BIOLOGY OF CHLOROSIS TOLERANCE, REPRODUCTIVE BEHAVIOUR, AND SEED PROPAGATION IN *ACER GINNALA* MAXIM.

by

Martha A. Barwinsky

A Thesis Submitted to

the Faculty of Graduate Studies

University of Manitoba

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

Department of Plant Science

ISBN 0-612-12973-X

Name MARTHA A. BARWINSKY

Dissertation Abstracts International is arranged by broad, general subject categories. Please select the one subject which most nearly describes the content of your dissertation. Enter the corresponding four-digit code in the spaces provided.

AGRICULTURE - GENERAL

SUBJECT TERM

0473

SUBJECT CODE

U·M·I

Subject Categories

THE HUMANITIES AND SOCIAL SCIENCES

COMMUNICATIONS AND THE ARTS
Architecture 0729
Art History 0377
Cinema 0900
Dance 0378
Fine Arts 0357
Information Science 0723
Journalism 0391
Library Science 0399
Mass Communications 0708
Music 0413
Speech Communication 0459
Theater 0465

EDUCATION
General 0515
Administration 0514
Adult and Continuing 0516
Agricultural 0517
Art 0273
Bilingual and Multicultural 0282
Business 0688
Community College 0275
Curriculum and Instruction 0727
Early Childhood 0518
Elementary 0524
Finance 0277
Guidance and Counseling 0519
Health 0680
Higher 0745
History of 0520
Home Economics 0278
Industrial 0521
Language and Literature 0279
Mathematics 0280
Music 0522
Philosophy of 0998
Physical 0523
Psychology 0525
Reading 0535
Religious 0527
Sciences 0714
Secondary 0533
Social Sciences 0534
Sociology of 0340
Special 0529
Teacher Training 0530
Technology 0710
Tests and Measurements 0288
Vocational 0747

LANGUAGE, LITERATURE AND LINGUISTICS
Language
- General 0679
- Ancient 0289
- Linguistics 0290
- Modern 0291

Literature
- General 0401
- Classical 0294
- Comparative 0295
- Medieval 0297
- Modern 0298
- African 0316
- American 0591
- Asian 0305
- Canadian (English) 0352
- Canadian (French) 0355
- English 0593
- Germanic 0311
- Latin American 0312
- Middle Eastern 0315
- Romance 0313
- Slavic and East European 0314

PHILOSOPHY, RELIGION AND THEOLOGY
Philosophy 0422
Religion
- General 0318
- Biblical Studies 0321
- Clergy 0319
- History of 0320
- Philosophy of 0322

Theology 0469

SOCIAL SCIENCES
American Studies 0323
Anthropology
- Archaeology 0324
- Cultural 0326
- Physical 0327

Business Administration
- General 0310
- Accounting 0272
- Banking 0770
- Management 0454
- Marketing 0338

Canadian Studies 0385
Economics
- General 0501
- Agricultural 0503
- Commerce-Business 0505
- Finance 0508
- History 0509
- Labor 0510
- Theory 0511

Folklore 0358
Geography 0366
Gerontology 0351
History
- General 0578
- Ancient 0579
- Medieval 0581
- Modern 0582
- Black 0328
- African 0331
- Asia, Australia and Oceania 0332
- Canadian 0334
- European 0335
- Latin American 0336
- Middle Eastern 0333
- United States 0337

History of Science 0585
Law 0398
Political Science
- General 0615
- International Law and Relations 0616
- Public Administration 0617

Recreation 0814
Social Work 0452
Sociology
- General 0626
- Criminology and Penology 0627
- Demography 0938
- Ethnic and Racial Studies 0631
- Individual and Family Studies 0628
- Industrial and Labor Relations 0629
- Public and Social Welfare 0630
- Social Structure and Development 0700
- Theory and Methods 0344

Transportation 0709
Urban and Regional Planning 0999
Women's Studies 0453

THE SCIENCES AND ENGINEERING

BIOLOGICAL SCIENCES
Agriculture
- General 0473
- Agronomy 0285
- Animal Culture and Nutrition 0475
- Animal Pathology 0476
- Food Science and Technology 0359
- Forestry and Wildlife 0478
- Plant Culture 0479
- Plant Pathology 0480
- Plant Physiology 0817
- Range Management 0777
- Wood Technology 0746

Biology
- General 0306
- Anatomy 0287
- Biostatistics 0308
- Botany 0309
- Cell 0379
- Ecology 0329
- Entomology 0353
- Genetics 0369
- Limnology 0793
- Microbiology 0410
- Molecular 0307
- Neuroscience 0317
- Oceanography 0416
- Physiology 0433
- Radiation 0821
- Veterinary Science 0778
- Zoology 0472

Biophysics
- General 0786
- Medical 0760

EARTH SCIENCES
Biogeochemistry 0425
Geochemistry 0996
Geodesy 0370
Geology 0372
Geophysics 0373
Hydrology 0388
Mineralogy 0411
Paleobotany 0345
Paleoecology 0426
Paleontology 0418
Paleozoology 0985
Palynology 0427
Physical Geography 0368
Physical Oceanography 0415

HEALTH AND ENVIRONMENTAL SCIENCES
Environmental Sciences 0768
Health Sciences
- General 0566
- Audiology 0300
- Chemotherapy 0992
- Dentistry 0567
- Education 0350
- Hospital Management 0769
- Human Development 0758
- Immunology 0982
- Medicine and Surgery 0564
- Mental Health 0347
- Nursing 0569
- Nutrition 0570
- Obstetrics and Gynecology 0380
- Occupational Health and Therapy 0354
- Ophthalmology 0381
- Pathology 0571
- Pharmacology 0419
- Pharmacy 0572
- Physical Therapy 0382
- Public Health 0573
- Radiology 0574
- Recreation 0575
- Speech Pathology 0460
- Toxicology 0383

Home Economics 0386

PHYSICAL SCIENCES
Pure Sciences
Chemistry
- General 0485
- Agricultural 0749
- Analytical 0486
- Biochemistry 0487
- Inorganic 0488
- Nuclear 0738
- Organic 0490
- Pharmaceutical 0491
- Physical 0494
- Polymer 0495
- Radiation 0754

Mathematics 0405
Physics
- General 0605
- Acoustics 0986
- Astronomy and Astrophysics 0606
- Atmospheric Science 0608
- Atomic 0748
- Electronics and Electricity 0607
- Elementary Particles and High Energy 0798
- Fluid and Plasma 0759
- Molecular 0609
- Nuclear 0610
- Optics 0752
- Radiation 0756
- Solid State 0611

Statistics 0463

Applied Sciences
Applied Mechanics 0346
Computer Science 0984
Engineering
- General 0537
- Aerospace 0538
- Agricultural 0539
- Automotive 0540
- Biomedical 0541
- Chemical 0542
- Civil 0543
- Electronics and Electrical 0544
- Heat and Thermodynamics 0348
- Hydraulic 0545
- Industrial 0546
- Marine 0547
- Materials Science 0794
- Mechanical 0548
- Metallurgy 0743
- Mining 0551
- Nuclear 0552
- Packaging 0549
- Petroleum 0765
- Sanitary and Municipal 0554
- System Science 0790

Geotechnology 0428
Operations Research 0796
Plastics Technology 0795
Textile Technology 0994

PSYCHOLOGY
General 0621
Behavioral 0384
Clinical 0622
Developmental 0620
Experimental 0623
Industrial 0624
Personality 0625
Physiological 0989
Psychobiology 0349
Psychometrics 0632
Social 0451

BIOLOGY OF CHLOROSIS TOLERANCE, REPRODUCTIVE BEHAVIOUR AND SEED PROPAGATION IN *Acer ginnala* MAXIM

BY

MARTHA A. BARWINSKY

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

ACKNOWLEDGEMENTS

I would like to thank Louis Lenz for accepting me as his last graduate student and for giving me considerable leeway in the development of this project. I appreciate his support and advice, and particularly his patience and confidence in me, as I took on other responsibilities during this time, including motherhood! This project was a positive, challenging and eye-opening experience.

To the members of my advisory committee, Dr. W.R. Remphrey, Dr. W. Woodbury and Dr. Tee Boon Goh, I wish to extend my thanks for their suggestions, support and encouragement. A special acknowledgement must be made of the time spent by Dr. Goh in travelling with me to Brookdale, Mb to collect soil and to Teulon, Mb to provide me with a soil profile description for this document.

There are several people who contributed to this project and who I would like to thank: Mr. Ralph Moeller of Teulon, Mb, for his kindness and generosity in providing me with a field site and with mature trees for sampling; Neil Slykerman, for his assistance in various aspects of this project; Ian Brown, for his practical advice and encouragement; and the people of the Horticulture lab (past and present), for their friendship, assistance and good humour, and for providing me with some workspace at times.

Also, I would like to thank and express a great appreciation to my parents and parents-in-law for their continuous support, and for the many hours and days they committed to caring for their new grandson. I would not have been able to complete this thesis without their help.

Lastly, thanks to my husband, Jeff Blanchard, for his patience, for listening to my trials and tribulations and for sampling flowers for me during a very busy time of year. To my son, Nicholas, thank you for being a patient and forgiving child.

TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

ABSTRACT

Barwinsky, Martha A. M.Sc., Department of Plant Science, University of Manitoba. Biology of Chlorosis Tolerance, Reproductive Behaviour and Seed Propagation in *Acer ginnala* Maxim.. Major Professor: Prof. L. M. Lenz.

The biology of chlorosis tolerance, reproductive behaviour and seed propagation, in *Acer ginnala* Maxim., were investigated in order to develop a basis for breeding for chlorosis tolerance. This species has considerable ornamental value in north temperate zone landscapes, but its use is limited due to its susceptibility to lime-induced iron chlorosis, when grown on calcareous soil.

Little information is available regarding genetic variability, diagnosis and evaluation of tolerance to lime-induced iron chlorosis in *A. ginnala.* Considerable variability in chlorosis tolerance existed in seedling populations of *A. ginnala* grown on highly calcareous soil. Eleven chlorosis-tolerant seedlings, which expressed red fall colour, were selected from this study. The use of a visual rating scale (0 - healthy, green plant; 8 - severely necrotic/dead plant) was found to be an effective diagnostic tool for chlorosis tolerance in *A. ginnala.* A SPAD-502 leaf chlorophyll meter accurately measured chlorosis, based on leaf chlorophyll content, but was less efficient in diagnosing chlorosis tolerance in *A. ginnala.* A pot-test, in which plants were grown in pots of calcareous and non-calcareous soils, under high and low water treatments, was an effective, alternative evaluation method for chlorosis tolerance in *A. ginnala.*

In order to understand the handling of plants for breeding purposes, flowering and pollination behaviour of *A. ginnala* were investigated. Distinct phases of functionally staminate and functionally pistillate flowers were observed during the

flowering period of sample plants. The expression of staminate flowers was highly variable in comparison to that of pistillate flowers. Seed set from self- and open-pollinations indicated that cross-pollination was the dominant mechanism in these plants.

In order to produce plants for selection purposes, after-ripening treatments to overcome seed dormancy and optimize germination in *A. ginnala* were investigated. The highest germination (54% and 41%, in two seed lots) occurred in 21 days, after moist stratification at 22° C for 60 days followed by 4° C for 150 days.

INTRODUCTION

The amur maple (*Acer ginnala* Maxim.) is a small tree grown in the north temperate zone landscape. It is valued for its size, form, colourful fruit, autumn leaf colouration and its tolerance to urban conditions. However, its use is limited in various landscapes with calcareous soil due to its susceptibility to lime-induced iron chlorosis. This form of chlorosis results from an iron deficiency within the plant and is a common disorder in plants growing on calcareous soils.

Lime-induced iron chlorosis appears to be a problem in *Acer* species growing throughout North America. There is some uncertainty as to whether this disorder is unique only to those *A. ginnala* plants which grow in Manitoba. However, due to the existence of highly calcareous soil in various landscapes in the province and to the limited seed sources of this plant for commercial production, this disorder is a common occurrence which requires attention.

Iron-deficiency chlorosis has received considerable attention in plant research. The majority of research dealing with this problem in landscape plants has focused on developing fertilizer treatments to correct the chlorosis. An alternative method, as demonstrated in agricultural crops, is to develop plants tolerant of this disorder.

Preliminary research conducted on *A. ginnala* at the University of Manitoba has suggested that selection, and possibly plant breeding, may yield tolerant forms (LaCroix and Lenz, 1974). This current study was undertaken to assist in the development of a breeding program for chlorosis tolerance in *A. ginnala.* The objectives of this research are:

1) to investigate and document the genetic variability of chlorosis tolerance in *A. ginnala*, by studying chlorosis expression in seedling populations growing on highly calcareous soil,

2) to analyze methods for evaluation of chlorosis tolerance,

3) to assess chlorosis tolerance, under greenhouse conditions, in plants that express varying degrees of chlorosis in the field,

4) to study the reproductive behaviour of *A. ginnala*, in an attempt to understand its breeding mechanism,

5) to determine optimum after-ripening requirements of *A. ginnala* seed to overcome seed dormancy, for propagation purposes.

LITERATURE REVIEW

Lime-Induced Iron Chlorosis

Introduction

Lime-induced iron chlorosis is a common iron-deficiency disorder in plants growing on calcareous soils. Calcareous soils cover 25-30% of the earth's land surface, providing many conditions under which iron chlorosis can occur (Chen and Barak, 1982). A wide range of plants suffer from lime-induced iron chlorosis, including agronomic crops, such as soybeans (*Glycine max* (L.) Merr.) (Weiss, 1941), horticultural crops, such as grapes (*Vitis* species L.) (Mengel et al, 1984a), and ornamental plants, such as oaks (*Quercus* species L.) (Berrang and Steiner, 1980) and maples (*Acer* species L.) (Neely, 1976).

Extensive research in iron-deficiency chlorosis has involved: 1) the identification of the disorder in various species, 2) the formulation and application of fertilizers to correct chlorosis, 3) the determination of species and cultivar differences in their efficiency of iron-uptake, and 4) the study of the physiology and biochemistry of iron-uptake in plants, including the function of the root and rhizosphere (Korcak, 1987). Past research in horticultural plants has differed from that in agronomic crops. Horticultural research has focused on the development of fertilizers to correct the problem, whereas agronomic research has focused on breeding and selecting genetically resistant plants (Korcak, 1987; Ross, 1986). More recently, research in horticultural plants has involved the study of genetic aspects of this disorder, particularly regarding differences among species and cultivars in their response to iron-deficiency chlorosis (Berrang and Steiner,

1980).

In order to conduct any study of lime-induced iron chlorosis, it is necessary to understand the basic causes of the disorder, that is, the conditions in the soil environment and the plant's reaction to these conditions. This particular problem essentially does not arise from a lack of iron in the soil, but rather from a combination of the non-availability of iron in the soil, the plant's inability to translocate iron from the roots to the leaves and the reaction of iron within the plant (Brown, 1978).

Soil Condition

In general, the occurrence of iron chlorosis may be influenced by environmental factors, such as light and temperature, and by factors related to the conditions in the soil, such as low available iron, soil pH, occurrence of carbonates and bicarbonates, high water levels and soil nutrient composition (Korcak, 1987). The term 'lime-induced iron chlorosis' is used to describe the specific iron-deficiency disorder occurring in plants growing on calcareous soils. Calcareous soils typically have a pH range of 7.4-8.5 (Loeppert, 1986). These soils are characterized as containing sufficient calcium carbonate ($CaCO_3$) and/or calcium-magnesium carbonate (Ca-$MgCO_3$) to effervesce visibly when treated with hydrochloric acid (Korcak, 1987).

The incidence of lime-induced iron chlorosis may be widespread or it may occur in localized areas referred to as 'hot spots' (Loeppert, 1986). The extent and severity of chlorosis in 'hot spots' may be highly variable. Loeppert (1986) classifies these 'hot spots' into two categories: 'true hot spots' and 'mini hot spots'. 'True hot spots' are those

in which chlorosis is observed each year in the same plants, and is not greatly affected by soil management and various environmental factors, such as soil moisture and temperature. The major soil factors which influence chlorosis in these areas are pH and $CaCO_3$ (Loeppert et al, 1984). 'Mini hot spots' are those in which soil management and environmental conditions influence the occurrence of chlorosis and the degree to which it occurs. Factors which may induce chlorosis in 'mini hot spots' include compaction, drainage, soil moisture and fertilizer.

Chemically, iron occurs as 2 forms: Fe^{3+} (ferric), the oxidized form, and Fe^{2+} (ferrous), the reduced form. Fe^{2+} is that form which is soluble and is taken up by plants (Chaney, 1972; Lindsay and Schwab, 1982). In high pH soils, Fe^{2+} is unstable and is readily oxidized to Fe^{3+} (Loeppert and Clarke, 1984). The concentration of soluble iron in calcareous soils is relatively very low, resulting in 'iron-stress' conditions (Loeppert, 1986).

The relative dissolution and precipitation of Fe oxides influences the solubility of iron in soils (Lindsay and Schwab, 1982; Loeppert and Clarke, 1984). In high pH soils, iron reacts with OH^- ions, which results in the precipitation of insoluble hydrous Fe oxides, demonstrated as follows:

$$2Fe^{3+} + 6OH^- \rightarrow 2Fe(OH)_3 \rightarrow Fe_2O_3 \cdot 3H_2O$$

(Salisbury and Ross, 1985). Lindsay and Schwab (1982) stated that soil pH greatly influences the activity of Fe^{3+} maintained by these Fe oxides. For example, for each unit increase in pH, Fe^{3+} solubility decreases 10^3 and can reach levels below 10^{-20} M as pH increases above 7.5.

According to Loeppert et al (1988), in oxidizing calcareous soils, exchangeable iron is not retained by clay as Fe^{3+} or Fe^{2+}, but is readily precipitated as Fe oxides and/or hydroxides. Fe^{2+} and Fe^{3+} salts react with $CaCO_3$ in oxidizing environments to form solid phase Fe oxides, shown as follows:

$$4Fe^{2+} + O_2 + 4CaCO_3 + 2H_2O \rightarrow 4FeOOH + 4Ca^{2+} + 4CO_2$$

$$2Fe^{3+} + 3CaCO_3 + 3H_2O \rightarrow 2Fe(OH)_3 + 3Ca^{2+} + 3CO_2$$

(Loeppert,1986). Iron availability to plants in calcareous soils is influenced further by the crystalline form, particle size and reactive surface area of the soil Fe oxide (Lindsay and Schwab, 1982; Loeppert, 1986). Amorphous or poorly crystalline Fe oxides have large reactive surface areas and may be more readily dissolved, thus making iron more available to the plant (Loeppert and Hallmark, 1985).

The reactive surface area of soil $CaCO_3$ may also influence the reaction of iron (Loeppert, 1986; Loeppert et al, 1988). The most reactive form of $CaCO_3$ has been termed "active lime" or "active carbonate", which is $CaCO_3$ in the clay or fine silt fractions of a soil, as opposed to total $CaCO_3$ of a soil (Loeppert, 1986). Several researchers have stated that the "active lime" content of a soil provides a better indication of the occurrence of iron chlorosis in some plants, than does total $CaCO_3$ (Carter, 1981; Inskeep and Bloom, 1986; Loeppert, 1986; Loeppert et al, 1984; Yaalon, 1957). It should be noted that Loeppert and Hallmark (1985) found that carbonate composition (Mg : Ca+Mg carbonate ratio) was positively correlated with the incidence of iron chlorosis, and that

this factor may be more important than total $CaCO_3$ in influencing chlorosis.

The presence of bicarbonate in the soil may enhance the iron-stress condition already present (Loeppert, 1986). In calcareous soils, a plant's ability to alleviate iron stress may be influenced by the rate of dissolution of $CaCO_3$ and the rate at which HCO_3^- enters the soil solution, both factors being dependent on the reactive surface area of the carbonate phase (Loeppert et al, 1988). An increase of bicarbonate may prevent the decrease in rhizosphere pH and the reduction of Fe^{3+} at the root surface (Loeppert, 1986). Chen and Barak (1982) stated that if a plant obtains a significant amount of iron from Fe oxides, which require reduction near the root surface to solubilize iron, then the pH-buffering effect of bicarbonate would inhibit this activity.

High soil moisture and high CO_2 pressure favour the hydrolysis of $CaCO_3$ to form HCO_3^-, which resulted in chlorosis of apples (*Malus* species Mill.), pears (*Pyrus* species L.) and roses (*Rosa* species L.) growing on wet calcareous soil (Boxma, 1972). In grape vine, Mengel et al (1984) suggested that high water saturation reduced soil gas exchange and resulted in HCO_3^- accumulation, giving rise to increased chlorosis. Inskeep and Bloom (1986) found that high CO_2 pressure and increased HCO_3^- were correlated with chlorosis development in soybeans. However, incidence of chlorosis due to HCO_3^- varied among different calcareous soils tested.

Therefore, the cause of lime-induced iron chlorosis is complicated. The most important soil-related factor in the incidence of iron chlorosis in many plant species may be the relationship between pH, carbonates, active $CaCO_3$, HCO_3^-, and CO_2 pressure in the soil (Chen and Barak, 1982).

Plant Response

Symptomology

Iron-deficiency chlorosis is expressed as interveinal yellowing of young leaves, with the veins remaining green. Chlorosis expression may be confounded by the occurrence of simultaneous micronutrient deficiencies or toxicities, such as Mn or Zn (Korcak, 1987). Chlorosis that is due to an iron deficiency alone, is determined by any regreening of yellow leaves that occurs when treated with ferrous sulfate or iron chelates, such as FeEDDHA (ethylene diamine di-o-hydroxyphenyl acetic acid), but not when treated with N, S, Zn, Mn, Cu, Co or other nutrients alone or in combination (Chaney, 1984). A chelate is an organic compound which can form strong soluble complexes with metal ions (Salisbury and Ross, 1985).

In trees, iron deficiency occurring throughout a growing season may result in smaller leaves formed as the season progresses, and necrotic areas between the veins (Neely, 1976). If the deficiency continues for two or more years, the plant may exhibit stunted growth and twig dieback (Neely, 1976).

Metabolic Function of Iron

Approximately 63% of total leaf iron is incorporated in Fe-proteins, in heme and non-heme forms, functioning in electron transport in many enzymatic reactions during photosynthesis and respiration (Miller et al, 1984; Salisbury and Ross, 1985). Heme iron constitutes approximately 9% of total iron in leaves and occurs largely in cytochromes, which function in electron transport (Mengel and Kirkby, 1978). Approximately 19% of total leaf iron occurs in non-heme Fe-proteins such as ferredoxin, thylakoid and

mitochondrial complexes, aconitase, nitrite reductase and sulfite reductase, with the remaining iron (35%) occurring in ferretin (Mengel and Kirkby, 1978; Miller et al, 1984).

Miller et al (1984) stated that there is limited knowledge regarding the role of iron in the formation and maintenance of chlorophyll. They suggested that the porphyrin biosynthesis pathway involved in chlorophyll formation may require iron (Fig. 1). Their hypothesis was that chlorophyll biosynthesis may require ferredoxin, which may function in the reduction and activation of ALA-synthase. ALA (δ-amino-levulinic acid) is the precursor of chlorophyll biosynthesis.

In photosynthesis, the role of iron is related to the function of ferredoxin in electron transport, which begins with the reduction of ferredoxin by electrons from chloroplast photosystems I and II. Miller et al (1984) stated that "iron deficiency would not only limit the total amount of ferredoxin, but also through limited chlorophyll and electron flow, decrease the total ferredoxin convertible to the reduced state."

With respect to chloroplast structure, Terry and Abadia (1986) cited evidence that iron deficiency reduces the amount of thylakoid membranes per chloroplast, and is associated with reduced amounts of electron carriers. Subsequently, electron transport in photosynthesis is affected by iron deficiency, and it has been found that there is a greater reduction in activity in photosystem I than in photosystem II (Terry and Abadia, 1986).

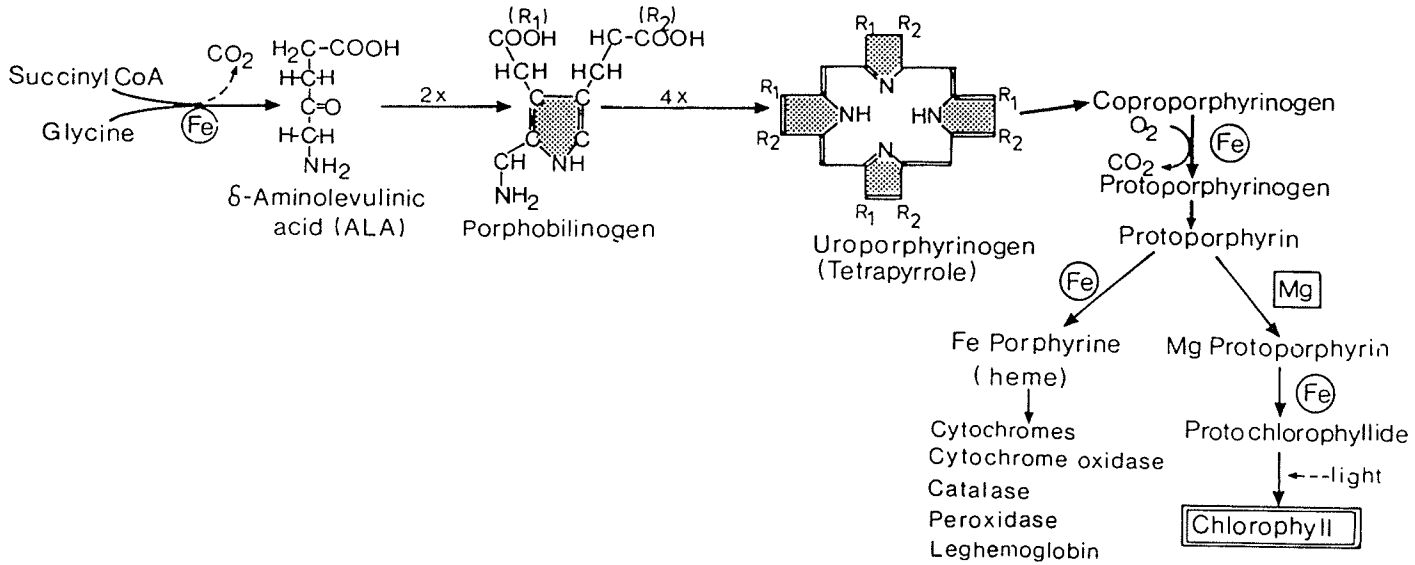


Fig. 1. The suggested role of iron (Fe) in the chlorophyll biosynthesis pathway (Römheld and Marschner, 1991).

Iron-Uptake and Transport

At the root-Fe oxide interface, Fe^{2+} is obtained from a chelate complex, by pH reduction and/or a drop in the redox potential, for plant uptake (Chaney, 1972; Lindsay, 1984; Olsen and Brown, 1980). In many dicotyledonous plants, Bienfait (1987) stated that ferric chelates are reduced at short rootzone areas, a few millimeters behind the root tip and directly behind the zone of elongation. Chaney et al (1972) suggested that reduction occurs at the outer plasmalemma, including the root hairs.

Iron is relatively immobile once in the plant and is translocated in the xylem by chelation with citric acid, forming ferric citrate (Chaney et al, 1972). Iron distribution in leaf tissue occurs in the veins and in the apoplast, followed by the "critical" transport across the plasma membrane (Mengel and Guertzen, 1986).

Fe-Stress Response Mechanism

In general, it has been determined that the critical level of Fe^{2+} in soil solution for optimum plant growth is 10^{-8} M (Lindsay, 1984; Lindsay and Schwab, 1982). If the concentration of soluble iron drops below this level then iron deficiency can occur. If iron is deficient, some plants may initiate physiological and morphological changes to improve iron mobilization, which has been termed the 'Fe-stress response mechanism'. In iron-deficient bean plants, iron-uptake is enhanced by pH reduction or a decrease in redox potential in the rhizosphere (Sijmons and Bienfait, 1984). Morphological changes may occur where the zone of elongation in roots may thicken and root hair formation is stimulated, increasing the area for iron-uptake (Bienfait et al, 1983). In addition, translocation of iron to the leaves can be higher in plants under iron stress, possibly due

to an increase in citrate in the xylem exudate (Brown, 1978; Chaney et al, 1972).

Plants may be differentiated by their response to iron stress. 'Fe-efficient' plants respond to iron deficiency by the induction of biochemical reactions, whereas 'Fe-inefficient' plants do not (Brown, 1978). The induced reactions of the Fe-stress response mechanism involve: 1) release of H^+ from the roots, 2) release of reducing compounds (i.e. organic acids) from the roots, 3) reduction of Fe^{3+} to Fe^{2+} at the roots, and 4) increases in organic acids (especially citrate) in the roots (Brown et al, 1972). Under conditions of iron stress, only the Fe-efficient root will make iron more available to the plant (Brown, 1978). Fe-efficient plants may respond to the stress without any visual symptoms, whereas Fe-inefficient plants develop chlorosis (Brown, 1978).

Olsen and Brown (1980) found that dicots and certain monocots differ in their response to iron stress. Iron-uptake can occur as one of 2 species-dependent strategies, as proposed by Römheld and Marschner (1985). Strategy I involves iron mobilization by Fe-efficient dicots and nongraminaceous monocots, in response to iron-deficiency stress. Strategy II involves the release of phytosiderophores by roots of grasses growing under iron-stress conditions. Phytosiderophores are chelating substances, believed to be non-protein amino acids which bind Fe^{3+} in the rhizosphere and transport the iron into the root cells (Korcak, 1987). For the purpose of the present study, only Strategy I will be discussed in this text.

Strategy I (Fig. 2) includes 3 main components: 1) an ATP-ase driven proton efflux pump, 2) a plasma membrane-bound inducible reductase and 3) the release of chelators and reductants. All higher plants contain a "basic reductase" in the plasma

membrane of root cells (Marschner, 1988). In Strategy I plants, root cells transfer electrons over the plasma membrane by a second plasma membrane-bound enzyme system ("inducible" or "turbo" reductase), which accepts electrons from cytosolic NAD(P)H (Chaney et al, 1972; Marschner, 1988 ; Sijmons and Bienfait, 1984). Rhizosphere acidification by the proton pump is believed to stimulate the inducible reductase (Marschner, 1988). All of the above factors described for Strategy I increase the solubility of iron in the rhizosphere and enhance its uptake at the plasma membrane (Marschner, 1988).

Several studies have indicated that the presence of HCO_3^- in the soil, particularly in the rhizosphere (Mengel et al, 1984a), may influence the effectiveness of the Fe-stress response mechanism (Strategy I). Bicarbonate-induced stress may indirectly affect iron metabolism by the plant (Brown, 1978; Loeppert, 1984).

If present in a sufficient concentration, HCO_3^- exhibits a high buffering capacity in solution and the Fe-inefficient plant is unable to lower the solution pH (Olsen and Brown, 1980). Olsen and Brown found that the Fe-efficient plant is able to remove the HCO_3^- and significantly lower the solution pH.

Mengel and Guertzen (1986) stated that HCO_3^- may affect the physiological availability of iron within the plant. In grape vine growing on calcareous soil, Mengel et al (1984a,b) suggested that HCO_3^- is absorbed and may either directly or indirectly affect iron transport from the veins to the intercostal cells in leaf tissue. Alkalinization of the leaf apoplast may cause precipitation of iron and may inhibit the plasma membrane-bound reductase responsible for iron transfer (Mengel and Guertzen, 1986).

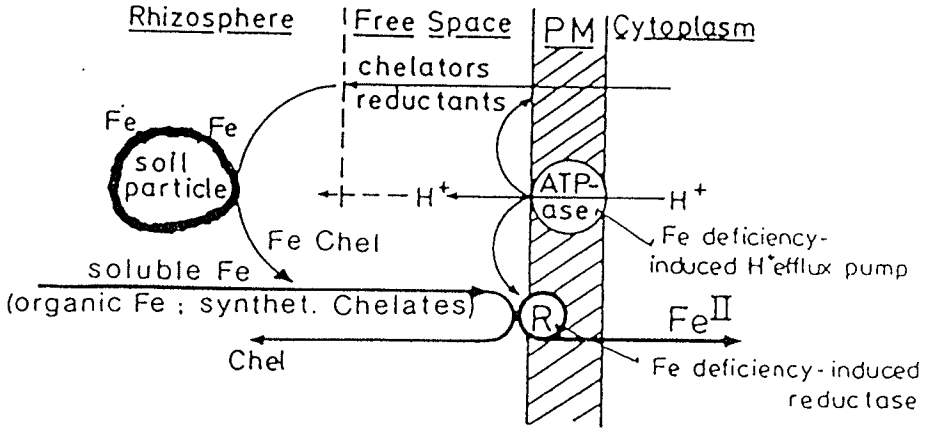


Fig. 2. A model of root responses for Strategy I, an Fe-stress response mechanism for mobilization and uptake of iron (Fe) (Marschner, 1988).

Fleming et al (1984) found distinct varietal differences in the inhibitory effect of HCO_3^- on iron metabolism in soybeans. Solution HCO_3^- significantly decreased translocation of chelated iron (FeEDDHA) to plant tops in chlorosis susceptible soybean cultivars, compared to that in chlorosis resistant cultivars. Inhibition of the Fe-stress response mechanism was the major factor involved in differences of chlorosis expression between cultivars.

Solutions to the Problem of Lime-Induced Iron Chlorosis

Introduction

Research dealing with finding solutions to the iron chlorosis problem has ranged from the development of appropriate fertilizers to correct the disorder, to understanding the genetic aspects involved in this disorder and the development of chlorosis-tolerant cultivars. The use of fertilizers has been applied mostly to landscape plants and horticultural crops. However, in grapes and in citrus, selections of resistant rootstocks have been used in crop production for several years (Korcak, 1987).

Fertilizer Treatments

Different fertilizer treatments have been tested, with respect to their ability to correct iron deficiency chlorosis in ornamental plants. Such treatments have included foliar sprays, soil applications, and trunk injections and implants. The effectiveness of these treatments has been analyzed extensively on landscape plants, focusing on such species as pin oak (*Quercus palustris* Muench.), silver maple (*Acer saccharinum* L.), sugar maple (*Acer saccharum* Marsh.), and red maple (*Acer rubrum* L.) (Neely, 1976).

The least effective treatments have been foliar applications of iron chelates, which only partially correct the chlorosis (Neely, 1976).

Studies regarding soil treatments have focused on soil acidification and on application of iron chelates. Various treatments to acidify the soil surrounding the root system, to increase iron availability, have produced variable results. To compare soil acidification and fertilizer treatments for increasing iron availability, Schoeneweiss (1973) applied liquid soil injections, consisting of suspensions of ferrous sulfate, sulfur, soluble fertilizers or combinations of these, to chlorotic *Q. palustris* growing on alkaline soil. These treatments proved to be ineffective in correcting the chlorosis. However, liquid soil injections of FeEDDHA, resulted in regreening of foliage within 30 days of application and this effect lasted for 2 growing seasons. Topsoil application of sulfuric acid rapidly lowered topsoil pH from 6.2 to 4.0, and remained at this level 14 months after treatment. Schoeneweiss (1973) speculated that subsoil acidification with sulfuric acid can maintain low pH for approximately 4 years, reducing the occurrence of chlorosis. Messenger (1984) similarly reduced or eliminated chlorosis in *Q. palustris* with subsoil acidification, but the effect was maintained for only a short period.

Harrell et al (1984) found soil treatments of Fe-sulfuric acid (Iron Sul™), liquid chelated iron (FeHEDTA) or ferrous sulfate-sulfur mixture were ineffective in correcting chlorosis immediately in *Q. palustris* and *A. saccharinum.* However, it was suggested that chlorosis may be corrected in subsequent years following application, and the effect may continue for a long period. Whitcomb (1986) applied granular sulfur to root zones of *Q. palustris* growing on high pH clay soil. The plants regreened at the end of the growing

season and no chlorosis was expressed after two growing seasons.

It has been suggested that subsoil acidification may be detrimental to plant growth. Watson et al (1991) found that sulfuric acid subsoil treatments for chlorosis of *Q. palustris* resulted in substantial root damage. Damage occurred soon after treatment, with significant reductions in fine root density and significant increases in root tip injury. Reduction in pH was confined to a small area and was short-lived, however, root damage occurred past the treated area and was long term. Watson et al (1991) suggested that subsequent treatments to maintain low soil pH could result in increased root injury and possible crown damage.

Harrell et al (1984) also treated *Q. palustris* and *A. saccharum* with trunk injections of ferrous sulfate solution (Medi-Ject™), trunk implants of ferric ammonium citrate (Medi-Cap Fe™), Mn sulfate (Medi-Cap MN™), a combination of nutrients (Medi-Cap MD™), and trunk injections containing chelated zinc with iron and other nutrients (Stemix™). Trunk injections and implants containing iron, applied in the spring, were most effective in correcting chlorosis in *Q. palustris*. Spring application of Medi-Ject™ was most effective for *A. saccharum*, however, no treatment was statistically different from the untreated control. Any correction of chlorosis was only short term for all applications.

Neely (1973) found trunk implants of gelatin capsules containing ferric citrate and ferric ammonium citrate to be most effective in correcting chlorosis of *Q. palustris*. Plants regreened 2-4 weeks following treatment, but this response was inconsistent throughout young trees. One side of the trees remained chlorotic while another side was green, as the

result of a single implantation site. The regreening effect was only temporary and Neely (1973) speculated that the trees would revert back to a chlorotic condition, once the iron from the implant was depleted. No phytotoxic symptoms were observed from the salts implanted, although injury may occur in some species from this practice (Neely, 1973).

Himelick and Himelick (1980) found trunk injections of ferric citrate and ferric ammonium citrate into *Q. palustris* were effective for 2 growing seasons, following a slow response. Timing of treatments was considered to be important for correcting chlorosis, with the effect of early spring and summer treatments (March, April, May) lasting for 3-4 years in *Q. palustris*.

Morris and Swanson (1980) treated *A. saccharinum* with trunk implants of encapsulated ferric ammonium citrate, FeEDTA (ferric ethylenediaminetetraacetic acid), and FeDTPA (ferric diethylenetriaminepentaacetic acid) to determine their effects on mineral and chlorophyll content of leaf tissue. Comparisons with chlorotic controls revealed no improvement in twig growth or leaf chlorophyll content, and no significant changes in iron levels, in treated plants.

Although callus closure generally occurs in one growing season, there are some objections to the use of trunk injections and implants. Neely (1976) cautioned the use of these treatments due to the numerous holes routinely drilled into the trunk, and to problems of sap leakage, toxicity to the cambium from the insertion of Fe-salts and phytotoxicity from excess iron applied at the wrong time to sensitive plants.

In general, fertilizer treatments are expensive, produce variable results and are effective for only a few years (Himelick and Himelick, 1980). However, highly valued

established landscape trees, which are chlorotic and left untreated, can continue to decline. Treatment may prolong the life, improve vigour and increase the aesthetic value of these plants (Himelick and Himelick, 1980). A more effective alternative solution for dealing with iron chlorosis in the landscape is to select and plant chlorosis-tolerant trees.

Genetic Tolerance - Variability and Inheritance

Mineral nutrition traits are under genetic control and iron-uptake differs among and within plant species (Brown, 1978). In a review on improving plants for tolerance to iron-deficiency chlorosis, Ross (1986) states that the principles for such improvement are similar to improvements in agronomic and quality traits or in pest resistance. According to Ross (1986), the degree of variability and the complexity of inheritance of a trait are major factors to consider in plant improvement.

Genetic variability limits selection (Ross, 1986). Therefore, one of the first steps in developing tolerant plants is to determine the extent of variability that exists in the plant material of interest.

Gupton and Spiers (1992) found variability in tolerance to iron-deficiency chlorosis in rabbiteye blueberry (*Vaccinium ashei* Reade), based on visual ratings, plant vigour and leaf mineral content (excluding iron). Chlorosis-resistant genotypes were obtainable through selection with the use of visual ratings.

Berrang and Steiner (1980) studied progenies of *Q. palustris* selected for high and low resistance from natural and cultivated populations. Seedlings were grown in solution culture and in soil environments. Highly significant differences in chlorosis expression were observed among progenies from the high and low resistance groups. None of the

progenies within each group were significantly different. Rankings were inconsistent among experimental environments, hindering the selection of the 'best' parents overall. However, the most consistently resistant populations were from a region where chlorosis was a common occurrence in *Q. palustris*. Berrang and Steiner suggested that consistently superior progenies, from several highly resistant populations, may be obtained through long-term testing.

Ross (1986) stated that in the early stages of plant breeding, it may be more effective to determine the extent of genetic variability in a population, using large numbers of genotypes, and to apply recurrent selection to concentrate favourable genes. The term, recurrent selection, is used to describe a breeding system which involves repeated cycles of selection, in order to increase the frequency of genes for certain quantitatively inherited traits (Poehlman, 1987). Recurrent selection may be practised in an unselected population to isolate desirable genotypes. This would involve repeated selection of desired genotypes, intermating and evaluation of progenies (either individuals or families). Ross (1986) stated that this method has been applied in sorghum and soybean for improving tolerance to iron chlorosis.

Weiss (1943) studied variability and inheritance of chlorosis resistance in soybean cultivars. This study revealed distinct differences in iron-deficiency chlorosis in soybean varieties and determined that iron-utilization efficiency was under monogenic control. Performance of the F1 generations indicated complete dominance and absence of maternal inheritance. Several decades later, Fehr (1982) found iron-deficiency chlorosis in soybean was controlled by a single dominant gene and multiple modifying genes, indicating

quantitative inheritance. The modifying genes are believed to influence the degree of resistance expressed by the genotypes growing on calcareous soils (Fehr, 1982). A soybean line A7 has been selected specifically for chlorosis resistance (Ross, 1986). Zaiter et al (1987) hypothesized that 2 major complementary dominant genes control resistance to iron-deficiency chlorosis in dry bean (*Phaseolus vulgaris* L.). Maternal inheritance is not involved.

In *A. ginnala*, LaCroix and Lenz (1974) determined that the frequency of tolerant types was 2%, based on one population of seedlings. A more extensive analysis is required, regarding the occurrence and behaviour of chlorosis tolerance in *A. ginnala*, using several seed sources. This information is necessary to determine how tolerance to lime-induced iron chlorosis may be obtained through breeding and selection.

Diagnosis of Iron-Deficiency Chlorosis

Various methods have been employed to evaluate Fe-efficiency, including visual ratings, foliar mineral nutrient composition, enzyme tests and leaf chlorophyll content. Identification of the cause of chlorosis is complicated, due to the many factors that may be involved in this disorder, and is not required for diagnosis (Chaney, 1984; Gupton and Spiers, 1992).

Total leaf iron content has proven to be a poor indicator of chlorosis, since chlorotic leaves may contain as much or more iron than healthy leaves (Carter, 1980; Chen and Barak, 1982; Mengel et al, 1984b). Other researchers have measured 'active iron' or HCl-extractable iron, and have found lower HCl-extractable iron in chlorotic leaves than in green leaves (Elgala and Maier, 1964; Mengel et al, 1984a,b), perhaps

serving as an indicator of iron deficiency. However, Chaney (1984) claimed that this type of analysis may not improve the sensitivity and reliability of diagnosis.

Other attempts at quantifying iron deficiency have involved the determination of the presence and amount of other nutrients, such as, Mn, P, Zn, Ca, Mg, or K, and their ratios to Fe (Chen and Barak, 1982; Gupton and Spiers, 1992; Mengel et al, 1984b). However, ratios or nutrient imbalances differ among and within species, and under stress conditions, availability and uptake of nutrients other than iron may be affected (Korcak, 1987).

Analysis of foliar enzymes, such as peroxidase and catalase, has been performed for the diagnosis of iron chlorosis. Peroxidase assays have been useful in citrus, however, this form of diagnosis is not suitable in many crops (Chaney, 1984). Chaney stated that "any enzyme test has to be calibrated with yield response and visual symptoms, and shown to be specific".

The use of visual ratings involves scoring plants on their expression of chlorosis. Visual scores have proven to be reliable and effective diagnostic tools in the selection of chlorosis-resistant soybean (de Cianzo et al, 1979), dry bean (Zaiter et al, 1987), rabbiteye blueberry (Gupton and Spiers, 1992), and pin oak (Berrang and Steiner, 1980). But, the accuracy of visual ratings may be reduced if evaluation is performed in different environments and by different persons (Chen and Barak, 1982; de Cianzo et al 1979). In this situation, determination of total leaf chlorophyll content is considered to be a more accurate and objective measurement of iron chlorosis. Chen and Barak (1982) stated that "chlorophyll content of leaves is a suitable indicator of iron chlorosis, since chlorophyll

is related to the iron supply and chlorotic appearance of plants".

Although chlorophyll extraction effectively measures iron chlorosis, this technique possesses significant disadvantages in that it is tedious and requires destructive sampling of plant material. A portable leaf chlorophyll meter, which measures leaf greenness, provides a more rapid, convenient and non-destructive measure of leaf chlorophyll content (Yadava, 1986). Chlorophyll extractions from several plant species were significantly related to readings from a Minolta portable leaf greenness meter (SPAD-501), with R^2 values of 0.64 to 0.79 (Campbell et al, 1990) and 0.478 (Yadava, 1986) using 80% acetone as the solvent, and 0.83 to 0.97 (Marquard and Tipton, 1987) using N,N-dimethylformamide (DMF) as the solvent. It was suggested from these studies that the relationship between SPAD-501 readings and leaf chlorophyll content must be determined for each plant species analyzed and possibly for each experiment (Campbell et al, 1990; Yadava, 1986).

Therefore, in order to select plants which are chlorosis-tolerant, it is necessary to be able to identify and to measure the symptoms of the disorder. An appropriate method of diagnosis of lime-induced iron chlorosis needs to be devised for *A. ginnala*, which may easily detect tolerant types.

Evaluation Procedures

Evaluation and selection of Fe-efficient plants has involved the use of conventional field tests and controlled environment experiments. Fe-efficiency classification and selection of soybean genotypes, under field conditions, have proven to be inconsistent due to soil heterogeneity and environmental fluctuations (Fairbanks et al, 1987). Evaluation

of plants in the field may pose problems, in that often chlorosis will not be expressed until the root system penetrates highly calcareous subsoil layers (LaCroix and Lenz, 1967). LaCroix and Lenz suggested field selection should be performed on an area with highly calcareous topsoil. The plants under study may be planted adjacent to chlorosis-susceptible PI soybeans, to serve as indicator plants. It was also suggested that these soybeans may be used to measure the soil's potential to induce chlorosis and the variability in the test area. Field testing may be useful in initially evaluating populations or large numbers of genotypes and for recurrent selection, followed by improvement of chlorosis tolerance under controlled environment experiments (Dragonuk et al, 1989).

Dragonuk et al (1989) evaluated Fe-efficiency of soybean by growing plants in nutrient solution. Plants that expressed chlorosis in solution culture showed little or no symptoms in the field, demonstrating that nutrient solution cultures do not duplicate field conditions. This procedure may be selecting for different Fe-stress response mechanisms when compared to field tests (Berrang and Steiner, 1980; Dragonuk et al, 1989). Inconsistencies in chlorosis expression of pin oak progenies were observed when results from solution culture and soil culture were compared (Berrang and Steiner, 1980).

Procedures involving plants grown in potted calcareous soils correlate well with field tests and have proven to be more representative of field conditions. In growth chamber and greenhouse studies, Fairbanks et al (1987) grew soybean plants in pots of calcareous soil, obtained from field test sites. Chlorosis scores from growth chamber experiments were significantly correlated with field scores in 3 out of 4 comparisons. However, some soils that produced chlorosis in the field did not produce chlorosis in pots.

Fairbanks et al (1987) speculated that these results may have occurred due to the heterogeneity of calcareous soils and the natural variation in iron chlorosis that can occur among genotypes.

Gildersleeve and Ocumpaugh (1980) found that growing 'Yuchi' arrowleaf (*Trifolium vesiculosum* Savi.) and 'Dixie' crimson (*Trifolium incarnatum* L.) clover seedlings in pots of different calcareous soils in a greenhouse, was an effective method of evaluation for chlorosis tolerance. Chlorosis was induced in susceptible plants by soil saturation with water for a minimum of 2 weeks. In both varieties of clover, the frequency distribution of chlorosis scores differed among the soils used in the study. Although the calcareous soils differed in their chemical and physical properties, Gildersleeve and Ocumpaugh (1980) stated that differences in chlorosis expression among the soils may have been due to genetic variation in the seedling populations as a result of cross-pollination.

Barak and Chen (1982) developed a bioassay-type test in growth chambers to evaluate iron deficiency using peanut plants (*Arachis hypogaea* L.) grown in pots of various soils ranging in $CaCO_3$ content. This procedure was found to be effective for estimating iron deficiency in various soils and for screening cultivars for chlorosis tolerance. This type of pot test may be applicable to evaluating chlorosis tolerance in plants of *A. ginnala*. Barak and Chen (1982) stated that experiments conducted under controlled environments ensure reproducibility between experiments, without complications of environmental factors and year-to-year variation.

Biology of *Acer ginnala* Maxim.

Introduction

Acer ginnala appears to be adaptable to many situations in the landscape. However, in areas of Manitoba with highly calcareous soil, the susceptibility of *A. ginnala* to lime-induced iron chlorosis reduces its adaptability and aesthetic value in the landscape (LaCroix and Lenz, 1974). Although selections have been made for form, and foliage and fruit colour, plants tolerant of lime-induced iron chlorosis have not been selected and made available for use (Dirr, 1990b; Snyder, 1980). In order to improve chlorosis tolerance in *A. ginnala* through breeding and selection, it is necessary to understand the reproductive behaviour and propagation of this plant.

Origin and Characteristics

Acer ginnala Maxim. belongs to the Aceraceae Juss. family. The genus *Acer* L. contains approximately 148 species occurring in North America, Asia, Europe and northern Africa (Olson and Gabriel, 1974). *Acer*, commonly called maple, is comprised of deciduous woody plants, many of which are considered to be important in the area of horticulture (Bailey and Bailey, 1976). The use of maples is wide and varied ranging from lumber, to maple sugar and syrup, to ornamental plantings (Olson and Gabriel, 1974). Many species are aesthetically valued due to their attractive foliage characteristics, plant form, flowers and fruit (Olson and Gabriel, 1974).

A. ginnala, commonly called amur maple, is indigenous to central and northern China, Manchuria and Japan, and was introduced to North America in 1860 (Dirr, 1990b;

Snyder, 1980). *A. ginnala* is considered to be hardy to USDA zones 2 to 8 (Dirr, 1990b), and is widely adaptable to many climates (Flint, 1985). This plant requires full sun or light shade and moist, fertile, well-drained soil for optimum growth, although it does exhibit some shade and drought tolerance (Dirr, 1990b; Flint, 1985; Snyder, 1980). It is believed that *A. ginnala* has few pest problems (Dirr, 1990b).

A. ginnala is often confused with *A. tataricum* L. because they are very similar in many aspects of growth and appearance (Dirr, 1990b; Snyder, 1980). *A. ginnala* has actually been described as a variety of *A. tataricum* - *A. tataricum* var *aidzuense* Franch. (Bailey and Bailey, 1976). *A. tataricum*, commonly called tatarian maple, originates from southeast Europe and western Asia, and was introduced to North America in 1759 (Dirr, 1990b). The two species may be differentiated primarily by their leaf shape - leaves of *A. ginnala* are distinctly 3-lobed whereas those of *A. tataricum* are usually unlobed (Dirr, 1990b; Snyder, 1980).

A. ginnala is well-suited to urban use and possesses considerable ornamental value (Flint, 1985). It may be grown as a multi-stemmed large shrub or as a small tree, ranging in height from 5 to 8 meters and similarly in width (Dirr, 1990b; Snyder, 1980). This plant has multiple uses in landscapes as a small specimen, as a visual screen, and in group or mass plantings, such as shelterbelts (Dirr, 1990b; Flint, 1985).

Its ornamental characteristics include leaf shape, autumn leaf colouration, and fruit form and summer colouration. The fruit is classified as a samara and may be red in colour, in June and July (Dirr, 1990b). Autumn leaf colouration ranges from yellow to orange to red, with red being of highest value in the landscape (Dirr, 1990b; Snyder,

1980). In addition, the flowers are scented, which is of rare occurrence in the genus (Dirr, 1990b). Various plants have been selected based on their compact growth habit, red fall colour, and/or fruit colour, and include such varieties as 'Compactum', 'Durand Dwarf' and 'Flame' (Dirr, 1990b; Snyder, 1980).

Sexual Reproduction

An understanding of the mode of reproduction of a given plant is an essential factor in a breeding program. The mode of reproduction, sexual and/or asexual, dictates the use of specific breeding methodologies and greatly influences the handling of material.

Reproductive Biology

Research and breeding of trees has been hampered by problems of long generation time and limited flowering periods, due to the perennial habit of these plants (Sedgley and Griffin, 1987). Nevertheless, research on flowering has been performed in many species in Aceraceae, probably due to the peculiarities in flowering habits exhibited in members of this family. The breeding system within a species is influenced by its flowering habit, which refers to the structural and functional relationship of the male and female reproductive organs (Sedgley and Griffin, 1987).

Flowers of *A. ginnala* are yellowish-white in colour, scented, have a 5-merous perianth, 8 stamens, a bicarpellate pistil and are perigynous with an extrastaminal honey disc (Dirr and Heuser, 1987; Jong, 1976). The presence of a honey disc and the scent in flowers of *A. ginnala* suggests that this is an entomophilous species, as are most *Acer* species (Dirr and Heuser, 1987; Jong, 1976; Percival, 1965). The flowers occur in a

compound inflorescence, in the form of small panicles, 2.5-4 cm in diameter (Dirr, 1990b) (Fig.3). These panicles may occur terminally or laterally on shoots (Jong, 1976). Fruit is a dry, indehiscent samara, 2-2.5 cm long, which may develop parthenocarpically (Jong, 1976).

Monoecy and dioecy are outcrossing mechanisms which provide spatial separation of the sexes. Both mechanisms have been observed in the genus *Acer*. In monoecy, the androecium and gynoecium occur in separate flowers on the same plant. In dioecy, the androecium and gynoecium occur in separate plants. *A. ginnala* exhibits a form of monoecy, in that the flowers are functionally unisexual, however, structurally are perfect or hermaphroditic at inception (Jong, 1976; Sedgley and Griffin, 1987). Snyder (1980) describes *A. ginnala* as an andromonoecious plant, bearing staminate and hermaphroditic (bisexual) flowers on the same plant. In specimens of *A. ginnala* studied by Jong (1976), individual flowers were all structurally hermaphroditic, but functionally, were either staminate or pistillate. Descriptions of the flowering habit of *A. ginnala* appear to be inconsistent in the literature.

Although the flowering habit of *A. ginnala* may be considered as functional monoecy, Jong (1976) stated that it is more appropriately described as dichogamy. Dichogamy is another outcrossing mechanism, in which male and female reproductive structures mature at different times (Frankel and Galun, 1977). This asynchronous maturation may occur in a hermaphrodite flower (single flower dichogamy) or in a monoecious plant (plant dichogamy).

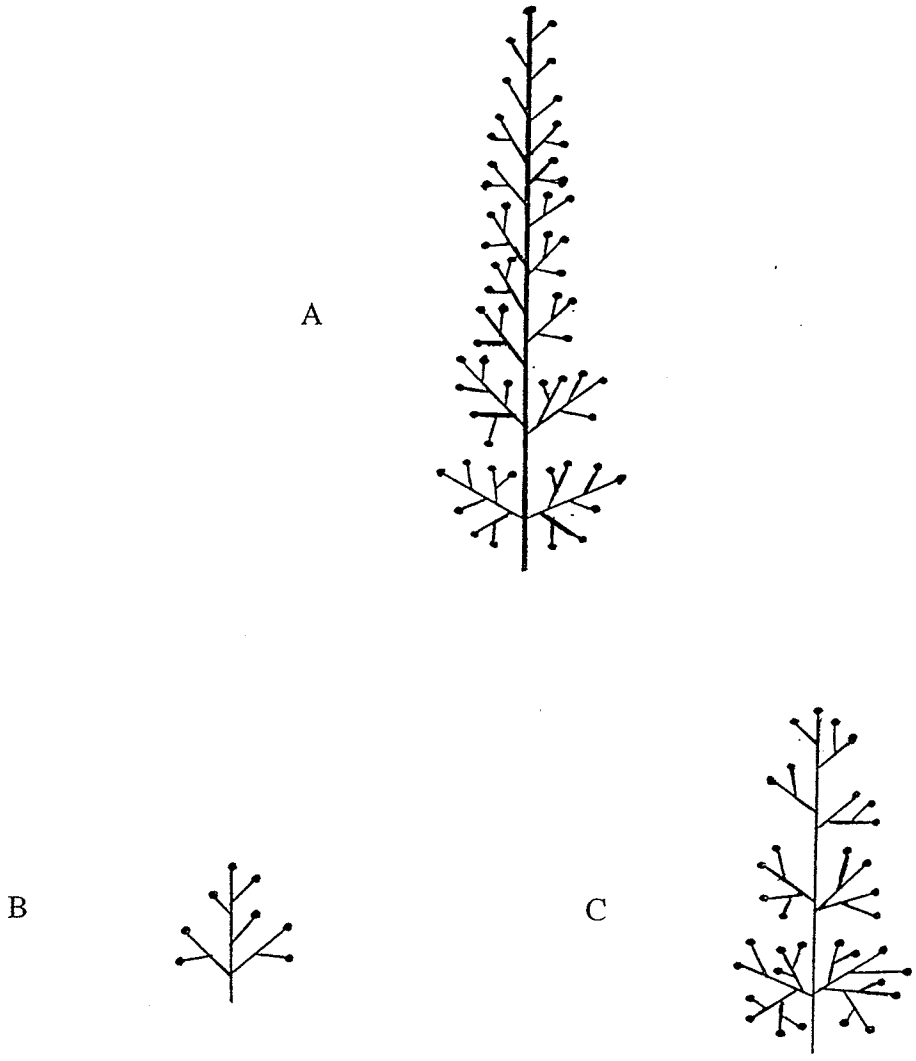


Fig. 3. Forms of inflorescences in *A. ginnala* as adapted from Jong (1976). (A, basic form; B, reduced rachis; C, elongated rachis)

It is important to distinguish between plant dichogamy or single flower dichogamy when considering breeding mechanisms (Frankel and Galun, 1977). According to Jong (1976), *A. ginnala* exhibits plant dichogamy, in which functionally staminate flowers mature at different times from functionally pistillate flowers in inflorescences, within an individual plant. Functionally staminate flowers are denoted as such because the pistil does not appear to develop past the rudimentary stage. Functionally pistillate flowers in *Acer* are characterised by inhibition of stamen development during the last few weeks before anthesis (Jong, 1976). Jong (1976) observed that filaments failed to elongate and the anthers did not open in functionally pistillate flowers. Anthers of pistillate flowers contained heterogeneous pollen, which was irregular and smaller than that of functionally staminate flowers (Jong, 1976). Throughout the remainder of the discussion, functionally staminate and functionally pistillate flowers will be simply referred to as staminate and pistillate flowers, respectively.

Dichogamous individuals may be further classified as protandrous or protogynous (Jong, 1976). Protandry involves the maturation of the male organs before the female organs, whereas protogyny involves the opposite sequence of maturation (Frankel and Galun, 1977). A species which exhibits both protandry and protogyny is denoted as heterodichogamous, and according to Jong (1976), heterodichogamy occurs in *A. ginnala*, with protandry predominating (≥50%) in the individuals studied.

Another variation of dichogamy may exist within a species, in which a sequence of flowering phases occurs over the flowering period, for example, staminate-pistillate-staminate. This condition is referred to as duodichogamy (Fig. 4) and was observed in *A.*

ginnala specimens studied by Jong (1976). The two staminate phases are denoted as primary (1°) and secondary (2°). The onset and anthesis of the 1° staminate phase occurs prior to that of the pistillate phase, whereas the onset and anthesis of the 2° staminate phase occurs after that of the pistillate phase.

Jong (1976) performed extensive research on flowering and sex expression in the genus *Acer*. A characterisation of differential sex expression in *Acer*, with respect to staminate and pistillate phases was developed primarily by Wittrock, as cited and adapted by Jong (1976) (Fig. 5). Flowering types observed in *A. ginnala* by Jong (1976) were that of type C (staminate-pistillate-staminate) and occasionally type D (staminate-pistillate). In one plant, a 2° pistillate phase was observed on the east and west sides of the plant (type J). Some inflorescences produced the 2° staminate flowers at the same time as the 2° pistillate phase. In one year, some inflorescences exhibited type K flowering (pistillate-staminate-pistillate-staminate). Variations in flowering and sex expression were observed in several *A. ginnala* plants over the 3-4 year study. Specimens exhibited similar flowering or combination of flowering types, with annual variations often occurring (Jong, 1976). Therefore, the flowering habit of a species may be complicated, and knowledge of the timing and sex of flowering is necessary to optimize pollination for breeding purposes.

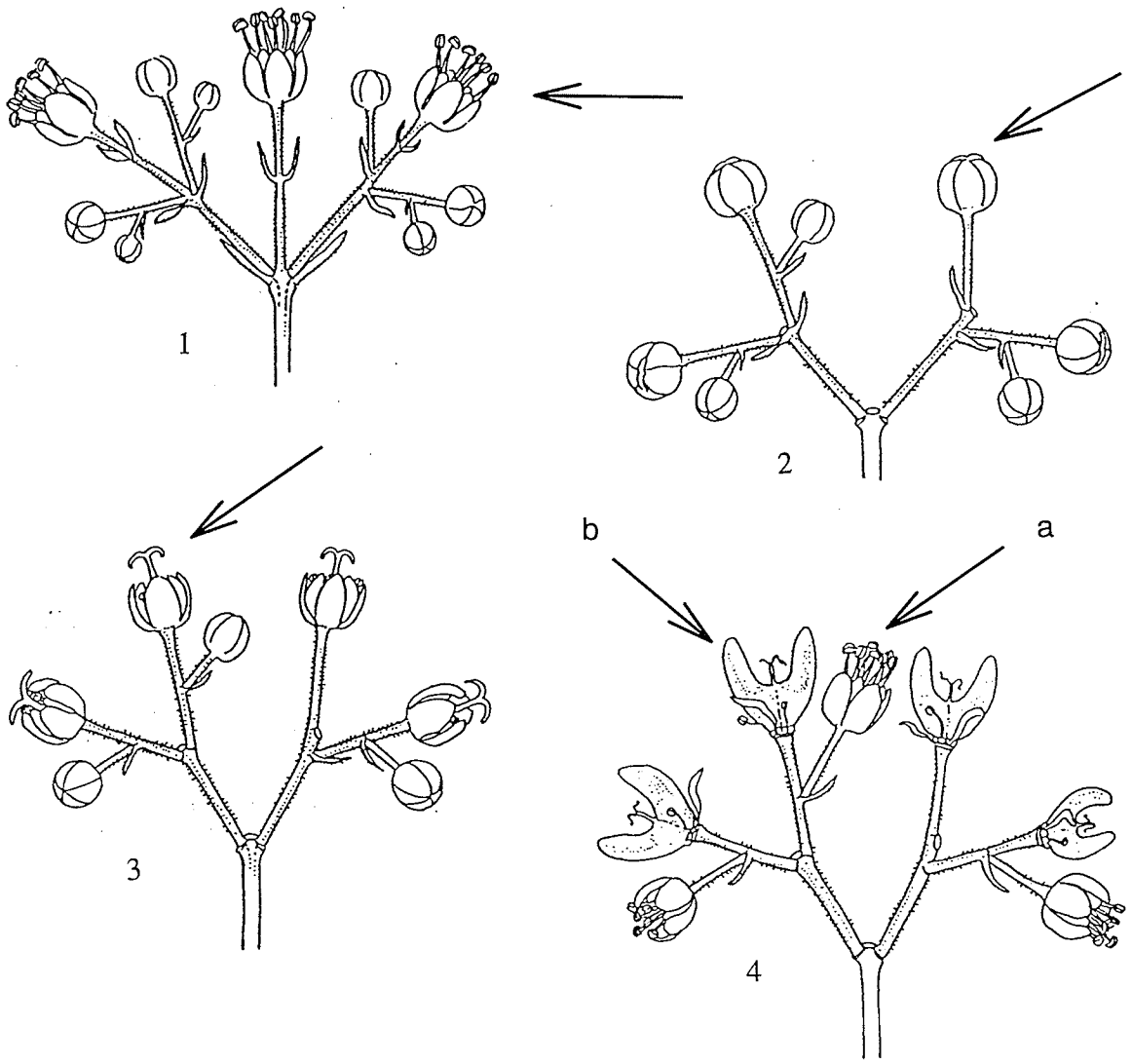


Fig. 4. Duodichogamy in *A. ginnala,* demonstrated in a single inflorescence. The sequence of development in all inflorescences throughout a plant is as follows: 1) 1° staminate phase, 2) onset of pistillate phase with stigmas becoming visible, 3) pistillate phase, and 4) 2° staminate phase (a) with withered stigmas of pistillate phase (b) (adapted from Jong, 1976).

Flowering Type	Consecutive Phases of Staminate and Pistillate Flowers During Anthesis				
	S	P	S	P	S
A		[----------]			
B		[----------]			
C	[----------]				
D	[----------]				
E	[----------]				
F			[----------]		
G	[----------]		[----------]		
H		[----------]			
J	[----------]				
K		[----------]			
L	[----------]				

Fig. 5. Flowering types of *Acer* inflorescences as cited in Jong (1976). (S, staminate; P, pistillate) ([--------] denotes the sequence of development in a single inflorescence)

Flower development in *A. ginnala* was also studied by Jong (1976), as a means of understanding the sex expression. Jong hypothesized that flower buds of monoecious *Acer* species were initially hermaphrodite and differentiated at various times, before anthesis, into pistillate or staminate flower buds. The staminate flowers showed pistils of various sizes and at various stages of development at anthesis. The initiation period of floral bud primordia occurred over 6-10 weeks, depending on the branching of the floral axes. In *A. ginnala*, floral buds had been initiated before winter, but floral organ initiation was not complete. Generally, in dichogamous species of *Acer*, sex differentiation of primary (1°) staminate flowers occurred between 1-5 months before anthesis in spring. The pistillate phase was largely influenced by environmental factors, resulting in annual variations, and variations among and within individual plants (Jong, 1976).

Environmental influences on sex expression may be due to edaphic conditions, light and temperature (Heslop-Harrison, 1972; Frankel and Galun, 1977). Jong (1976) summarized that in protandrous *Acer* individuals, environmental conditions just before and during anthesis appeared to influence the length of the pistillate phase. Relatively high temperatures reduced the number of pistillate flowers. Temperature variation during floral induction was also involved. Yearly and local fluctuations in mean size of inflorescences were observed, which resulted in variations in the number of phases and frequency of flowering types. The effect of certain environmental factors on sex expression in trees, specifically, may be difficult to analyze, since woody plants interact with environmental conditions throughout each year (Sedgley and Griffin, 1987), and flowering occurs over a period of two years - initiation in one year followed by flowering the next year.

Breeding Mechanism

Generally, in order for pollination to occur, the stigma must be receptive to pollen, which in turn must be viable and compatible (Gabriel, 1966). The asynchronous maturation of the male and female reproductive structures, as observed in studies of *A. ginnala*, reduces the occurrence of self-pollination (Jong, 1976). Fryxell (1957) denotes *A. ginnala* as "probably" a partially self-fertilized and partially cross-fertilized species. There is very little information regarding the extent of selfing and cross-pollination in *A. ginnala.* In *A. saccharum*, Gabriel (1966) indicated that selfing may occur in a dichogamous individual, although it may be limited, depending on the duration of stigma receptivity and the supply of pollen at the appropriate time. Geitonogamy (pollen received from another flower on the same plant) is possible, as noted for *A. spicatum*, when both staminate and pistillate conditions occur in inflorescences in one plant (Sullivan, 1983). Jong (1976) observed that isolated protandrous *Acer* species produced fruit as a consequence of geitonogamy. Self-fertility and self-pollination create an advantage for a particular species, if insect populations are low and individual plants are growing far apart (Percival, 1965).

The breeding mechanism of a particular species can be influenced by such conditions as self-incompatibility, apomixy, and parthenocarpy, which can occur in various *Acer* species (Fryxell, 1957; Gabriel, 1967; Gustafson, 1942; Sullivan, 1983). Of these conditions, parthenocarpy was observed in specimens of *A. ginnala* studied by Jong (1976). Parthenocarpy is the production of seedless fruit (Gustafson, 1942). Parthenocarpic fruit development may result from: 1) ovary development without pollination, 2) fruit

growth stimulated by pollination but without fertilization, or 3) fertilization followed by early embryo abortion (Gustafson, 1942; Salisbury and Ross, 1985). It is difficult to distinguish between parthenocarpic fruit developed without fertilization, or, with fertilization and embryo abortion, since in either situation, the ovules may be very small or empty and partially developed seeds may be produced (Gustafson, 1942). This situation was observed by Kurdiuk (1984), who obtained seed-bearing fruit, fruit with underdeveloped embryos and fruit which was empty from various plants of *A. ginnala.*

Jong (1976) denotes *A. ginnala* as exhibiting a moderate parthenocarpic tendency. In *Acer*, the term moderate parthenocarpy is used to describe the condition in which the seedless mericarp of fully grown fruits is relatively smaller in wing and nutlet size than the seed-bearing carpel (Jong, 1976).

Parthenocarpic fruit development is influenced by such factors as climate and plant growth hormones (Gustafson, 1942). Gustafson hypothesized that developing seeds aid in the conduction of substances into fruits. Seedless fruits are less capable of obtaining required substances for development and thus, develop only under conditions of high nutrition or little competition. Therefore, it is suggested that more favourable growing conditions are required for a plant to produce parthenocarpic fruit rather than seed-bearing fruit.

Auxin may influence the production of parthenocarpic fruit (Salisbury and Ross, 1985). Based on a study of varieties of orange, lemon and grape, Gustafson (1939) suggested that plants which contain large amounts of auxin in the ovaries at flowering may exhibit ovary growth without fertilization. The formation of an abscission layer

between the ovary and the pedicel, resulting in fruit abortion, may be prevented by the presence of auxin. Ovary development may continue as a result of auxin production in the ovary itself, or auxin translocation into the ovary from the leaves. Jong (1976) reported that pistillate flowers of all *Acer* species have a tendency to show parthenocarpic development of the ovary initially (that is, fruit development in certain pistillate flowers may begin before withering of the stigma), but may or may not continue developing parthenocarpically depending on whether seed production occurs. A small proportion of parthenocarpic fruit are shed within a few weeks of flowering and some may remain until autumn, if there are few or no fertilized flowers.

Propagation by Seed

Introduction

Genetic variability is obtained through sexual reproduction in conventional plant breeding. Effective propagation methods are important tools in producing plant materials for evaluation and selection of progeny. Often different techniques must be developed to overcome certain obstacles, such as seed dormancy. This condition exists in *A. ginnala*, which makes propagation difficult and often results in poor germination.

Seed Dormancy

Seeds are considered to be in a dormant state when they fail to germinate, even when external conditions are favourable (Salisbury and Ross, 1985). Two types of dormancy have been classified: primary and secondary dormancy. Primary dormancy refers to a delay in germination of mature seeds due to internal conditions, which exist

or develop during ripening and for a period of time after harvest. Secondary dormancy refers to the prevention of germination or a reduction in the rate of germination, induced under unfavourable environmental conditions (Crocker, 1916; Hartmann et al, 1990; Khan and Samimy, 1980).

Various types of primary seed dormancy exist and have been classified as follows: 1) immaturity of the embryo, 2) impermeability of the seed coat to water, 3) mechanical resistance of the seed coat to embryo growth, 4) impermeability of the seed coat to gases, 5) a state of dormancy in the embryo itself, and 6) combinations of the above (Crocker, 1916).

The main forms of dormancy found in the genus *Acer* are true embryo dormancy and testa- or integument-imposed dormancy (Pinfield and Dungey, 1985). There are arguments as to whether these two types of dormancy may actually be distinct categories in *Acer* (Pinfield et al, 1987). Mechanical restraint to embryo growth does not appear to be a major factor in *Acer* species (Pinfield and Dungey, 1985).

In *A. ginnala*, Dumbroff and Webb (1970) stated that seed dormancy is mainly controlled by the covering structures, which impede water uptake. The pericarp was found to be a more significant barrier to imbibition than the testa. Presoaking seeds stimulated germination, but extracts from the pericarp were non-inhibitory. The pericarp did not interfere with gas exchange. Embryo dormancy was also referred to in this study as a "metabolic block in the embryo", which required sufficient water uptake to overcome the dormant state. McMillan-Browse (1979) stated that seed of *A. ginnala* develop a hard seed coat and exhibit embryo dormancy.

Methods for Germination

Seed exhibiting various forms of dormancy may require a period of after-ripening in order to germinate (Raven et al, 1981). This period may be viewed as a survival mechanism often seen in temperate-zone plants with late seed dispersal, where germination is delayed until spring, when chances of survival are greatest (Powell, 1987). The term after-ripening refers to any changes that occur within the seed leading to the alleviation of dormancy (Salisbury and Ross, 1985). Various techniques are used as after-ripening treatments for seed propagation. Seed of the genus *Acer* are typically stratified to overcome dormancy (Hartmann et al, 1990; McMillan-Browse, 1979). For seed of *A. ginnala*, recommendations for after-ripening treatments appear to be inconsistent in the literature, and results in some studies are based on methods which are inappropriate for large scale propagation.

In *A. ginnala*, Dumbroff and Webb (1970) obtained 74% germination in 2 months following stratification of whole seeds at 5° C sown in Petri dishes containing distilled water. Presoaking seeds in distilled water for 24 days decreased germination time and increased germination percentage. Removal of the pericarp increased germination to 87% and tearing the testa increased germination to 93%. The after-ripening period required for whole seed, seed with the pericarp removed and seed with torn testa was reduced from 30, to 15, to 9 days, respectively.

Norton (1987) studied the effects of post-harvest ageing of *A. ginnala* seed on the alleviation of dormancy. Various after-ripening treatments were applied to 1-, 6-, and 9-month-old seed, which included cool, moist stratification and use of gibberellic acid (GA)

and ethephon. In general, storage of seed at 20-25° C resulted in the development of dormancy, in which untreated 9-month-old seed did not germinate under appropriate conditions. Untreated fresh seed (1-month-old) provided 44% germination, but germination did not increase following any after-ripening treatments. However, moist stratification at 4° C for 30 days increased germination of 6-month-old seed and of 9-month-old seed to 46% and 26%, respectively. A combination of GA (100 $mg \cdot L^{-1}$), ethephon (100 $mg \cdot L^{-1}$) and a 30 day cold treatment increased germination of 9-month-old seed to 86%. It was concluded from this study that post-harvest ageing may reduce germination due to drying and hardening of the testa and pericarp. McMillan-Browse (1979) stated that long-term dry storage of *Acer* seed can have deleterious effects on seed viability and results in the development of a hard seed coat, which subsequently, can result in the failure of seed to germinate.

According to McMillan-Browse (1979) and to Norton (1987), early collection of samaras, prior to pericarp drying, may improve germination if sown immediately, since the seed will exhibit little dormancy. McMillan-Browse (1979) advised that storage of samaras in this 'green' condition may become a problem due to overheating.

Although the above propagation methods were used mainly for the purpose of studying seed dormancy, the use of Petri dishes, pericarp removal, and growth regulators are not practical for large scale propagation. There are various suggestions for handling seed of *A. ginnala*, which may be applied to large scale or commercial production. For *A. ginnala* seed, Olson and Gabriel (1974) suggested scarification of the pericarp before warm and cold stratification treatments, in Kimpac medium, of 30-60 days at 21°-29° C

followed by 90-150 days at 5°C. Germination test conditions of stratified seed provided 50% germination in 10 days under day/night temperatures of 30°/20°C. Dirr (1990b) suggested several stratification treatments for *A. ginnala* seed sown in media: 1) 21°-29°C for 30-60 days followed by 5°C for 150+ days, 2) light scarification and then 5°C for 90 days, or 3) 3-4 months at 5°C. However, in these two pieces of literature (Olson and Gabriel, 1974; Dirr, 1990b), no scientific evidence has been provided.

Vegetative Propagation

Vegetative propagation by cuttings is considered to be a simple means of increasing numbers of plants in *A. ginnala*, which is described as an 'easy-to-root' species (Dirr, 1990b). The propagative material is primarily in the form of softwood cuttings, although hardwood cuttings have been capable of rooting (Enright, 1958). Enright (1958) found that softwood cuttings of *A. ginnala* taken in June and July exhibited 100% rooting in sand, provided that they were treated with rooting hormone. Chapman (1979) similarly obtained high rooting percentages (80%) in cuttings taken near the end of June. Both studies by Chapman (1979) and by Enright (1958) suggested that cuttings may be taken over a longer period of time during the growing season with good results, allowing flexibility in harvesting propagative material and in use of propagation facilities. However, Dirr and Heuser (1987) cautioned taking cuttings too late in the growing season, stating that timing is critical and that there is a significant decline in rooting as the tissue matures.

The use of rooting hormones, particularly of IBA (1*H*-indole-3-butyric acid)

stimulates rooting in *A. ginnala* (Enright, 1958). A relatively new chemical P-ITB (phenyl indole-3-thiolobutyrate) has also been found to stimulate rooting of softwood cuttings of *A. ginnala* (Dirr, 1990a). Enright (1958) found that concentrations of IBA at 10-20 $mg \cdot ml^{-1}$, increased rooting of *A. ginnala* cuttings in sand to 92% and 100%, respectively, over a period of 22 days.

Overall, Dirr and Heuser (1987) stated that 90% rooting could be obtained in cuttings of *A. ginnala*, which were treated with 1000 - 5000 ppm IBA-talc, stuck in peat:perlite (v/v) and placed in a mist propagation system with bottom heat of 23° C. Enright (1958) specified that the ideal bottom heat to apply is 23° C day/ 17° C night.

CHAPTER 1

Biology of Chlorosis Tolerance

in *Acer ginnala* Maxim.

ABSTRACT

In order to develop a basis for selection for chlorosis tolerance in *Acer ginnala* Maxim., genetic variability, diagnosis and evaluation of tolerance to lime-induced iron chlorosis in *A. ginnala* were investigated.

Seedling populations from tolerant and susceptible plants of *A. ginnala*, and from one plant of *A. tataricum*, were grown in a field of highly calcareous soil. Germination was highly variable and very low, ranging from 8.2% to 25.5% among parents. The expression of chlorosis tolerance and seedling growth were highly variable among and within the populations. Chlorosis tolerant parents generally did not produce a greater frequency of tolerant progeny. Eleven chlorosis-tolerant seedlings, which expressed red fall colour, were selected.

Visual ratings, SPAD-502 meter readings and total leaf chlorophyll content were obtained from individual plants of *A. ginnala*, to determine the most efficient method for diagnosing and measuring chlorosis. Correlations among the three methods were all significant ($P<0.0001$). The meter readings and chlorophyll extraction provided objective measurements of chlorosis, but the rating scale was a more efficient method. Consequently, a visual rating scale (0 - healthy, green plant; 8 - severely necrotic/dead plant) was developed in this study for the diagnosis of chlorosis tolerance in *A. ginnala*.

Evaluation of chlorosis tolerance, under a controlled environment, was investigated in *A. ginnala* as an alternative procedure for selection purposes. Clones of susceptible and tolerant plants were grown in a greenhouse in pots filled with calcareous and non-calcareous soils, with high and low soil water contents. Tolerant and susceptible plants

were detected in the pot-test. A significant soil x plant interaction (P=0.0275) indicated that the calcareous soil was effective in inducing chlorosis in susceptible types. Differences between water contents were significant (P=0.0084) in chlorosis expression. Plant height (cm) was greater in the non-calcareous soil/low water treatment (P=0.0010) and there was a significant plant x soil x water interaction (P=0.035).

INTRODUCTION

Tolerance to lime-induced iron chlorosis in *A. ginnala* has been observed to occur at a frequency of 2% (LaCroix and Lenz, 1974). Although some tolerant plants have been selected (LaCroix and Lenz, 1974), they do not express red fall colour. In *A. ginnala*, the evaluation of tolerance to lime-induced iron chlorosis, its variability within the species and the process of selection of tolerant types have not been documented. Therefore, these factors were investigated in an attempt to develop procedures for selecting chlorosis-tolerant plants which express red fall colour.

A/ GENETIC VARIABILITY

INTRODUCTION

Selection of chlorosis-tolerant plants requires the existence of genetic variability for that desired trait. Therefore, this study had the following objectives:

1) to document genetic variability in the expression of lime-induced iron chlorosis in seedling populations of *A. ginnala*, grown on highly calcareous soil,

2) to determine if plants, previously selected for chlorosis tolerance, produce a higher percentage of tolerant progeny,

3) to determine if seedling vigour is associated with chlorosis tolerance.

MATERIALS AND METHODS

The experimental plot for this study was situated at a farm (NW¼-23-16-2E) located on provincial highway 17, east of Teulon, Mb. The plant material consisted of seedling populations from 13 plants of *A. ginnala* and from one plant of *A. tataricum*. *A.*

tataricum was included in this study due to its similarities of growth and appearance to *A. ginnala* (Dirr, 1990b; Snyder, 1980). The soil at this experimental site is highly calcareous (Appendix A) and has been observed to induce chlorosis in susceptible plants in a previous study (LaCroix and Lenz, unpublished). The parent plants utilized in this study were selected on the basis of their expression of chlorosis (ranging from tolerant types to susceptible types) and/or on the basis of their expression of red fall colour (Table 1). Of the 13 parent plants of *A. ginnala*, 5 are located at the University of Manitoba campus, 4 are located near Teulon, Mb and 4 are located at the Agriculture Canada Research Station in Morden, Mb. The plant, UMParent, is located by the Dairy Science Building on campus and is the parent plant which was utilized in the study by LaCroix and Lenz (1974). Three of the plants growing at the University of Manitoba campus (UM7301, UM7306, UM7308) are selections from the open-pollinated UMParent. The four plants at Teulon, Mb, are also progeny from the open-pollinated UMParent used in the study by LaCroix and Lenz (1974) (Appendix B). UMParent and UMWalker located on the University campus are believed to be progeny from two seed sources, growing at the Agriculture Canada Research Station in Morden, Mb. The plant of *A. tataricum* is located at the Agriculture Canada Research Station in Morden, Mb.

Samaras were harvested in September, 1992 and were dewinged by rubbing them over a wire screen. The fruit were sorted visually according to whether they were filled with seed or were incompletely developed or empty. Fruit which contained seed were selected for the study. On October 7, 1992, the fruit were planted using a V-belt seeder. Experimental design was a RCBD with 14 treatments replicated 3 times. Each treatment

Table 1. Parent plants of *A. ginnala* and *A. tataricum* utilized in progeny study near Teulon, Mb, 1992 - 1993.

Location	Plant	Chlorosis Rating	Red Fall Colour
University of Manitoba			
Dept. of Plant Science	UM7301	tolerant	no
Experimental Site	UM7306	"	no
	UM7308	"	no
Old Arboretum	UMWalker	susceptible	yes
Dairy Science Building	UMParent	somewhat tolerant	yes
Teulon, Mb	UMTeulon-1	observed tolerant	yes
	UMTeulon-2	"	no
	UMTeulon-3	"	no
	UMTeulon-5	"	yes
Morden, Mb	Morden 4/51/271	susceptible	yes
	Morden 60-0009	unknown	yes
	Morden 'Compact'1078-67	unknown	yes
	Morden 60-0013	unknown	yes
	A. tataricum 73-2961	unknown	yes

consisted of one row, which represented progeny from one parent. The experimental plot consisted of a total of 42 rows, 45 feet in length with spacing between rows at 3 feet. The seeding rate was 500 fruit/row.

Seedling emergence was recorded on May 14, May 27 and June 18, 1993. Total emergence for each treatment was calculated as a percentage of fruit planted. ANOVA was performed on the data to compare emergence among parents.

Random samples of 25 seedlings per row were tagged during the first week of July, 1993 for visual ratings of chlorosis tolerance and height measurements. Sample seedlings were distributed along the entire length of each row. Some rowshad very poor emergence, hence, fewer than 25 samples were obtained in these rows. Visual ratings for chlorosis tolerance were recorded on August 20, 1993. Leaves from the top half portion of each sample seedling were visually rated to obtain a chlorosis rating for that seedling. The rating scale used is described as follows:

0 - no chlorosis

1 - interveinal tissue becoming light green

2 - light green interveinal tissue with darker green veins

3 - greenish-yellow tissue with green veins

4 - yellowish- to whitish-green tissue with green veins

5 - slight necrosis beginning

6 - yellow tissue with necrosis

7 - yellowish-white tissue with severe necrosis

8 - severe necrosis/dead seedling.

Those plants with ratings of 0 or 1 were considered to be chlorosis-tolerant. ANOVA was performed on the rating scale data, with the model for analysis as rating = rep + treatment + rep x treatment. Sample variances were used to determine the extent of variability within and among treatments.

During the first week of September, 1993, growth of seedling samples was measured as plant height (cumulative length of internodes) at the end of the season, to determine the amount of growth attainable by seedlings during their first growing season. Correlation of chlorosis rating and plant height was also determined.

At the end of September, 1993, 11 seedlings which were determined to be chlorosis-tolerant (i.e. had ratings of 0 or 1) and expressed red fall colour, were selected from the experimental plot. These plants were transplanted in October, 1993, to the University of Manitoba Dept. of Plant Science Experimental Site.

RESULTS

Emergence

Emergence was very low throughout the experimental plot, ranging from 8.2% to 25.5% among parents. There were no significant differences in emergence between replicates and between parent plants. Chlorosis-tolerant and chlorosis-susceptible parents did not differ significantly for percent seedling emergence. But, the Morden selection for red fall colour (Morden 4/51/271), which is considered to be susceptible to lime-induced iron chlorosis, had one of the poorest emergence percentages at 8.7%, averaged among replicates. Parents believed to be chlorosis-tolerant had emergence percentages ranging

from 9.3% to 15.9%. Seedling emergence and distribution throughout the plot were inconsistent and highly variable within treatments among replicates (Table 2).

Table 2. Mean percent seedling emergence (± standard error) in the field, from parents represented in experiment near Teulon, Mb, 1993.

Parent plant	Mean emergence (%)
UMParent	12.5 ± 1.28
UM7301	11.1 ± 2.78
UM7306	9.3 ± 3.01
UM7308	13.1 ± 2.40
UMTeulon-1	13.7 ± 7.81
UMTeulon-2	9.9 ± 2.61
UMTeulon-3	15.9 ± 9.98
UMTeulon-5	15.0 ± 3.84
UMWalker	18.7 ± 7.19
Morden 4/51/271	8.7 ± 5.05
Morden 60-0009	17.1 ± 5.37
A. tataricum	15.3 ± 3.35
Morden 'Compact'	25.5 ± 6.21
Morden 60-0013	8.2 ± 3.24

Chlorosis

There were no significant replicate or treatment effects for chlorosis ratings of seedling populations. Differences in chlorosis ratings of seedlings between parents within replicates were highly significant ($P<0.0001$), indicating a genotype-by-environment interaction. These differences were evident between UM7308 and UMTeulon-3 in replicate 2, in which mean ratings of progeny were 2.1 and 5.2, respectively (Table 3). Means of individual treatments across replicates were inconsistent and chlorosis ratings of progeny were highly variable between treatments (Table 3). For example, there was a two-fold increase in sample variances from parents UM7301 to UM7306, indicating greater variability in chlorosis tolerance among progeny from UM7306.

In general, chlorosis ratings were normally distributed with approximately half of the progeny from most of the parents obtaining chlorosis ratings of 4 and 5 (Figs. 6a-6d). UMTeulon-1 exhibited the most narrow distribution of progeny ratings, from 0 - 5 (Fig. 6b). The frequencies of certain chlorosis ratings varied among parents. For example, UM7306 had a greater percentage of progeny in the most susceptible range (ratings of 7 and 8), relative to all other parents (Fig. 6a). The frequencies of tolerant progeny (ratings of 0 and 1) produced from chlorosis-tolerant parents ranged from 1.3% to 22.7%, and were not greater than those produced from parents believed to be chlorosis-susceptible (Figs. 6a-6d, Table 1). One chlorosis-tolerant parent (UM7308) did produce the highest frequency of tolerant progeny in 2 out of the 3 replicates. Overall, eleven seedlings, which exhibited chlorosis tolerance and red fall colour, were selected from the experimental plot (Table 4). Five of these seedlings are progeny from three chlorosis-tolerant parents.

Table 3. Mean iron-deficiency chlorosis ratings and variances of progeny from *A. ginnala* parents represented in the study near Teulon, Mb, 1993. From ANOVA, significance levels were: rep, p=0.7533, treatment (parent), p=0.3745, rep x treatment, p<0.0001.

	Mean Chlorosis Rating				
Parent	Rep 1	Rep 2	Rep 3	Overall	Variance
UMParent	4.9	3.5	4.6	4.3	1.58
UM7301	3.5	4.0	4.0	3.8	1.67
UM7306	4.9	4.9	4.9	4.9	3.76
UM7308	3.4	2.1	4.7	3.5	3.38
UMTeulon-1	4.0	3.8	3.7	3.9	1.50
UMTeulon-2	3.3	3.1	4.1	3.4	3.43
UMTeulon-3	4.1	5.2	4.4	4.5	2.02
UMTeulon-5	3.8	3.6	3.1	3.5	3.36
UMWalker	3.6	4.4	4.3	4.1	2.32
Morden 4/51/271	4.0	3.1	3.0	3.4	2.67
Morden 60-0009	3.2	4.0	5.3	4.2	2.90
A. tataricum	4.0	4.4	3.8	4.1	2.38
Morden 'Compact'	4.8	3.5	3.2	3.8	2.73
Morden 60-0013	5.3	4.5	3.0	4.2	2.97

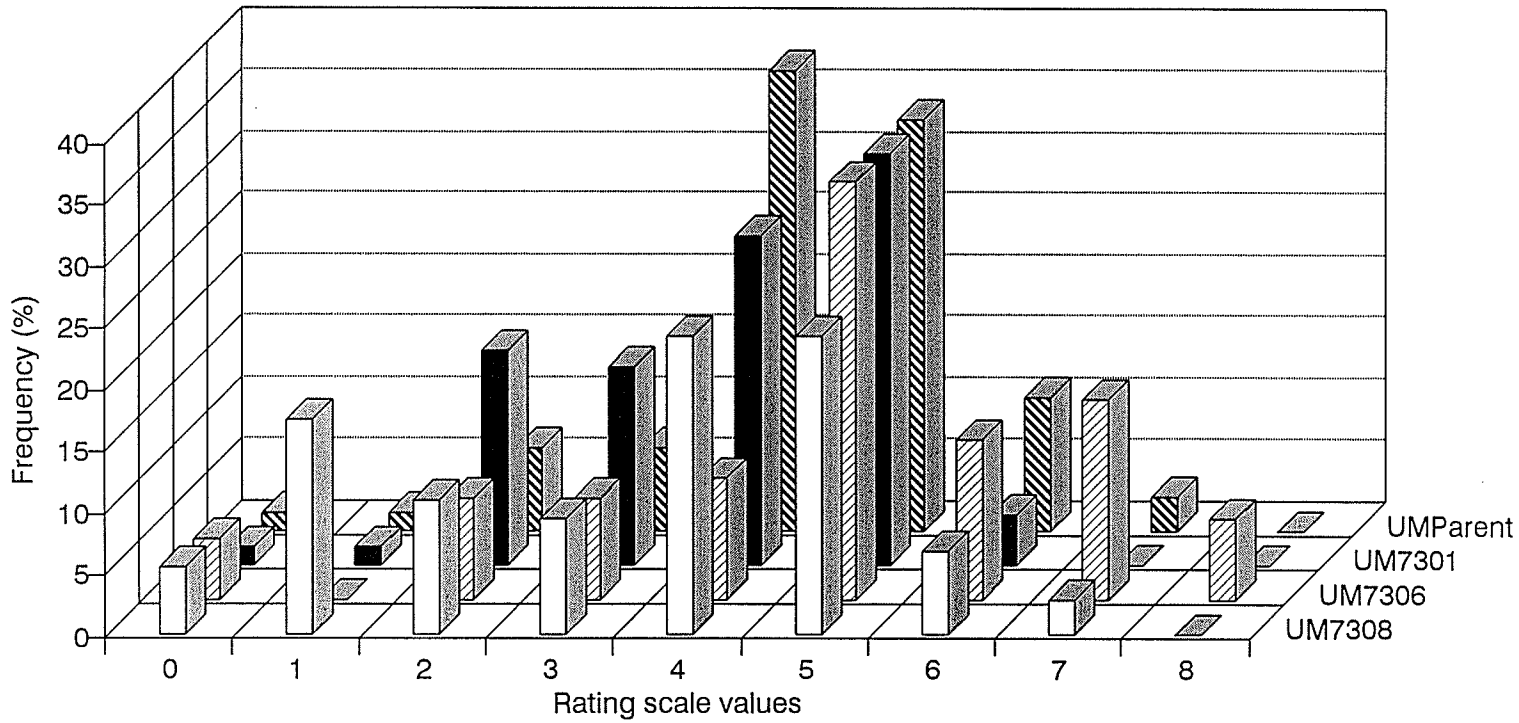


Fig. 6a. Mean frequency distribution of chlorosis ratings from progeny of UMParent, UM7301, UM7306 and UM7308, near Teulon, Mb, 1993.

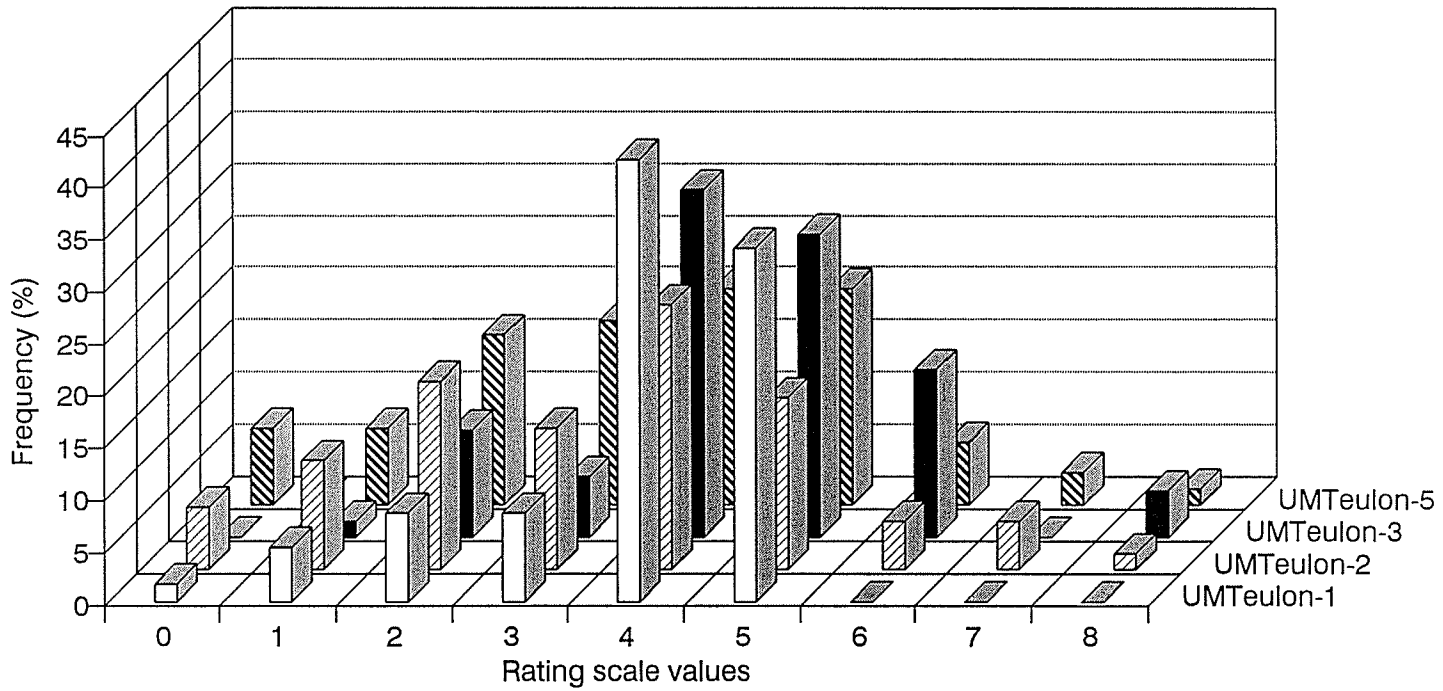


Fig. 6b. Mean frequency distribution of chlorosis ratings from progeny of UMTeulon-1, UMTeulon-2, UMTeulon-3 and UMTeulon-5, near Teulon, Mb, 1993.

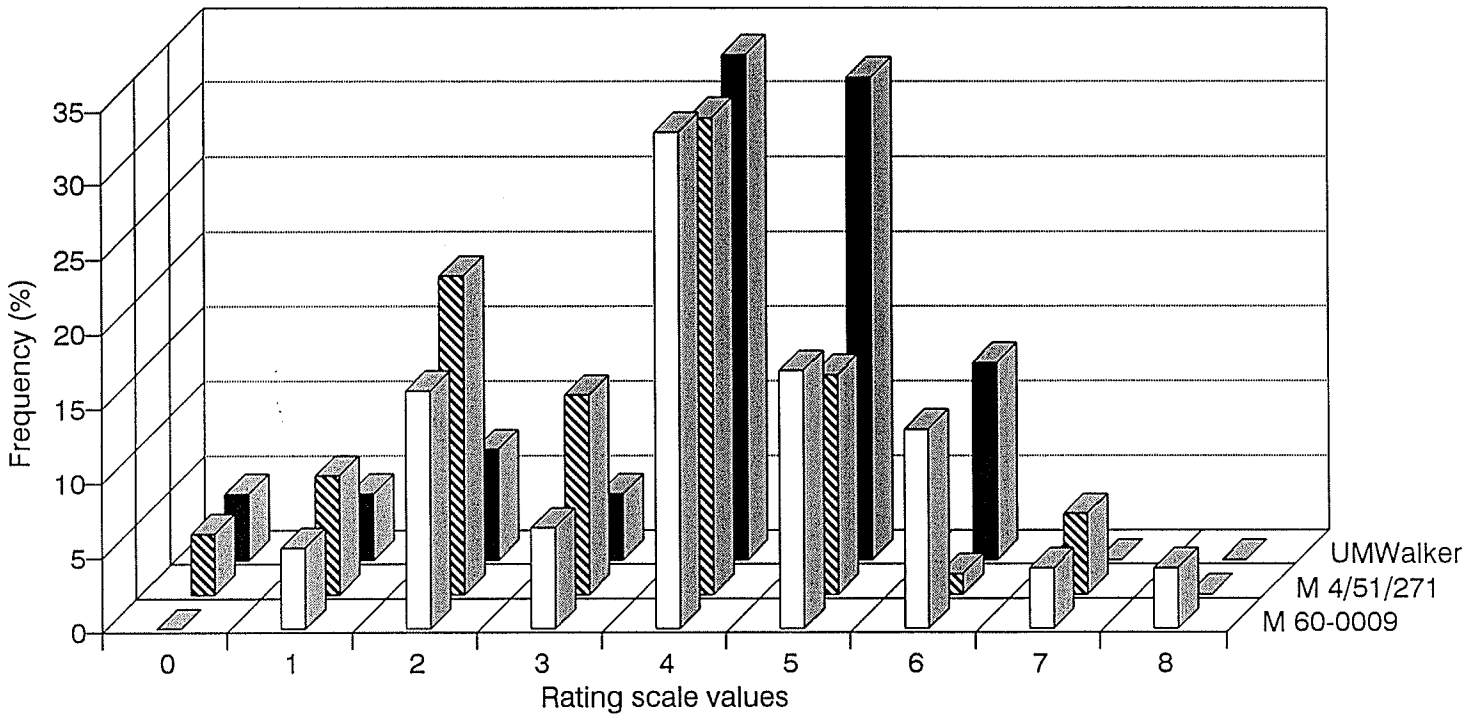


Fig. 6c. Mean frequency distribution of chlorosis ratings from progeny of UMWalker, Morden 4/51/271, Morden 60-0009, near Teulon, Mb, 1993.

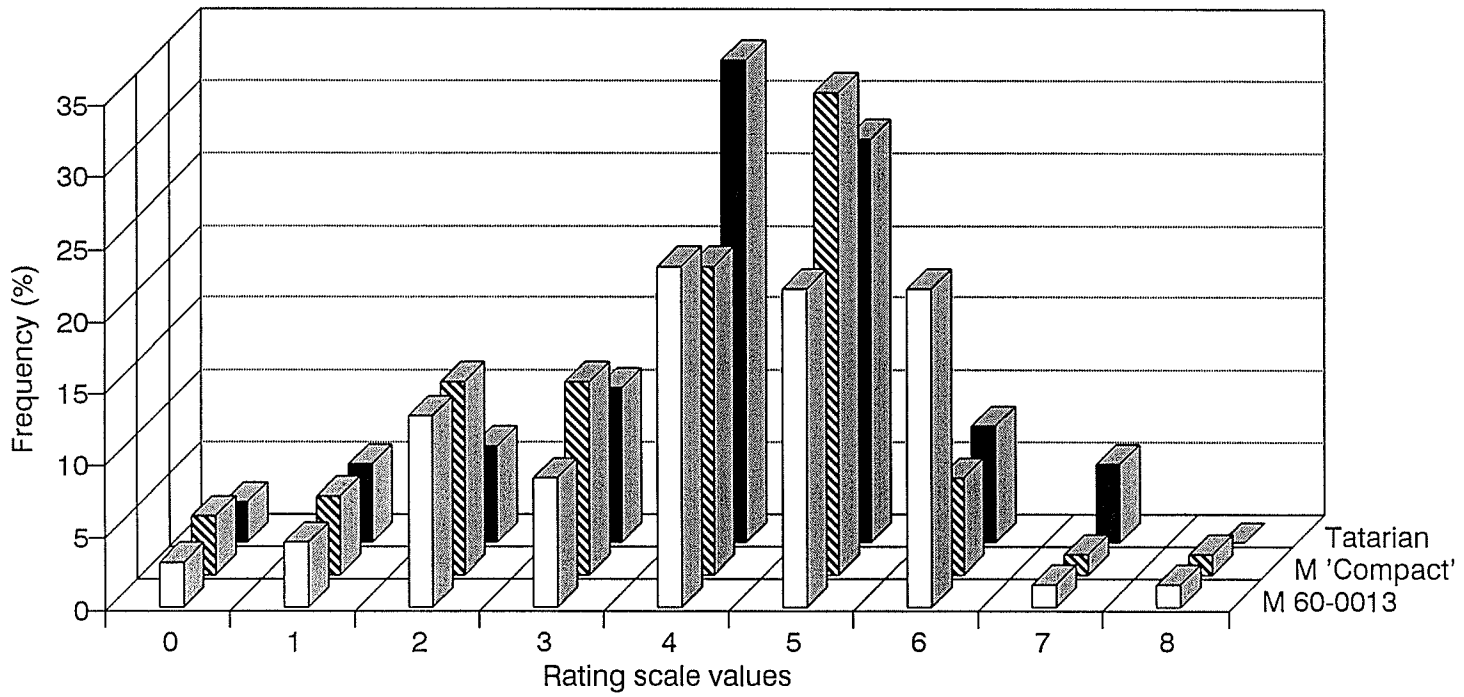


Fig. 6d. Mean frequency distribution of chlorosis ratings from progeny of *A. tataricum*, Morden 'Compact', and Morden 60-0013, near Teulon, Mb, 1993.

Table 4. Seedlings of *A. ginnala* selected for tolerance to lime-induced iron-deficiency chlorosis and for red fall colour, from the experimental plot, near Teulon, Mb, 1993.

Parent Plant	Tolerant Seedling Selection
UM7308	UM93-004-005
UMTeulon-1	UM93-005-006 UM93-005-007
UMTeulon-5	UM93-008-008 UM93-008-009
UMWalker	UM93-009-010 UM93-009-011
Morden 60-0009	UM93-011-002
Morden *A. tataricum* 73-2961	UM93-012-003 UM93-012-004
Morden 'Compact' 1078-67	UM93-013-001

Height

Seedling height did not differ significantly among replicates nor among parent plants. There were significant differences in seedling height among parents within replicates ($P<0.0001$). Plant height was highly variable throughout the experimental plot, as demonstrated by the sampling variances (Table 5). Visual ratings of chlorosis were correlated with seedling height ($r=0.36$) ($P<0.0001$).

Table 5. Mean height (cm) and variance of progeny from *A. ginnala* parents represented in the study near Teulon, Mb; 1993. From ANOVA, significance levels were: rep, p=0.3931, treatment (parent), p=0.7042, rep x treatment, p<0.0001.

	Mean Height (cm)				
Parent	Rep 1	Rep 2	Rep 3	Overall	Variance
UMParent	3.9	6.1	3.0	4.3	16.2
UM7301	6.7	3.8	4.8	5.1	7.2
UM7306	4.2	5.7	3.1	4.6	19.1
UM7308	5.1	5.4	3.9	4.8	4.9
UMTeulon-1	4.6	7.4	4.5	5.8	7.9
UMTeulon-2	4.2	6.4	3.8	4.9	10.0
UMTeulon-3	7.6	3.9	2.7	4.8	14.9
UMTeulon-5	5.1	5.9	5.4	5.5	11.0
UMWalker	7.2	5.3	7.0	6.6	9.7
Morden 4/51/271	3.4	4.5	5.4	4.4	8.7
Morden 60-0009	7.3	8.1	3.2	6.2	19.8
A. tataricum	7.3	3.1	7.6	6.0	15.9
Morden 'Compact'	5.5	7.1	7.2	6.6	16.7
Morden 60-0013	3.6	5.5	4.8	5.5	11.0

DISCUSSION

The results indicate that variability in tolerance to lime-induced iron chlorosis exists in the parents of *A. ginnala* utilized in this study. Variation in chlorosis tolerance was observed among and within progeny populations upon germination.

Overall, seedling emergence was low and highly variable within treatments among replicates, indicating an interaction between replicates and parents. The low emergence percentages may have occurred due to poor germination as a result of the complicated seed dormancy that exists in this species (Dumbroff and Webb, 1970), which was not overcome under the existing field conditions. Sufficient water may not have been available in the field for imbibition during the after-ripening period. Various factors may have been involved in actual germination in the spring, such as low soil moisture and poor seed viability. In order for a seed to be capable of germination, it must be viable (Hartmann et al, 1990). The inconsistent distribution of seedlings throughout the plot may have resulted from variability in chlorosis-tolerance of seedlings upon emergence. Numerous seedlings died within a few days of emergence, while adjacent seedlings continued to grow. Sufficient iron may have been available from seed reserves, but when that iron was depleted, seedlings not tolerant of deficient levels of available iron either in the soil or within the plant, subsequently died (Berrang and Steiner, 1980). Perhaps if the iron in the seed reserves was immediately depleted, susceptible seedlings may have died before emerging from the soil. It may be proposed that the iron content of seed reserves is related to the uptake and transport of iron in the parent plant, suggesting that a susceptible parent growing under iron stress would produce seed with a low iron content

and poorer seedling emergence may occur under iron-stress conditions.

The variability of chlorosis ratings throughout the plot may have been due to the fact that the progenies studied in this experiment represented heterogeneous populations, produced from outcrossing of genetically different parents. Environmental effects could have resulted from the possible existence of 'hot spots' due to high lime content (Loeppert, 1986). There were very distinct areas (possible hot spots) throughout the plot where seedlings did not grow. Extensive soil testing throughout the plot would be required to determine if these hot spots actually occur.

Genetic effects were believed to be the most probable cause of variability of chlorosis ratings among the seedlings. The existence of genetic tolerance of iron-deficiency chlorosis in *A. ginnala* was evident in several instances in the field, where a healthy, green seedling grew adjacent to a chlorotic seedling. If any reduction or soil acidification occurred at the rhizosphere of the resistant seedling, it was very localized and only benefited that resistant seedling, and did not appear to enhance iron-uptake or iron utilization for the susceptible seedling (Bienfait, 1987).

Numerous seedlings died during the middle to end of the growing season. This occurrence may have been due to excess moisture and exclusion of oxygen from the soil. The experimental plot near Teulon, Mb, received very large amounts of precipitation during the growing season of 1993 (Appendix C). Although soil saturation generally results in reducing conditions in which Fe^{2+} would be more available, the excess soil moisture may have resulted in a reduction of root growth and activity, thus inhibiting the uptake of iron (Lindsay, 1984). The majority of symptoms of living, but unhealthy, plants

were typically those of iron deficiency and not of excess water (i.e., leaf scorch).

Height measurements were found to be even more variable than chlorosis scores. The significant genotype x environment interaction may have resulted from the heterogeneity of progeny populations and from the variable conditions in the soil. The significant correlation between height measurements and chlorosis ratings, indicates an association between chlorosis tolerance and greater internodal extension or plant vigour.

Overall, it was difficult to determine which parents were of greatest value in producing tolerant progeny, due to the highly variable results and to the lack of significant differences between parents. Those parents considered to be tolerant were inconsistent among replicates in producing tolerant offspring. One chlorosis-tolerant parent, UM7308, produced the highest percentage of tolerant progeny in two out of the three replicates. But, since red fall colour was a secondary selection factor, only one of these tolerant seedlings was selected. Several seedlings were selected from parents that are considered to be chlorosis-susceptible. These parents were chosen for this study because they express red fall colour. The manner in which seedling selection was performed indicates that complications may arise by selecting for more than one desirable characteristic from heterogeneous populations, at the beginning of a breeding program. It is suggested from this study that the primary goal is to develop or obtain an initial collection of chlorosis-tolerant specimens, and subsequently, if tolerant lines can be developed then other desirable characteristics, such as red fall colour, could be included as selection factors.

The frequency of tolerance in progeny from UMParent was 2.7% in this present study and 2% in the study by LaCroix and Lenz (1974). The progeny from UMTeulon-1

exhibited a 7.7% frequency of chlorosis tolerance, averaged among replicates. UMTeulon-1 is a first generation progeny of UMParent, based on the study by LaCroix and Lenz (1974). The seedlings from UMTeulon-1 were considered to be second generation progeny of UMParent, in this present study. Two seedlings were selected from UMTeulon-1. These two selections should be monitored to determine if their progeny exhibit an increased frequency of chlorosis tolerance. UMTeulon-1 grows on the highly calcareous soil at the Teulon site, and has red fruit in the summer and intense red fall colour. This plant may be a valuable seed source for chlorosis tolerance and red fall colour.

The analysis of variance indicates a high coefficient of variation (c.v.) (38.6%) and a very low R^2 (0.18) for chlorosis ratings. Future studies would require more effective experimental design, for example a lattice design, and a more effective model for analysis, in order to detect actual differences and to reduce random experimental error. Also, the number of replicates should be increased due to soil variability and the number of locations should be increased to test populations in different environments.

B/ DIAGNOSIS OF IRON-DEFICIENCY CHLOROSIS

INTRODUCTION

In order to improve upon a plant's characteristics, it is necessary to be able to identify and measure the desired trait, using a simple and accurate method. Diagnostic methods for iron-deficiency chlorosis that have been used are visual observations, laboratory analysis of leaf chlorophyll content and, more recently, the use of a leaf chlorophyll meter. The objectives of this study were:

1) to determine the accuracy of the Minolta SPAD-502 leaf chlorophyll meter, in estimating chlorophyll content in leaves of *A. ginnala*,

2) to evaluate its use in assessing chlorosis expression of *A. ginnala.*

MATERIALS AND METHODS

This experiment involved sampling leaves from specimens of *A. ginnala* and determining chlorophyll content of leaves ranging from healthy, green to chlorotic. Two plants, which grow at the University of Manitoba campus, were utilized in this study. The plant sampled in 1992 is situated by the Physical Plant on Freeman Crescent, whereas the plant sampled in 1993 is situated in the parking lot by the Plant Science building. Chlorophyll content was measured with the use of a leaf chlorophyll meter and by chlorophyll extraction.

On August 18, 1992, 25 leaves were selected from the south side of a plant of *A. ginnala.* Leaves were fully expanded on recent growth and showed varying degrees of chlorosis. Visual ratings were recorded for each leaf. The rating scale used is described

as follows:

1 - healthy green leaf

2 - slight chlorosis

3 - light green leaf with darker green veins

4 - yellow interveinal areas with darker green veins

5 - white interveinal areas with dark green veins.

Twenty meter readings were taken per leaf, using a Minolta SPAD-502 leaf chlorophyll meter. Major veins were avoided when taking meter readings because these areas often provided very high readings compared to the rest of the leaf tissue. In addition, leaf margins were avoided for meter readings because inexplicable readings of zero were often obtained at the margins, even if the tissue was green. Once readings were obtained for each leaf, an average reading was calculated for that leaf using the SPAD-502 meter.

Leaves were removed from the plant for chlorophyll extraction. Four leaf discs were randomly punched from the same areas as used above for meter readings. The area of each leaf disc was 0.385 cm^2. Leaf discs were weighed and placed in test tubes containing 7 ml of N,N-dimethyl-formamide (DMF). Due to limited leaf material, replication of leaf disc samples was not performed. The test tubes were placed in a dark cabinet, to prevent chlorophyll degradation, for 24 hours, in a room held at 22° C. Wavelength absorbance of leaf disc samples was measured at 648 nm and 664 nm. Total chlorophyll ($mg \cdot L^{-1}$) was calculated using extinction coefficients for DMF (Inskeep and Bloom, 1985), and was expressed on a fresh weight basis and leaf disc area basis.

On August 26, 1993, 20 leaves were sampled from a different plant of *A. ginnala*. A different plant was chosen for 1993, since the plant used in 1992 showed no chlorosis in 1993, making it difficult to select leaves expressing a range of chlorosis. Visual ratings were determined for each leaf, using the rating scale from 1992. Ten meter readings were taken from each leaf, avoiding leaf margins and major veins. Average readings were calculated for each leaf using the SPAD-502 meter. These leaves were then removed from the plant.

Chlorophyll extraction was performed using 80% acetone. The extraction solvent was changed from DMF to acetone, because acetone is relatively less toxic to handle than DMF. DMF and 80% acetone are both considered to be effective solvents for chlorophyll extraction. Ten leaf discs were randomly punched from the interveinal tissue of each leaf. Five leaf discs per leaf were weighed and placed in test tubes containing 2 ml of 80% acetone, with two replicates per leaf. The test tubes were placed in a box to exclude light and were agitated for 16 hours at 22° C. Wavelength absorbance was measured at 652 nm. Total chlorophyll ($mg \cdot L^{-1}$) was determined for each sample using the extinction coefficient for 80% acetone (Holden, 1976), and was expressed on a fresh weight basis and leaf disc area basis. For each year of study, the relationship between total chlorophyll extracted and SPAD-502 meter readings was evaluated using linear regression analysis. Correlation analysis was used to evaluate the relationships between visual ratings, SPAD-502 meter readings and total chlorophyll.

RESULTS

Although the two years of study yielded different regression models, there was a significant linear relationship (P=0.0001) between SPAD-502 meter readings and total chlorophyll ($mg \cdot L^{-1}$), based on leaf disc area (cm^2), in both 1992 and 1993, with R-square values of 0.91 and 0.90, respectively (Table 6). R-square values were lower when total chlorophyll was expressed on a fresh weight basis, and are not included in these results. This difference in R-square values was also observed by Marquard and Tipton (1987).

The ranges in SPAD readings were similar for both years (Table 6). However, the differences in leaf sample sets and in chlorophyll extraction solvents yielded considerably different ranges of total chlorophyll. There was at least a two fold increase in maximum total chlorophyll from 1992 to 1993 (5.12 to 12.22 $mg \cdot cm^{-2}$, respectively), even though the maximum SPAD readings were the same. In general, SPAD-502 readings varied with leaf greenness as did total extracted chlorophyll from the same leaves (Table 6). In 1992 and 1993, there were significant negative correlations between visual scores and SPAD-502 meter readings, and between visual scores and total chlorophyll, explaining 73-77% and 65-75% of the variation, respectively (Table 7).

Table 6. Relationship between the SPAD-502 leaf chlorophyll meter and total extracted chlorophyll for *A. ginnala*, 1992 and 1993.

Year	Regression equation	R^2	SPAD		TCHL ($mg \cdot cm^2$)	
			Min	Max	Min	Max
1992[1]	TCHL = -0.38 + 0.12 SPAD	0.91	11.4	40.7	1.28[2]	5.12
1993	TCHL = -0.89 + 0.31 SPAD	0.90	1.1	40.9	0.63	12.22

[1]Plants sampled were different for 1992 and 1993.
[2]Chlorophyll extracted with DMF in 1992 and with 80% acetone in 1993.

Table 7. Correlations among visual ratings, SPAD-502 meter readings and total chlorophyll ($mg \cdot cm^2$), from 1992 and 1993. (r, Pearson correlation coefficient; p, probability of a larger r).

	meter		chlorophyll	
	r	p	r	p
1992				
visual	-0.857	0.0001	-0.864	0.0001
1993				
visual	-0.876	0.0001	-0.802	0.0001

DISCUSSION

The SPAD-502 leaf chlorophyll meter appears to be an effective tool in measuring chlorosis in leaves of *A. ginnala.* The procedure is simple, non-destructive, and considerably less time-consuming than laboratory extraction of chlorophyll.

Due to differences in solvents and plants used between the two years of study, direct comparisons of results from both years cannot be made. However, similar relationships between meter readings and total chlorophyll extracted were obtained from both experiments, regardless of solvent and plant used.

A smaller range of total chlorophyll was obtained in 1992, but the ranges of SPAD-502 meter readings between the two years were similar. Campbell et al (1990) had similar findings in different forms of 'Delicious' apple cultivars (*Malus domesticus* Borkh.) growing under various conditions. Leaf disc samples taken in 1992 were lower in fresh weight than those taken in 1993, which may have been due to differences in leaf thickness. Variations in leaf thickness also may have contributed to the different ranges of total chlorophyll and may have influenced the linear relationships between SPAD-502 readings and extracted chlorophyll. Campbell et al (1990) stated that differences in leaf thickness may result in different regression models from year to year, among different plants and among various growing environments.

If meter readings are to be taken from plants, perhaps samples should be taken from the same side of the plant (i.e., the south-facing side) due to the presence of sun and shade leaves, which vary in thickness and may result in inconsistent measurements of total chlorophyll. If chlorophyll is to be determined in an experiment with the use of the

SPAD-502 meter, it is necessary to initially obtain a regression model with meter readings and extracted total chlorophyll, in order to obtain accurate measurements.

Despite its effectiveness, certain limitations regarding the operation of the SPAD-502 meter were realized in this study. It was necessary to avoid major veins and margins of the leaves in order to obtain reliable meter readings. Also, only a very small area of leaf tissue (2 mm x 3 mm) was measured at any one time, requiring several readings from each leaf of *A. ginnala.* Over the two years of study, it was found that calculating the average of 10 meter readings from a single leaf provided an adequate measurement of chlorophyll for that leaf. Regression analysis (not shown) between a single meter reading and total chlorophyll gave very low R^2 values.

In general, the SPAD-502 leaf chlorophyll meter would be very useful in physiological studies, in which an objective measurement of total chlorophyll is desired, and particularly where chlorophyll is to be measured over time. However, in plant breeding and selection studies, for example, where plants are evaluated based on their expression of lime-induced iron chlorosis, the simplest method is the use of visual ratings. This conclusion is supported by the significant correlations between visual ratings, SPAD-502 meter readings and chlorophyll extraction (Table 7). The SPAD-502 meter provides a relative measure of chlorosis, but it does not actually measure chlorosis due to iron deficiency. The plant breeder would still be required to visually diagnose the type of chlorosis being expressed. If selection is primarily based on plant appearance, as in this study of *A. ginnala*, then visual selection may be all that is necessary.

C/ EVALUATION PROCEDURES

INTRODUCTION

Selection of plants tolerant to lime-induced iron chlorosis requires evaluation of that trait. Screening and evaluation of resistant types has been performed in field nurseries, in greenhouses and in growth chambers, for soybean (*Glycine max* (L.) Merr.) (Dragonuk et al, 1989; Fairbanks et al, 1987) and for peanuts (*Arachis hypogea* L.) (Barak and Chen, 1982). Various forms of growing media have been used in controlled environment studies, such as nutrient solutions and potted calcareous soils. Greenhouse or growth chamber procedures have proven to be effective for performing recurrent selection and evaluation of tolerant types, without the constraints of the growing season. Problems have arisen with the use of nutrient solutions in that these tests may select for a different mechanism of Fe-stress response than those tests performed in the field (Dragonuk et al, 1989). The purpose of this study was to assess a pot-test method for evaluating tolerance to lime-induced iron chlorosis in *A. ginnala.*

MATERIALS AND METHODS

From June 23 - June 30, 1992, softwood cuttings were taken from four plants of *A. ginnala*, Morden 4/51/271, UMParent, UMWalker, and UM7308 (Table 8). These plants were selected for this present study based on their expression of varying degrees of chlorosis in the field.

Table 8. Plants of *A. ginnala* evaluated in a pot-test and their tolerance ratings in their field environments.

Plant	Chlorosis Expression
UM7308	tolerant
UMParent	moderately tolerant
UMWalker	susceptible
Morden 4/51/271	susceptible

Cuttings were dipped in Stim-Root No. 2 rooting powder and were stuck into a rooting medium of 1:1 (v/v) peat moss:perlite. A mist chamber, with 90% relative humidity, located in a growth room set at 22° C day/ 18° C night, with a 16-hour photoperiod, provided the appropriate rooting environment. After roots developed, these plants were removed from the mist chamber and held in the growth room to increase root mass. On October 28, 1992, 16 rooted cuttings from each of the four plants to be evaluated (64 rooted cuttings in total) were transplanted into pots filled with either highly calcareous soil (Lakeland Series) or non-calcareous soil (Newdale Association) (Appendix A). Each of the two types of soil was obtained from the A horizon.

Both soils were air-dried and passed through a 2 mm sieve. The large clay particles of the Lakeland soil needed to be broken down to smaller particles, in order to

pass through the soil sieve. The pots were plastic 2 L containers, which did not contain drainage holes, in order to maintain specific water content and to prevent leaching of soil. Dry weight of each individual soil varied by no more than 20 g, among the pots. Water-holding capacity, expressed as grams of water per gram of soil, was determined as 0.323 for the Lakeland soil and as 0.601 for the Newdale soil. Upon transplanting, pots were weighed and water was added to reach 100% water-holding capacity. Transplants were held in a growth room at 22° C day/ 18° C night, with a 16-hour photoperiod for 6 weeks. On December 9, 1992, these plants were moved to a cold room held at a constant 4° C, for a period of chilling to overcome bud dormancy. Pots were weighed, and water was added if necessary, to maintain 100% water-holding capacity, every week for 4 weeks and then every 2 weeks until April, 1993.

Transplants began breaking bud in early April, 1993. The pots were removed from the cold room on April 17, 1993, and were moved into the greenhouse. From May 1 - July 7, two water treatments were applied to the pots - a high water treatment of 110-120% water-holding capacity and a low water treatment of 80-90% water-holding capacity. Pots were weighed at 2-day intervals to maintain appropriate water levels. Experimental design was a split split plot, with the 4 plants growing in the field comprising the main plot and the 2 soil types and 2 water treatments as the subplots. Each treatment was replicated four times for each plant, with one rooted cutting/plant representing one replicate for each treatment (Fig. 7).

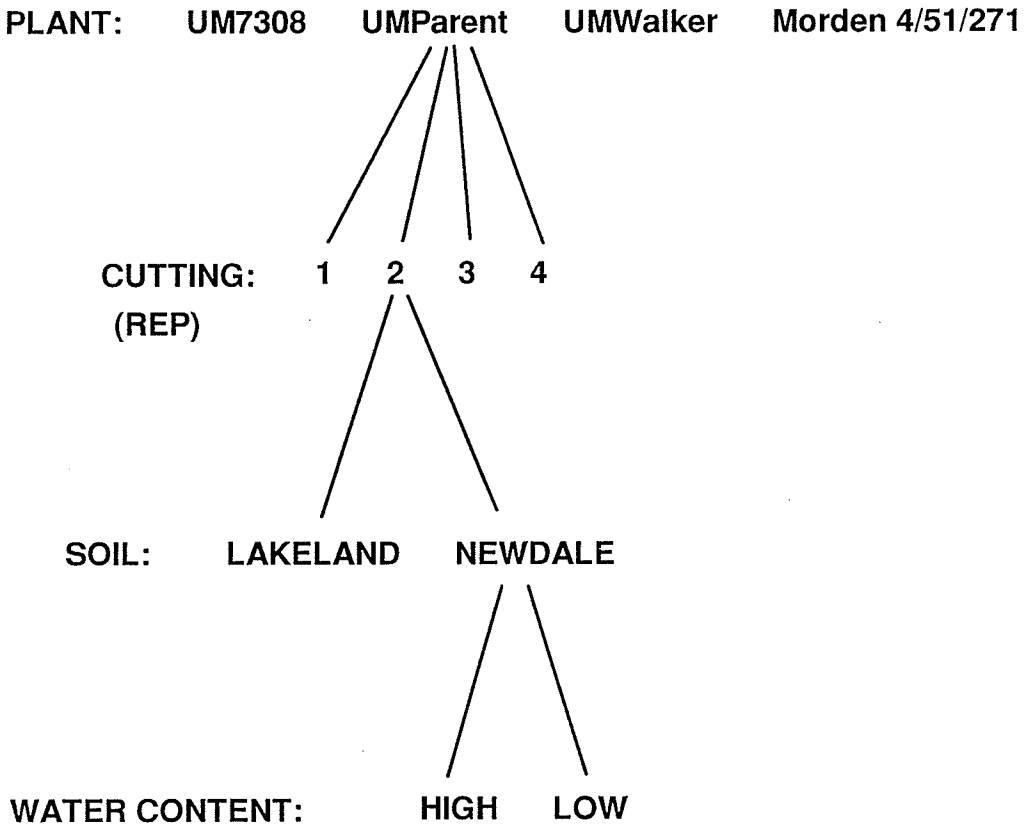


Fig. 7. The general design for the pot-test experiment, showing the relationship between plants, cuttings and treatments.

On May 10, 1993, light transmission was measured as 400 microeinsteins m^{-2} sec^{-1} at the top of the plant canopy. Plants were fertilized on May 11 and May 25. The fertilizer used was a formulation of three separate Sudbury™ granular fertilizers: nitrogen (urea) 44-0-0, phosphorus (phosphate) 0-44-0, and potassium (potash) 0-0-44. The concentration of the fertilizer solution was 15 ml of each chemical/litre of water. Each pot received equal amounts of the solution.

On June 28 and July 7, visual ratings for chlorosis expression were recorded, using the same 9-point rating scale as that used in Chapter 1, Part A. Growth was measured as plant height (cm) on August 28. At the end of the experiment all plants were treated with a foliar application of ferrous sulfate in order to observe any regreening of chlorotic tissue, indicating that iron deficiency was responsible for the chlorosis.

ANOVA was performed on the data obtained test differences between plants, between soils and between water treatments, and to determine if any treatment interactions occurred. The model used for analysis of variance was as follows:

rating (or height) = rep + plant + (rep x plant) + soil + (plant x soil) + (rep x soil(plant))
+ water + (plant x water) + (soil x water) + (plant x soil x water).

The error term used to test differences between replicates and between plants was (rep x plant). The error term used to test differences between soil and between (plant x soil) was (rep x soil(plant)).

RESULTS

Chlorosis

Those plants which developed iron-deficiency chlorosis began expressing symptoms 4 weeks after water treatments commenced. The most severe iron-deficiency chlorosis symptoms were observed in susceptible plants growing in the Lakeland soil (Table 9). There was a distinct separation of plants into chlorosis-tolerant and chlorosis-susceptible categories, based on visual ratings. Differences between plants were highly significant ($P<0.0001$). Mean comparisons of plants using a LSD-test indicated that tolerant plants were UMParent and UM7308, and susceptible plants were Morden 4/51/271 and UMWalker (Table 9).

Analysis of visual ratings also indicated that the two soils were significantly different in inducing chlorosis ($P<0.0001$). There was a significant plant x soil interaction ($P=0.0275$). The Lakeland soil was effective in inducing chlorosis in Morden 4/51/271, and particularly in UMWalker. Differences between water treatments were significant ($P=0.0084$) where high soil water content resulted in iron-deficiency chlorosis in susceptible plants. Interactions of soil x water and plant x water were not significant ($P=0.6614$ and $P=0.7318$, respectively).

Table 9. Mean chlorosis ratings (± standard error) of plants of *A. ginnala* grown in potted calcareous (Lakeland Series) and non-calcareous (Newdale Association) soils, with high (110-120%) and low (80-90%) soil water contents.

	Lakeland Series			Newdale Association			
Plant	High Water	Low Water	Mean	High Water	Low Water	Mean	Overall Mean
UM7308	3.0	1.5	2.3 ± 0.37b[1]	1.0	0.0	0.4 ± 0.26a	1.3 ± 0.33
UMParent	4.5	1.0	2.9 ± 0.90b	0.5	0.0	0.4 ± 0.26a	1.6 ± 0.55
Morden 4/51/271	6.0	5.0	5.4 ± 0.68c	3.0	2.0	2.5 ± 0.63b	3.9 ± 0.58
UMWalker	7.0	7.5	7.3 ± 0.31d	3.0	1.0	1.7 ± 0.57ab	4.7 ± 0.80

[1] Treatment means followed by the same letter are not significantly different according to LSD test at the 1% level.

Height

Analysis of height measurements indicated significant differences between plants (P=0.0145) , between soil types (P<0.0001), and between water treatments (P<0.0001). In general, UMParent and UMWalker produced the greatest internodal extension and were significantly different from Morden 4/51/271, but not from UM7308 (Fig. 8). The Newdale soil and the low water treatment generally produced the greatest increases in growth among all plants (P=0.0010). There was a significant plant x soil x water interaction (P=0.035). This interaction was observed in UMParent and UMWalker growing in the Newdale soil under the low water treatment, which exhibited considerable increases in growth (Fig. 8). Correlation analysis for visual ratings and height was significant (r=-0.432, P=0.0004), indicating that chlorotic plants were less vigorous than plants which did not express chlorosis.

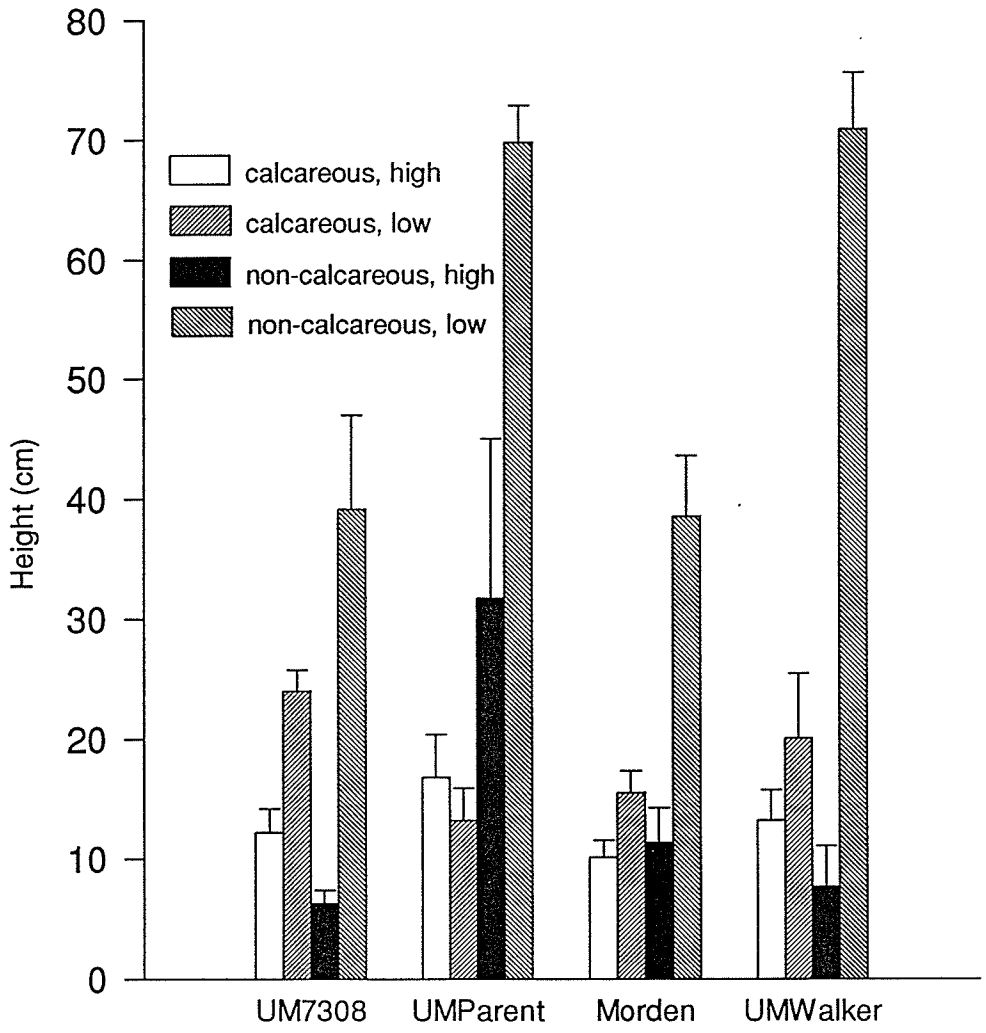


Fig. 8. Mean height (cm) of *A. ginnala* plants grown in potted calcareous and non-calcareous soils, with high (110-120%) and low (80-90%) soil water content. From ANOVA, significance levels were: plant, $p=0.0145$, soil, $p<0.0001$, water, $p<0.0001$, plant x soil, $p=0.0085$, plant x water, $p=0.0342$, soil x water, $p<0.0001$, plant x soil x water, $p=0.035$.

DISCUSSION

Bioassay-type pot tests have been used to evaluate iron-deficiency problems in various plant species and to evaluate various soils for their ability to induce iron-deficiency chlorosis (Barak and Chen, 1982; Fairbanks et al, 1987; Gildersleeve and Ocumpaugh, 1989). Greenhouse or growth chamber screening methods for iron-deficiency chlorosis using potted calcareous soils have shown mixed results. A seemingly common problem in these experiments is that plants which expressed chlorosis in the field failed to express iron-deficiency symptoms in the potted soils (Fairbanks et al, 1987). Fairbanks et al (1987) stated that the heterogeneity of calcareous soils in the experimental sites and difficulties in obtaining and maintaining soil water contents to induce chlorosis in soybeans, accounted for low correlations of field and pot studies. But, these experiments were still considered to be effective for the classification of tolerant and susceptible genotypes.

Results from this study indicate that a pot-test method, using Lakeland Series soil, for evaluating lime-induced iron chlorosis in *A. ginnala* is effective in distinguishing between tolerant and susceptible types. Those plants that have expressed chlorosis in the field also expressed chlorosis growing on highly calcareous soil in the greenhouse. Those plants believed to be tolerant were evaluated as tolerant in the pot test.

Following preparation of plant material and soil, the duration of the experiment itself was short, which allowed rapid evaluation of plants after the treatments had begun. Those plants that were chlorosis-susceptible began developing symptoms within 4 weeks. A few plants of Morden 4/51/271 and of UMWalker developed patches of necrotic tissue

and/or marginal necrosis early in the experiment, simultaneously with the development of chlorosis. These symptoms were not consistent among these plants, but did seem to occur in the high water treatments in both soils. Consequently, these plants also exhibited poor growth throughout the experiment. The high water treatment may have affected other aspects of plant growth, possibly by the exclusion of oxygen in the potted soils. Those plants with severe chlorosis symptoms did not regreen after foliar application of ferrous sulfate. Plants with less severe symptoms appeared to regreen, indicating that they had been suffering from an iron deficiency (Chaney, 1984).

There was concern regarding the effect of high soil water content on root growth and development. Plants generally exhibited greater internodal extension under the low water treatments. After completion of the experiment, plants were removed from the pots to observe the root mass. Those plants growing under the high water treatments, particularly those growing in the Lakeland soil, appeared to have somewhat smaller root masses than plants growing under the low water treatment. However, these differences did not appear to be great.

Considerable research has focused on the role of bicarbonate as the most important factor influencing iron-deficiency chlorosis (Fairbanks et al, 1987; Gildersleeve and Ocumpaugh, 1989; Inskeep and Bloom, 1986; Mengel et al, 1984a). Studies have indicated that high soil water content can result in greater soil bicarbonate content (Inskeep and Bloom, 1986; Mengel et al, 1984a). In this present study the effects of the high water treatment generally resulted in the expression of chlorosis in susceptible plants. In order to determine if bicarbonate influences the occurrence of lime-induced iron

chlorosis in *A. ginnala*, a measurement of bicarbonate in the treatments of this pot-test would be required.

This study indicates that the incidence of iron chlorosis in *A. ginnala* is greatly influenced by the soil lime content. The Lakeland soil was effective in producing chlorosis in susceptible plants, regardless of soil water content. Although high soil water content induced chlorosis in susceptible types, it appears that the $CaCO_3$ and/or Ca-$MgCO_3$ content of the soil were more important factors. This was demonstrated particularly in UMWalker, where all plants growing in the Lakeland soil were severely chlorotic, whereas those in the Newdale soil were only slightly chlorotic, regardless of water treatment. Barak and Chen (1982) found the most important soil properties influencing lime-induced iron chlorosis in peanuts were $CaCO_3$ and active lime contents.

In general, the factors of bicarbonate and high lime may simultaneously affect different points in producing iron-deficiency chlorosis in plants. High soil moisture may reduce soil gas exchange, resulting in an increase in CO_2 and consequently an accumulation of bicarbonate (Korcak, 1987). As the plant absorbs bicarbonate, its presence within the plant may affect the amount of iron that is active in chlorophyll formation (Elgala and Maier, 1964; Mengel et al, 1984a). The $CaCO_3$ and/or Ca-$MgCO_3$, and the reactivity of the carbonate phase, may be influencing the availability and uptake of iron in the rhizosphere (Loeppert, 1986).

Therefore, it can be concluded that a pot test, in which plants of *A. ginnala* are grown in calcareous and noncalcareous soil, under high and low soil water contents, can be useful for evaluating tolerance to lime-induced iron chlorosis. It is recommended that

the Lakeland soil, or a soil with similar properties (i.e. high pH and high carbonate content), be used due to its ability to induce chlorosis in susceptible plants.

SUMMARY

Basic components of the evaluation and selection of *A. ginnala* plants, tolerant to lime-induced iron chlorosis, have been developed. It appears that the primary cause of this chlorosis, in *A. ginnala* growing in our landscape, originates in highly calcareous soil and may be influenced by soil moisture. A simple and efficient means of diagnosis of the iron deficiency, and of chlorosis tolerance, is the use of a detailed visual rating scale, developed in this present study. Soil variability can be a complicating factor in the evaluation of and selection for chlorosis tolerance. The pot test, assessed in this study, may be a useful procedure to perform more extensive evaluation of field selected plants, without the complications of varying climatic and soil conditions. This study has indicated that chlorosis tolerant plants of *A. ginnala* may be obtained by selection. Eleven seedlings, which exhibited chlorosis tolerance and red fall colour, were selected from the field study.

CHAPTER 2

Flowering Behaviour, Pollination and

Fruit Set of *Acer ginnala* Maxim.

ABSTRACT

Flowering and pollination behaviour in *A. ginnala* were investigated in order to determine means by which plants should be handled for breeding purposes. Inflorescences from plants of *A. ginnala* were sampled to observe and document the expression of functionally staminate and functionally pistillate flowers. Distinct phases of primary (1°) staminate, pistillate and secondary (2°) staminate flowers occurred sequentially, during the flowering period of these plants. The staminate phases contained variable numbers of functionally staminate flowers within and between plants, whereas the number of pistillate flowers was consistent among all plants sampled. The expression of staminate flowers may have been influenced by temperature changes, which occurred during the flowering period. Row x column contingency tests for independence indicated that there was an association between expression of flower types and plants, in one of the 2 years of study.

Seed set from manual self-pollinations and from open-pollinations were monitored and recorded. The plants studied were capable of producing seed from selfing, although seed set was generally low, ranging from 0% to 45%. Seed set from open-pollination ranged from 15% to 91%. Results indicated that cross-pollination was the dominant mechanism in these plants. Row x column contingency tests for independence indicated that there were associations between the degrees of selfing and open-pollination and sampled plants. Varying degrees of parthenocarpic fruit development were observed in all plants. All flowers developed fruit regardless of seed production.

INTRODUCTION

Genetic variability of desired characteristics is obtained by sexual reproduction. An essential component of a breeding program is knowledge of the mode of reproduction of the plant, in order to obtain the desired trait or to combine traits. *A. ginnala* has been shown to exhibit a complex flowering behaviour, involving different flowering phases, which may vary from plant to plant (Jong, 1976). Knowledge of the timing of the various phases, is required in order to determine appropriate scheduling for pollinations. Although *A. ginnala* is described as 'probably' primarily cross-pollinated (Fryxell, 1957; Jong, 1976), the extent of selfing and crossing needs to be determined, in order to understand how to handle the flowers for pollinations. The success of pollinations is generally measured by the degree of seed set and fruit development. This assessment can be difficult if a plant exhibits parthenocarpy. Jong (1976) provided a general description of the occurrence of parthenocarpy in *Acer*, and described parthenocarpy in *A. ginnala* as moderate, based on the appearance of the samaras. Additional information is needed regarding the extent to which this plant develops parthenocarpic fruit, since seedless fruit is not desired for breeding purposes.

Therefore, the objectives of this study were :

1) to determine the flowering behaviour of *A. ginnala* growing in the north temperate zone landscape,

2) to determine the degree of self-pollination and cross-pollination, and,

3) to determine the expression and the degree of parthenocarpy.

MATERIALS AND METHODS

This experiment was conducted at the University of Manitoba Campus and at a farm (NW¼-23-16-2E) situated on provincial highway 17, east of Teulon, Mb. A total of eight plants of *A. ginnala* were chosen to study flowering behaviour, pollination and seed set, over a period of two years. The 4 plants studied in 1992 are located at the University of Manitoba. Three of these plants, UM7301, UM7306, and UM7308, are selections for chlorosis tolerance from a previous study (LaCroix and Lenz, 1974), and are located in Block 25, at the Dept. of Plant Science Experimental Site, on the east side of the campus. The plants in Block 25 are situated adjacent to one another in the following order: UM7301 (east), UM7308 (centre), UM7306 (west). The fourth plant studied in 1992, UMWalker, is located in the Old Arboretum adjacent to the stadium, on the west side of the campus. The four plants studied in 1993, UMTeulon-1, UMTeulon-2, UMTeulon-3, and UMTeulon-5, are located at the Teulon site (Appendix B), and were obtained from the previous study by LaCroix and Lenz (1974). These plants are situated in a row, along a driveway, in the northwest section of the farmyard, in the following order: UMTeulon-1 (east and somewhat isolated with greater exposure), UMTeulon-2, UMTeulon-3, and UMTeulon-5 (west and somewhat sheltered by mature Colorado spruce (*Picea pungens* Engelm.).

In the third week of May, 1992, twenty-five inflorescences were randomly selected in each plant and tagged, in order to observe and document the expression and timing of functionally staminate and functionally pistillate flowers. In 1993, 20 inflorescences were

randomly selected in each plant and removed at sampling dates, during May and June, to similarly observe and document flowering behaviour. Dates of anthesis and stigma withering were not recorded in 1993.

In 1992 and 1993, other inflorescences were randomly selected in each of the four plants, respectively, in order to study pollination and seed set. Within each plant a minimum of 5 inflorescences was selected for controlled self-pollination, and a minimum of 5 inflorescences was selected for open-pollination.

For the self-pollinations, inflorescences were covered with glycine bags, prior to stigma emergence and for a short period of time after pollination, to avoid contamination by foreign pollen. Pollen was obtained from staminate flowers, in each plant, by collecting fully developed anthers, placing them in glass vials, and dehiscing them in a desiccator overnight. When stigmas emerged from the pistillate flowers of a selected inflorescence, the glycine bag was removed to perform pollination. Pollen was applied to the stigmas using 'bee sticks', which essentially are honeybee thoraxes glued onto toothpicks (Williams, 1980). In 1993, UMTeulon-5 was not self-pollinated due to an insufficient number of flowers available for pollen collection and application. Once pistillate flowers of an inflorescence were pollinated, the glycine bag was replaced on the inflorescence. The bags were removed from the inflorescences when stigmas had withered, which was considered as the time when the stigma was no longer receptive (Gabriel, 1966).

Parthenocarpic fruit development was observed in self-and open-pollinated inflorescences in each plant, and that fruit was counted at the end of the growing season. Parthenocarpic fruit was considered to be that which was not fully developed and did not appear to contain seed.

Average maximum frequencies of flower types and average number of flowers per inflorescence were determined for each individual plant. A Chi-square test for independence was performed on the flower data in 4 x 3 contingency tables, to test the null hypothesis that the frequency of different flower types is independent of specimen plant, within the two sites. Seed set was determined in individual inflorescences as (# seed-bearing fruit / total # expected fruit x 100), and means were calculated for each respective plant. The value of total expected fruit in individual inflorescences was determined as the number of pistillate flowers expressed in the inflorescence, multiplied by two, since each pistil is bicarpellate (Jong, 1976). A Chi-square test for independence was performed in a 4 x 2 contingency table in 1992, and in a 3 x 2 contingency table in 1993, to test the null hypothesis that the pollination mechanism is independent of specimen plant, within the two sites.

RESULTS

Flowering Behaviour

In 1992 and 1993, all plants of *A. ginnala* studied exhibited similar flowering behaviour. Within all inflorescences, each flower was structurally hermaphroditic, or perfect, but functionally were either staminate or pistillate. Observations made from this present study were similar to those made by Jong (1976) - in the functionally staminate flowers, the stigmas did not emerge, and in the functionally pistillate flowers, the filaments did not elongate and the anthers did not dehisce. UMWalker exhibited irregularly formed anthers, which did not contain pollen, in the 1° staminate and the pistillate flowers. The 2° staminate flowers contained anthers, which appeared to be functional, that is, they contained pollen and dehisced. The same flowering sequence was observed in all plants from the University and near Teulon, Mb. This sequence consisted of a primary (1°) staminate phase, followed by a pistillate phase, followed by a secondary (2°) staminate phase. These phases were distinct in occurrence, although there was some overlap in timing from one phase to another (Figs. 9-16). In 1992, plants began flowering on May 29 and continued until June 19, with the exception of UMWalker, which flowered from June 8-29 (Figs. 9-12). In 1993, plants began flowering on June 9 and continued until June 28 (Figs. 13-16).

There was considerable variation in the total number of flowers per inflorescence within trees, in 1992 and in 1993 (Tables 10, 11). In 1992, there were significant differences between plants and frequency of flower types, based on a Chi-square test for

independence (χ^2=82.07, P<0.01). The primary difference occurred in UM7306 which expressed a considerably higher frequency of 1° staminate flowers and a considerably lower frequency of 2° staminate flowers, than the other plants (Table 10). UMWalker exhibited significantly smaller inflorescences and expressed significantly different frequencies of all phases relative to the other plants studied in 1992 (Table 10). In 1993, differences in the frequency of flower types occurred among the plants, but were not highly significant based on a Chi-square test for independence (χ^2=2.98, P>0.80) (Table 11). The frequency of pistillate flowers ranged from 11 - 18 in 1992 (excluding UMWalker) and from 9 - 14 in 1993, indicating relatively little variation between plants within each year of study (Tables 10, 11).

Pollination and Fruit Set

There were significant differences (P<0.01) in seed set from self- and open-pollinations among plants, within their respective sites, based on a Chi-square test for independence (Tables 12, 13). In 1992, the poorest seed set occurred from self-pollinations. UM7308 exhibited the highest seed production from selfing (29%) and this seed set was considerably greater than the other plants studied in 1992. UM7301 exhibited the highest seed set from open-pollination (83%), and exhibited one of the lowest seed set from selfing (6%) (Table 12). UM7306 had one of the lowest seed sets from selfing (6%), and the lowest seed set from open-pollination (29%) compared to the other plants studied in 1992. The plants in Teulon, Mb, also had higher percentages of seed-bearing fruit from open-pollinations than from selfing, in general (Table 13). UMTeulon-2 was

the exception, where selfing and open-pollination resulted in similar percentages of seed obtained (45% and 43%, respectively). Seed set was relatively very low in UMTeulon-5 (0% from selfing and 15% from open-pollination). The highest seed set (91%) was obtained in UMTeulon-1 from open-pollination.

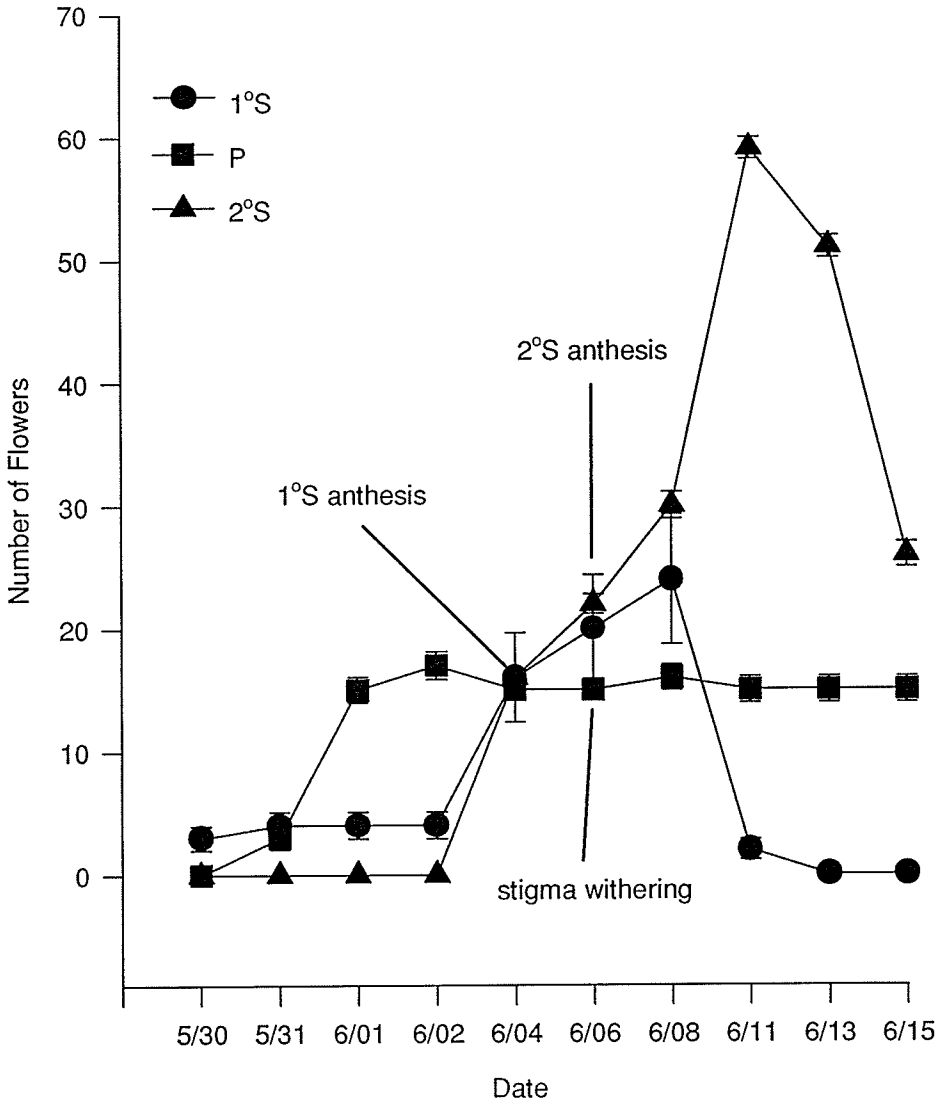


Fig. 9. Flowering behaviour of UM7301, 1992. Means (with standard error bars) were based on samples of 25 inflorescences. (1°S, primary staminate flowers; P, pistillate flowers; 2°S, secondary staminate flowers)

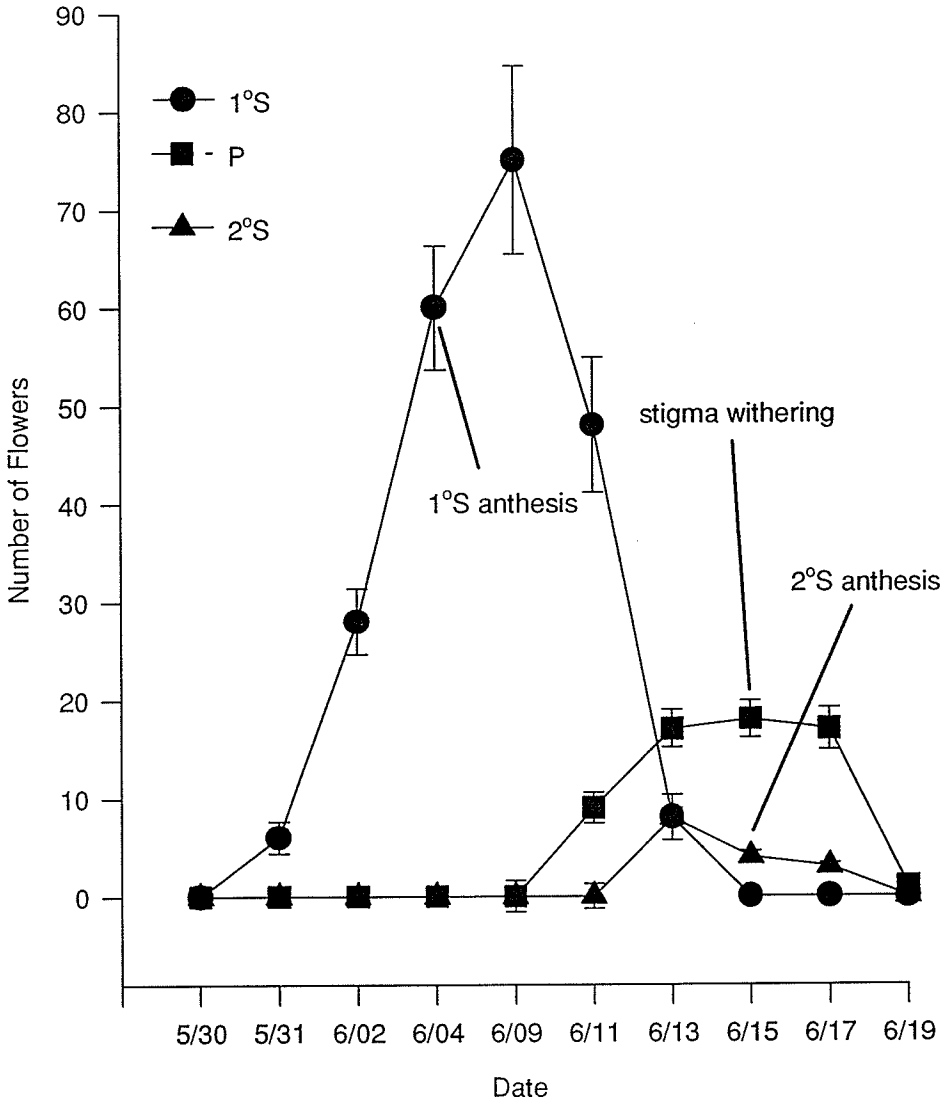


Fig. 10. Flowering behaviour of UM7306, 1992. Means (with standard error bars) were based on samples of 25 inflorescences. (1°S, primary staminate flowers; P, pistillate flowers; 2°S, secondary staminate flowers)

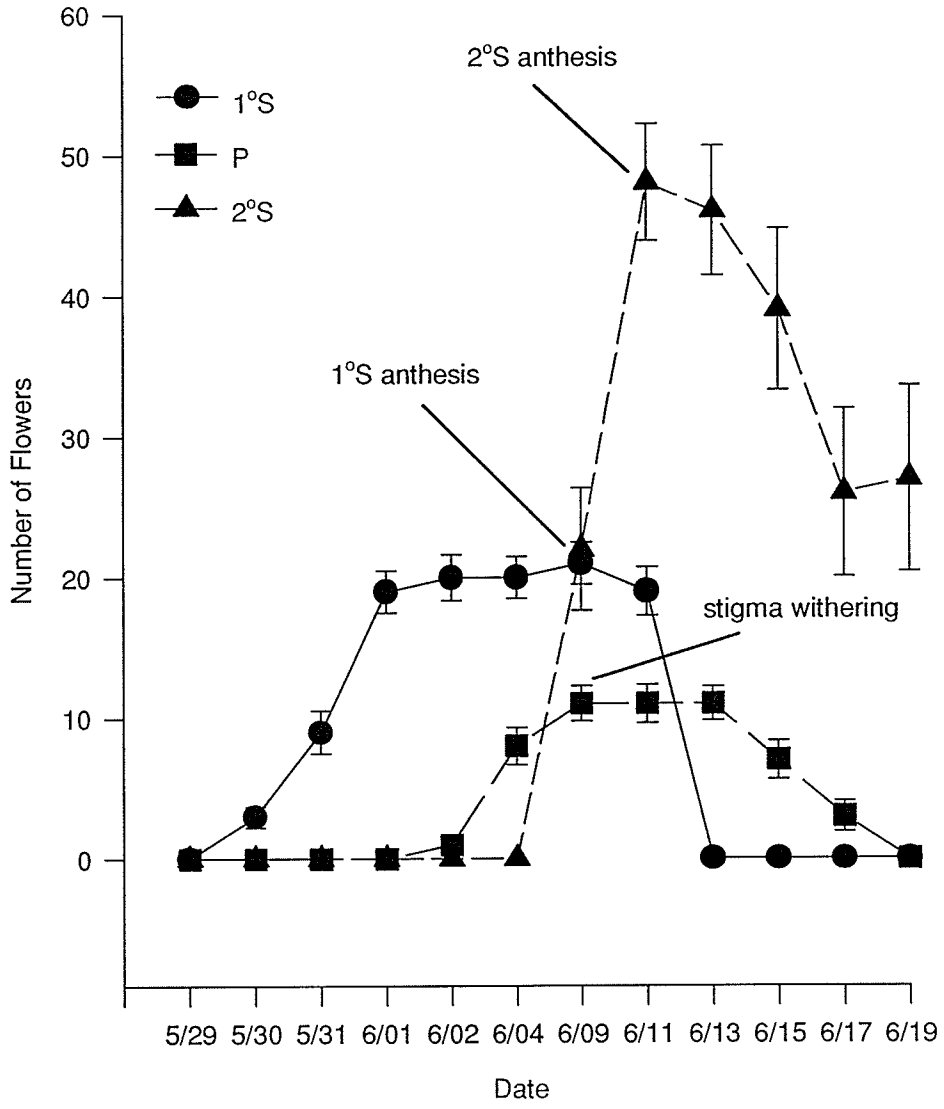


Fig. 11. Flowering behaviour of UM7308, 1992. Means (with standard error bars) were based on samples of 25 inflorescences. (1°S, primary staminate flowers; P, pistillate flowers; 2°S, secondary staminate flowers)

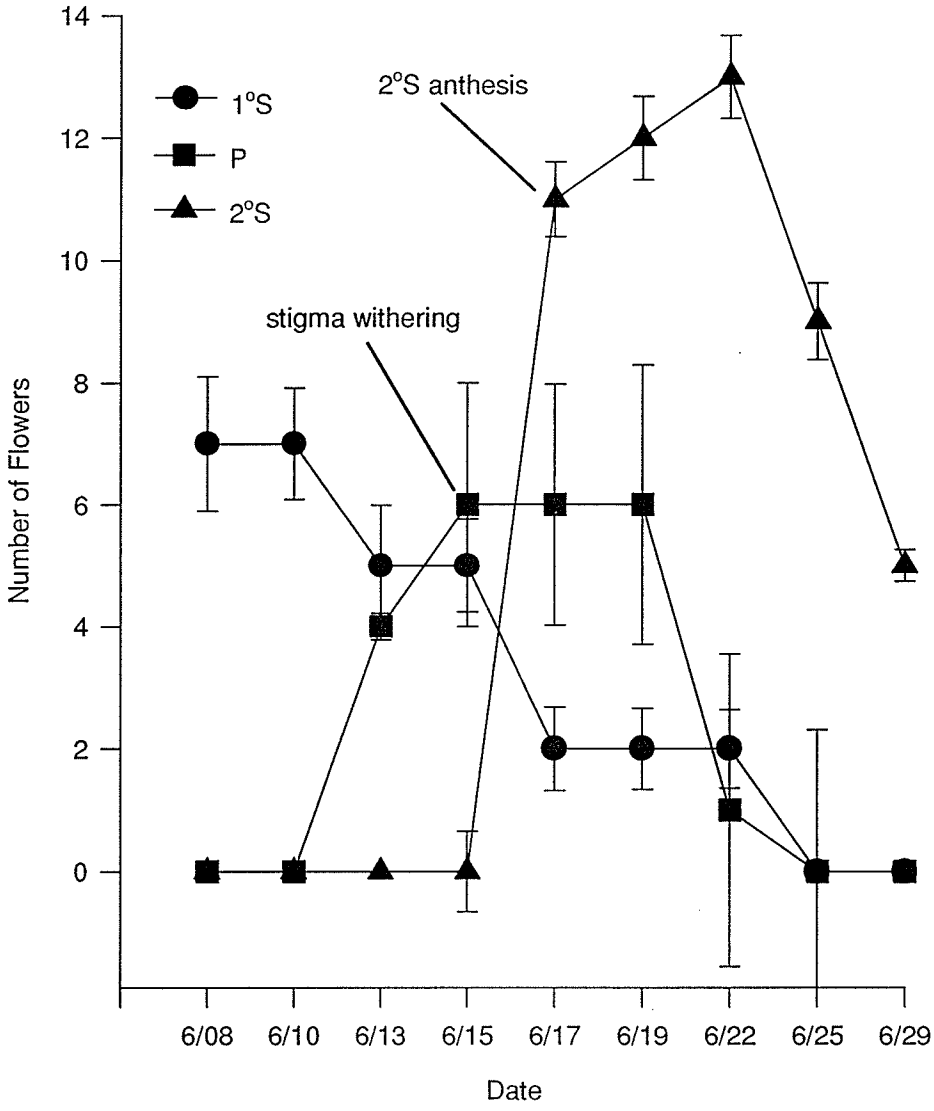


Fig. 12. Flowering behaviour of UMWalker, 1992. Means (with standard error bars) were based on samples of 25 inflorescences. (1°S, primary staminate flowers; P, pistillate flowers; 2°S, secondary staminate flowers)

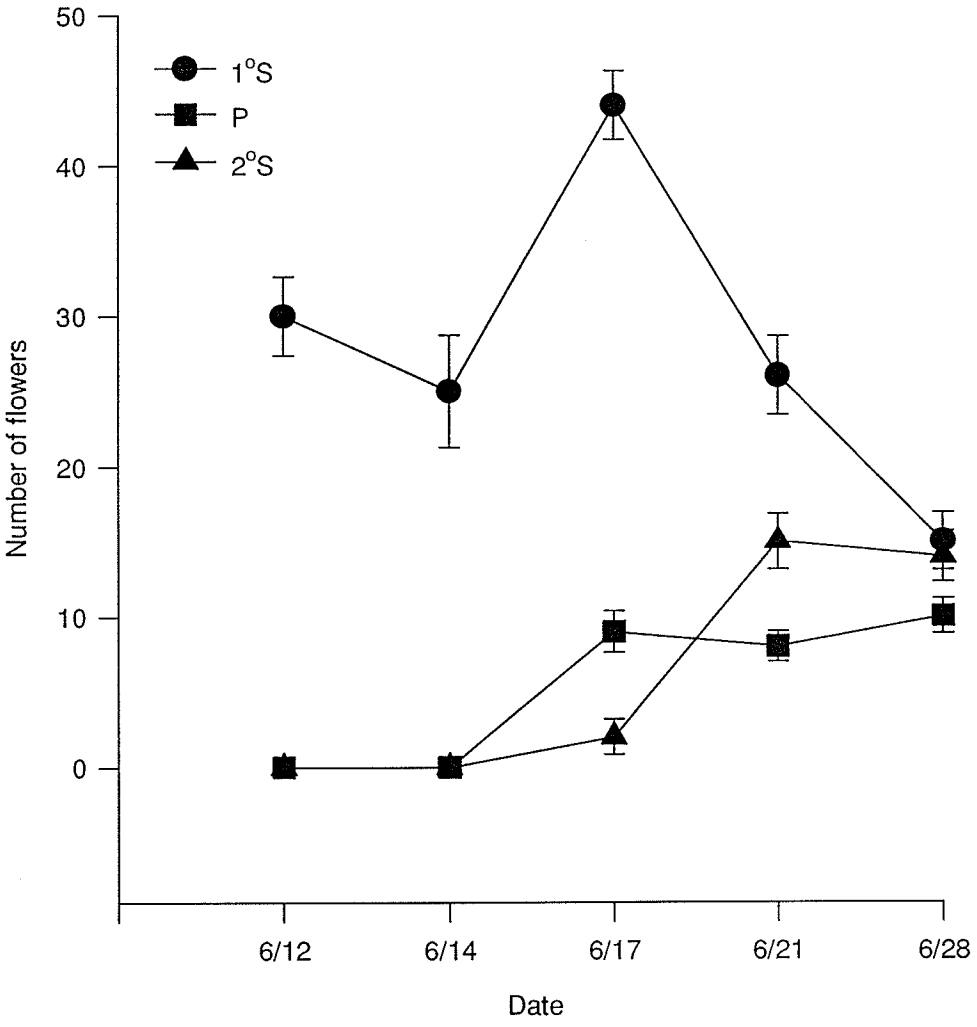


Fig. 13. Flowering behaviour of UMTeulon-1, 1993. Means (with standard error bars) for each date were based on samples of 20 inflorescences. (1°S, primary staminate flowers; P, pistillate flowers; 2°S, secondary staminate flowers)

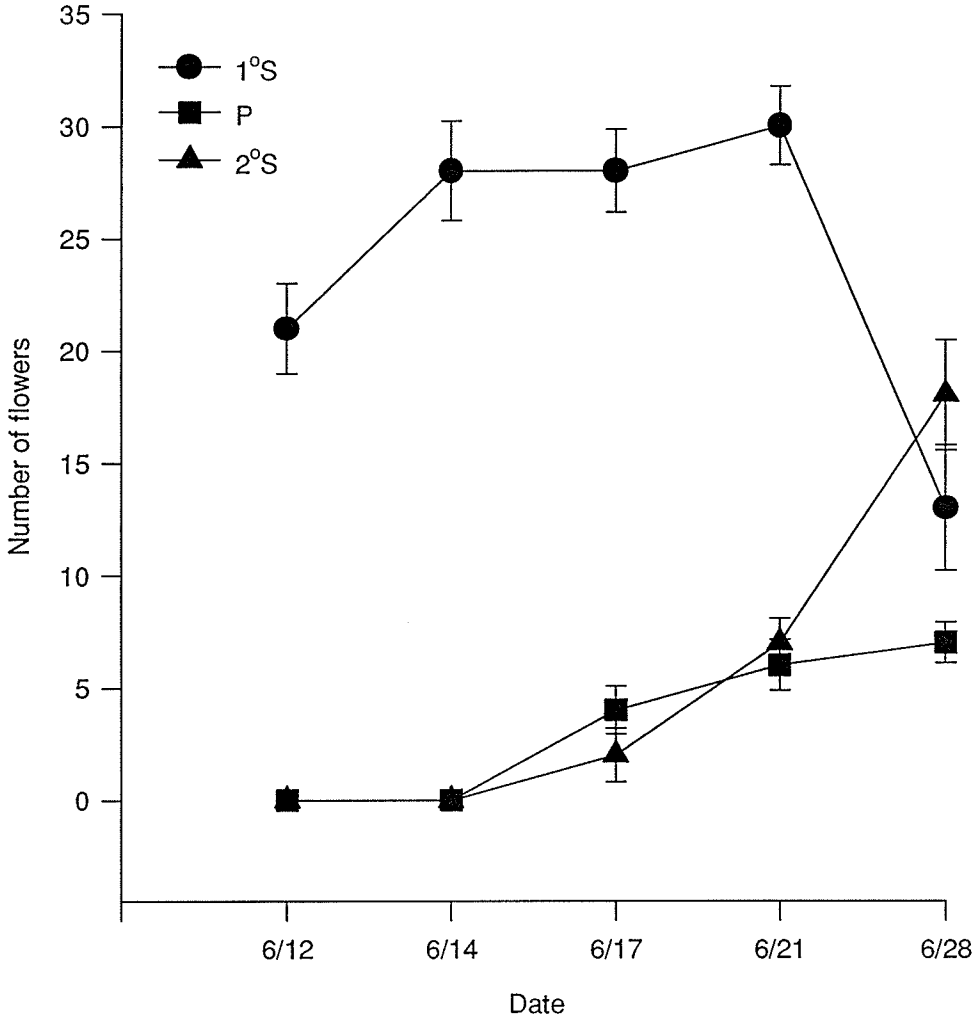


Fig. 14. Flowering behaviour of UMTeulon-2, 1993. Means (with standard error bars) for each date were based on samples of 20 inflorescences. (1°S, primary staminate flowers; P, pistillate flowers; 2°S, secondary staminate flowers)

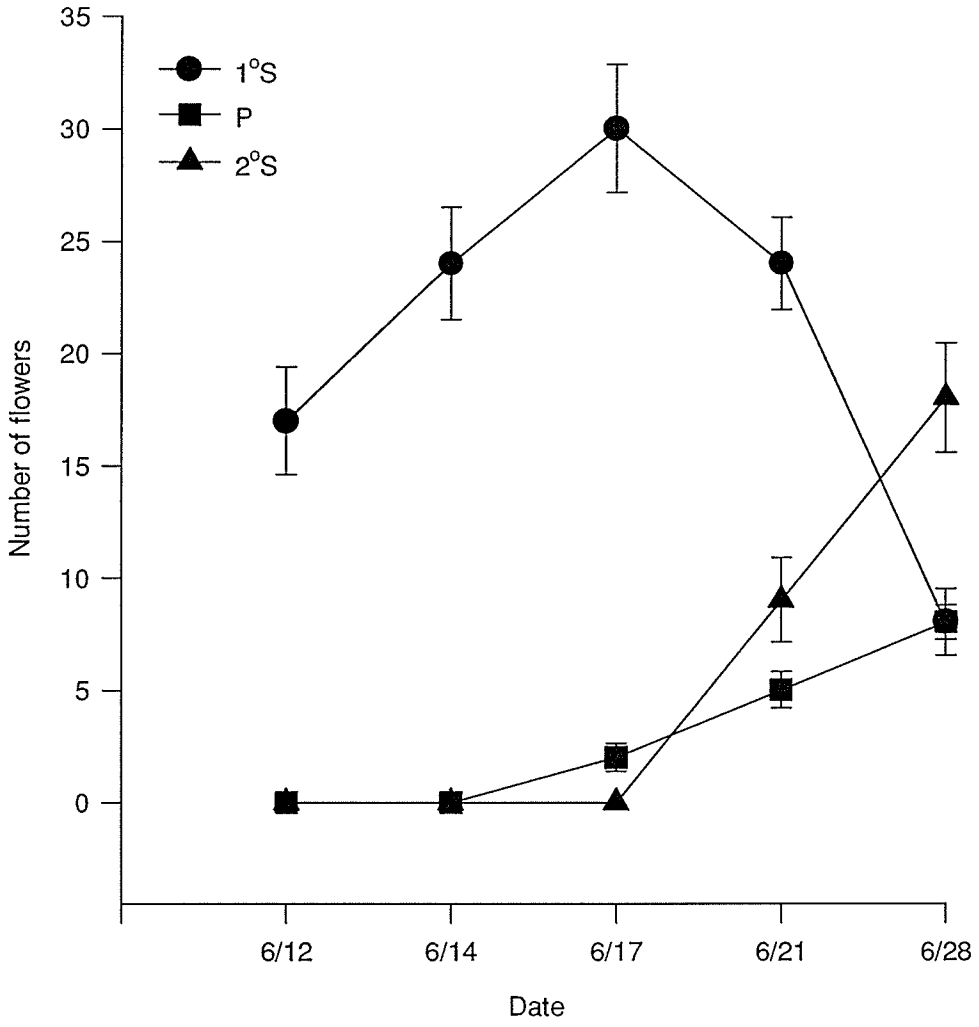


Fig. 15. Flowering behaviour of UMTeulon-3, 1993. Means (with standard error bars) for each date were based on samples of 20 inflorescences. (1°S, primary staminate flowers; P, pistillate flowers; 2°S, secondary staminate flowers)

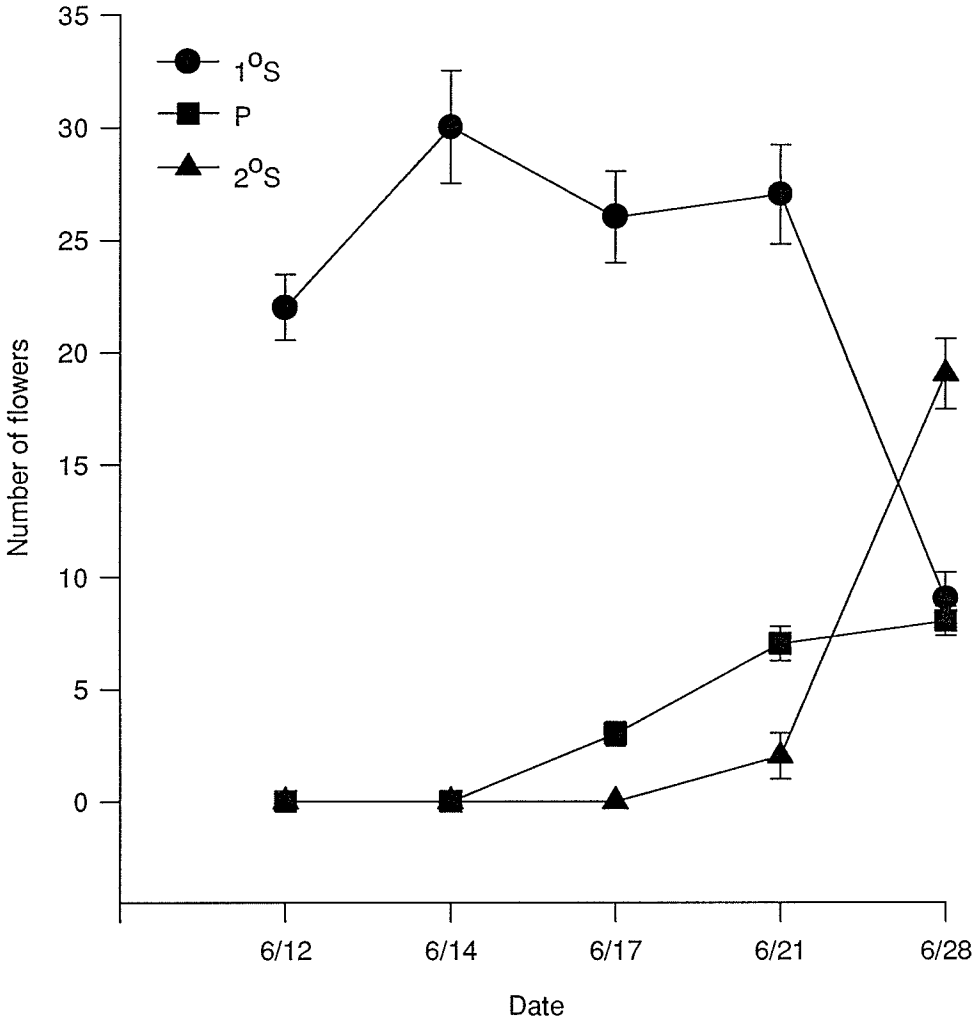


Fig. 16. Flowering behaviour of UMTeulon-5, 1993. Means (with standard error bars) for each date were based on samples of 20 inflorescences. (1°S, primary staminate flowers; P, pistillate flowers; 2°S, secondary staminate flowers)

Table 10. Average number of flowers (± standard error) observed per inflorescence and the maximum average frequencies (± standard error) of 1° staminate (1°S), pistillate (P) and 2° staminate (2°S) flowers, in *A. ginnala*, at the University of Manitoba, 1992.

Plant	Mean no. of flowers per inflorescence	Frequency		
		1°S	P	2°S
UM7301	101 ± 6.32	24 ± 4.71 (41)[1]	17 ± 1.01 (17)	59 ± 4.84 (42)
UM7306	119 ± 9.84	75 ± 9.12 (42)	18 ± 1.88 (17)	8 ± 1.41 (42)
UM7308	89 ± 5.39	21 ± 1.45 (33)	11 ± 1.26 (14)	48 ± 4.03 (80)
UMWalker	23 ± 2.43	7 ± 0.99 (11)	6 ± 0.48 (4)	13 ± 2.12 (11)

[1] Numbers in brackets represent expected values in a row x column (4 x 3) contingency test of independence using χ^2 statistics (df = 6) for flowering behaviour. H_0 = flower expression is independent of plant type; $\chi^2 = 82.07$, $p<0.01$.

Table 11. Average number of flowers (± standard error) observed per inflorescence and the maximum average frequencies (± standard error) of 1° staminate (1°S), pistillate (P) and 2° staminate (2°S) flowers, in *A. ginnala*, near Teulon, Mb, 1993.

Plant	Mean no. of flowers per inflorescence	Frequency		
		1°S	P	2°S
UMTeulon-1	65 ± 3.98	46 ± 2.67 (43)[1]	14 ± 0.66 (12)	19 ± 1.41 (23)
UMTeulon-2	45 ± 2.61	36 ± 1.79 (36)	10 ± 0.81 (10)	19 ± 2.30 (19)
UMTeulon-3	57 ± 5.71	37 ± 2.27 (40)	9 ± 0.65 (11)	25 ± 5.40 (21)
UMTeulon-5	52 ± 3.31	37 ± 1.94 (37)	10 ± 0.51 (10)	19 ± 1.60 (19)

[1] Numbers in brackets represent expected values in a row x column (4 x 3) contingency test of independence using χ^2 statistics (df = 6) for flowering behaviour. H_0 = flower expression is independent of plant type; $\chi^2 = 2.98$, $p>0.80$).

Table 12. Percent seed set from self- and open-pollinations, in *A. ginnala*, at the University of Manitoba, 1992.

	Percent Seed Set	
Plant	Self-pollination	Open-pollination
UM7301	6 (11)[1]	83 (78)
UM7306	6 (4)	29 (31)
UM7308	21 (12)	71 (80)
UMWalker	0 (5)	41 (36)

[1] Numbers in brackets represent expected values in a row x column (4 x 2) contingency test of independence using χ^2 statistics (df = 3) for pollination mechanism. H_0 = seed set is independent of plant type; $\chi^2 = 17.18$, $p<0.01$).

Table 13. Percent seed set from self- and open-pollinations, in *A. ginnala*, near Teulon, Mb, 1993.

	Percent Seed Set	
Plant	Self-pollination	Open-pollination
UMTeulon-1	31 (42)[1]	91 (80)
UMTeulon-2	45 (31)	43 (57)
UMTeulon-3	21 (24)	48 (45)
UMTeulon-5[2]	--	15

[1] Numbers in brackets represent expected values in a row x column (3 x 2) contingency test of independence using χ^2 statistics (df = 2) for pollination mechanism. H_0 = seed set is independent of plant type; $\chi^2 = 14.73$, $p<0.01$).

[2] UMTeulon-2 was not included in the χ^2 statistical analysis.

DISCUSSION

In 1992 and 1993, all plants of *A. ginnala* studied exhibited similar flowering behaviour, in that they were all protandrous and duodichogamous, supporting observations made by Jong (1976). Protandry is the term used to describe the maturation of male organs before that of female organs in plants (Frankel and Galun, 1977). Dichogamy is the condition in which anthesis occurs at a different time from stigma receptivity, and duodichogamy more specifically refers to the occurrence of alternating staminate and pistillate phases (Jong, 1976).

The flowering sequence exhibited in all plants studied (1° staminate, pistillate, 2° staminate) may be classified as flowering type 'C' (refer to Fig. 5), according to Jong (1976). Each individual phase was classified as such based on its time of occurrence within a plant. The different times of occurrence of the flowering phases are most evident in Figs. 10, 11, 13-16. The first flowers to open were staminate and were classified as 1° staminate. Anthesis of the 1° staminate flowers was observed as the anthers changed in appearance and condition, that is, they changed from a bright yellow to a yellowish-brown colour and appeared mealy. Analysis of anthers under a dissecting microscope over the duration of the staminate phase indicated that this observed change marked dehiscence. The next set of flowers to open were pistillate. A second set of staminate flowers began opening shortly after the onset of the pistillate phase and were classified as 2° staminate. In UM7301, UM7308 and UMWalker the 1° and 2° staminate flowers were both present at a certain point after the onset of the pistillate phase, suggesting that it may be difficult to distinguish between the two staminate phases (Figs. 9, 11, 12). However, the separate

phases were evident in the field in that the two sets of staminate flowers could be differentiated by their appearance and condition based on their maturation to anthesis, as described above. Shortly after anthers had dehisced on a staminate flower the entire flower quickly deteriorated and aborted, which provided another means of visually separating the 1° and 2° staminate phases. In UMWalker, the two staminate phases were also distinguishable due to the fact that the anthers in the 1° staminate flowers were irregularly formed and dysfunctional, whereas those in the 2° staminate flowers were functional. In 1993, the separation of the two staminate phases with respect to time of occurrence was more evident than in 1992 (Figs. 13-16).

Some differences occurred in the timing of opening and of maturation of the different phases, between the plants studied in 1992 (Figs. 9-12). This occurrence may have been due to genetic variation, since the plants studied in each separate year grow adjacent to one another (excluding UMWalker in 1992), and would have been exposed to common environmental conditions within the sites. But, slight differences in the relative positions of UM7301, UM7306 and UM7308, may have affected the flowering in these plants. In 1992, UM7301 was more advanced in its flowering time than the other plants (Figs. 9-12), possibly due this plant having relatively the least exposure to environmental extremes. Flowering was somewhat delayed and the 1° staminate phase was of longer duration, in UM7306, as compared to UM7301 and UM7308, possibly due to its greater exposure to environmental extremes.

In 1993, there was very little difference in the behaviour of the different phases among the plants near Teulon, Mb (Figs. 13-16). These plants generally flowered later

than those observed on campus, since they are situated 70 km north of the university. However, occurrence and duration of phases in the plants near Teulon, Mb, were similar to those studied on campus in 1992.

In 1992, the significant differences in frequencies of flower types among plants, based on a Chi-square test, were primarily due to the occurrence of the different phases of staminate flowers. There was little variation in the frequencies of pistillate flowers among all plants studied in both sites, suggesting that the expression of pistillate flowers may be a common trait in the plants of *A. ginnala* studied and may not be significantly affected by climatic conditions. In contrast, Jong (1976) stated that in *Acer*, the occurrence of pistillate flowers is highly variable between plants and between years, and is largely influenced by the environment.

The present study found that the expression of staminate flowers varied between plants, and that there were differences between the staminate phases within plants. These differences may have been due to genetic variability, but climatic conditions may have played a more influential role. It should be noted that environmental conditions present at the time of floral induction can influence sex expression in plants (Jong, 1976; Sedgley and Griffin, 1987). In this study, differences in sex expression also may have been influenced by temperature during flowering. By relating temperatures (Appendix D) to the period of expression of flower types (Figs. 9-12) in 1992, it appeared that an increase in the expression of staminate flowers coincided with cooler temperatures. In 1992, it was observed that on June 4, the average temperature began decreasing and remained relatively low for a period of 5 days. From June 5 to June 8, the minimum temperatures

were considerably lower than in the previous days. During this period there was some increase in the number of 1° staminate flowers expressed in UM7306, even though this phase was already at the maturation stage (Fig. 10). It is possible that the cooler temperatures extended the 1° staminate phase and delayed the onset of the pistillate phase, resulting in greater numbers of flowers within an inflorescence to differentiate into 1° staminate flowers (Table 10) (Jong, 1976). Considerably greater numbers of 2° staminate flowers were expressed in UM7301 and UM7308, as the onset of this phase occurred during the period of cooler temperatures (Figs. 9, 11) (Appendix D). It is possible that unopened buds may have a tendency to differentiate into staminate flowers during periods of cooler temperatures (Jong, 1976).

Pollinations performed in 1992 and 1993 indicated that *A. ginnala* can be self- and cross-pollinated, but cross-pollination is of primary significance (Tables 12, 13). Cross-pollination appeared to be the dominating mechanism in open-pollinated flowers, since the manual self-pollinations, performed in the plants, generally resulted in poor seed set. The use of glycine bags for flowers which were self-pollinated could have affected the function of the stigma and pollen, however this is difficult to determine from this study. In open-pollinated flowers, pollination occurred through the activity of honey bees, bumble bees and serphid flies, in both sites. Leaf cutter bees were also pollen vectors in Teulon, Mb. The occurrence and duration of the various flowering phases may have influenced cross-pollination in the plants growing adjacent to one another. UM7306 had low seed set from open-pollination (29%), relative to UM7301 and UM7308 (Table 12). In UM7306, the pistillate phase occurred late relative to anthesis of the staminate phases

in UM7301 and UM7308 (Figs. 9-12), indicating that perhaps sufficient pollen was not available for cross-pollination of that plant. It was assumed in this study that these three plants were the only known pollen sources in the area surrounding Block 25. In UMTeulon-5, seed set from open-pollination was very low (15%) relative to the other plants, in both years of study (Table 13). This plant produced fewer flowers than the others growing nearby at the Teulon site, possibly resulting in reduced bee activity and thus, reduced pollination overall. Seed set from open-pollination was lower in UMWalker, relative to UM7301 and UM7308 (Table 12), which primarily resulted from cankerworm damage.

In this study, the plants of *A. ginnala* exhibited some self-compatibility, in that some seed was produced through self-pollinations (Tables 12, 13). Since these plants could be artificially self-pollinated, natural self-pollination could have occurred in the open-pollinations through insect activity within the plants, as a result of geitonogamy. Geitonogamy is a form of self-pollination in which pollen from one flower fertilizes a different flower within the same plant (Sullivan, 1983). Geitonogamy may have occurred due to the overlapping of staminate and pistillate phases within individual plants (Figs. 9-16). The stigmas may have been receptive at the time of anthesis, although this would have occurred in a considerably short period of time.

All pistillate flowers began developing the winged pericarp upon stigma withering. This fruit development was observed to continue throughout the growing season, although some fruit aborted before they ripened. It was not known if parthenocarpic fruit development occurred without fertilization, or if fertilization occurred but the embryo

aborted (Gustafson, 1942). Regardless, any fruit that did not contain seed, or that was not fully developed, including aborted fruit, was classified as parthenocarpic fruit. Generally, awareness of the behaviour of parthenocarpic fruit development is important in performing pollinations for breeding purposes, since it is difficult to determine if pollinations are successful shortly after they have occurred.

CHAPTER 3

Seed Propagation of *Acer ginnala* Maxim.

ABSTRACT

In order to propagate plants of *A. ginnala* for selection purposes, after-ripening treatments to overcome seed dormancy for germination were investigated. Two different seed lots were used. Seed were after-ripened and germinated in pots filled with pre-moistened Metro Mix 220, a soilless mix. The germination temperature was 22° C day/ 18° C night. Treatments in the first year of study consisted of 15, 30, 45, 60, 75,and 90 days at either 22° C or 4° C. Germination was very poor (maximum 6%). In the second year of study, treatments consisted of the following combinations of time periods at 22° C and 4° C: 150/0, 0/120, 0/150, 30/90, 30/120, 30/150, 60/90, 60/120, and 60,150. At least 120 days of 4° C were required for both seed lots to begin to overcome dormancy and germinate. Highest germination (54% and 41% for both seed lots) occurred in the 60/150 treatment, 21 days following removal from treatment. This treatment was significantly different from all other treatments in the seed lot with 54% germination ($P<0.05$). The duration of the cold temperature treatment, specifically, was the significant factor for after-ripening in both seed lots.

INTRODUCTION

In the development of a breeding program, it is necessary to understand how to propagate the plant material, in order to obtain plants for selection. If genetic variability of the desired trait is obtained through sexual reproduction, then knowledge of seed propagation for that species is required. *A. ginnala* exhibits a complicated seed dormancy which requires a period of after-ripening to germinate. Recommendations for after-ripening treatments are available, but they differ in procedure, claim variable percentages of germination and do not cite scientific evidence for the results. This study was performed to determine the best seed after-ripening treatment to overcome dormancy in *A. ginnala*, in order to optimize germination.

MATERIALS AND METHODS

This study was performed during the periods of September 1991 - March 1992 and September 1992 - July 1993, using fruit harvested from two plants of *A. ginnala*, UM7301 and UMTeulon-2. UM7301 is located in Block 25 at the Dept. of Plant Science Experimental Site at the University of Manitoba and UMTeulon-2 is located near Teulon, Mb (Appendix B).

In 1991 and 1992, fruit were harvested on September 23 and 24, and on September 9 and 10, respectively. Fruit were dewinged by rubbing them over a wire screen, which also provided some scarification of the pericarp. The fruit were visually sorted based on those which appeared plump and filled with seed versus those which were flat, or incompletely developed, and empty. Fruit which contained seed were stored for

two months at approximately 4° C, before planting.

Fruit were sown in pots filled with Metro-Mix '220'. Metro-Mix is a soilless medium consisting of peat moss, vermiculite and perlite. The medium had been pre-moistened by adding sufficient water to moisten, but not saturate it. Each pot contained 25 fruit.

In 1991, after-ripening treatments consisted of 15, 30, 45, 60, 75, and 90 days at 4° C or at 22° C. In 1992, after-ripening treatments consisting of combinations of 22° C and 4° C, for various time periods, were applied (Table 14). Following treatments, pots were moved to an environmentally controlled growth room, which was set on a 16 hour photoperiod at 22° C day and 18° C night, for seed germination.

Experimental design was CRD. In 1991 and 1992, treatments were replicated 2 and 4 times, respectively, for each plant. One replicate consisted of one pot containing 25 fruit. In 1992 and 1993, percent seed germination per pot and time to germinate were recorded. ANOVA was performed on the data obtained. Mean comparisons were performed using Duncan's Multiple Range Test, to detect differences between treatments. In 1993, a t-test was performed to detect significant differences in treatment response between the two plants.

Table 14. After-ripening treatments applied to seed of *A. ginnala*, 1992.

Treatment Name	Days at 22° C	Days at 4° C
control	0	0
150/0[1]	150	0
0/120	0	120
0/150	0	150
30/90	30	90
30/120	30	120
30/150	30	150
60/90	60	90
60/120	60	120
60/150	60	150

[1]First number denotes days at 22° C; second number denotes days at 4° C.

RESULTS

Results generally indicate that *A. ginnala* seed require a long after-ripening period in order to germinate. In 1992, highest germination was 6%, obtained from the 60, 75, and 90 day cold treatments for UM7301 (Fig. 17). There were no significant differences among replicates and among treatments. All germination occurred within 14 days following removal from the 60 day cold treatment and within 28 days from the 75 daycold treatments (Fig. 17). In the 90 day cold treatment, seeds began germinating before the treatment was completed. However, germination ceased when the seeds were removed from the treatment and placed in the germination environment. Seed from UMTeulon-2 did not germinate following any of the treatments.

In 1993, treatment effects were significantly different ($p<0.01$) within both parent plants (Tables 15, 16). There were no significant differences in response to after-ripening treatments between the two parent plants, based on a t-test.

No germination occurred for seeds in the control and those subjected to treatments 150/0, 30/90 and 60/90, for UM7301 (Table 15). For UMTeulon-2, 3% germination occurred in treatment 30/90. However, this result was not significantly different from the 0% germination observed in the control and in treatments 150/0 and 60/90 (Table 16).

In both parent plants, greatest germination occurred following treatment 60/150, and thus was the most effective after-ripening treatment applied in this experiment. Treatment 60/150 was significantly different from all other treatments in UM7301 ($p<0.05$). However, for UMTeulon-2, treatment 60/150 was not significantly different from 30/150 and 0/150. Average maximum germination from treatment 60/150 was 54%

for UM7301 and 41% for UMTeulon-2, occurring 21 days following removal from after-ripening treatments (Figs. 18, 19). In UM7301, the majority of the seed germinated in 14 days following removal from after-ripening treatments, but the majority of the seed from UMTeulon-2 required 21 days to germinate.

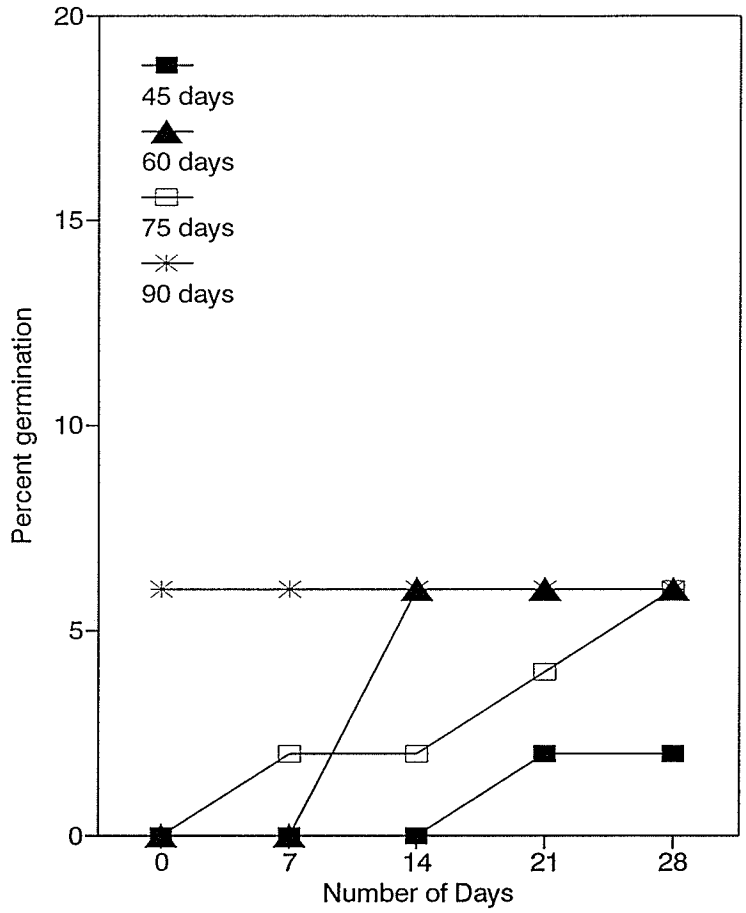


Fig. 17. Percent seed germination of UM7301, following after-ripening treatments at 4° C, 1992.

Table 15. Average percent seed germination (± standard error) from UM7301 following after-ripening treatments, 1993.

Treatment	Percent germination
control	0 ± 0d[1]
150/0	0 ± 0d
0/120	18 ± 1.39c
0/150	20 ± 1.31c
30/90	0 ± 0d
30/120	10 ± 1.03cd
30/150	39 ± 1.65b
60/90	0 ± 0d
60/120	16 ± 2.85c
60/150	54 ± 3.84a

[1]Means followed by different letters differ significantly ($p<0.05$) using Duncan's Multiple Range Test.

Table 16. Average percent seed germination (± standard error) from UMTeulon-2 following after-ripening treatments, 1993.

Treatment	Percent germination
control	0 ± 0d[1]
150/0	0 ± 0d
0/120	13 ± 2.10bc
0/150	32 ± 1.31a
30/90	3 ± 0.77cd
30/120	18 ± 1.39b
30/150	33 ± 2.89a
60/90	0 ± 0d
60/120	7 ± 1.01cd
60/150	41 ± 1.65a

[1]Means followed by different letters differ significantly ($p<0.05$) using Duncan's Multiple Range Test.

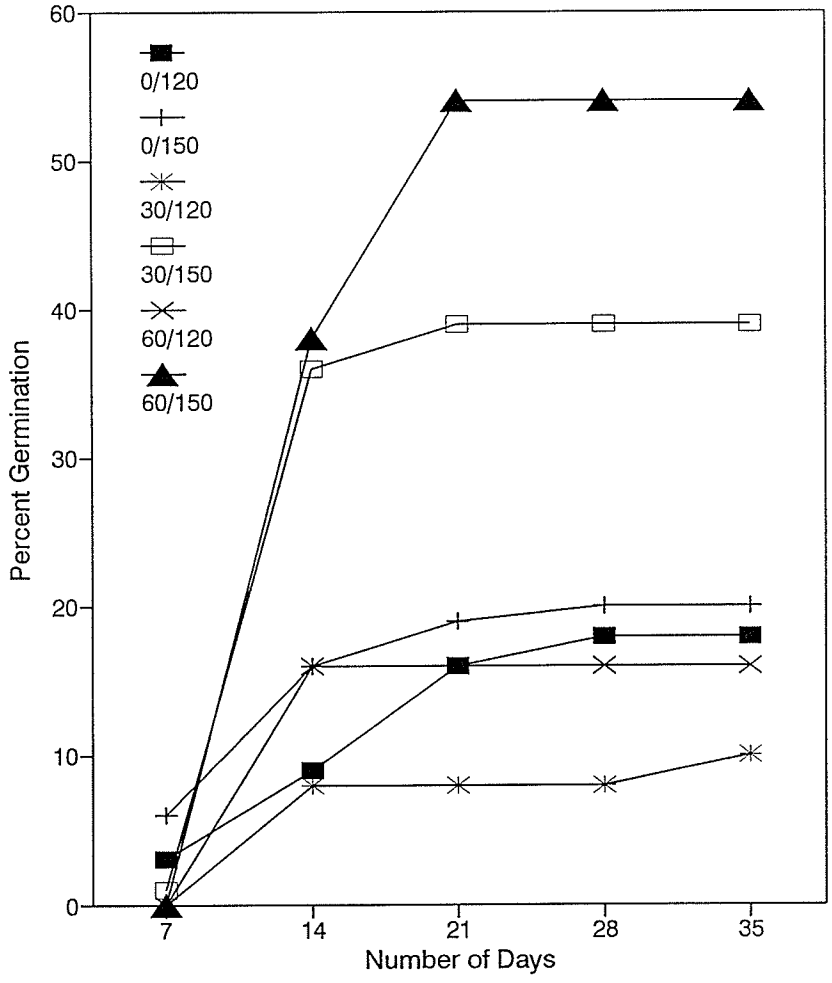


Fig. 18. Percent seed germination of UM7301, following after-ripening treatments at 22° C followed by 4° C, 1993.

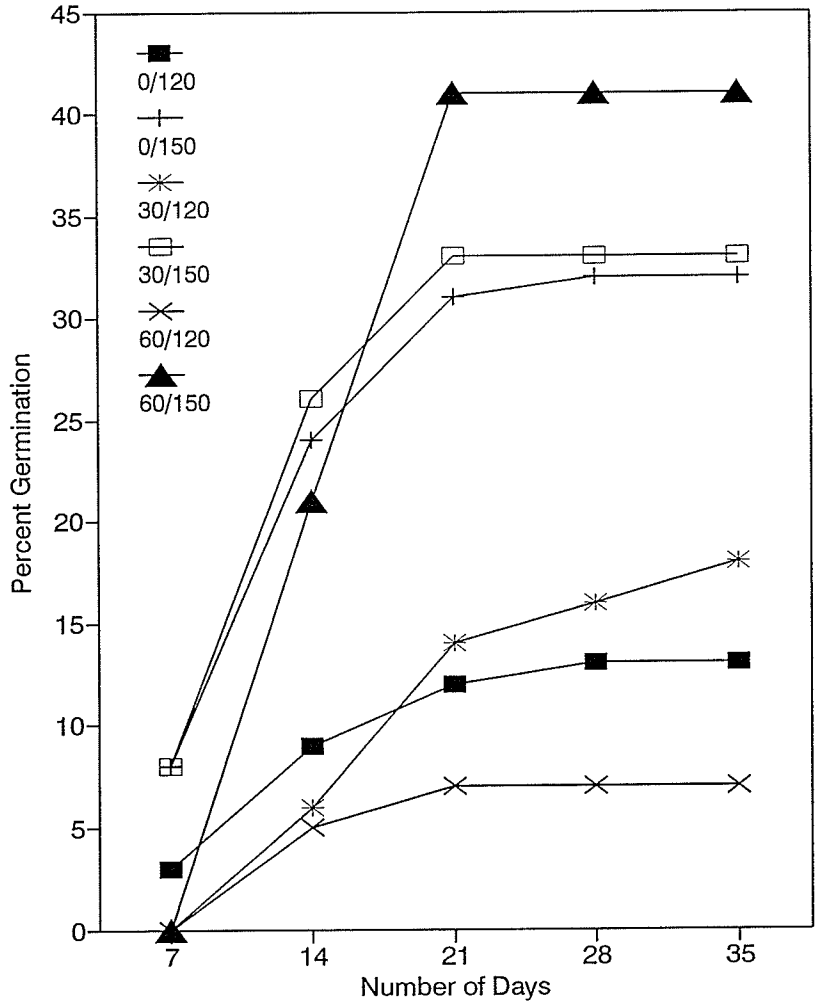


Fig. 19. Percent seed germination of UMTeulon-2, following after-ripening treatments at 22° C followed by 4° C, 1993.

DISCUSSION

In the *A. ginnala* seed used in this study, the length and type of after-ripening treatment appeared to influence two factors, testa- and pericarp-imposed dormancy and embryo dormancy. Dumbroff and Webb (1970), Norton (1987), and McMillan-Browse (1979) all refer to the drying and hardening of the pericarp as a major barrier to seed germination in *A. ginnala.* In the present study, it is possible that a hard pericarp developed over 2 months of storage, as suggested by McMillan-Browse (1979) and Norton (1987). This condition may have resulted in the seed generally requiring a considerably long period of time to imbibe sufficient water for the germinative process.

McMillan-Browse (1979) stated that embryo chilling is required to overcome dormancy in *A. ginnala* seed, which implies that the length of time the seed is exposed to cold temperatures will influence germination. The duration of the cold temperature in all treatments was a significant factor in overcoming dormancy in the seed lots used in this study. Based on the poor germination results in 1992, it was assumed that the duration of the longest cold treatment (90 days) was not sufficient to overcome seed dormancy, although poor seed viability may have been a contributing factor. Similarly, in 1993, treatments including 90 days of cold treatment were not effective, and at least 120 days at 4° C were required for seeds to begin to overcome dormancy and germinate. In 1993, comparisons of treatments of 150 days, in total, and of 180 days, in total, also indicate that the duration of exposure to the cold temperature, specifically, was the significant factor in after-ripening, particularly in the seed of UMTeulon-2 (Tables 15, 16).

In the present study, germination percentages were considerably lower and the seed required considerably longer after-ripening periods to germinate, in comparison with results obtained in *A. ginnala* by Dumbroff and Webb (1970) and by Norton (1987). Dumbroff and Webb (1970) obtained 74% germination following just 30 days stratification at 5° C. Norton (1987) obtained 46% in 1-month-old and in 6-month-old seed, and 26% germination in 9-month-old seed, following just 30 days stratification at 4° C. Although various factors could have influenced the differences in results among these studies and the present study, it appears that an important factor could be the difference in media used. In the studies by Dumbroff and Webb (1970) and Norton (1987), seeds were sown in Petri plates containing water for after-ripening and germination, whereas a pre-moistened soilless mix was used as the medium in the present study. Seed sown in Petri plates containing water may have a more direct and uniform supply of moisture for the imbibing seed, which may result in higher rates of germination. However, seed planted in soil or in a soilless mix may have a less consistent supply of moisture, as the media nearest to the seed dries and needs to be replenished by water from more distant pores (Hartmann et al, 1990). Proper seed-soil contact within the media is also a factor in adequate supply of moisture.

The use of Petri plates is not practical for the purpose of large-scale propagation. Commercially, if seeds are stratified as an after-ripening treatment prior to planting, the medium would typically consist of a soilless mix, sand or topsoil (Hartmann et al, 1990). The practical application of procedures derived from germination studies, which have not used an actual medium, may not produce the desired results.

The inconsistent results between the present study and previous studies (Dumbroff and Webb, 1970; Norton, 1987) may also be due to the use of different seed sources. Seed obtained from different sources have acquired different after-ripening requirements, depending on the growing conditions in their respective environments (Powell, 1987). It is important to note that *A. ginnala* is an introduced species, and has adapted to various growing conditions, which, particularly in the north temperate zone, are harsher than in its native habitat of eastern Asia. Perhaps it is necessary to determine after-ripening treatments for individual seed lots obtained from different climatic regions.

GENERAL DISCUSSION

The presence of stress in a plant's growing environment affects its productivity and general growth. More specifically, a plant growing under mineral stress may respond by a reduction in quantity and quality of plant parts, "which is of concern to the scientist and consumer alike" (Ross, 1986). Lime-induced iron chlorosis is one such stress which occurs in many plant species, including *A. ginnala.*

This present study investigated this disorder from a breeding viewpoint, to assess the potential for genetic control and to determine viable methods for developing tolerant plants. An important factor in the development of tolerant plants of *A. ginnala* is the retention of other desirable characteristics, particularly red fall colour. Chlorosis tolerance and red fall colour were found in seedling populations of *A. ginnala* growing on highly calcareous soil. The desirable characteristics are present in the seed sources of *A. ginnala* utilized in the present study, and through breeding and selection, an improved individual or improved population may be produced. Further discussion will concentrate on how these results may be obtained.

General suggestions, regarding breeding and selection for chlorosis tolerance in *A. ginnala*, may be made from the basic information obtained in the present study. In order to conduct plant breeding, it is necessary to determine a basic strategy. The general steps of this strategy may be outlined as follows (Poehlman, 1987):

1) recognize the desired trait(s),

2) develop techniques to measure and evaluate the genetic potential for the desired trait(s),

3) identify sources of genes for the desired trait(s), and

4) determine means of combining the genetic potential of the desired trait into an improved cultivar or an improved population.

The first factor to consider in plant improvement is how the desired trait is expressed by the plant. Part of the ability to recognize mineral stress is to obtain a basic understanding of its cause and the plant's response to the stress. Lime-induced iron chlorosis is an iron deficiency that occurs in susceptible plants growing on highly calcareous soil. Symptoms may be diagnosed by various methods. Certain laboratory procedures may detect exudation of reductants by the roots in Fe-efficient soybeans (Olsen and Brown, 1980), or changes in chlorophyll and mineral composition of leaf tissue in grape vine (Mengel et al, 1984b). In rabbiteye blueberry, Gupton and Spiers (1992) found that the use of visual observations was effective in determining plant response to iron-deficiency stress and was a useful diagnostic tool for selection of chlorosis resistant plants. In this present study, a leaf chlorophyll meter was useful in detecting chlorosis in *A. ginnala.* However, visual observations were also effective and were more rapid and simple than the chlorophyll meter. In *A. ginnala*, lime-induced iron chlorosis is readily identified in susceptible plants as interveinal yellowing of leaf tissue.

Measurement of iron chlorosis in *A. ginnala* is relatively simple compared to that in agronomic and edible horticultural crops, since the major characteristics of concern in ornamental plants are related to quality and appearance. In this case, visual ratings provide sufficient measurements of iron chlorosis. In the other crops, yield under stress is of major concern and requires more extensive analysis.

Methods by which the desired trait is evaluated should be simple, inexpensive, and

repeatable (Poehlman, 1987). In addition, in this case of lime-induced iron chlorosis, tolerant types should be identifiable. Field evaluation generally possesses all of these characteristics, but certain problems can arise. The expression of symptoms can vary from year to year, as has generally been observed in mature specimens of *A. ginnala.* Evaluation can be difficult due to variable conditions in the field for the stress, or soil heterogeneity, and other environmental factors. Mineral stress in plants can be highly influenced by the environment (Ross, 1986). In soybean, Fehr (1982) concluded that evaluation and selection of individuals for chlorosis tolerance in the field could be effective, provided that the calcareous soil is uniform in producing symptoms in susceptible types. In this present study of *A. ginnala* seedlings growing near Teulon, Mb, soil variability may have affected the expression of iron chlorosis within the populations, throughout the experimental plot. Distinct pockets of chlorotic types observed in the field may have been due to areas of particularly high lime content (hot spots) in the soil (Loeppert, 1986). Fehr (1982) also stated that if evaluation is performed on a single plant basis in the field, then the frequency of misclassifications for chlorosis tolerance can be high.

It is proposed from this present study, that field evaluation and selection be accompanied by screening procedures performed in the greenhouse. This method could provide a more direct evaluation of tolerance to lime-induced iron chlorosis in *A. ginnala* based on soil factors, while reducing the effect of other environmental conditions. The pot test method which was assessed in this present study can be effective in detecting chlorosis-tolerant types. Plants should be grown in highly calcareous soil, preferably that

from the site near Teulon, Mb, and in noncalcareous soil, to serve as a check. Water treatments may not be necessary, since results show that the calcareous soil itself was sufficient in producing chlorosis in susceptible types, and there was no soil x water interaction for chlorosis expression.

Once techniques for measuring and evaluating chlorosis tolerance are devised, then they may be applied to identify gene sources for chlorosis tolerance and to select parents for breeding purposes. A primary factor in this step is the existence of genetic variability for the trait, so that tolerant plants may be identified. Considerable variability was observed within certain *A. ginnala* populations grown near Teulon, Mb. Chlorosis-tolerant seedlings which exhibited red fall colour were selected. However, these plants were selected after only one season of growth under extremely wet soil conditions and require further evaluation as sources of tolerance. It is proposed that when these selected individuals produce sufficient growth, they should be propagated by cuttings and undergo subsequent evaluation under the pot test method, to assess their actual tolerance to lime-induced iron chlorosis under controlled conditions. This method may be used before the plants reach seed-bearing age (minimum 5 years (Olson and Gabriel, 1974)) to further assess their potential use as gene sources for breeding. Clones of these selections may also be tested in the site near Teulon, Mb, and other field locations, over at least two years, to assess their adaptability to various environmental conditions.

Following more extensive testing, the manner in which these present selections are handled may proceed in two different directions. These plants themselves may undergo subsequent evaluation with respect to plant form and fruit colour. Then, they may have

the potential to become new cultivars which are chlorosis tolerant, exhibit red fall colour and other desirable characteristics. Alternatively (or even simultaneously), when these plants reach seed-bearing age they may be considered as parents for the development of populations of *A. ginnala* with improved chlorosis tolerance. However, as was observed in the study near Teulon, Mb, plants selected for chlorosis tolerance in the past did not necessarily produce a greater frequency of tolerant progeny. These progeny would have originated from natural open-pollinations involving unknown pollen parents. Perhaps, if chlorosis-tolerant types were intermated, then the desired genes could be concentrated to develop lines with increased chlorosis tolerance. The result could be an improved seed source for commercial production. In order to obtain these lines, it would be necessary to determine an appropriate breeding and selection method.

The inheritance of a trait influences the type of breeding and selection method to be used (Ross, 1986). Methods used for simply inherited traits are different from those used for traits with complex inheritance. In soybean, inheritance of chlorosis resistance is believed to be controlled by multiple genes (Fehr, 1982). In general, tolerance to mineral stress often exhibits quantitative inheritance (Ross, 1986). It is possible that chlorosis tolerance in *A. ginnala* is quantitatively inherited. A characteristic of quantitative inheritance is continuous variation in the expression of the trait, where discrete classes do not exist (Poehlman, 1987). This characteristic was observed in *A. ginnala* seedlings, showing varying degrees of chlorosis.

For a trait that is controlled by many genes, breeding methods can be determined based on the heritability of that trait. In general, heritability is an estimate of the degree

to which a trait is passed on from a parent to its offspring. Information regarding heritability of a trait may be used to predict the success of a particular selection procedure. Dragonuk et al (1989) determined that the heritability for chlorosis resistance in soybean for field tests was 10±2%. Fehr (1982) performed recurrent selection by S_1 line testing, where plants are self-pollinated and selected on the basis of the performance of their progeny. This method of selection has been successful in producing chlorosis-resistant lines of soybean. Recurrent selection is the general procedure that is recommended for use in a breeding program for improved chlorosis tolerance in *A. ginnala*, but more information is required regarding the heritability of this trait, to determine a specific selection procedure.

The mode of reproduction of a plant is also a significant factor in devising procedures for breeding and selection, since it determines how the plants may be manipulated (Ross, 1986). *A. ginnala* is primarily cross-pollinated, but does exhibit some selfing (Chapter 2, Tables 3, 4). Recurrent selection is a general procedure used for breeding cross-pollinated crops. Several suggestions may be made regarding the handling of *A. ginnala*, for selfing and intermating chlorosis tolerant types. In performing crosses, inflorescences need to be bagged to prevent contamination from undesired pollen, and staminate flowers should be removed from the inflorescence, since selfing may occur. For self-pollinations, success may be increased by manual application of pollen, rather than relying solely on natural selfing in bagged inflorescences. Pollen of *A. ginnala* is of sticky substance (Jong, 1976) and may require some manipulation to come in contact with a stigma. For open-pollination, selected parents should be grown in isolation, if possible,

or a considerable distance from other plants of *A. ginnala*, to avoid pollen contamination from undesirable sources. It was observed at the University of Manitoba and the site near Teulon, Mb, that plants were pollinated primarily by honeybees, which could travel from other pollen sources of *A. ginnala*.

A major factor to consider for manipulating pollinations in *A. ginnala* is external, in the form of spatial isolation, although internal factors, such as incompatibility, may exist (Gabriel, 1967). Functionally staminate flowers mature at different times than do functionally pistillate flowers, within inflorescences throughout a plant. Appropriate timing of pollen collection and application is critical for successful pollination. Anthers from the 1° staminate flowers can be removed, dehisced in a desiccator and stored, ensuring adequate supply of pollen when pistillate flowers open. More information is needed regarding storage of pollen and maintenance of pollen viability. Additional information is also required regarding the duration of stigma receptivity in the pistillate flowers, to determine optimal timing for pollination.

Another important factor of a breeding program is the length of time required to obtain the desired results. Since more information is required regarding the inheritance of chlorosis tolerance in *A. ginnala*, it is difficult to predict the duration of such a program. The number of selection cycles is limited to the long period before these plants reach seed-bearing age, to the chilling requirement to overcome bud dormancy ('rest') and to a complicated seed dormancy.

The period of bud dormancy or rest, which occurs naturally in the field, needs to be considered in the greenhouse pot-test screening procedure. Plants growing in the field

were vegetatively propagated by cuttings, for evaluation in the greenhouse. Following transplanting, the clones appeared to have reached vegetative maturity for that growing cycle, by ceasing internodal extension and setting terminal buds. Chilling of the transplants was required, in order for the plants to overcome bud dormancy and initiate new top growth (Powell, 1987). Therefore, only one selection cycle per year may be performed, under field and under controlled conditions.

Past studies and this present study have shown that seed of *A. ginnala* requires a long after-ripening period to overcome a complicated dormancy. Germination and emergence in the field can be very low. In the present study, a maximum average seedling emergence of 25.5% was obtained in the experimental plot near Teulon, Mb. Results from this study indicate that an improved method of seed propagation may be required for field conditions. Germination experiments performed under controlled conditions indicated that at least 41% seed germination could be obtained by dewinging and scarification of samaras, followed by moist stratification at 22° C for 60 days and at least 150 days at 4° C. This after-ripening treatment may be applied to improve seed propagation in the field, however, stratification would need to be timed appropriately for planting. One problem that can arise is that seed may begin germinating under after-ripening treatments before planting.

SUMMARY AND CONCLUSION

The majority of *A. ginnala* grown in commercial ornamental nurseries in Manitoba, appear to originate from two seed sources at the Agriculture Canada Research Station in Morden, Mb. These sources are selections for red fall colour but do not exhibit tolerance to lime-induced iron chlorosis. This study has provided basic information and offers suggestions for the development of chlorosis-tolerant plants through breeding and selection.

Chlorosis-tolerant cultivars and/or improved seed sources of *A. ginnala* would benefit the nursery industry and the public by providing healthier, more attractive plants with improved adaptability to various soil conditions. These plants would greatly benefit the homeowner by eliminating the time and money spent on cultural control of lime-induced iron chlorosis in *A. ginnala.*

REFERENCES

Bailey, L.H. and Bailey, E.Z. 1976. Hortus Third: A Concise Dictionary of Plants Cultivated in the United States and Canada. MacMillan Publishing Co., New York. 1290 pp.

Barak, P. and Chen, Y. 1982. The evaluation of iron deficiency using a bioassay-type test. Soil Sci. Soc. Am. J. 46:1019-1022.

Berrang, P. and Steiner, K.C. 1980. Resistance of pin oak progenies to iron chlorosis. J. Amer. Soc. Hort. Sci. 105(4):519-522.

Bienfait, F. 1987. Biochemical basis of iron efficiency reactions in plants. *In* Iron Transport in Microbes, Plants and Animals. (G. Winkleman, D. van der Helm and J.B. Neilands, ed.), pp.339-349. VCH Verlagsgesellschaft, Germany.

Bienfait, H.F., Bino, R.J., van der Bliek, A.M., Duivenvoorden, J.F. and Fontain, J.M. 1983. Characterization of ferric reducing activity in roots of Fe deficient *Phaseolus vulgaris*. Physiologia Plantarum 59:196-202.

Boxma, R. 1972. Bicarbonate as the most important soil factor in lime-induced chlorosis in The Netherlands. Plant and Soil 37:233-243.

Brown, J.C. 1978. Mechanism of iron uptake by plants. Plant, Cell and Environment. 1:249-257.

Brown, J.C., Ambler, J.E., Chaney, R.L. and Foy, C.D. 1972. Differential responses of plant genotypes to micronutrients. *In* Micronutrients in Agriculture. (J.J. Mortvedt, P.M. Giordano and W.L. Lindsay, ed.), pp. 389-418. Soil Science Society of America, Inc., Madison, Wisc.

Campbell, R.J., Mobley, K.N., Marini, R.P. and Pfeiffer, D.G. 1990. Growing conditions alter the relationship between SPAD-502 values and apple leaf chlorophyll. HortScience 25(3):330-331.

Carter, M.R. 1980. Association of cation and organic anion accumulation with iron chlorosis of Scots pine on prairie soils. Plant and Soil 56:293-300.

_____. 1981. Association of total $CaCO_3$ and active $CaCO_3$ with growth of five tree species on chernozemic soils. Can. J. Soil Sci. 61:173-175.

Chaney, R.L., Brown, J.C. and Tiffin, L.O. 1972. Obligatory reduction of ferric chelates in iron uptake by soybeans. Plant Physiol. 50:208-213.

Chaney, R.L. 1984. Diagnostic practices to identify iron deficiency in higher plants. J. Plant Nutr. 7:47-67.

Chapman, D.J. 1979. Propagation of *Acer campestre*, *Acer platanoides*, *Acer rubrum*, and *Acer ginnala* by cuttings. Comb. Proc. Int. Plant Prop. Soc. v.29, p. 345-348.

Chen, Y. and Barak, P. 1982. Iron nutrition of plants in calcareous soils. Advances in Agronomy 35:217-420.

Crocker,W. 1916. Mechanics of dormancy in seeds. Am. J. of Botany. 3:99-120.

de Cianzo, S.R., Fehr, W.R. and Anderson, I.C. 1979. Genotypic evaluation for iron deficiency chlorosis in soybeans by visual scores and chlorophyll concentration. Crop Sci. 19:644-646.

Dirr, M.A. 1990a. Effects of P-ITB and IBA on the rooting response of 19 landscape taxa. J. Environ. Hort. 8(2):83-85.

Dirr, M.A. 1990b. Manual of Woody Landscape Plants: Their Identification, Ornamental Characteristics, Culture, Propagation and Uses. Fourth edition. Stipes Publishing Co., Illinois. 1007 pp.

Dirr, M.A. and Heuser, C.W. 1987. The Reference Manual of Woody Plant Propagation: From Seed to Tissue Culture. Varsity Press, Inc., Athens, Georgia. 239 pp.

Dragonuk, M.B., Fehr, W.R. and Jessen, H.J. 1989. Effectiveness of nutrient-solution evaluation for recurrent selection for Fe efficiency of soybeans. Crop Sci. 29:952-955.

Dumbroff, E.B. and Webb, D.P. 1970. Factors influencing the stratification process in seeds of *Acer ginnala*. Can. J. Bot. 48:2009-2015.

Ehrlich, W.A., Poyser, E.A. and Pratt, L.E. 1957. Report of Reconnaissance Soil Survey of Carberry Map Sheet Area. Soils Report No. 7. Manitoba Dept. of Agriculture and Immigration and Canada Dept. of Agriculture.

Elgala, A.M. and Maier, R.H. 1964. Chemical forms of plant and soil iron as influenced by soil moisture. Plant and Soil 21:201-212.

Enright, L.J. 1958. Propagation of several species of *Acer* by cuttings. J. Forestry v. 56, p. 426-428.

Fairbanks, D.J., Orf, J.H., Inskeep, W.P. and Bloom, P.R. 1987. Evaluation of soybean genotypes for iron-deficiency chlorosis in potted calcareous soil. Crop Sci. 27:953-957.

Fehr, W.R. 1982. Control of iron-deficiency chlorosis in soybeans by plant breeding. J. Plant Nutr. 5:611-621.

Fleming, A.L., Chaney, R.L. and Coulombe, B.A. 1984. Bicarbonate inhibits Fe-stress response and Fe uptake-translocation of chlorosis-susceptible soybean cultivars. J. Plant Nutr. 7:699-714.

Flint, H.L. 1985. Plants showing tolerance of urban stress. J. Environ. Hort. 3(2):85-89.

Frankel, R. and Galun, E. 1977. Pollination Mechanisms, Reproduction and Plant Breeding. Springer-Verlag, New York. 281 pp.

Fryxell, P.A. 1957. Mode of reproduction of higher plants. Bot. Rev. 23(3):135-233.

Gabriel, W.J. 1966. The onset and duration of stigma receptivity in sugar maple flowers. Forest Science. 12:14-18.

_____. 1967. Reproductive behaviour in sugar maple: self-compatibility, cross-compatibility, agamospermy and agamocarpy. Silvae Genetica 16:165-168.

Gildersleeve, R.R. and Ocumpaugh, W.R. 1989. A greenhouse technique for screening *Trifolium* seedlings for iron-deficiency chlorosis. J. Plant Nutr. 12(10):1141-1152.

Gupton, C.L. and Spiers, J.M. 1992. Inheritance of tolerance to mineral element-induced chlorosis in rabbiteye blueberry. HortScience 27(2):148-151.

Gustafson, F.G. 1939. The cause of natural parthenocarpy. Am J. Botany 26:135-138.

Gustafson, F.G. 1942. Parthenocarpy: natural and artificial. Bot. Rev. 8:599-654.

Harrell, M.O., Pearce, P.A., Mooter, D.P. and Webster, B.L. 1984. A comparison of treatments for chlorosis of pin oak and silver maple. J. Arboric. 10(9):246-249.

Hartmann, H.T., Kester, D.E. and Davies, Jr. F.T. 1990. Plant Propagation : Principles and Practices. Prentice-Hall, Inc., New Jersey. 647 pp.

Heslop-Harrison, J. 1972. Sexuality of Angiosperms. *In* Plant Physiology vol. VI C, Physiology of Development: from Seeds to Sexuality. (F.C. Steward, ed.), pp. 133-289. Academic Press, New York.

Himelick, E.B. and Himelick, K.J. 1980. Systematic treatment for chlorotic trees. J. Arboric. 6(7):192-196.

Holden, M. 1976. Chlorophylls. *In* Chemistry and Biochemistry of Plant Pigments, vol. 2. (T. W. Goodwin, ed.), pp. 1-37. Academic Press Inc., London.

Inskeep, W.P. and Bloom, P.R. 1985. Extinction coefficients of chlorophyll a and b in N,N-dimethylformamide and 80% acetone. Plant Physiol. 77:483-485.

_____. 1986. Effects of soil moisture on soil pCO_2, soil solution bicarbonate, and iron chlorosis in soybeans. Soil Sci. Soc. Am. J. 50:946-952.

Jong, P.C. de. 1976. Flowering and sex expression in *Acer* L. A biosystematic study. Meded. Landbouwhogeschool Wageningen 76-2.

Khan,A.A. 1982. The Physiology and Biochemistry of Seed Development, Dormancy and Germination. Elsevier Biomedical Press, Amsterdam.

Korcak, R.F. 1987. Iron deficiency chlorosis. HortReviews v.9, p. 133-186.

Kurdiuk, O.M. 1984. Peculiarities of flowering and fruiting of East-Asian maples in Ukraine (*Acer* species). Ukrains'kii Botanichnii Zhurnal v.3, p. 44-47.

LaCroix, L.J. and Lenz, L.M. 1967. The use of PI soybeans as an indicator plant for the selection of chlorosis resistant horticultural plants. HortScience 2:114.

_____. 1974. Iron chlorosis - a review of recent developments in Manitoba. Rep. Proc. Annu. Meet. West. Can. Soc. Hortic. 30th : 114-117.

Lindsay, W.L. 1984. Soil and plant relationships associated with iron deficiency with emphasis on nutrient interactions. J. Plant Nutr. 7:489:500.

Lindsay, W.L. and Schwab, A.P. 1982. The chemistry of iron in soils and its availability to plants. J. Plant Nutr. 5:821-840.

Loeppert, R.H. 1986. Reactions of iron and carboantes in calcareous soils. J. Plant Nutr. 9(3-7):195-214.

Loeppert, R.H. and Clarke, E.T. 1984. Reactions of Fe^{2+} and Fe^{3+} in calcareous soils. J. Plant Nutr. 7:149-163.

Loeppert, R.H., Geiger, S.C., Hartwig, R.C. and Morris, D.R. 1988. A comparison of indigenous soil factors influencing the Fe-deficiency chlorosis of sorghum and soybean in calcareous soils. J. Plant Nutr. 11:1481-1492.

Loeppert, R.H. and Hallmark, C.T. 1985. Indigenous soil properties influencing the availability of iron in calcareous soils. Soil Sci. Soc. Am. J. 49:597-603.

Loeppert, R.H., Hossner, L.R. and Chmielewski, M.A. 1984. Indigenous soil properties influencing the availability of Fe in calcareous hot spots. J. Plant Nutr. 7:135-149.

Marquard, R.D. and Tipton, J.L. 1987. Relationship between extractable chlorophyll and an in situ method to estimate leaf greenness. HortScience 22(6):1327.

Marschner, H. 1986. Mineral Nutrition in Higher Plants. Academic Press Inc., London. 674 pp.

_____. 1991. Root-induced changes in the availability of micronutrients in the rhizosphere. *In* Plant Roots: The Hidden Half. (Y. Waisel, A. Eshel and U. Kafkafi, ed.), pp.503-528. Marcel Dekker, Inc., New York.

McMillan-Browse, P.D.A. 1979. Hardy Woody Plants from Seed. Growers Books, London. 163 pp.

Mengel, K., Breininger, M.Th. and Bübl, W. 1984a. Bicarbonate, the most important factor in inducing iron chlorosis in vine grapes on calcareous soil. Plant and Soil 81:333-344.

Mengel, K., Bübl, W. and Scherer, H.W. 1984b. Iron distribution in vine leaves with HCO_3^- induced chlorosis. J. Plant Nutr. 7:715-724.

Mengel, K. and Geurtzen, G. 1986. Iron chlorosis on calcareous soils. Alkaline nutritional condition as the cause for the chlorosis. J. Plant Nutr. 9(3-7):161-173.

Mengel, K. and Kirkby, E.A. 1978. Principles of Plant Nutrition. International Potash Institute, Switzerland. 593 pp.

Messenger, A.S. 1984. Treatment of chlorotic oaks and red maples by soil acidification. J. Arboric. 10(4):122-128.

Miller, G.W., Pushnik, J.C. and Welkie, G.W. 1984. Iron chlorosis, a world wide problem, the relation of chlorophyll biosynthesis to iron. J. Plant Nutr. 7:1-22.

Morris, R.L. and Swanson, B.T. 1980. Mineral and chlorophyll changes in leaf tissue of silver maple after treatment with iron chelates. J. Amer. Soc. Hort. Sci. 105(4):551-555.

Neely, D. 1973. Pin oak chlorosis. J. Forestry 71:340-342.

_____. 1976. Iron deficiency chlorosis of shade trees. J. Arboric. 2(7):128-130.

Norton, C.R. 1987. Post harvest age-induced seed dormancy of *Acer ginnala* and its alleviation by growth regulator and low temperature treatments. Ann. Appl. Biol. 110:169-174.

Olsen, R.A. and Brown, J.C. 1980. Factors related to iron uptake by dicotyledonous and monocotyledonous plants. I. pH and reductant. J. Plant Nutr. 2(6):629-645.

Olson, Jr. D.F. and Gabriel, W.J. 1974. *Acer* L. Maple. *In* Seeds of Woody Plants in the United States. pp.187-194. U.S. Dept. Agric. Handbook. USDA Forest Service.

Percival, M. 1965. Floral Biology. Pergamon Press Ltd., London. 243 pp.

Pinfield, N.J. and Dungey, N.O. 1985. Seed dormancy in *Acer*: An assessment of the role of the structures covering the embryo. J. Plant Physiology 120:65-81.

Pinfield, N.J., Stuchbury, P.A. and Bazaid, S.M. 1987. Seed dormancy in *Acer*: Is there a common mechanism for all *Acer* species and what part is played in it by abscisic acid? Physiologia Plantarum 71:365-371.

Poehlman, J.M. 1987. Breeding Field Crops. Third edition. AVI Publishing Co., Inc., Westport, Connecticut. 724 pp.

Powell, L.E. 1987. Hormonal aspects of bud and seed dormancy in temperate-zone woody plants. HortScience 22(5):845-850.

Raven, P.H., Evert, R.F. and Curtis, H. 1981. Biology of Plants. Third edition. Worth Publishers, Inc., USA. 686 pp.

Römheld, V. and Marschner, H. 1986. Mobilization of iron in the rhizosphere of different plant species. *In* Advances in Plant Nutrition, vol.2. (B. Tinker and A. Läuchli, ed.), pp. 155-204. Praeger Scientific, New York.

_____. 1991. Function of micronutrients in plants. *In* Micronutrients in Agriculture. (J.J. Mortvedt, F.R. Cox, L.M. Shuman and R.M. Welch, ed.), pp. 297-328. Soil Science Society of America, Inc., Madison, Wisc.

Ross, W.M. 1986. Improving plants for tolerance to iron deficiency and other mineral nutrition problems : Breeding and genetics point of view. J. Plant Nutr. 9(3-7):309-333.

Salisbury, F.B. and Ross, C.W. 1985. Plant Physiology. Third edition. Wadsworth Publishing, Inc., USA. 540 pp.

Schoeneweiss, D.F. 1973. Correction of lime-induced chlorosis of pin oak by liquid soil injection. HortSci. 8(4):333-334.

Sedgley, M. and Griffin, A.R. 1989. Sexual Reproduction of Tree Crops. Academic Press Inc., San Diego, California. 378 pp.

Sijmons, P.C. and Bienfait, H.F. 1984. Mechanism of iron reduction by roots of *Phaseolus vulgaris* L. J. Plant Nutr. 7:687-693.

Snyder, L. 1980. Trees and Shrubs for Northern Gardens. University of Minnesota Press, Minneapolis. 411 pp.

Sullivan, J.R. 1983. Comparative reproductive biology of *Acer pensylvanicum* and *A. spicatum* (Aceraceae). Am. J. Botany 70(6):916-924.

Terry, N. and Abadia, J. 1986. Function of iron in chloroplasts. J. Plant Nutr. 9:609-646.

Watson, G.W., Kupkowski, G. and von der Heide, K. 1991. Pin oak root injury from soil acidification with sulfuric acid. J. Environ. Hort. 9(2):102-105.

Weiss, M.G. 1943. Inheritance and physiology of efficiency in iron utilization in soybeans. Genetics 28:251-268.

Whitcomb, C.E. 1986. Solving the iron chlorosis problem. J. Arboric. 12(2):44-48.

Williams, P.H. 1980. Bee-sticks, an aid in pollinating Cruciferae. HortScience 15(6):802-803.

Yaalon, D.H. 1957. Problems of soil testing in calcareous soils. Plant and Soil. 3:257-288.

Yadava, U.L. 1986. A rapid and nondestructive method to determine chlorophyll in intact leaves. HortScience 21(6):1449-1450.

Zaiter, H.Z., Coyne, D.P. and Clark, R.B. 1987. Genetic variation and inheritance of resistance of leaf iron-deficiency chlorosis in dry beans. J. Amer. Soc. Hort. Sci. 112(6):1019-1022.

APPENDICES

APPENDIX A. The Description and Selected Physical and Chemical Properties of Experimental Soils (Chapter 1)

Profile Description of Pedon #1 near Teulon, Mb

Soil Series:	Lakeland
Subgroup:	Gleyed Rego-Black (carbonated phase)
Location:	Moeller Farm, 23-16-2E
Date:	July 21, 1992; bright day with clear skies
Described by:	Tee Boon Goh and Martha Barwinsky
Topography:	The pedon is located in the south part of the farmyard with flat to gently undulating topography
Drainage:	Imperfect to moderately well drained
Vegetation:	Bluff of Colorado spruce (*Picea pungens* Engelm.) with underbrush of redosier dogwood (*Cornus sericea* L.) and Manitoba maple (*Acer negundo* L.); crested wheatgrass, mustard (*Brassica* sp.), stinkweed (*Thlaspi arvense* L.), Shepherds purse (*Capsella bursa-pastoris* (L.) Medic.) were also present
Parent material:	Mixture of (fine) lacustrine, and (mixed textured, usually medium) till

Ahk_1 0 - 18 cm. Black (10YR 2.5/1) silty clay loam; fine, strong granular; abundant medium-sized fibrous grass roots; aggregates strongly held by root mat; friable, slightly plastic; strong evidence of earthworm activity in the form of uniform biochannels approximately 2 to 4 mm in diamter; 5% stones of 1 to 2 cm diameter; friable, slightly plastic when wet; weak to moderate effervescence; pH 7.1 (1 soil:1 water suspension); indistinct boundary to:

Ahk_2 18 - 36 cm. Black (10YR 3/1) silty clay loam; coarse, strong granular grading to medium blocky; stones absent; abundant roots, 0.5 to 2 cm in diameter, non-fibrous from trees; no evidence of earthworm activity; firm; strong effervescence; pH 7.1; sharp, straight boundary to:

Ckg 36 - 110 cm. Brownish-grey (2.5YR 5/2-5/3) silty clay loam; weak, fine blocky; fine roots from trees extending to 81 cm; reddish brown to dark brown staining on walls of root channels possibly due to iron or manganese and decomposing roots; powdery charcoal fragments evident; firm; slightly sticky and plastic when wet; because of the lower organic matter, the soil was more dense than the Ahk_2; very strong effervescence; pH 7.5;

Auger boring to:

Cca 110+ cm. Grey; very strong to violent effervescence.

Table A-1. Laboratory Analysis of Pedon #1 near Teulon, Mb - Tilled Field (Norwest Labs, March 16, 1992).

Depth (cm)	Iron (ppm)	Carbonate Content	Total CO_3 (%)	Calcite (%)	Dolomite (%)
0-15	19	medium	20.3	10.4[1]	9.1[1]
16-60	--	very high	41.3	17.2	22.2

[1]Quantitative manometric determination of calcite and dolomite.

Depth (cm)	pH	Nitrate (ppm)	Available Phosphorus (ppm)	Available Potassium (ppm)
0-15	8.0	10.2	13.6	200
15-60	---	9.2	----	---

Profile Description of Pedon #2 near Brookdale, Mb (Ehrlich et al, 1957)

Soil Series:	Newdale Association - smooth phase
Subgroup:	Black-Meadow
Location:	McKee Farm, 5-13-16
Topography:	Smooth to slightly undulating
Drainage:	Imperfect to poorly drained
Vegetation:	Wheat and prairie grasses.
Parent material:	Medium-textured, moderately calcareous boulder till of mixed shale, limestone and granitic rock

A 10 - 20 cm. Very dark grey loam to clay loam; finely granular; friable when moist, hard when dry, slightly alkaline in reaction.

B 2.5 - 10 cm. Very dark greyish brown loam to clay loam; subangular blocky; friable when moist, hard when dry; alkaline in reaction.

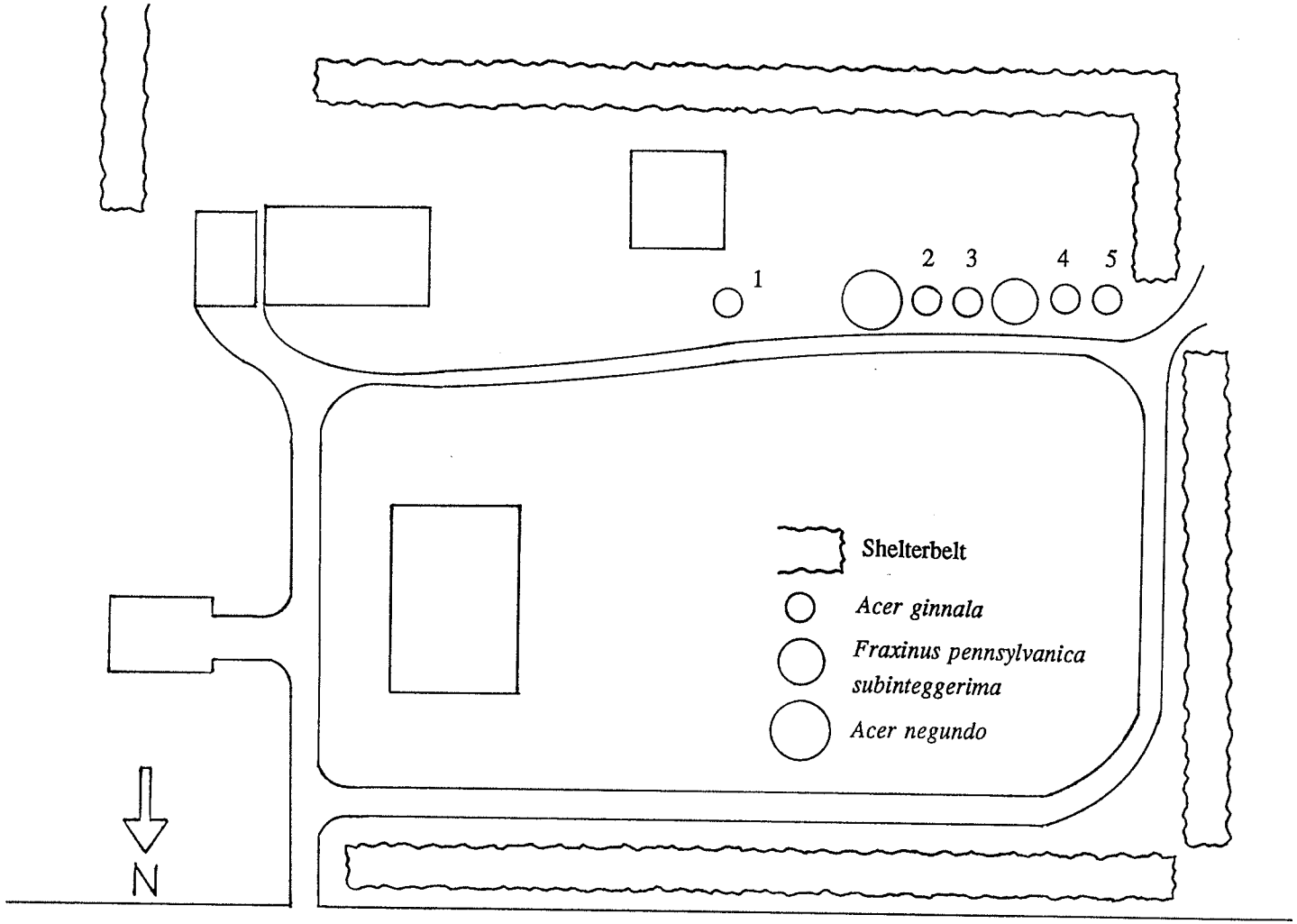
Cca Very pale brown loam to clay loam, carbonate accumulation of variable thickness; fades gradually into:

C Light yellowish brown loam to clay loam boulder till; amorphous; weakly cemented when dry, plastic and sticky when wet; some iron staining and may contain gypsum crystals.

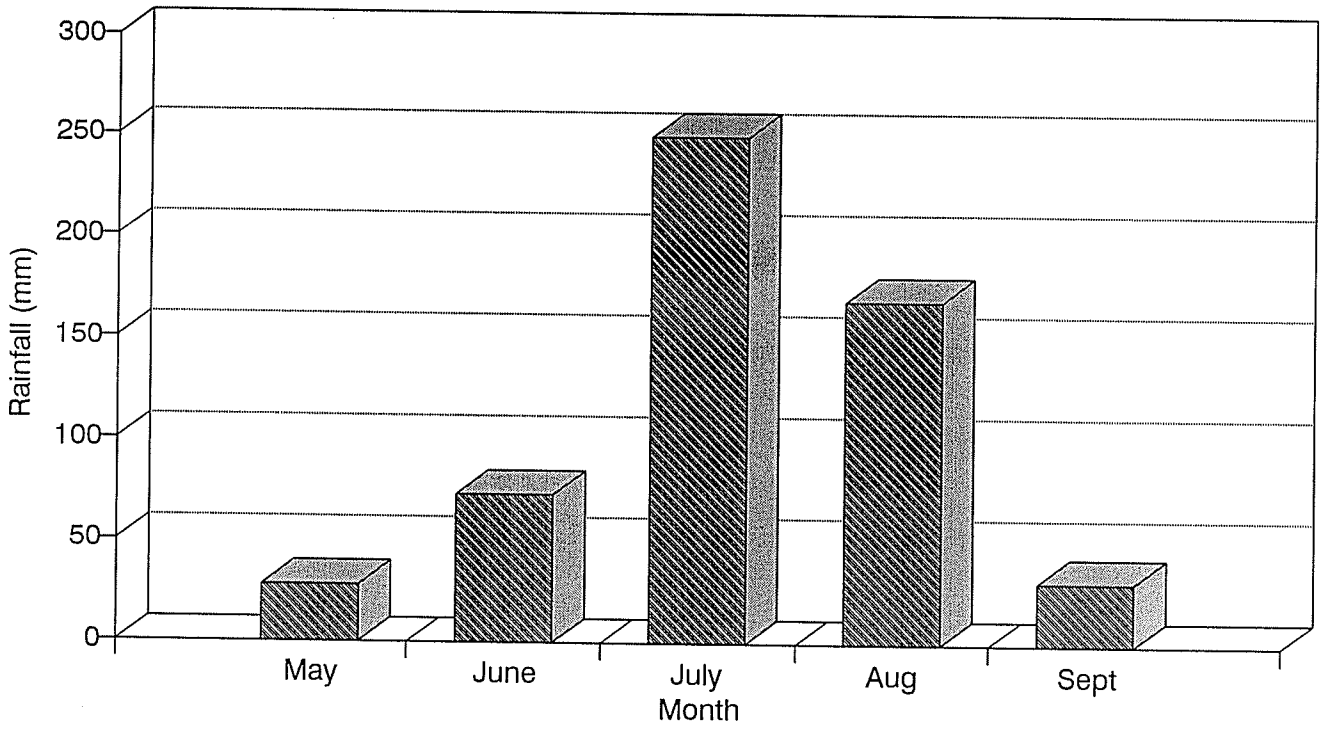
Table A-2. Chemical and Physical Analyses of Pedon #2 near Brookdale, Mb (Ehrlich et al, 1957)

Depth (cm)	pH	Total CO_3 %	Nitrate %	Available Phosphorus (ppm)	Available Potassium (ppm)
surface	7.6	---	0.37	5-12	150-200
0-7.5	7.1	0.4	0.73		
7.5-22.5	7.1	0.2	0.34		
22.5-30	7.5	3.0	0.14		
30-37.5	7.6	12.1	0.11		
37.5-45	7.6	13.2	0.08		
45-60	7.6	15.5	0.07		

APPENDIX B. Location of Plants of *A. ginnala*, near Teulon, Mb.



APPENDIX C. Precipitation (mm) at Experimental Site near Teulon, Mb, 1993 (Chapter 1)



APPENDIX D. Ambient Temperature (°C) during Flowering of *A. ginnala*, 1992 (Chapter 2).