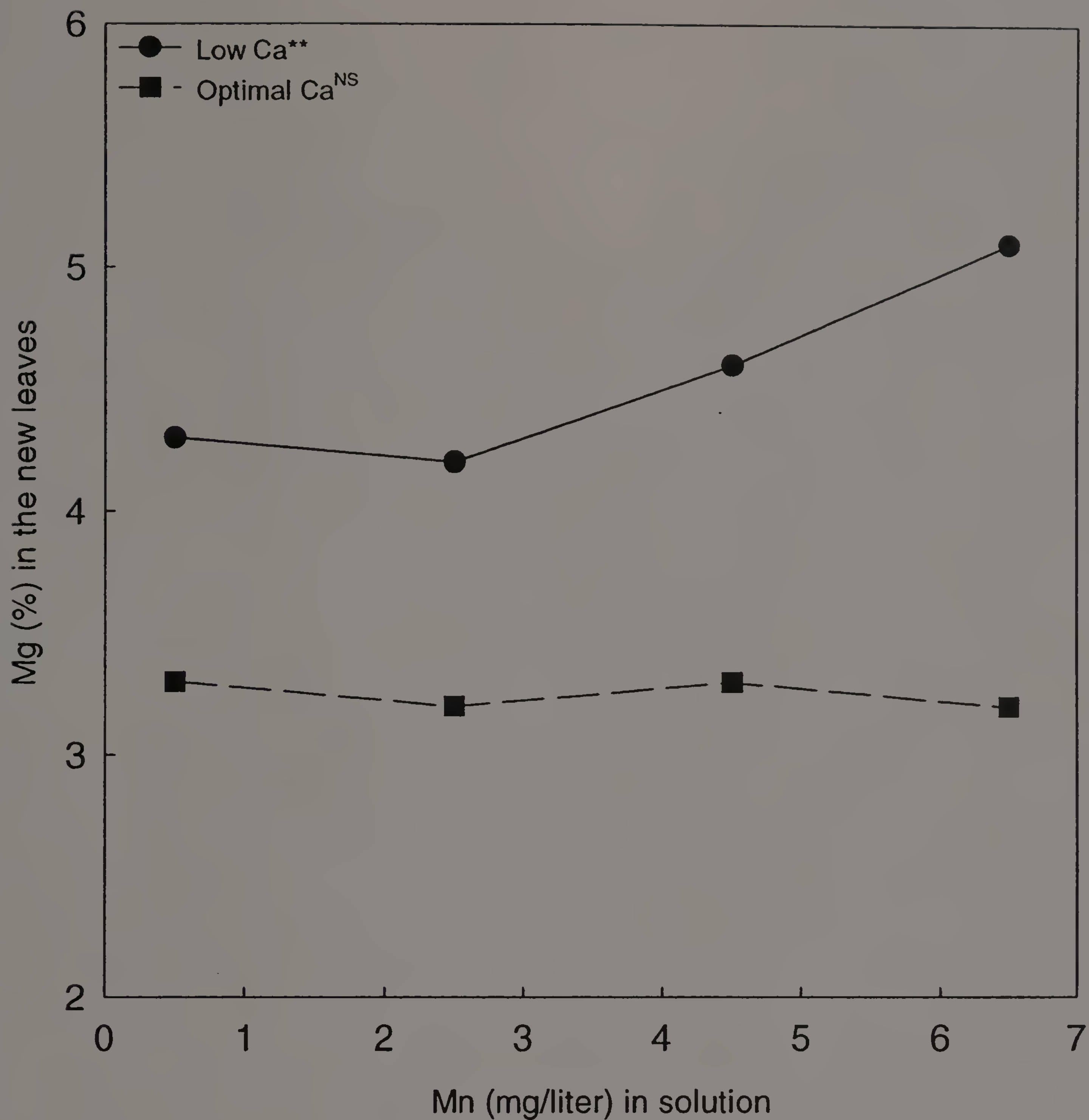

Figure 8.37: Effect of Mn level in solution on Mg level in the new leaves under low and optimal levels of Ca
(see Appendix H, Table 2)

competition between Mg and Mn for binding sites in the roots during absorption, inhibits Mg absorption, since Mn competes more effectively than Mg and even blocks the binding sites for Mg (Horst and Marshner, 1990).

Calcium concentration in the solution had no significant effect on Mg in the roots but did have a highly significant effect on Mg in the stem, old leaves and young leaves. More Mg was contained by the stem, old leaves and young leaves when Ca in the solution was low (Figure 8.38).

These results may be explained by the inhibitory effect that Ca has on Mg uptake. Indeed, a large concentration of Ca in the medium inhibits Mg influx (Bergmann, 1992). Moor et al. (1961) and Maas and Ogata (1971) found that high Ca concentration in the medium reduced Mg uptake rate by suppressing the Mg transport capacity of the roots rather than competing with Mg for absorption sites.

Magnesium in the solution had a highly significant effect on Mg in the roots, stems, old leaves and young leaves. Magnesium in the different plant parts was greater when Mg level in the medium was high rather than low (Figure 8.39).

8.5 CONCLUSION

Low Ca and Mg in the solution (incipient deficiency concentrations 20 mg/l for Ca and 10 mg/l for Mg) reduced the critical toxicity concentration of Mn in marigold from 4.6 to 0.5 mg/l. High concentration of Mn in solution (critical toxicity solution concentration 6.5 mg/l) combined with low Ca and Mg resulted in stunted plants with curled deformed leaves. Small brown spots covered the leaves. The tips of the leaves and edges were necrotic and the roots were brown.

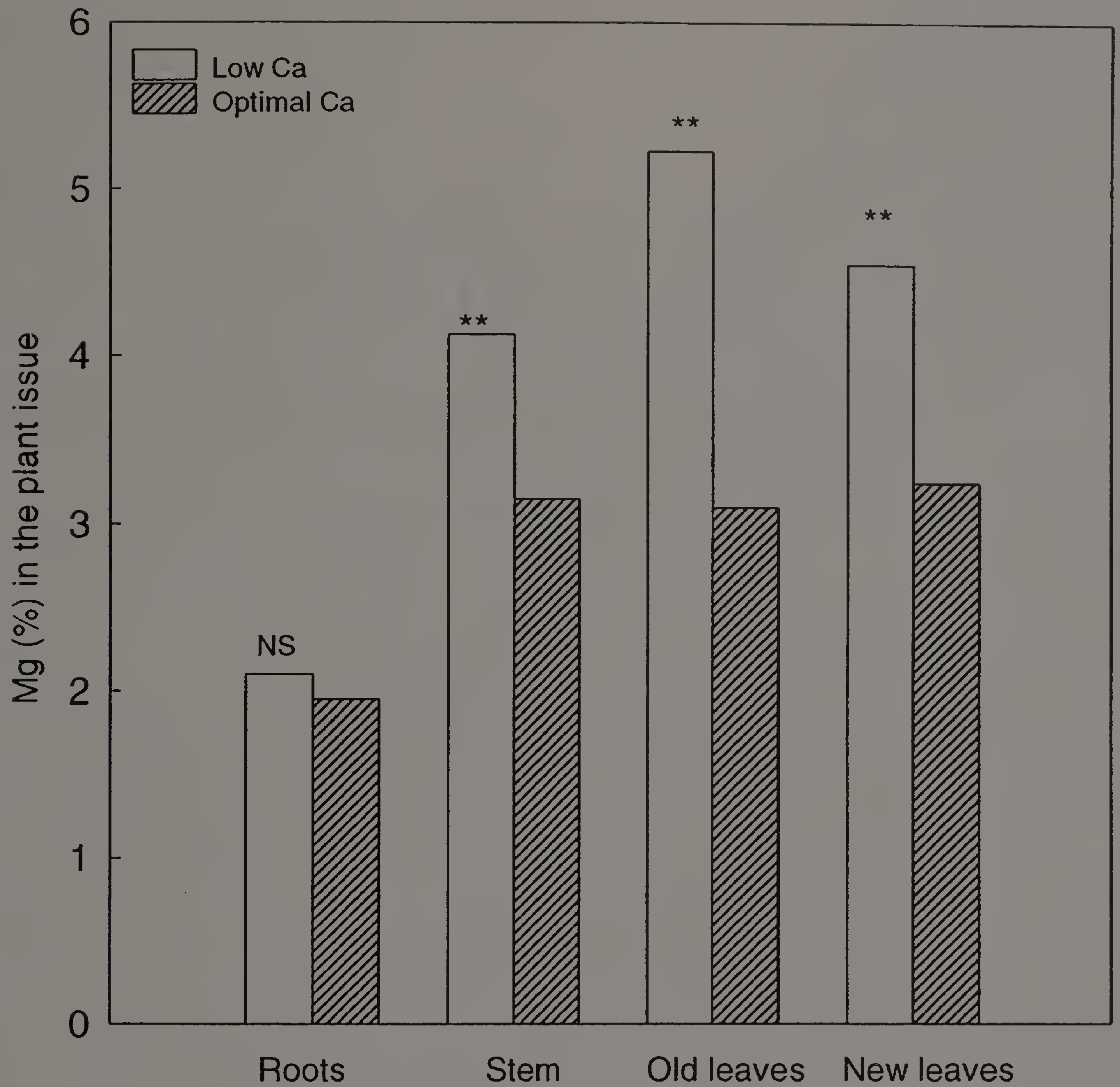

Figure 8.38: Effect of Ca level in solution on the level of Mg in the plant tissue (see Appendix H, Table 2)

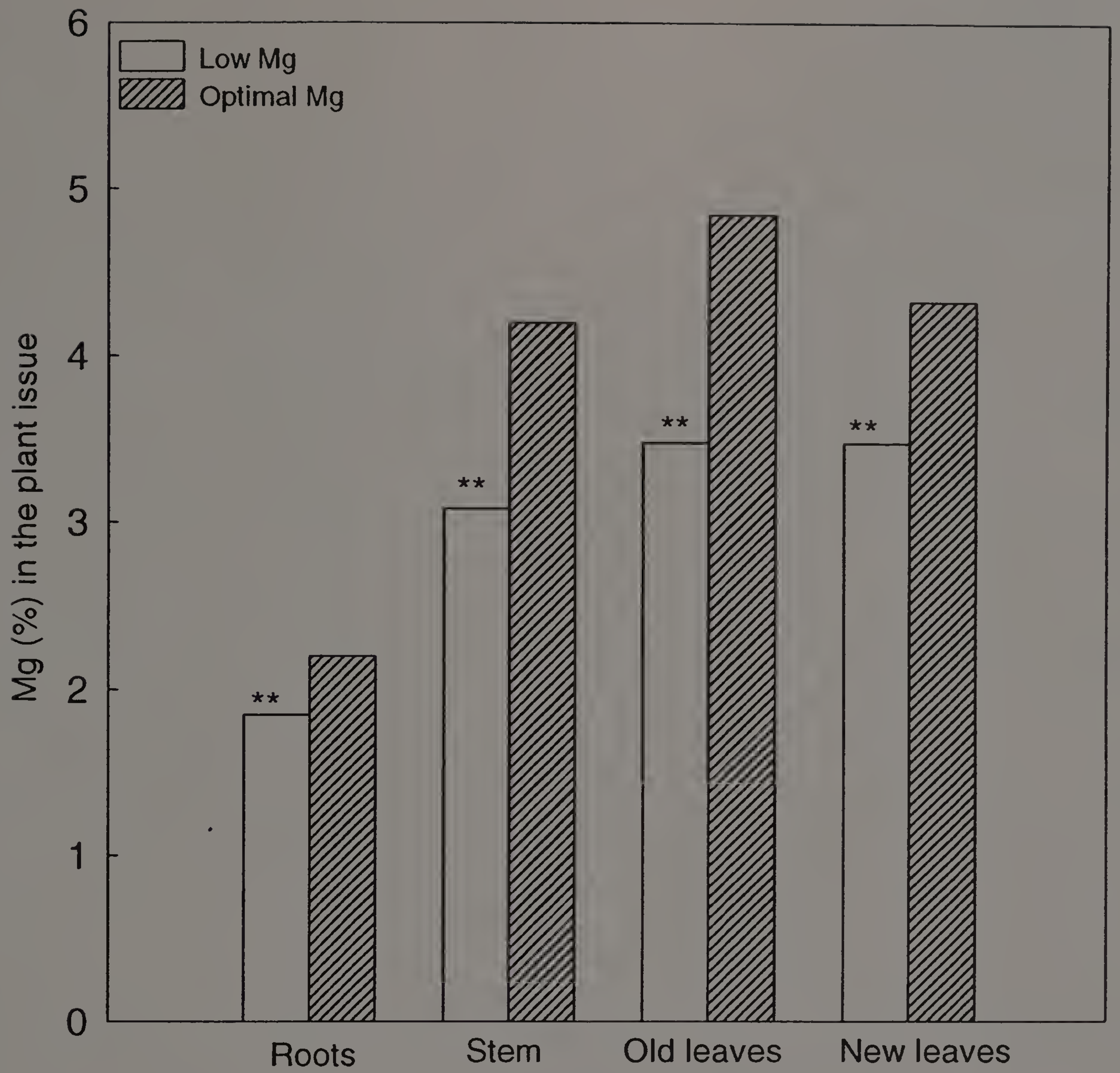

Figure 8.39: Effect of Mg level in solution on the level of Mg in the plant tissue (see Appendix H, Table 2)

The small brown spots and the brown color of the leaves may have resulted from localized precipitations of Mn as Mn oxide (MnO_2). The chlorosis on the leaves may have been due to Mn-induced Fe deficiency. The curling of the leaves may have been due to Mn-induced Ca deficiency.

High Mn in the solution resulted in increased Mn in all the plant parts. This increase was greater when Ca level was low. These results may be explained by the nature of the interaction between Mn and Ca. High levels of Ca in the growth medium decrease Mn absorption. Low Mg in the solution did not have as great an effect on Mn as that of Ca.

High Mn in the solution had no effect on Fe in the stems and leaves, but resulted in increased Fe in the roots. This increase was greater when Ca in the solution was low, but was not affected by Mg in the solution.

Despite this increase in Fe in the roots, high Mn in the solution may have resulted in Fe deficiency in the plant (the cause of the chlorotic areas on the leaves). Excess Mn did not inhibit Fe absorption, but it appeared to reduce Fe movement to the shoots.

High Mn in the solution caused an increase in Ca in the roots, stems, and new leaves regardless of the concentration of Mg. This increase in Ca was greater when Mg in the solution was low.

These results may be explained by the fact that high Mn in the medium may have inhibited Mg uptake, and that reduced Mg uptake may have favored Ca uptake. Indeed, Mg can reduce Ca uptake by plants.

In the old leaves, excess Mn in the solution caused an increase of Ca when Mg level was low and caused a decrease in Ca when Mg was high in the solution. These results may be explained by the antagonism between Mg and Ca.

High Mn in the solution increased the overall concentration of Mg in the plants. This increase was greater when Mg and Ca levels in the solution were high. These results may be explained by the interaction between Mn, Ca and Mg. High Mn in the medium may have inhibited Mg uptake; it may also have inhibited Ca uptake. The inhibition of Ca uptake may have favored Mg uptake over Ca uptake.

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CHAPTER 9

CONCLUSION

9.1 INCIPIENT DEFICIENCY CONCENTRATIONS FOR Ca AND Mg

Under the experimental conditions provided here, the incipient deficiency concentration of Mg in 'First Lady' marigold was determined to be about 10 mg/liter nutrient solution. This concentration was the turning point of Mg nutrition from deficiency to sufficiency. It still does not fulfill the requirement of Mg in marigold for optimal growth, but it was enough to prevent foliar deficiency symptoms.

Magnesium lower than 10 mg/liter resulted in stunted plants with severely damaged leaves. The symptoms on the leaves were typical symptoms of Mg deficiency: chlorosis and necrosis.

The incipient deficiency concentration of Ca in marigold was determined to be about 20 mg/liter nutrient solution. Concentrations of Ca lower than 20 mg/liter resulted in stunted bushy looking plants. Chlorotic spots and blotches developed along the margins and particularly at the tips of the leaves. Necrotic lesions appeared on the leaf blade as well. The leaves died from the tip to the base, as also did the apical meristematic tissue of the terminal buds and the roots.

9.2 CRITICAL TOXICITY CONCENTRATION OF Mn

The critical toxicity concentration of Mn in 'First Lady' marigold was determined to be 4.5 mg/liter nutrient solution. This concentration was the turning point of Mn nutrition from sufficiency to toxicity. It seems that 4.5 mg/l Mn was not high enough to cause localized precipitation of Mn in the leaves or to induce Fe or Ca deficiency, but it was high enough to reduce growth.

Concentrations of Mn higher than 4.5mg/liter resulted in stunted plants with damaged leaves. These leaves developed chlorotic patches that may be related to Fe deficiency induced by Mn toxicity. They also showed brown spots that may have been the result of localized precipitations of Mn.

Calcium and/or Mg deficiency decreased the critical toxicity solution concentration of Mn in marigold. When the concentration of Ca and/or Mg was low in the nutrient solution, the symptoms of Mn toxicity developed at lower concentrations of Mn in the solution.

9.3 NUTRIENT CONCENTRATION IN THE PLANT TISSUE

9.3.1 Manganese Concentration in the Plant Tissue

The present data suggested that Mn was evenly distributed in the plant shoots. There was no significant difference between the concentration of Mn in the growing point and the concentration of Mn in the older part of the shoots.

Low Mg in the solution resulted in increased Mn in the shoot tissue.

Low Ca in the solution had no significant effect on Mn in the old part of the shoots but resulted in increased Mn in the growing point (terminal bud, young leaves and young stem). Manganese in the growing point decreased as Ca in the solution increased. Calcium regulates Mn toxicity by reducing Mn absorption and transport to the shoot.

High Mn in the solution resulted in increased Mn in all the plant parts. Manganese accumulated in high concentrations in the roots perhaps reflecting a means reducing excess translocation to the shoots. It seemed that when the concentration of Mn exceeded certain amount, Mn was then translocated to the shoots where it may have precipitated as Mn oxide in the leaves (some of the symptoms of Mn toxicity).

The increase in Mn in the plant as a result of excess Mn in the solution was greater when Ca in the solution was low. Calcium tended to reduce Mn absorption and transport in the plant.

Magnesium did not affect Mn increase in the plant resulting from excess Mn in the solution.

When both Ca and Mg in the solution were low, high Mn in the solution resulted in increased Mn the plant. This increase was greater when Ca in the solution was low. These results may be explained by the nature of the interaction between Mn and Ca in the medium. High levels of Ca in the solution reduced Mn absorption. Low Mg in the solution did not have as great an effect on Mn increase as that of Ca.

9.3.2 Iron Concentration In The Plant Tissue

Magnesium in the solution had no significant effect on Fe in the shoots (old leaves, new leaves and stems).

Calcium deficiency had no significant effect on Fe in the growing point (new leaves, stems and buds) but resulted in a significant increase of Fe in the older part of the shoots (old leaves and stems). As Ca in the solution increased, Fe in the older part of the shoots decreased.

High Mn in the solution resulted in increased Fe in the roots. Increased Fe in the roots as a result of high Mn in the solution may have been caused by reduced translocation of Fe from the roots to the shoots. Excess Mn catalyses the oxidation of the physiologically active form of Fe (Fe^{2+}) to the inactive form (Fe^{3+}). Also, the formation of insoluble ferric-phosphate-organic complex in the roots due to high Mn in the roots may have prevented Fe translocation to the shoots.

The increase in Fe in the roots as a response to excess Mn in the solution was greater when Ca in the solution was low.

Magnesium in solution did not alter the effect of Mn in the solution on Fe in the plant.

When both Ca and Mg were low in the solution, high Mn in the solution had no effect on Fe in the stems and leaves, but resulted in increased Fe in the roots. This increase was greater when Ca in the solution was low, but was not affected by Mg. Despite the increase of Fe in the roots, high Mn in solution may have resulted in Fe deficiency in the plant (the cause of the chlorotic areas on the leaves). In fact, excess Mn did not inhibit Fe absorption, but it unfavorably affects Fe action in the plant tissues.

9.3.3 Calcium Concentration in the Plant Tissue

Low Mg in solution resulted in increased Ca in the shoots. An increase in Mg in solution caused a decrease in Ca in the shoots (old leaves, new leaves and stem). Reduced Ca in the plant as a response to increased Mg in the solution may be attributed to decreased Ca binding to absorption sites or carrier sites in the root.

Low Ca in the solution caused a decrease in Ca in the shoots. As Ca in the solution increased, Ca in the shoots increased.

High Mn in the solution resulted in a slight increase in the overall concentration of Ca in the plant. Increased Ca in the plant as a response to excess Mn in the medium may be attributed to reduced dry weight rather to an increased Ca uptake by the plants.

The interaction between Mn and Ca in the solution had no significant effect on Ca in the plant tissue.

The interaction between Mn and Mg in the solution had no significant effect on Ca in the plant.

When both Ca and Mg were low in the solution, high Mn in the solution caused an increase in Ca in the roots, stems, and new leaves regardless of the concentration of Mg in the solution. This increase may have been due to the fact that high Mn in the solution inhibited Mg uptake, which in turn favored Ca uptake. Indeed, Mg can reduce Ca uptake by plants.

In the old leaves, excess Mn in the solution caused an increase of Ca when Mg was low in the solution and caused a decrease in Ca when Mg was high. These results may be due to the antagonism between Mg and Ca in the solution.

9.3.4 Magnesium Concentration in the Plant Tissue

Magnesium deficiency resulted in decreased Mg in the plant shoots. Magnesium in the shoots increased with increased Mg in the solution.

Low Ca in the solution caused an increase of Mg in the shoots. As Ca in the solution increased, Mg in the shoot tissues decreased. Reduced Ca in the plant shoots as a response to increased Mg in the solution may have been attributed to decreased Ca binding to absorption sites or carrier sites in the roots.

High Mn in the solution resulted in a slight increase in the overall Mg of the plant. Increased Mg as a response to excess Mn in the solution may have resulted from a reduced dry weight rather to an increased Mg uptake by the plants.

The interaction between Mn and Ca in the solution had no significant effect on Mg in the plant tissue.

The interaction between Mn and Mg in the solution had no significant effect on Mg in the plant tissue.

When both Ca and Mg are low in the solution, high Mn in the solution resulted in increased overall Mg in the plant. This increase was greater when Mg and Ca levels in the solution were high. These results may have been due to the interaction between Mn, Ca and Mg in the solution. High Mn in the solution may inhibit Ca uptake. Since Ca inhibits Mg uptake, high Mn ends up favoring Mg uptake to the detriment of Ca.

As a conclusion, Fe/Mn toxicity disorder in marigold can be attributed to Mn toxicity. Low Ca supply or low Ca and Mg supplies are factors favoring the occurrence of Mn toxicity in marigold. Low Mg supply does not seem to have great an effect on this disorder.

Based on these results, large concentrations of Ca in the medium could alleviate the harmful effects of Mn toxicity in marigold. Low Mn supply could reduce or prevent the toxicity problems. Agricultural practices and nutritional regimes that reduce the availability of Mn and favor the availability of Ca could reduce the occurrence of Fe/Mn toxicity disorder in marigold and similar physiological disorders in other bedding plants grown in soilless media. I would recommend monitoring Mn supply and fertilizing with Ca to prevent or reduce Mn toxicity to floriculture plants.

APPENDIX A

TISSUE ANALYSIS BY ATOMIC ABSORPTION FLAME SPECTROPHOTOMETRY

PART I: HIGH TEMPERATURE OXIDATION (DRY ASHING)

Half of a gram of dried (70°C) and ground (20 mesh) plant tissue were weighed into a 30 ml high form porcelain crucibles. The crucibles were then placed in a cool furnace. The temperature control of the furnace was set at 500 °C. After approximately 6 hours of muffling at 500oC, the crucibles were removed from the furnace and allowed to cool. Ten ml dilute aqua regia (300 ml hydrochloric (HCl) and 100 ml nitric (HNO_3) acids in 1 liter distilled water) to dissolve the ash (solution A). The digest was diluted as necessary to bring an element concentration within the range of the analytical instrument.

PART II: ATOMIC ABSORPTION PROCEDURE

1. Calcium and Magnesium Analysis

1.1 Reagents

- Lanthanum solution, 12.5%: 147 g of Lanthanum oxide (La_2O_3) were dissolved in a minimum amount of concentrated HCl. 100 ml of HCl was added in excess. The content was mixed, diluted to 1 liter with distilled water and mixed again.
- Lanthanum solution, 5%: 1 volume of "Lanthanum solution, 12.5%" was diluted with 24 volumes of 1 + 9 HCl.
- Calcium reference solution (diluted to 100 ppm).
- Magnesium reference solution (diluted to 10 ppm).
- Calcium and magnesium standards: The standards were

- prepared in distilled water as directed bellow and used to establish a calibration curve for the atomic absorption spectrophotometer.

- Magnesium standards:

Final concent-ration Of Mg	Volume of Mg reference Solution	Volume of La^{2+} solution (12.5%)	Final volume ml
0	0	4 ml	100
0.1	1	4 ml	100
0.2	2	4 ml	100
0.3	3	4 ml	100
0.4	4	4 ml	100

- Calcium standards:

Final concent-ration of Ca	Volume of Ca reference Solution	Volume of La^{2+} solution (12.5%)	Final volume ml
0	0	4 ml	100
1	1	4 ml	100
2	2	4 ml	100
3	3	4 ml	100
4	4	4 ml	100

1.2 Procedure

One ml aliquot of solution A was transferred into a 25 ml volumetric flask. One ml of the 12.5% lanthanum solution was added and the solution was diluted to volume with distilled water. Calcium and magnesium concentrations were determined with the atomic absorption spectrophotometer, following manufacturer's instructions. When it was necessary to dilute the solutions, 0.5% lanthanum solution was used as the diluent.

1.3 calculations

[Ca] in the plant tissue = [Ca] read (ppm) X Dilution Factor
in mg/kg dry weight 0.5

[Mg] in the plant tissue = [Mg] read (ppm) X Dilution Factor
in mg/kg dry weight 0.5

2. Iron and Manganese Analysis

2.1 Reagents

- Iron reference solution (diluted to 100 ppm).
- Manganese reference solution (diluted to 100 ppm).
- 0.2N HNO_3.
- Manganese standards:

Final concent-ration of Mn	Volume of Mn reference Solution	Final volume ml
0	0	100 ml
1	1	100 ml
2	2	100 ml
3	3	100 ml

- Iron standards

Final concent-ration of Mn	Volume of Mn reference Solution	Final volume ml
0	0	100 ml
1	1	100 ml
2	2	100 ml
3	3	100 ml
4	4	100 ml
5	5	100 ml

2.2 Procedure

As for Calcium and magnesium, solution A was used to determine iron and Manganese concentrations on the atomic absorption spectrophotometer, following the manufacturer's instructions. All dilutions were made with 0.2N HNO_3.

2.3 Calculations

$$[Mn] \text{ in the plant tissue in mg/kg dry weight} = \frac{[Mn] \text{ read (ppm)} \times \text{Dilution Factor}}{0.5}$$

$$[Fe] \text{ in the plant tissue in mg/kg dry weight} = \frac{[Fe] \text{ read (ppm)} \times \text{Dilution Factor}}{0.5}$$

APPENDIX B

TISSUE ANALYSIS BY INDUCTIVE COUPLED PLASMA (ICP)

Procedure

Half of a gram of dried (70°C) and ground (20 mesh) plant tissue were weighed into a 30 ml high form porcelain crucibles. The crucibles were then placed in a cool furnace. The temperature control of the furnace was set at 500°C. After approximately 6 hours of muffling at 500oC, the crucibles were removed from the furnace and allowed to cool. Ten ml dilute Nitric acid (1liter 15 N (HNO_3) + 1 liter H_2O) was added to the crucibles to dissolve the ash . The digest was diluted as necessary to bring an element concentration within the range of the analytical instrument.

Manganese, Fe, Ca and Mg were determined with the ICP following manufacturer's instructions.

Calculations

[Ca] in the plant tissue in mg/kg dry weight = $\frac{\text{[Ca] read (ppm) X Dilution Factor}}{0.5}$

[Mg] in the plant tissue in mg/kg dry weight = $\frac{\text{[Mg] read (ppm) X Dilution Factor}}{0.5}$

[Mn] in the plant tissue in mg/kg dry weight = $\frac{\text{[Mn] read (ppm) X Dilution Factor}}{0.5}$

[Fe] in the plant tissue in mg/kg dry weight = $\frac{\text{[Fe] read (ppm) X Dilution Factor}}{0.5}$

APPENDIX C

STATISTICAL ANALYSIS

MAGNESIUM DEFICIENCY IN MARIGOLD

Abbreviation used in statistics

SDW : Shoot dry weight in grams
RDW : Root dry weight in grams
TDW : Total dry weight in grams
M : Magnesium concentration in the medium
Mg : Magnesium concentration of the plant shoots
Ca : Calcium concentration of the plant shoots
Mn : Manganese concentration of the plant shoots
Fe : Iron concentration of the plant shoots

Degree of significance

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

PART 1: ANALYSIS OF VARIANCE

Table 1: Effect of Mg level in the solution on the plant dry weight (Figure 3.1)

SDW

Source	DF	Anova SS	Mean Square	F Value	Pr > F
M	6	1469.91	244.98	168.40**	0.0001
Error	33	48.01	1.45		
Correct.T.	39	517.92			

RDW

Source	DF	Anova SS	Mean Square	F Value	Pr > F
M	6	14.53	2.42	35.21**	0.0001
Error	33	2.27	0.07		
Correct.T.	39	16.80			

TDW

Source	DF	Anova SS	Mean Square	F Value	Pr > F
M	6	1771.43	295.30	183.39**	0.0001
Error	33	53.13	1.61		
Correct.T.	39	1824.56			

Table 2: Effect of Mg level in the solution on the nutrient levels in the plant shoots (Figure 3.2)

Mn

Source	DF	Anova SS	Mean Square	F Value	Pr > F
M	6	09568.28	18261.38	4.17**	0.0077
Error	19	83218.50	4379.92		
Correct.T.	25	192786.78			

Fe

Source	DF	Anova SS	Mean Square	F Value	Pr > F
M	6	150916.00	25152.67	0.88 NS	0.53
Error	19	546134.95	28743.95		
Correct.T.	25	697050.95			

Ca

Source	DF	Anova SS	Mean Square	F Value	Pr > F
M	6	2.94	0.49	11.22**	0.0001
Error	19	0.83	0.04		
Correct.T.	25	3.77			

Mg

Source	DF	Anova SS	Mean Square	F Value	Pr > F
M	6	0.08	0.01	15.88**	0.0001
Error	19	0.02	0.01		
Correct.T.	25	0.09			

PART 2: GENERAL LINEAR MODEL ANALYSIS

Table 3 (Figures 3.1, 3.2)

Variable	SDW	RDW	TDW	Mn	Ca	Mg
Linear	**	**	**	**	**	**
Quadratic	**	**	**	*	**	NS
Cubic	**	**	**	NS	NS	NS

PART 3: REGRESSION ANALYSIS

Table 4 (Figures 3.1, 3.2)

Variable	Equation	R^2
SDW	$SDW = -19.5 + 25.7\ M - 5.2\ M^2 + 0.3\ M^3$	94%
RDW	$RDW = -1.7 + 2.4\ M - 0.5\ M^2 + 0.03\ M^3$	85%
TDW	$TDW = -21.3 + 28.2\ M - 5.7\ M^2 + 0.4\ M^3$	95%
Mn	$Mn = 303.1 - 86\ M + 7.7\ M^2$	44%
Ca	$Ca = 2.7 - 0.5\ M + 0.05\ M^2$	70%
Mg	$Mg = 0.1 + 0.02\ M$	71%

APPENDIX D

STATISTICAL ANALYSIS

CALCIUM DEFICIENCY IN MARIGOLD

Abbreviation used in statistics

SDW : Shoot dry weight in grams
RDW : Root dry weight in grams
TDW : Total dry weight in grams
C : Calcium concentration in the medium in mg/l solution
GP/SHMg : Magnesium concentration of the growing point or the old part of the shoots in mg/kg dry weight
GP/SHCa : Calcium concentration of the growing point or the old part of the shoots in mg/kg dry weight
Gp/SHMn : Manganese concentration of the growing point or the old part of the shoots in mg/kg dry weight
GP/SHFe : Iron content of the growing point or the old part of the shoots in mg/kg dry weight
P1 : The old part of the shoots
P2 : Growing point (new leaves, new stem, terminal buds)

Degree of significance

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

PART 1: ANALYSIS OF VARIANCE

Table 1: Effect of [Ca] in the medium on the plant dry Weight (Figure 4.1)

RDW

Source	DF	Anova SS	Mean Square	F Value	Pr > F
C	10	32.67	3.26	19.93**	0.0001
Error	52	8.52	0.16		
Correct.T.	62	41.19			

SDW

Source	DF	Anova SS	Mean Square	F Value	Pr > F
C	10	1132.77	113.27	44.97**	0.0001
Error	52	130.98	2.51		
Correct.T.	62	263.76			

TDW

Source	DF	Anova SS	Mean Square	F Value	Pr > F
C	10	1533.51	153.35	45.45**	0.0001
Error	52	175.434667	3.37		
Correct.T.	62	1708.95			

Table 2: Effect of [Ca] in the medium on the nutrient concentration of the plant shoot (Figures 4.3, 4.4, 4.5, 4.6)

Mn

Source	DF	Anova SS	Mean Square	F Value	Pr > F
C	10	141162.46	14116.24	2.77^{**}	0.0001
P	1	2959.63	2959.63	2.68^{NS}	0.1046
C*P	10	128425.25	12842.52	11.62^{**}	0.0001
$C:P_1$	10	7194.18	719.41	0.65^{NS}	
$C:P_2$	10	262393.53	26239.35	23.74^{**}	
Error	110	121569.41	1105.1765		
Corr.T.	131	394116.76			

Fe

Source	DF	Anova SS	Mean Square	F Value	Pr > F
C	10	616101.28	61610.12	2.66^{**}	0.0060
P	1	1030869.45	1030869.45	44.54^{**}	0.0001
C*P	10	969915.64	96991.56	4.19^{**}	0.0001
$C:P_1$	10	1419567.05	141956.70	6.13^{**}	
$C:P_2$	10	166449.86	16644.98	0.72^{NS}	
Error	110	2545655.61	23142.32		
Corr.T.	131	162541.993			

Ca

Source	DF	Anova SS	Mean Square	F Value	Pr > F
C	10	29.00	2.90	30.97^{**}	0.0001
P	1	1.60	1.60	17.19^{**}	0.0001
C*P	10	3.96	0.39	4.24^{**}	0.0001
$C:P_1$	10	24.01	2.40	25.54^{**}	
$C:P_2$	10	8.95	0.89	9.53^{**}	
Error	110	10.29	0.09		
Corr.T.	131	44.87			

Mg

Source	DF	Anova SS	Mean Square	F Value	Pr > F
C	10	20.11	2.01	49.93**	0.0001
P	1	0.15	0.15	3.93*	0.0500
C*P	10	1.09	0.10	2.72**	0.0050
C:P1	10	13.67	1.36	34.18**	

Part 2: REGRESSION ANALYSIS

Table 3 (Figures 4.1, 4.3, 4.4, 4.5, 4.6)

Equation	R^2
RDW = -0.88 + 1.62 C - 0.25 C2 + 0.01 C3	69%
SDW = -2.49 + 7.09 C - 0.99 C2 + 0.05 C3	86%
TDW = -3.37 + 8.71 C - 1.24 C2 + 0.06 C3	85%
GPMn = 450.68 - 162.85 C + 23.85 C2 - 1.08 C3	72%
SHFe = 333.58 + 174.61 C - 42.33 C2 + 2.38 C3	30%
GPCa = -0.34 + 1.44 C - 0.46 C2 + 0.06 C3 - 0.002 C4	48%
SHCa = -0.40 + 0.97 C - 0.36 C2 + 0.05 C3 - 0.002 C4	88%
GPMg = 2.20 - 0.27 C + 0.01 C2	70%
SHMg = 2.38 - 0.24 C + 0.009 C2	85%

APPENDIX E

STATISTICAL ANALYSIS

MANGANESE TOXICITY IN MARIGOLD

Abbreviation used in statistics

SDW : Shoot dry weight in grams
RDW : Root dry weight in grams
TDW : Total dry weight in grams
M : manganese concentration in the medium (mg/l)
Mg : Magnesium concentration in the plant tissue
Ca : Calcium concentration in the plant tissue
Mn : Manganese concentration in the plant tissue
Fe : Iron concentration in the plant tissue

MnT : Manganese treatment

1 : 0.5 mg/l
2 : 2.5 mg/l
3 : 4.5 mg/l
4 : 6.5 mg/l

Plant parts:

1 : Roots
2 : Stem
3 : Old leaves
4 : New leaves

Degree of significance

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

PART 1: ANALYSIS OF VARIANCE

Table 1: Effect of [Mn] in the medium on the plant dry weight (Figure 5.1)

RDW

Source	DF	Anova SS	Mean Square	F Value	Pr > F
M	9	0.21	0.02	3.71**	0.0013
Error	50	0.31	0.01		
Correct.T.	59	0.51			

SDW

Source	DF	Anova SS	Mean Square	F Value	Pr > F
M	9	3.06	0.4	9.11**	0.0001
Error	50	1.86	0.04		
Correct.T.	59	4.92			

TDW

Source	DF	Anova SS	Mean Square	F Value	Pr > F
M	9	4.68	0.52	9.63**	0.0001
Error	50	2.70	0.05		
Correct.T.	59	7.37			

Table 2: Effect of [Mn] in the medium on the nutrient concentration in the plant tissue (Figures 5.2, 5.3, 5.4)

Manganese

Source	DF	Anova SS	Mean Square	F Value	Pr > F
M	9	168890638.1	18765626.5	81.75**	0.0001
P	3	131533588.8	43844529.6	190.99**	0.0001
M*P	27	55542212.1	2057119.0	8.96**	0.0001
M:P1	9	3398102.9	3775667.0	1.64NS	
M:P2	9	52416976.8	5824108.5	25.37**	
M:P3	9	29220110.0	3246678.9	14.14**	
M:P4	9	139397660.7	15488629.0	67.47**	
Error	200	45912159.3	229560.8		
Cor.T.	239	401878598.4			

Iron

Source	DF	Anova SS	Mean Square	F Value	Pr > F
M	9	5257994.60	584221.62	2.98**	0.0001
P	3	42559485.15	14186495.05	72.29**	0.0001
M*P	27	103102259.77	381861.47	1.95**	0.0001
M:P1	9	244647.74	27183.08	0.12 NS	
M:P2	9	105708.67	11745.41	0.05 NS	
M:P3	9	179092.48	19899.16	0.09 NS	
M:P4	9	15038805.51	1670978.39	7.28**	
Error	200	39250565.67	196252.83		
Cor.T.	239	97378305.18			

Calcium

Source	DF	Anova SS	Mean Square	F Value	Pr > F
M	9	26.00	2.89	3.16**	0.0014
P	3	113.46	37.82	41.31**	0.0001
M*P	27	23.14	0.86	0.94NS	0.5601
Error	200	183.09	0.92		
Cor.T.	239	345.69			

Magnesium

Source	DF	Anova SS	Mean Square	F Value	Pr > F
M	9	0.77	0.08	2.72**	0.0051
P	3	7.07	2.36	74.73**	0.0001
M*P	27	1.29	0.05	0.52NS	0.0558
Error	200	6.31	0.03		
Cor.T.	239	15.44			

PART 2: GENERAL LINEAR MODEL ANALYSIS

Table 3 (Figures 5.1, 5.2, 5.3, 5.4)

Variable	SDW	RDW	TDW	Mn	Fe	Ca	Mg
Linear	*	*	**	**	**	**	**
Quadratic	NS	*	*	**	**	**	**
Cubic	NS	NS	*	**	**	**	*

APPENDIX F

STATISTICAL ANALYSIS

MANGANESE TOXICITY IN MARIGOLD AS AFFECTED BY CALCIUM

Abbreviation used in statistics

SDW : Shoot dry weight in grams
RDW : Root dry weight in grams
TDW : Total dry weight in grams
M : manganese concentration in the medium (mg/l)
Mg : Magnesium concentration in the plant tissue
Ca : Calcium concentration in the plant tissue
Mn : Manganese concentration in the plant tissue
Fe : Iron concentration in the plant tissue

CaT : Calcium treatment

1 : 20 mg/l
2 : 100 mg/l

MnT : Manganese treatment

1 : 0.5 mg/l
2 : 2.5 mg/l
3 : 4.5 mg/l
4 : 6.5 mg/l

Plant parts:

1 : Roots
2 : Stem
3 : Old leaves
4 : New leaves

Degree of significance

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

PART 1: ANALYSIS OF VARIANCE

Table 1: Plant dry weight (Figure 6.1)

Shoot dry weight

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CAT	1	0.48803333	0.48803333	10.05**	0.0029
MNT	3	0.59055833	0.19685278	4.05*	0.0132
CAT*MNT	3	0.24780000	0.08260000	1.70^{NS}	0.1823

Root dry weight

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CAT	1	0.06526875	0.06526875	6.70*	0.0134
MNT	3	0.03022292	0.01007431	1.03^{NS}	0.3878
CAT*MNT	3	0.02317292	0.00772431	0.79^{NS}	0.5051

Total dry weight

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CAT	1	0.91025208	0.91025208	10.99**	0.0020
MNT	3	0.79920625	0.26640208	3.22*	0.0329
CAT*MNT	3	0.42007292	0.14002431	1.69^{NS}	0.1845

Table 2: Nutrient concentration in the plant tissue (Figures 6.2, 6.3, 6.4, 6.4a, 6.5, 6.6, 6.7, 6.8, 6.9)

Manganese

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CaT	1	2356981.92	2356981.92	19.71**	<.0001
MnT	3	4525916.56	11508638.85	96.24**	<.0001
Part	3	6826363.52	8942121.17	74.78**	<.0001
CaT*MnT	3	2776919.72	925639.91	7.74**	<.0001
MnT:Ca1	3	-	-	78.47**	<.0001
MnT:Ca2	3	-	-	25.51**	<.0001
CaT*Part	3	930404.43	310134.81	2.59^{NS}	0.0545
MnT*Part	9	5503715.01	611523.89	5.11**	<.0001
MnT:P1	3	-	-	29.34**	<.0001
MnT:P2	3	-	-	4.60**	0.0041
MnT:P3	3	-	-	50.80**	<.0001
MnT:P4	3	-	-	26.85**	<.0001
CaT*MnT*Part	9	493376.84	4819.65	0.46^{NS}	0.9004

Iron

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CaT	1	22598.38	22598.38	0.16^{NS}	0.6905
MnT	3	726359.97	242119.99	1.70^{NS}	0.1681
Part	3	25182782.31	8394260.77	59.11^{**}	<.0001
CaT*MnT	3	119440.31	39813.44	0.28^{NS}	0.8395
CaT*Part	3	170362.56	56787.52	0.40^{NS}	0.7533
MnT*Part	9	3269982.59	363331.40	2.56^{**}	0.0090
MnT:P1	3	-	-	8.94^{**}	<.0001
MnT:P2	3	-	-	0.11^{NS}	0.9523
MnT:P3	3	-	-	0.11^{NS}	0.9537
MnT:P4	3	-	-	0.21^{NS}	0.8863
CaT*MnT*Part	9	324207.67	36023.07	0.25^{NS}	0.9854

Calcium

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CaT	1	9.82265052	29.82265052	1481.69^{**}	<.0001
MnT	3	0.53556823	0.17852274	8.87^{**}	<.0001
Part	3	27.42130156	9.14043385	454.13^{**}	<.0001
CaT*MnT	3	0.23615156	0.07871719	3.91^{**}	0.0099
MnT:Ca1	3	-	-	0.61^{NS}	0.6075
MnT:Ca2	3	-	-	12.17^{**}	<.0001
CaT*Part	3	7.24844323	2.41614774	120.04^{**}	<.0001
CaT:P1	1	-	-	11.48^{**}	0.0009
CaT:P2	1	-	-	697.56^{**}	<.0001
CaT:P3	1	-	-	713.77^{**}	<.0001
CaT:P4	1	-	-	419.01^{**}	<.0001
MnT*Part	9	0.60426302	0.06714034	3.34^{**}	0.0009
MnT:P1	3	-	-	0.25^{NS}	0.8590
MnT:P2	3	-	-	3.20^{*}	0.0250
MnT:P3	3	-	-	2.97^{*}	0.0334
MnT:P4	3	-	-	12.45^{**}	<.0001
CaT*MnT*Part	9	0.10336302	0.01148478	0.57^{NS}	0.8197

Magnesium

Source	DF	Anova SS	Mean Square	F Value	Pr > F
CaT	1	3.06282552	3.06282552	166.93**	<.0001
MnT	3	0.02705156	0.00901719	0.49 NS	0.6887
Part	3	3.11554740	1.03851580	56.60**	<.0001
CaT*MnT	3	0.01699323	0.00566441	0.31 NS	0.8191
CaT*Part	3	0.34353906	0.11451302	6.24**	0.0005
CaT:P1	1	-	-	8.03**	0.0052
CaT:P2	1	-	-	48.42**	<.0001
CaT:P3	1	-	-	72.12**	<.0001
CaT:P4	1	-	-	57.08**	<.0001
MnT*Part	9	0.32209219	0.03578802	1.95*	0.0485
MnT:P1	3	-	-	1.88 NS	0.1355
MnT:P2	3	-	-	0.63 NS	0.5973
MnT:P3	3	-	-	0.89 NS	0.4480
MnT:P4	3	-	-	2.95*	0.0346
CaT*MnT*Part	9	0.06390052	0.00710006	0.39 NS	0.9401

APPENDIX G

STATISTICAL ANALYSIS

MANGANESE TOXICITY IN MARIGOLD AS AFFECTED BY MAGNESIUM

Abbreviation used in statistics

SDW : Shoot dry weight in grams
RDW : Root dry weight in grams
TDW : Total dry weight in grams
M : manganese concentration in the medium (mg/l)
Mg : Magnesium concentration in the plant tissue
Ca : Calcium concentration in the plant tissue
Mn : Manganese concentration in the plant tissue
Fe : Iron concentration in the plant tissue

MGTRT: Magnesium treatment

5 mg/l
10 mg/l
15 mg/l
20 mg/l

MNTRT: Manganese treatment

0.5 mg/l
2.5 mg/l
4.5 mg/l
6.5 mg/l

PART : Plant parts

1 : Roots
2 : Stem
3 : Old leaves
4 : New leaves

Degree of significance

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

PART 1: ANALYSIS OF VARIANCE

Table 1: Plant dry weight

Root dry weight

Source	DF	Anova SS	Mean Square	F Value	Pr > F
MNTRT	3	0.17615375	0.05871792	4.44**	0.0067
MGTRT	3	0.14162375	0.04720792	3.57*	0.0187
MNTRT*MGTRT	9	0.05475125	0.00608347	0.46NS	0.8956

Shoot dry weight

Source	DF	Anova SS	Mean Square	F Value	Pr > F
MNTRT	3	3.79088500	1.26362833	4.32**	0.0078
MGTRT	3	1.07254500	0.35751500	1.22NS	0.3092
MNTRT*MGTRT	9	3.10404500	0.34489389	1.18NS	0.3241

Total dry weight

Source	DF	Anova SS	Mean Square	F Value	Pr > F
MNTRT	3	5.30981375	1.76993792	4.52**	0.0062
MGTRT	3	1.98813375	0.66271125	1.69NS	0.1777
MNTRT*MGTRT	9	3.72024125	0.41336014	1.05NS	0.4078

Table 2: Nutrient concentration in the plant tissue

Manganese

Source	DF	Anova SS	Mean Square	F Value	Pr > F
MgT	3	3673.58	1224.52	0.02NS	0.9954
MnT	3	24765876.83	8255292.27	152.01**	0.0001
PART	3	28767565.16	9589188.38	176.57**	0.0001
MgT*MnT	9	438899.49	48766.61	0.90NS	0.5276
MgT*PART	9	193771.04	21530.11	0.40NS	0.9362
MnT*PART	9	6587120.62	731902.29	13.48**	0.0001
MnT:P1	3	-	-	63.54**	0.0001
MnT:P2	3	-	-	2.13NS	0.0971
MnT:P3	3	-	-	96.81**	0.0001
MnT:P4	3	-	-	29.96**	0.0001
MGT*MNT *PART	27	481056.64	17816.91	0.33NS	0.9995

Iron

Source	DF	Anova SS	Mean Square	F Value	Pr > F
MgT	3	167556.46	55852.15	7.27^{**}	0.0001
MnT	3	116950.64	38983.547	5.07^{**}	0.0020
PART	3	2210196.84	736732.28	95.84^{**}	0.0001
MgT*MnT	9	106339.92	11815.54	1.54^{NS}	0.1351
MgT*PART	9	190668.58	21185.39	2.76^{**}	0.0043
MgT:P1	3	-	-	13.09^{**}	0.0001
MgT:P2	3	-	-	0.44^{NS}	0.7230
MgT:P3	3	-	-	1.05^{NS}	0.3690
MgT:P4	3	-	-	0.94^{NS}	0.4195
MNT*PART	9	164116.63	18235.18	2.37^{*}	0.0137
MnT:P1	3	-	-	4.45^{**}	0.0046
MnT:P2	3	-	-	2.84^{*}	0.0383
MnT:P3	3	-	-	3.52^{*}	0.0158
MnT:P4	3	-	-	1.38^{NS}	0.2491
MGT*MNT*PART	27	175637.47	6505.09	0.85^{NS}	0.6884

Calcium

Source	DF	Anova SS	Mean Square	F Value	Pr > F
MGT	3	27.45	9.15	1.14^{NS}	0.3335
MNT	3	204.14	68.04	8.48^{**}	0.0001
PART	3	17897.92	5965.97	743.17^{**}	0.0001
MGT*MNT	9	95.67	10.63	1.32^{NS}	0.2244
MGT*PART	9	67.92	7.54	0.94^{NS}	0.4908
MNT*PART	9	202.29	22.47	2.80^{**}	0.0038
MnT:P1	3	-	-	0.56^{NS}	0.6395
MnT:P1	3	-	-	1.36^{NS}	0.2554
MnT:P1	3	-	-	12.22^{**}	0.0001
MnT:P1	3	-	-	2.74^{*}	0.0441
MGT*MNT*PART	27	215.68	7.98	1.00^{NS}	0.4760

Magnesium:

Source	DF	Anova SS	Mean Square	F Value	Pr > F
MgT	3	47.16	15.72	110.40**	0.0001
MnT	3	2.16	0.72	5.08**	0.0020
PART	3	57.98	19.32	135.71**	0.0001
MgT*MnT	9	1.99	0.22	1.55NS	0.1297
MgT*PART	9	3.65	0.40	2.85**	0.0032
Mg:P1	3	-	-	10.55**	0.0001
Mg:P2	3	-	-	33.50**	0.0001
Mg:P3	3	-	-	44.56**	0.0001
Mg:P4	3	-	-	30.33**	0.0001
MnT*PART	9	0.39	0.04	0.31NS	0.9725
MgT*MnT*PART	27	2.73	0.10	0.71NS	0.8548

APPENDIX H

STATISTICAL ANALYSIS

MANGANESE TOXICITY IN MARIGOLD
AS
AFFECTED BY CALCIUM AND MAGNESIUM

Abbreviation used in statistics

SDW : Shoot dry weight in grams
RDW : Root dry weight in grams
TDW : Total dry weight in grams
M : manganese concentration in the medium (mg/l)
Mg : Magnesium concentration in the plant tissue
Ca : Calcium concentration in the plant tissue
Mn : Manganese concentration in the plant tissue
Fe : Iron concentration in the plant tissue

Calcium levels:

20 mg/l
100 mg/l

Magnesium levels:

10 mg/l
20 mg/l

Manganese levels:

0.5 mg/l
2.5 mg/l
4.5 mg/l
6.5 mg/l

Plant parts:

1 : Roots
2 : Stem
3 : Old leaves
4 : New leaves

Degree of significance

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

PART 1: ANALYSIS OF VARIANCE

Table 1: Plant dry weight

Root dry weight

Source	DF	Anova SS	Mean Square	F Value	Pr > F
MNTRT	3	1.51	0.50	32.64**	0.0001
CATRT	1	0.32	0.32	21.21**	0.0001
MGTRT	1	0.05	0.05	3.26^{NS}	0.0759
MNTRT*CATRT	3	0.26	0.08	5.66**	0.0017
MNTRT*MGTRT	3	0.01	0.01	0.26^{NS}	0.8558
CATRT*MGTRT	1	0.01	0.01	0.10^{NS}	0.7544
MNTRT*CATRT*MGTRT	3	0.01	0.01	0.28^{NS}	0.8372

Shoot dry weight

Source	DF	Anova SS	Mean Square	F Value	Pr > F
MNTRT	3	15.50	5.16	42.86**	0.0001
CATRT	1	4.47	4.47	37.11**	0.0001
MGTRT	1	0.77	0.77	6.41*	0.0138
MNTRT*CATRT	3	2.47	0.82	6.85**	0.0004
MNTRT*MGTRT	3	0.51	0.17	1.42^{NS}	0.2461
CATRT*MGTRT	1	0.01	0.01	0.00^{NS}	0.9591
MNTRT*CATRT*MGTRT	3	0.41	0.13	1.14^{NS}	0.3411

Total dry weight

Source	DF	Anova SS	Mean Square	F Value	Pr > F
MNTRT	3	26.71	8.90	46.16**	0.0001
CATRT	1	7.23	7.23	37.48**	0.0001
MGTRT	1	1.21	1.21	6.31**	0.0145
MNTRT*CATRT	3	4.07	1.35	7.04**	0.0004
MNTRT*MGTRT	3	0.61	0.20	1.06^{NS}	0.3730
CATRT*MGTRT	1	0.01	0.01	0.02^{NS}	0.8971
MNTRT*CATRT*MGTRT	3	0.55	0.18	0.97^{NS}	0.4144

Table 2: Nutrient concentration in the plant tissue

Manganese

Source	DF	Anova SS	Mean Square	F Value	Pr > F
MnT	3	121064375.31	40354791.77	1127.80**	0.0001
CaT	1	35267696.10	35267696.10	985.63**	0.0001
MgT	1	1196583.20	1196583.20	33.44**	0.0001
PART	3	82786466.60	27595488.80	771.21**	0.0001
MnT*CaT	3	1730149.06	7243383.02	202.43**	0.0001
MnT:Ca1	3	-	-	1130.3**	0.0001
MnT:Ca2	3	-	-	199.89**	0.0001
MnT*MgT	3	1748559.17	582853.05	16.29**	0.0001
MnT:Mg1	3	-	-	676.40**	0.0001
MnT:Mg2	3	-	-	467.69**	0.0001
MnT*PART	9	4429145.81	2714349.53	75.86**	0.0001
MnT:PART1	3	-	-	375.44**	0.0001
MnT:PART2	3	-	-	34.74**	0.0001
MnT:PART3	3	-	-	696.17**	0.0001
MnT:PART4	3	-	-	249.03**	0.0001
CaT*MgT	1	532358.44	532358.44	14.88**	0.0001
CaT:Mg1	1	-	-	46.46**	0.0001
CaT:Mg2	1	-	-	1.85NS	0.1745
CaT*PART	3	7975218.16	2658406.05	74.29**	0.0001
CaT:PART1	1	-	-	192.45**	0.0001
CaT:PART2	1	-	-	40.70**	0.0001
CAT:PART3	1	-	-	740.52**	0.0001
CAT*PART4	1	-	-	234.85**	0.0001
MgT*PART	3	256532.57	85510.85	2.39NS	0.0693
MnT*CaT*MgT	3	719027.87	239675.95	6.70**	0.0002
MNT*MGT:Ca1	7	-	-	500.34**	0.0001
MNT*MGT:Ca2	7	-	-	86.51**	0.0001
MNT:(Ca1*Mg1)	3	-	-	680.13**	0.0001
MNT:(Ca1*Mg2)	3	-	-	471.84**	0.0001
MNT:(Ca2*Mg1)	3	-	-	116.75**	0.0001
MNT:(Ca2*Mg2)	3	-	-	84.49**	0.0001
MnT*CaT*PART	9	4531406.56	503489.61	14.07**	0.0001
(MNT*CAT):PART1	7	-	-	217.75**	0.0001
(MNT*CAT):PART2	7	-	-	23.60**	0.0001

(MNT*CAT):PART3	7	-	-	454.82**	0.0001
(MNT*CAT):PART4	7	-	-	162.20**	0.0001
MNT:(CAT1*PART1)	3	-	-	371.45**	0.0001
MNT:(CAT1*PART2)	3	-	-	35.77**	0.0001
MNT:(CAT1*PART3)	3	-	-	687.48**	0.0001
MNT:(CAT1*PART4)	3	-	-	261.94**	0.0001
MNT:(CAT2*PART1)	3	-	-	72.48**	0.0001
MNT:(CAT2*PART2)	3	-	-	5.72**	0.0008
MNT:(CAT2*PART3)	3	-	-	126.93**	0.0001
MNT:(CAT2*PART4)	3	-	-	38.25**	0.0001
MnT*MgT*PART	9	618608.55	68734.28	1.92*	0.0494
(MNT*MGT):PART1	7	-	-	163.66**	0.0001
(MNT*MGT):PART2	7	-	-	15.66**	0.0001
(MNT*MGT):PART3	7	-	-	308.36**	0.0001
(MNT*MGT):PART4	7	-	-	108.45**	0.0001
MNT:(MGT1*PART1)	3	-	-	214.48**	0.0001
MNT:(MGT1*PART2)	3	-	-	22.46**	0.0001
MNT:(MGT1*PART3)	3	-	-	433.15**	0.0001
MNT:(MGT1*PART4)	3	-	-	143.50**	0.0001
MNT:(MGT2*PART1)	3	-	-	165.71**	0.0001
MNT:(MGT2*PART2)	3	-	-	13.36**	0.0001
MNT:(MGT2*PART3)	3	-	-	277.82**	0.0001
MNT:(MGT2*PART4)	3	-	-	106.94**	0.0001
CaT*MgT*PART	3	423528.92	141176.30	3.95**	0.0089
(CAT*MGT):PART1	3	-	-	65.86**	0.0001
(CAT*MGT):PART2	3	-	-	14.51**	0.0001
(CAT*MGT):PART3	3	-	-	262.91**	0.0001
(CAT*MGT):PART4	3	-	-	82.00**	0.0001
CAT:(MGT1*PART1)	1	-	-	3.24**	0.0728
CAT:(MGT1*PART2)	1	-	-	2.64NS	0.1052
CAT:(MGT1*PART3)	1	-	-	48.17**	0.0001
CAT:(MGT1*PART4)	1	-	-	10.67**	0.0012
CAT:(MGT2*PART1)	1	-	-	1.88NS	0.1711
CAT:(MGT2*PART2)	1	-	-	0.20NS	0.6575
CAT:(MGT2*PART3)	1	-	-	0.05NS	0.8294
CAT:(MGT2*PART4)	1	-	-	0.48NS	0.4900
MnT*CaT*MgT*PART	9	371190.84	41243.42	1.15NS	0.3262

Iron

Source	DF	Anova SS	Mean Square	F Value	Pr > F
MnT	3	1628957.80	542985.93	32.13**	0.0001
CaT	1	349074.25	349074.25	20.66**	0.0001
MgT	1	12713.40	12713.40	0.75NS	0.3866
PART	3	16400236.53	5466745.51	323.50**	0.0001
MnT*CaT	3	802776.23	267592.07	15.84**	0.0001
MnT:Ca1	3	-	-	46.19**	0.0001
MnT:Ca2	3	-	-	1.78NS	0.1518
MnT*MgT	3	68672.58	22890.86	1.35NS	0.2572
MnT*PART	9	5949968.57	661107.61	39.12**	0.0001
MnT*PART1	3	-	-	47.95**	0.0001
MnT*PART2	3	-	-	0.19NS	0.9034
MnT*PART3	3	-	-	1.31NS	0.2704
MnT*PART4	3	-	-	0.04NS	0.9890
CaT*MgT	1	5502.90	5502.90	0.33NS	0.5687
CaT*PART	3	1680877.85	560292.61	33.16**	0.0001
CaT*PART1	1	-	-	118.03**	0.0001
CaT*PART2	1	-	-	0.00NS	0.9849
CaT*PART3	1	-	-	1.97NS	0.1619
CaT*PART4	1	-	-	0.12NS	0.7247
MgT*PART	3	2245.20	748.40	0.04NS	0.9876
MnT*CaT*MgT	3	37346.03	12448.67	0.74NS	0.5310
MnT*CaT*PART	9	1824132.00	202681.33	11.99**	0.0001
(MNT*CAT):PART1	7	-	-	101.88**	0.0001
(MNT*CAT):PART2	7	-	-	0.20NS	0.9853
(MNT*CAT):PART3	7	-	-	1.32NS	0.2418
(MNT*CAT):PART4	7	-	-	0.04NS	0.9999
MnT:(Ca1*PART1)	3	-	-	184.35**	0.0001
MnT:(Ca1*PART2)	3	-	-	0.32NS	0.8088
MnT:(Ca1*PART3)	3	-	-	0.47NS	0.7055
MnT:(Ca1*PART4)	3	-	-	0.00NS	0.9996
MnT:(Ca2*PART1)	3	-	-	14.02**	0.0001
MnT:(Ca2*PART1)	3	-	-	0.14NS	0.9337
MnT:(Ca2*PART1)	3	-	-	1.95NS	0.1215
MnT:(Ca2*PART1)	3	-	-	0.06NS	0.9820
MnT*MgT*PART	9	96823.15	10758.12	0.64NS	0.7653
CaT*MgT*PART	3	10772.35	3590.78	0.21NS	0.8877
MnT*CaT*MgT*PART	9	171316.8	19035.20	1.13NS	0.3443

Calcium

Source	DF	Anova SS	Mean Square	F Value	Pr > F
MNT	3	94.83	31.61	10.49**	0.0001
CAT	1	2726.11	2726.11	904.93**	0.0001
MGT	1	43.51	43.51	14.44**	0.0002
PART	3	7585.71	2528.57	839.36**	0.0001
MNT*CAT	3	46.93	15.64	5.19**	0.0017
Mn:Ca1	3	-	-	7.64**	0.0001
Mn:Ca2	3	-	-	8.05**	0.0001
MNT*MGT	3	43.33	14.44	4.80**	0.0029
Mn:Mg1	3	-	-	14.06**	0.0001
Mn:Mg2	3	-	-	1.23^{NS}	0.2979
MNT*PART	9	43.63	4.84	1.61^{NS}	0.1127
CAT*MGT	1	27.61	27.61	9.17**	0.0027
Ca:Mg1	1	-	-	23.31**	0.0001
Ca:Mg2	1	-	-	0.30^{NS}	0.5851
CAT*PART	3	659.81	219.93	73.01**	0.0001
Ca:PART1	1	-	-	13.96**	0.0002
Ca:PART2	1	-	-	505.40**	0.0001
Ca:PART3	1	-	-	430.22**	0.0001
Ca:PART4	1	-	-	174.38**	0.0001
MGT*PART	3	5.36	1.78	0.59^{NS}	0.6199
MNT*CAT*MGT	3	30.63	10.21	3.39*	0.0186
(Mn*Ca):Mg1	7	-	-	7.01**	0.0001
(Mn*Ca):Mg2	7	-	-	6.59**	0.0001
Mn:(Ca1*Mg1)	3	-	-	5.26**	0.0015
Mn:(Ca1*Mg2)	3	-	-	3.33*	0.0200
Mn:(Ca2*Mg1)	3	-	-	9.44**	0.0001
Mn:(Ca2*Mg2)	3	-	-	5.84**	0.0007
MNT*CAT*PART	9	67.83	7.53	2.50**	0.0093
(Mn*Ca):PART1	7	-	-	2.83**	0.0075
(Mn*Ca):PART2	7	-	-	76.27**	0.0001
(Mn*Ca):PART3	7	-	-	65.97**	0.0001
(Mn*Ca):PART4	7	-	-	27.51**	0.0001
Mn:(Ca1*PART2)	3	-	-	1.80^{NS}	0.1475
Mn:(Ca1*PART2)	3	-	-	3.40*	0.0185
Mn:(Ca1*PART3)	3	-	-	1.76^{NS}	0.1560
Mn:(Ca1*PART4)	3	-	-	4.30**	0.0055
Mn:(Ca2*PART1)	3	-	-	0.14^{NS}	0.9353

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Mn:(Ca2*PART2	3	-	-	6.09**	0.0005
Mn:(Ca2*PART3)	3	-	-	8.77**	0.0001
Mn:(Ca2*PART4)	3	-	-	1.76^{NS}	0.1560
MNT*MGT*PART	9	54.28	6.03	2.00*	0.0394
(Mn*Mg):PART1	7	-	-	0.71^{NS}	0.6625
(Mn*Mg):PART2	7	-	-	1.84^{NS}	0.0793
(Mn*Mg):PART3	7	-	-	7.95**	0.0001
(Mn*Mg):PART4	7	-	-	3.01**	0.0047
Mn:(Mg1*PART1)	3	-	-	1.01^{NS}	0.3903
Mn:(Mg1*PART2)	3	-	-	1.50^{NS}	0.2137
Mn:(Mg1*PART3)	3	-	-	15.90**	0.0001
Mn:(Mg1*PART4)	3	-	-	4.24**	0.0061
Mn:(Mg2*PART1)	3	-	-	0.45^{NS}	0.7149
Mn:(Mg2*PART2)	3	-	-	0.69^{NS}	0.5563
Mn:(Mg2*PART3)	3	-	-	1.05^{NS}	0.3717
Mn:(Mg2*PART4)	3	-	-	1.28^{NS}	0.2805
CAT*MGT*PART	3	23.96	7.98	2.65*	0.0492
MNT*CAT*MGT*PART	9	70.88	7.87	2.61**	0.0066
(Mn*Ca*Mg):PART1	15	-	-	1.41^{NS}	0.1435
(Mn*Ca*Mg):PART2	15	-	-	36.29**	0.0001
(Mn*Ca*Mg):PART3	15	-	-	36.15**	0.0001
(Mn*Ca*Mg):PART4	15	-	-	13.32**	0.0001

Magnesium

Source	DF	Anova SS	Mean Square	F Value	Pr > F
MNT	3	3.73	1.24	4.77**	0.0030
CAT	1	103.51	103.51	396.69**	0.0001
MGT	1	68.45	68.45	262.32**	0.0001
PART	3	221.96	73.98	283.54**	0.0001
MNT*CAT	3	1.93	0.64	2.48^{NS}	0.0620
MNT*MGT	3	2.30	0.76	2.94*	0.0338
Mn:Mg1	3	-	-	7.31**	0.0001
Mn:Mg2	3	-	-	0.41^{NS}	0.7480
MNT*PART	9	2.18	0.24	0.93^{NS}	0.4982
CAT*MGT	1	0.20	0.20	0.77^{NS}	0.3821
CAT*PART	3	40.06	13.35	51.18**	0.0001
Ca:PART1	1	-	-	1.72^{NS}	0.1903
Ca:PART2	1	-	-	72.86**	0.0001
Ca:PART3	1	-	-	346.11**	0.0001
Ca:PART4	1	-	-	129.53**	0.0001

MGT*PART	3	11.57	3.85	14.79**	0.0001
Mg:PART1	1	-	-	9.39**	0.0024
Mg:PART2	1	-	-	97.01**	0.0001
Mg:PART3	1	-	-	144.91**	0.0001
Mg:PART4	1	-	-	55.38**	0.0001
MNT*CAT*MGT	3	2.95	0.98	3.77*	0.0113
MNT*CAT*PART	9	6.68	0.74	2.85**	0.0033
(Mn*Ca):PART1	7	-	-	0.63NS	0.7313
(Mn*Ca):PART2	7	-	-	13.35**	0.0001
(Mn*Ca):PART3	7	-	-	51.35**	0.0001
(Mn*Ca):PART4	7	-	-	21.24**	0.0001
Mn:(Ca1*PART1)	3	-	-	0.26NS	0.8574
Mn:(Ca1*PART2)	3	-	-	3.16NS	0.0252
Mn:(Ca1*PART3)	3	-	-	1.88NS	0.1327
Mn:(Ca1*PART4)	3	-	-	6.26**	0.0004
Mn:(Ca2*PART1)	3	-	-	0.64NS	0.5907
Mn:(Ca2*PART2)	3	-	-	3.70*	0.0123
Mn:(Ca2*PART3)	3	-	-	2.55NS	0.0559
Mn:(Ca2*PART4)	3	-	-	0.13NS	0.9436
MNT*MGT*PART	9	3.47	0.38	1.48NS	0.1556
CAT*MGT*PART	3	0.52	0.17	0.67NS	0.5707
MNT*CAT*MGT*PART	9	2.12	0.23	0.90NS	0.5215

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