NUTRITIONAL AND PHYSIOLOGICAL STUDIES OF *OXALIS REGNELLII*

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Shamrock Plant (*Oxalis regnellii*) is an ornamental plant grown primarily for St. Patrick's Day. Interveinal chlorosis is a production problem and has been hypothesized to be caused by micronutrient deficiencies, either iron (Fe) or manganese (Mn) or virus. Several experiments were conducted testing these hypotheses. A virus screen was conducted and positive identification was found and plants were rouged.

Oxalis plants were grown hydroponically on five NO_3^--N:NH_4^+-N ratio treatments. Increasing proportions of NO_3^--N increased total leaf, root, flower, and bottom shoot biomasses. Other hydroponic studies were conducted removing Fe and Mn from nutrient solutions to characterize the respective deficiencies. Iron deficiency, characteristic interveinal chlorosis on newly developed leaves was observed as early as three weeks after removing Fe. Plants grown without Mn did not exhibit interveinal chlorosis, but were slightly less green than control plants grown in complete nutrient solutions. Iron deficiency was also induced in a greenhouse media, using dolomitic lime. Again, typical interveinal chlorosis and reduced plant growth was observed after two weeks. Tissue analysis confirmed the chlorosis was due to reduced Fe as opposed to limited N or Mn concentrations. Our data suggest Fe influences interveinal chlorosis more than Mn.

Micronutrient chelate foliar and media applications were assessed as corrective measures for foliar chlorosis. Media drenches of Fe-EDDHA were effective in re-greening Fe deficient oxalis in 5 days. Foliar chelate applications were less effective.

Organic acids, such as oxalic acid often assist plants in nutrient acquisition through root exudation. Oxalic acid levels of recently matured leaves increase in Fe deficient *O. regnellii* plants.

Cultural practices such as temperature, irrigation, and fertilizer selection also influence plant growth and incidence of leaf chlorosis. Our research found that cooler temperatures (13˚ C) did not increase chlorosis and temperatures of at least 21˚ C produced more aesthetically pleasing and floriferous plants. Overhead irrigation, compared with subirrigation produced higher quality and larger *O. regnellii.* Plant growth and development is best when fertilized with N rates between 100 mg $N \cdot L^{-1}$ and 350 mg $N \cdot L^{-1}$.

BIOGRAPHICAL SKETCH

Chad Thomas Miller was born and raised in northwest Wisconsin, providing him a rich opportunity to grow and develop an interest for many things. He loved to hunt and fish and enjoy nature. The abundance of dairy farms along with a family of farming heritage, no doubt had an influence on his interest in plants and science. Chad was a hard worker and believed in his studies. Shortly before high school, he was introduced to horticulture**.** A neighbor lady gave him some bulbs, dahlias and glads, and it all grew from there. In high school and most of college, Chad worked at Rogers Concrete Creations painting ornamental concrete lawn ornaments.

Chad enrolled in the fall of 1997 at the University of Wisconsin—River Falls (UWRF), where he really developed a passion for horticulture. Chad was active in extra-curricular activities at UWRF, serving as the president of the horticulture society and was an active member of the Pi Alpha Xi Floral Crop Quality Evaluation team. As a member, he judged at the national level, and earned an overall individual award for judging potted plants, and later served as assistant coach for the team, under the direction of Dr. Terry Ferriss (his advisor)**.** He also served as the student chair for the national competition hosted by UWRF in 2002.

Chad was determined to make a trip to the Netherlands and did so in the spring of 2001 where he participated in an internship at Royal Van Zanten, working with alstroemeria and gerbera. This opportunity reassured his passion for the floriculture industry. He returned to UWRF and graduated with honors in May of 2002. Upon graduation, he interned as a plant breeder at Pan American Seeds, in California.

Upon completion of his internship at Pan American Seeds, Chad enrolled at Cornell University in the graduate program and received his M.S. with Dr. Mark Bridgen and his Ph.D. with Dr. Bill Miller. After his M.S. degree, Chad was awarded the Dreer Fellowship Award in which he spent another year in the Netherlands,

conducting lily and tulip breeding at Wageningen University under Dr. Jaap van Tuyl and also conducted physiology research at the PPO in Lisse with Dr. Henk Gude.

Chad loved education and always wanted to teach horticulture. It was his aspiration to be able to help others learn not only about plants, but help others learn about themselves and the world around them.

This is dedicated to my family and all my friends.

Thank you for all your love and support.
Really, this would not be possible without each and every one of you.

Love and compassion are necessities, not luxuries.
Without them humanity cannot survive.
- Dalai Lama

Doubt whom you will, but never yourself.
-C. Bovee

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I also want to thank Dr. Terry Ferriss, my undergraduate advisor. Dr. Ferriss is not only an advisor but a great friend, for whom I deeply respect. We shared many laughs and many times she listened and provided a shoulder for me to lean on—and she still does. I would not be writing this if it were not for her guidance and mentorship. I will always be grateful for that. Thank you so very much. I hope when I am a faculty member somewhere that I am able I am able to inspire a student like she has me.

I also want to express my gratitude for all of the Dutch friends and colleagues I have befriended working in the Flowerbulb Research Program and those I made during my Dreer Fellowship experience. My experience was more than I ever expected, both professionally and personally. Heel erg bedankt!

The first couple of years at Cornell were some of the most difficult, yet, some of the most exciting. The last ~5 years have also had their trials and tribulations. I have to thank all of the other graduate students who have provided assistance over the years, whether it was a statistics question or if they wanted to go grab a coffee or beer. I am thankful for their advice and willingness to listen when things seemed to be rough—I am a better person because of them. Thank you Amaya, Chris, Denise, Michael, Maren, Michelle, Obdulia, Raza, Rebecca, and Susan, and to the many others who have been a part of this experience.

And then there is Cheni, or China as we affectionately know her. The Filios family has been my Ithaca family. Where do I start? A little part of me is now Greek—even though I cannot speak a lick of Greek. I will not forget the Cabana nights or the great Greek Easters (and all of the wonderful lamb entrails...). At some point, I will attempt to make baklava and spanikopita, but it won't be as authentic as it from Candy's kitchen. I guess that just means I will have to come back to Trumansburg on occasion. Ευχαριστώ.

Whenever I see an African violet or miniature schnauzer, I will always be reminded of Dr. Tom Weiler. Tom served as an advisor on my Master's project and has become a great friend and mentor. We have cooked and shared countless dinners (and bottles of wine)—thanks so much, Tom.

The trips to DC and Montreal with Chris and Will won't be forgotten. I cannot forget about all the other teachers and many other countless friends who have been a

part of my life. There are many others who I have shared wonderful memories with--Thank you.

And last but not least, I need thank my family—Mom and Rick, Dad and Mary, Tommy, Derek, Adam, and Emily. Without their support and guidance, I don't know that I could have done this. They believed in me and have allowed me to follow my dreams and develop into the person I am. I thank them for instilling in me a strong work ethic and a kind, caring, open heart. And to Grandpa and Grandma Clark and Grandpa Miller, looking from above…I love you all.

> "All of life is a journey which paths we take, what we look back on, and what we look forward to is up to us. We determine our destination, what kind of road we will take to get there, and how happy we are when we get there."

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

Introduction and Literature Review

Growing minor or niche greenhouse crops often times is an art because little information is known about individual species' growth and development, let alone any number of cultivars that may be in production. Because production numbers are limited, limited resources are invested in gathering more information about the necessary greenhouse forcing requirements. There may be a need or desire to learn and know more about a specific crop, but time and resource investment to advance any understanding of the crop is necessary. Oxalis is just one example of a niche crop with greenhouse potential.

The interveinal chlorosis phenomenon in *Oxalis regnellii* has perplexed growers for years. Information regarding the chlorosis phenomenon in *Oxalis regnellii* and greenhouse cultural information is limited and many aspects are presented in this dissertation. Four objectives in particular were addressed through this research. They were as follows:

1) Determine if clonal stock of *O. regnellii* is virus infected.
2) Develop and establish a hydroponic system for culturing *O. regnellii*.
3) Characterize and describe Fe deficiency in *O. regnellii* through nutrient deletion and pH modification.
4) Evaluate irrigation type and fertilization rates for greenhouse forcing of *O. regnellii*

The following chapter provides a brief introduction to floriculture and its relevance, along with current information known about *O. regnellii* forcing. This chapter also provides background information into several factors that can be involved in chlorosis, including virus, roles of specific plant nutrients, along with a brief discussion about oxalic acid.

Floriculture

Floriculture is an important component of United States agriculture. In 2005, it was estimated that one third of the estimated $50 billion of agricultural sales were from greenhouse and nursery crops (ERS/USDA, 2007). Recent statistics from the USDA/NASS indicate that the value of potted flowering plants was valued at $632 million dollars from growers with sales of $100,000 or more across the United States (USDA, 2010). Product mix is a very important aspect in floriculture marketing and there has been a growing interest in new crops as consumer preferences are changing. Different, new and exciting crops for gift-giving holidays and other occasions are needed to meet consumer demands (Craig, 2003; Evans, 2002), in addition to increasing the diversity of materials for use in container or outdoor plantings.

Many of the commercially important floriculture species used for cut flowers and potted plants are geophytes, or bulbs. As global demand for flower bulbs increases, additional research into production and marketing of other interesting bulbous crops (Kamentsky et al., 2005) will be important for the floriculture industry, particularly in lesser known species. *Oxalis regnellii,* (oxalis) also known as "The Shamrock Plant" is a specialty potted bulb crop grown for its clover-like leaves and white flowers. In the United States, oxalis is marketed in the spring, primarily for the St. Patrick's Day holiday (Dole and Wilkins, 2005; Miller, 1997). Oxalis is susceptible to foliar disorders including wrinkled leaves, leaf edge burn, but most importantly, interveinal chlorosis. While the exact cause(s) of interveinal chlorosis remain unclear, two commonly hypothesized explanations are iron (Fe) deficiency and/or virused plant material (De Hertogh, 1996; Dole and Wilkins, 2005). However, there has been very little investigation to substantiate these claims. Preliminary studies indicate that Fe deficiency may be a contributing factor to the interveinal chlorosis in oxalis and merits further investigation.

Geophytes

Plants with modified storage organs derived from three main plant parts, namely the root, shoot, and stem are known as geophytes. In many geophytes, more than one of the above-mentioned organ modifications is involved in ensuring species survival (Rees, 1992). The common geophyte examples are (true) bulbs, tuberous roots, tuberous stems, rhizomes, corms, and enlarged hypocotyls. While botanically incorrect, gardeners often refer to these structures as 'bulbs.' Root tubers are modified roots, whereas stem tubers, rhizomes and corms are modifications of stem tissue. Bulbs are primarily composed of leaf and stem tissue (Rees, 1992). The possession of a geophytic structure provides a species independence from the periodicity of its habitat (Rees, 1992). Geophytes are endemic to all climactic regions of the world, with most geophytes found between 23° and 45° latitude (De Hertogh and Le Nard, 1993). Ornamental geophytes are a diverse group of plants, belonging to more than 800 different genera (Benschop et al., 2010). The unifying characteristic of geophytes is the ability of each respective storage organ to provide food reserves that enable the plant to grow and flower and survive during unfavorable periods of growth in its endemic environment, when natural synthesis of metabolites is not supported (Rees, 1992). With these stored food reserves, growth and development can be hastened, allowing rapid maturation during favorable environmental periods.

Rhizomes are specialized stems which grow at or just below the soil surface. There are two types of rhizomes, pachymorphic and leptomorphic. Pachymorph rhizomes are characterized by a large, fleshy horizontal stem with determinate growth. *Oxalis regnellii* rhizomes are leptomorphic rhizomes characterized by smaller, fleshy structures that remain vegetative and develop many flower shoots (Albrecht, 1998). *Oxalis regnellii* rhizomes are slender structures comprised of tiny modified leaf scales (Ingram, 1959) and maintain a supply of food reserves (presumably carbohydrates) for

perrenation, serving as a survival mechanism. These rhizomes, in the case of *Oxalis*, also function as a means of propagation, as the mother plant may develop several daughter rhizomes over the growing season, each capable of surviving on its own.

Dormancy is described as the (temporary) cessation of visible growth, particularly of the apical meristem (Lang, 1987). Sometimes cessation of growth or induced 'dormancy' can be due to external factors such as temperature or daylength. In some species, growth cessation may be due to innate or natural factors (Rees, 1992). In the horticultural sense, a bulb, corm, or tuber with no emergent shoot or roots is apparently 'dormant', and therefore protected from unfavorable environments.

However, examination of the apical meristem often reveals significant developmental activity. For example, in an apparently 'dormant' tulip, initiation of leaf, flower, and root primordia occurs continually during the summer after lifting. This activity illustrates the success of the survival strategy during the unfavorable period while simultaneously providing for the continuation of developmental processes despite adverse conditions (Rees, 1992).

Lang (1987) subdivided dormancy into endodormancy, paradormancy, and ecodormancy. Endodormancy is defined as an environmental or endogenously signaled perception by the organ itself; paradormancy is a response to a biochemical signal from other organ(s), and ecodormancy is a result of any number of environmental factors. Kamerbeek et al. (1970) described three types of dormancy: 1) "Deep dormancy", such as that seen in lily and gladiolus that require cold treatment to overcome dormancy and flowers are initiated after the start of shoot elongation and the initiation of a large number of leaves, [we now know the specific type in many *Lilium* to actually be vernalization (Langhans and Weiler, 1968)]; 2) "Tulip type", in which there is little if any true physiological dormancy. Flower initiation occurs before or shortly after aerial vegetation dies, and extension growth requires a period of cold,

with the timing of emergence and anthesis determined by ambient temperatures; 3) No physiological dormancy, as seen in bulbous iris, which is typically observed as summer dormancy, after high temperature. Growth resumes as temperatures decrease and leaves emerge in autumn and flowering occurs in the spring after initiation during the low temperature period. This third type of dormancy may be characteristic of *Oxalis regnellii*, however little is known about its dormancy, if any.

Oxalis

General Characteristics

Oxalis L. is a member of the Oxalidaceae. The name oxalis is derived from Greek meaning 'sour' or 'acid', due to the characteristic presence of oxalic acid in the family. There are reported to be between 600 and 900 species of *Oxalis* (Bahattacharyya and Johnri, 1998; Bryan, 2002; De Azkue, 2000; Dole and Wilkins, 2005; Wilkins, 1985) with only a few cultivated for ornamental purposes such as indoor potted flowering plants or patio containers. Some of the ornamental species include *O. adenophylla, O. bowiei, O. tetraphylla* (syn. *O. deppei*), *O. versicolor*, *O. regnellii* and *O. triangularis* (De Hertogh and Le Nard, 1993; Wikesjo and Shussler, 1981; Wilkins, 1985). Some species, such as *O. deppei* and *O. tuberosa* (oca) produce edible roots or tubers (Wilkins, 1985). *Oxalis* L. is native to all continents, with two main centers of diversity: coastal South Africa and subtropical America (Argentina, Bolivia, Brazil, Paraguay, and Peru) (De Azkue, 2000; De Hertogh and Le Nard, 1993; Dole and Wilkins, 2005). It is not entirely clear, but from limited accession data, *O. regnellii* appears to be native to at least four states in Argentina, and is also found in Bolivia, and Paraguay (Fig. 1.1). Bryan (2002) and Hollowell (1999) also

Figure 1.1. A map of *Oxalis regnellii* accessions on record at the Missouri Botanical Garden; St. Louis, Missouri.

report that *O. regnellii* is found in Brazil and Peru. Salter (1944) produced an extensive monograph of the South African species, however a modern South American species monograph has yet to be completed.

Nomenclature regarding *O. regnellii* varies, depending on the source referenced (Dole and Wilkins, 2005). Within this dissertation, I will refer to the green leaved variety as *O. regnellii*. However, *O. regnellii* is synonymous with *O. regnellii* Miq. (Dole and Wilkins, 2005; Miller, 1997), *O. cathariensis* (Bryan, 2002), *Acetosella papilionacea* Kuntze, *Acetosella regnellii* Kuntze; *O. palustris* A. St.-Hil.; *O. papilionacea* Hoffmans. Ex Zucc; and *O. vernalis* Fredr. Ex Norlind (Hollowell, 1999). *Oxalis triangularis* (synonymous to *O. triangularis* ssp. *papilionacea* Lourteig) refers to the purple leaved variety, and is considered to be a separate species (De Hertogh, 1996).

Oxalis regnellii is an acaulescent species (the stem rarely rises above the soil level) and exhibits a rosette of leaves and inflorescences that arise from the soil (Ingram, 1959). The compound leaves are most often trifoliate and alternately arranged. Leaf colors for species and selections range in color from green to purple and sometimes include leaf markings. Leaves are nyctanastic and also close during hot and/or cloudy conditions. Flowers are white or pink and are borne on an umbellate cyme, having 5 basally fused sepals and petals. Flower colors in other *Oxalis* species include red, yellow, and orange.

Propagation and Dormancy

Some *Oxalis* readily propagate sexually from seed, lending themselves to their invasive, weedy nature. *Oxalis* species are unique as they exhibit tristylic heteromorphy, in which two groups of five fertile stamens are found in two different planes (Fig. 1.2). This arrangement can reduce or prevent inbreeding. Tristyly morphology is unique and is only found in two other flowering plant families,

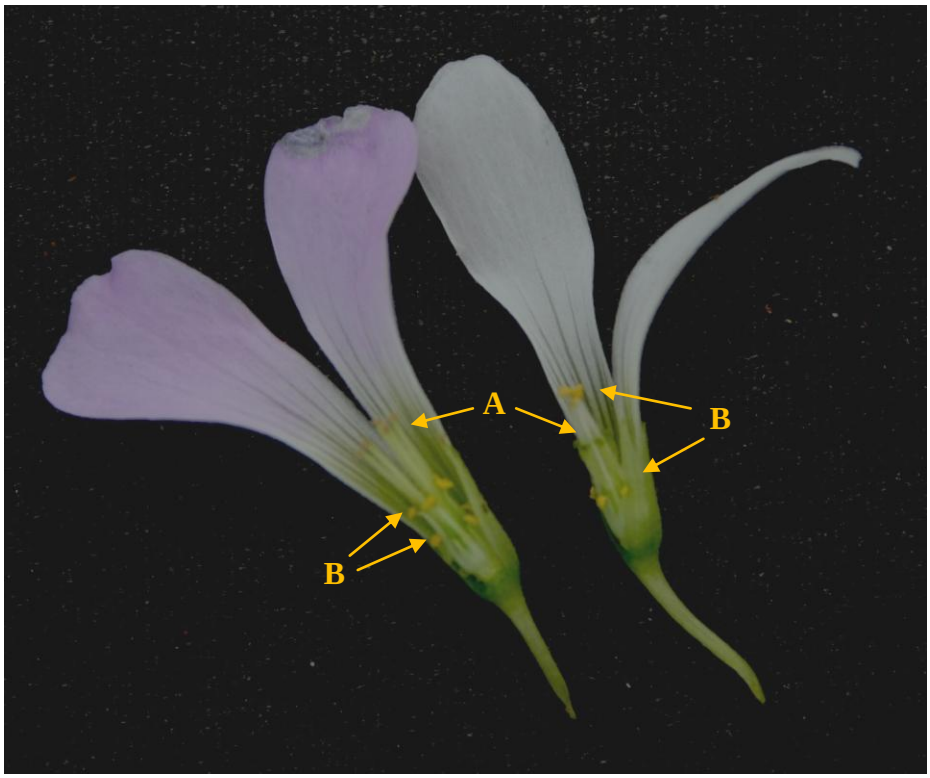

Figure 1.2. Flower anatomy of *O. triangularis* (left floret) and *O. regnellii* (right floret) exhibiting tristylous heteromorphy; one plane of pistils (A) and two planes of stamen (B).

Lythraceae and Ponteriaceae (Watson, 1993). *Oxalis regnellii* is a tetraploid (2n=4x=28) (De Azkue, 2000; Naranjo et al., 1982), but little information exists regarding sexual propagation in this species.

Asexual propagation from rhizomes, bulbs, tubers, and creeping rhizomatous rootstocks also occurs in *Oxalis*. Generally, South African species form bulbs, while South American species develop rhizomes or tubers (Dole and Wilkins, 2005). As previously described, *O. regnellii* develops underground scaly rhizomes (Dole and Wilkins, 2005). Although not reported in the literature, proliferation-like adventitious shoots occasionally develop at the junction of the three leaflets in *O. regnellii* and *O. triangularis* (personal observation).

Commercially, rhizomes are produced in The Netherlands and the United States (California and Oregon) (De Hertogh, 1996). In total, there are approximately 6,000 to 8,000 m^2 (P. Van Leeuwen, personal communication) of *O. regnellii* and *O. triangularis* produced in the Netherlands. There is likely similar production numbers in the United States (M.A. Mellano, personal communication). In commercial practice, rhizomes are harvested from as August to October. After lifting, rhizomes are separated, graded and stored in a cool environment at 1-5° C until shipment in November or December (De Hertogh, 1996; Dole and Wilkins, 2005). Dole and Wilkins (2005) report that rhizomes can be stored for 10+ months in moist peat, while Mellano (personal communication) suggested a much shorter storage period of one to two months. In our research, we have stored oxalis rhizomes longer than two months, up to four months with no ill effects on greenhouse growth.

Protocols for micropropagation of oxalis are limited. Teng and Ngai (1999) tested several different explant sources of *O. triangularis*, including leaves, petioles, bulb scales, and suspension cells (derived from regenerated bulbs) in different systems; bulb-derived suspension cells were most regenerative.

It is reported that moisture stress, cold temperatures, inadequate nutrition, or other unfavorable growing conditions may induce dormancy in oxalis. However, it is also known that growth may persist for long periods of time (years) if plants are provided with adequate water and nutrition (Dole and Wilkins, 2005; Wilkins, 1985). It is not known whether true dormancy exists in *O. regnellii* or whether a dormant period is beneficial. Growth observed from freshly harvested bulbs of *O. tetraphylla* was poor compared to growth from bulbs that were stored long term (storage period was not reported) (Dole and Wilkins, 2005).

Current Status of *Oxalis regnellii* Greenhouse Culture

Forcing Conditions and Flowering

Typically, two to three rhizomes per 10 cm pot are planted 1 cm deep in a well drained media with a pH of 6 to 7 (De Hertogh, 1996). Recommended forcing temperatures for *O. regnellii* are 21-24°C until plants are well rooted, and then adjusting the night temperature to 18-21°C is suggested (De Hertogh, 1996; Miller, 1997). A low to medium light intensity (1000 to 2500 foot candles; 200 to 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$) is adequate (De Hertogh, 1996; De Hertogh and Le Nard, 1993). *Oxalis regnellii* pots are typically marketable after 6 to 7 weeks.

Photoperiod effects have not been fully investigated for *O. regnellii*. In a published abstract, De Hertogh, et al. (1995) examined two different photoperiods on oxalis, but no data were actually reported. Photoperiod effects have been reported in other species such as *O. adenophylla*, *O. deppei*, *O. pes-caprae*, and *O. crassipes* 'Rosea', in which extended long days of 14 to 16 hours produced better flowering plants (Whitman et al., 2001; Wikesjo and Shussler, 1981).

Nutrition

Fertilization recommendations for oxalis are limited. De Hertogh and Le Nard (1993) recommend a 14-14-14 Osmocote application after visible growth or a weekly 200 ppm N of 20-20-20 liquid feed.

Pests, Diseases and Disorders

Oxalis is relatively free of pests and diseases. Dole and Wilkins (2005) report spider mites (*Tetranychus urticae*) and fungus gnat larvae (*Bradysiai* spp.) on oxalis. The major disease problem occurring during storage and shipment is *Penicillium* (Dole and Wilkins, 2005), which can be reduced with a fungicide dip (Mellano, personal communication). Powdery mildew (*Microsphaera russellii*) and rust (*Puccinia* sp.) have also been reported to affect oxalis (Horst, 1990). Oxalis is susceptible to several foliar disorders (Table 1.1) including wrinkled leaves, leaf edge burn, and interveinal chlorosis (De Hertogh, 1993; De Hertogh, 1996; Dole and Wilkins, 2005). De Hertogh et al. (1995) reported that wrinkling and chlorotic leaf disorders on *O. regnellii* were more prevalent when plants were grown under cool greenhouse conditions (less than 21 ºC) compared to those grown in a growth chamber at 26 ºC. The only virus to have been reported in *O. regnellii* is Shamrock Chlorotic Ringspot Virus (SCRV) and once infected, is lethal (Coyier, 1981).

Growth Regulation

When grown under ideal conditions, oxalis does not need growth regulators. However, low light situations often occur during typical *O. regnellii* forcing periods (December to March), especially in the northern latitudes, and can lead to increased petiole lengths, or "legginess". Bonzi sprays of 1 to 4 ppm or drench applications 0.05 to 0.1 mg/a.i. along with A-rest spray applications of 33 ppm were effective in controlling plant height in *O. regnellii* and *O. triangularis* (Miller, 1997; Miller, 2001).

Table 1.1 Physiological leaf disorders reported in *Oxalis regnellii*.

Disorder	**Symptom**	**Cause or hypothesized cause**
Chlorosis	Yellowing of leaves; sometimes interveinal	Nutrition deficiency Cool temperatures Virus
Mottling	White spotting	Thrips damage
	Yellow spotting; ringspots	Virus
Wrinkling or puckering	Edges of leaves curl up, pucker and cup	Nutritional disorder? Pests; i.e. Thrips (*Thrips tabaci)*, Aphids ?
Bronze leaves	Leaves turn bronzy-orange	Cool forcing temperatures
Rust spots	Leaves exhibit brown, chlorotic looking spots	Rust (*Puccinia* sp.)

Plant Nutrition

Seventeen nutrients; carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P), potassium (K), sulfur (S), magnesium (M), calcium (Ca), iron (Fe), manganese (Mn), boron (B), copper (Cu), zinc (Zn), molybdenum (Mo), chlorine (Cl), nickel (N) have been determined to be essential for proper plant growth and development. Understanding mineral nutrition and the role of each nutrient is important to produce high quality plants. For decades, researchers have conducted experiments in order to determine the critical minimum and maximum levels and the interaction of the minerals needed for plant growth. Paparozzi (2003) reviewed the progress that has been made in floriculture crops and nutrition. However, with the continued development and introduction of new cultivars/species and improvement of 'old' species, coupled with new production technologies, continued understanding of nutrition in relation to these factors is imperative to produce marketable crops.

Nitrogen Nutrition

Nitrogen is a major constituent of proteins and amino acids, which play essential roles in plant development. Nitrogen has a controlling effect on plant growth, fruiting, and plant quality and is the element required in greatest concentration for plant development, and is often 2 to 5% of dry matter depending on the plant species. Plants can utilize both nitrate (NO_3^-) and (NH_4^+) forms of N. Nitrate-N must be reduced before it can be directly used in synthesis of plant organic compounds such as amino acids, while NH_4^+-N can be utilized directly (Bloom et al., 1989). For most plants, a combination of N forms is most suitable for optimum growth and development (Gashaw and Mugwira, 1981; Salsac et al., 1987). The N form and availability may effect plant growth and development such as chlorophyll content, leaf area, plants size, and availability of other nutrients due to its influence on root-zone pH (Bar-Tal et al., 2001, Van Iersel et al., 1998a, 1998b).

The N form used affects root-zone pH. Ammonium leads to a more acidic root-zone due to two processes 1) NH_4^+uptake by roots with the release of protons by roots into the rhizosphere and 2) nitrification, or conversion of ammonium to nitrate, which results in a net release of two protons per molecule of ammonium converted to nitrate (Marschner, 1995). Excess nitrate can be absorbed and safely stored in vacuoles of plant cells; however ammonium/ammonia becomes toxic at low levels (Marschner, 1995). In greenhouse production NH_4^+toxicity occurs under high NH_4^+supply in combination with environmental conditions that hinder nitrification such as low light, low temperature, poorly aerated media conditions, and low root-zone pH (Reed, 1996). High NH_4^+supply can arrest root growth and can cause leaf chlorosis; both resulting in poor plant growth due to reduced carbohydrate supplies.

Iron Nutrition

Iron is an important element involved in many fundamental physiological processes such as photosynthesis, respiration, nitrogen fixation, and DNA synthesis. In soil and nutrient solutions, Fe exists primarily in the ferric form (Fe(III)) and occasionally in the reduced or ferrous form (Fe(II)). Ferrous Fe is preferentially taken up by plants, however, this is species dependent and on the plant's Fe uptake strategy (see below) (Marschner, 1995).

Fe Uptake Strategies

Plants use one of two strategies to obtain Fe from soils and media (Marschner and Roemheld, 1994). Strategy I Fe uptake is characterized by an increased efflux of H^+, increased ferric chelate reductase (FCR) activity in root cell plasma membranes, induction of Fe(II) transporter activity, and the release of Fe(III) chelating organic acids into the rhizosphere (Bienfait, 1987; Kochian, 1991).

The Strategy II mechanism is limited to the *Poaceae* (grasses). These species respond to Fe deficiencies by increasing the biosynthesis and secretion of Fe(III)

chelators (Bienfait, 1989). The entire new Fe complex is then transported across the plasma membrane (Romheld and Marschner, 1986).

Fe Deficiency and pH

Most substrate mixes used in the greenhouse industry do not contain mineral soil as a component; instead they are usually peat based, combined with other organic materials such as bark, coir (coconut byproduct), perlite, vermiculite, recycled tires, or other innovative 'recycled' materials. Another 'media' that can be used in greenhouse production is hydroponics, which is a nutrient water solution, without a solid substrate that contributes nutrient exchange capabilities. The individual characteristics of these media, namely the physical properties, nutrient exchange capacity, and water retention capabilities collectively have a significant impact on pH and nutrient availability.

Substrate pH affects nutrient solubility and the resulting uptake of nutrients into the plant (Nelson, 1998). As pH increases, the solubility of several elements, particularly, P, Fe, Mn, Z, Cu, and B decreases, while Mo solubility increases (Bierenbaum and Argo, 1995; Wright and Niemiera, 1987; Peterson, 1981; Lucas and Davis, 1961). The optimum pH range for many floriculture crops grown in soil-less media is 5.6 to 6.2, and outside of that range, deficiencies (higher pH) or toxicities (lower pH) may occur.

Iron deficiency is a common disorder that affects many plant species and Fe is usually the first micronutrient that becomes limiting in greenhouse media (Nelson, 1994). Iron deficiency is most often observed under high media pH. The most common visible symptom is interveinal yellowing or chlorosis of young leaves due to the fact that Fe is a non-mobile nutrient. Interveinal chlorosis has been reported in many species such as corn (*Zea mays* L.) (Stocking, 1975), soybean (*Glycine max* L.) (Wallace et al., 1982), peanut (*Arachis hypogaea* L.) (Romheld and Marschner, 1983), olive (*Olea europaea*) (Codeiro, et al., 1995), sugar beet (*Beta vulgaris*) (Morales, et

al., 1991), peach (*Prunus persica* L. Batsch), pear (*Pyrus communis* L.) (Morales et al., 1998) and in many ornamental and floriculture species such as crabapple (*Malus* sp.) (Wallace and Wallace, 1986), Ficus (*Ficus benjamina*) (Lang, H.J. et al., 1990), Piggy Back Plant (*Tolmiea menziesii*) (Smith, 1985), petunia (*Petunia xhybrida*) (Wallace and Wallace, 1986), marigold (*Tagetes erecta* L.) (Argo and Fisher, 2002), Holiday Cactus (*Schlumbergera* sp.) (Ramirez and Lang, 1997), calibrachoa (*Calibrachoa xhybrida*) (Argo and Fisher, 2002), and geranium (*Pelargonium x hortorum*) (Wallace and Wallace, 1986). To date, no information has been published describing Fe chlorosis in *Oxalis regnellii*.

As mentioned, a classic visual symptom of Fe deficiency is interveinal chlorosis of upper leaves. However, sometimes chlorotic plants will have higher leaf Fe levels when compared to green, non-chlorotic leaves. This phenomenon is known as the "Fe Chlorosis Paradox" (Romheld, 1997). This is most often associated with severe growth inhibition, resulting in 'higher' Fe concentrations (mg/g tissue) in reduced leaf tissue overall, when there is actually significantly less Fe content (mg) in an individual leaf.

Several biochemical and morphological properties are altered in Fe deficient plants. Iron is involved in the synthesis and development of chloroplasts and chlorophyll. Deficient plants exhibit smaller chloroplasts and reduced chlorophyll compared to non-Fe deficient plants (Marschner, 1995). Iron chlorosis in Mexican lime (*Citrus aurantifolia*) reduced levels of chlorophyll a, b, and a+b, and caused thickening of the palisade and spongy parenchyma cells (Maldonado-Torres et al., 2006). Price (1968) found that Fe deficiency caused a significant chloroplast derangement, both ultrastructurally and physiologically. Stocking (1975) found similar results in maize mesophyll cells. Albano and Miller (1996) found that Fe-reductase activity and rhizosphere acidification were inducible in marigold under Fe

deficient conditions. Iron chelate reductase (FCR) activity was also induced in papaya cultivars after 7 to 11 days of Fe stress (Marler et al., 2002); whereas FCR activity did not significantly increase under Fe stress in pond apple (*Annona* sp.) (Ojeda et al., 2003). Reduced photosynthesis levels due to decreased CO_2 uptake per unit of area (Briat et al., 1995) in Fe deficient plants have been reported in several species (Larbi et al., 2006).

Morphological root changes may also occur under Fe deficiencies. Cluster roots, characterized by root tip thickening and increased root hair formation, are adaptations that come about from a change in root initiation and physiology (Skene, 2000). These root adaptations assist in nutrient acquisition and are found in many plant species. Hagstrom et al., (2001) observed cluster root formation in *Lupinus albus* when grown under Fe (and P) deficient conditions.

Correcting Fe deficiency

There are two main procedures for correcting Fe deficiency in plants in the greenhouse. The first is pH management, one of the most challenging practices in any greenhouse nutrition program. As previously discussed, when pH levels increase or decrease, the availability of many micronutrients, including Fe, may become limited or toxic, respectively. Manipulation of pH is accomplished through addition of acids under high pH situations and in low pH situations a base, most often a form of limestone, can be incorporated or calcium applied in the fertilizer solution. Many factors need to be considered when altering the pH of any greenhouse media. These include the media and its properties (physical components, cation exchange capacity), crop(s) to be grown, fertilization practices, the characteristics of the type of acid or limestone used, etc. (Argo and Fisher, 2002). Media pH adjusting can be difficult, especially during crop production, thus it is often easier to manipulate media before planting.

A second strategy for correcting Fe deficiency is foliar and/or soil Fe applications. Previous research has shown that both foliar or soil applications of Fe chelates were beneficial in correcting Fe deficiency in calibrachoa and in fruit crops (Fisher et al., 2003; Swietlik and Faust, 1984). Miller (1997) reported that interveinal chlorosis was more prevalent at lower growing temperatures, and could be remedied with micronutrient applications. Hammer (2006) described successful re-greening of yellow plants from foliar applications of Fe chelates; however, plants grown in cooler temperatures were slower to green. In both methods of corrective procedures, the form of Fe applied had a significant effect on the overall application effectiveness. Common types of Fe forms used in water soluble fertilizers (from increasing solubility at pH solutions above 6.5) are $FeSO_4$, Fe-EDTA, Fe-DTPA, and Fe-EDDHA (Fisher et al., 2003).

Oxalic Acid and Oxalate

Oxalic acid is an organic acid found in algae, fungi, lichens, higher plants, and animals (Oke, 1969). It exists both as a free acid and mineral crystals, which may include insoluble salts of calcium, magnesium, iron, and soluble salts of potassium, sodium, and zinc, collectively known as "oxalates". Studies indicate that oxalates play various roles in plants including plant protection, calcium regulation, and ion balance (Liber and Franceshi, 1987; Franceshi and Nakata, 2005). Oxalates in buckwheat, taro and rice play a role in heavy metal (lead, aluminum, strontium, cadmium, and copper) detoxification (Ma et al., 1997; Ma and Miyasaka, 1998; Yang et al., 2000; Franceschi and Schueren, 1986; Choi et al., 2001; Mazen and Maghraby, 1997). Although oxalates play important functional roles in plants, the high oxalate content in plants, when consumed by humans, can have adverse effects. Namely, excess oxalate levels lower nutritional quality by reducing calcium and iron bioavailability and can lead to

kidney stone formation (Libert and Fraceschi, 1987; Horner and Wagner, 1995; Massey, 2003; Franceshi and Nakata, 2005; Bataille and Fournier, 2001).

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CHAPTER 2

Investigating Interveinal Chlorosis in *Oxalis regnellii*

Abstract

Oxalis regnellii is an ornamental pot plant grown primarily for its clover-like leaves. However often times the leaves become chlorotic for unknown reasons, including virus infection, iron (Fe) and/or manganese (Mn) deficiencies, and improper greenhouse forcing temperatures. We conducted a series of experiments to address these hypotheses. Shamrock Chlorotic Ringspot Virus (SCRV) has been reported before in *Oxalis regnellii*. Oxalis plants exhibiting virus symptoms were found in some commercially produced oxalis, and were disposed of to confidently test other hypotheses. In order to non-destructively measure the relative chlorophyll concentration in leaves we conducted a chlorophyll SPAD meter correlation. A strong correlation (r^2= .9784) was observed between the SPAD 502 meter and the total chlorophyll content in *Oxalis regnellii* leaves. A media drench of ferric ethylenediaminedi (*o*-hydroxyphenylacetic) acid (Fe-EDDHA) was applied to chlorotic Fe deficient oxalis plants and plants successfully re-greened. Foliar applications of ferric ethylenediamine tetraacetic acid (Fe-EDTA), ferric ethylenediaminedi(*o*-hydroxyphenylacetic) acid (Fe-EDDHA) and manganese ethylenediamine tetraacetic acid (Mn-EDTA) at concentrations of 0, 60, 120, and 240 mg $\cdot L^{-1}$ Fe or Mn were applied to interveinal chlorotic oxalis leaves and were not as successful. Only Fe-EDDHA applications at 240 mg $\cdot L^{-1}$ slightly increased the chlorophyll content after 9 days. Growing plants at 13˚ C reduced growth and development of the plants, however the incidence of leaf chlorosis did not increase compared to warmer temperatures of 21/16˚ C (Day/Night); 22˚ C constant; or 22 to 16˚ C (plants were moved to 16˚ C when 50% of the plants were in first flower).

Introduction

Oxalis regnellii, (oxalis) also known as "The Shamrock Plant" is a specialty potted bulb crop grown for its clover-like leaves and white flowers. In the United States, oxalis is marketed in the spring, primarily for the St. Patrick's Day holiday (Dole and Wilkins, 2005; Miller, 1997). Oxalis is susceptible to foliar disorders including wrinkled leaves, leaf edge burn, but most importantly, interveinal chlorosis. While the exact cause(s) of interveinal chlorosis remain unclear, two commonly hypothesized explanations are iron (Fe) deficiency and/or virused plant material (De Hertogh, 1996; Dole and Wilkins, 2005). Manganese (Mn) deficiency has also been considered to be a factor in chlorosis. However, there has been very little investigation to substantiate these claims. Preliminary studies indicate that Fe deficiency may be a contributing factor to the interveinal chlorosis in oxalis.

Iron is often the first micronutrient that becomes limiting in greenhouse media (Nelson, 1994). Iron deficiency affects many plants and is most often encountered with high soil or media pH, as solubility of Fe (and other micronutrients, with the exception of molybdenum) decreases with increasing pH. Iron is a major component of many photosynthetic pigments and is involved in the development and synthesis of chloroplasts and chlorophyll (Marshner, 1995). Iron deficiency leads to chlorophyll reduction and smaller chloroplasts and subsequent chlorosis. Manganese (Mn) is involved in the electron transport system and deficient levels result in reduced or stunted growth and interveinal chlorosis of young leaves**.**

Several factors can contribute to yellowing or chlorosis in plants. A major factor is nutrient availability, directly (quantity) and indirectly (pH). A second factor is viral infection, as infected plants may exhibit chlorotic symptoms. A third potential factor is an indirect temperature effect. As temperature decreases, plant metabolic

activity decreases, including potential nutrient uptake. These factors are potential reasons for leaf chlorosis in oxalis and merit further investigation.

In the limited published literature on *Oxalis regnellii*, it has been hypothesized that some of the observed foliar disorders in oxalis could be of viral origin (De Hertogh, 1993; De Hertogh, 1996; Dole and Wilkins, 2005). *Oxalis regnellii* is asexually propagated which carries a high risk of perpetuating any virus infection. We observed oxalis plants that exhibited virus-like symptoms in shipments from three different rhizome suppliers. In order to effectively evaluate and accurately determine and characterize interveinal chlorosis and confidently test the Fe deficiency hypothesis, identification and disposal of any potential virus infected oxalis was necessary. Therefore, we conducted an evaluation of plants suspected to have the Shamrock Chlorotic Ringspot Virus (SCRV).

Shamrock Chlorotic Ringspot Virus, suspected to be a potyvirus, was first reported in 1981 (Coyier) and is the only virus reported in *Oxalis regnellii*. Virus symptoms may look very similar to interveinal chlorosis (resulting from a nutritional deficiency) with the most identifiable virus symptom being the characteristic chlorotic ring spot surrounding an island of green tissue (Fig. 2.1). As the virus infection progresses, the chlorotic ring spots fade into indistinct chlorotic blotches and streaks and infected rhizome scales become dark brown or black. The virus is thought to be transmitted via aphid feeding and through mechanical contact between diseased roots and healthy plant roots. Ultimately the plants may die within two years after being infected with SCRV (Coyier, 1981). Careful and immediate rouging of symptomatic plants is the most effective control of SCRV. Oxalis plants with virus-like symptoms in our project were first tested at the Cornell University Diagnostic Laboratory for two

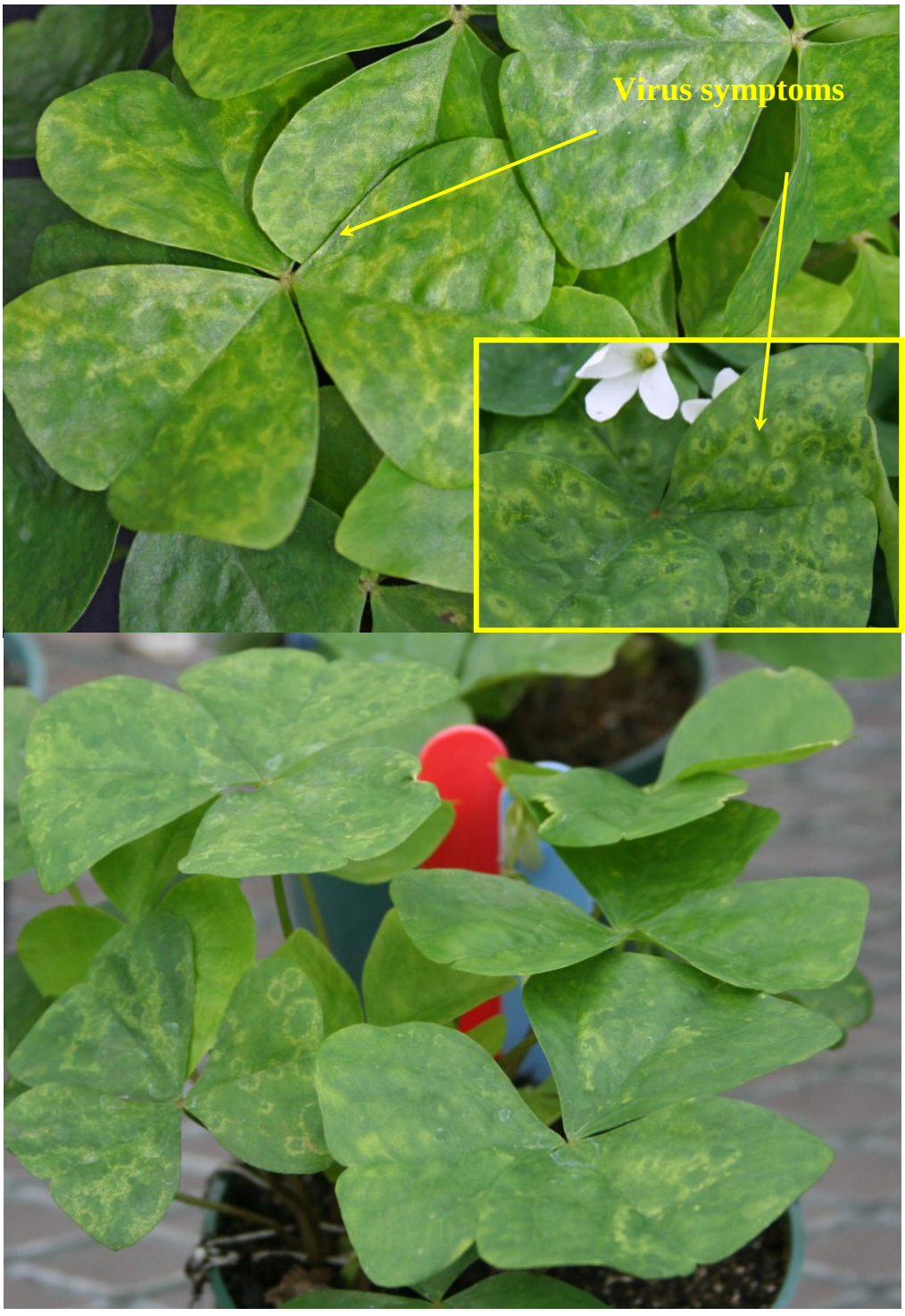

Figure 2.1. Virus symptoms in *Oxalis regnellii*.

common viruses that infect greenhouse ornamentals, Tobacco Mosaic Virus and Impatiens Necrotic Spot Virus. Results were negative for both viruses. After consulting with Dr. Margery Daughtrey, a collaborative virus evaluation was conducted with Dr. Benham Lockhart at the University of Minnesota.

Optimal greenhouse forcing temperatures or temperature regime is important in order to produce high quality, marketable plants. Information is limited for oxalis greenhouse production, including greenhouse forcing temperatures. Current forcing recommendations for *O. regnellii* suggest 21-24° C until plants are well rooted then reducing the night temperature to 18-21° C when flowering commences (De Hertogh, 1996; Miller, 1997). Oxalis is forced in the middle of winter, late December to early March for the St. Patrick's Day holiday and *O. triangularis* can be forced anytime from December to June, as it is typically used as a pot plant and/or for mixed containers. Since *Oxalis regnellii* is produced primarily during cooler months, the potential ability to reduce greenhouse heating costs is important. Further understanding and information of forcing temperature would be valuable relating to leaf chlorosis and rising production costs.

Foliar nutrient analysis is an accurate method to assess nutritional status in plants. However, tissue analysis can be costly, both monetarily and in the time needed from tissue sampling to the analysis report and any subsequent correction procedures. The SPAD-502 meter (Minolta Chlorophyll Meter SPAD-502; Spectrum Technologies, Plainfield, IL) is an efficient, non-destructive instrument that has been used to measure the relative chlorophyll concentration in leaves of many species (Loh et al., 2002; Kapotis et al., 2003; Uddling et al., 2007).

There are two main procedures to correct Fe deficiency in greenhouse production. The first (and one of the most challenging practices in any greenhouse nutrition program) is pH management. However, adjusting media pH can be difficult,

especially during crop production; therefore it is often easier to manipulate potting media before planting. The second strategy to correct Fe deficiency is Fe chelate foliar sprays and/or media drenches. In both corrective procedures, the form of Fe applied has a significant effect on the overall application effectiveness. Common Fe chelate forms used (from increasing solubility at pH solutions above 6.5) are ferrous iron sulfate ($FeSO_4$), ferric ethylenediamine tetraacetic acid (Fe-EDTA), diethylenetriaminepentaacetic acid (Fe-DTPA), and ferric ethylenediaminedi (o-hydroxyphenylacetic) acid (Fe-EDDHA) (Fisher et al., 2003). Hammer (2006) described successful re-greening of yellow oxalis plants from foliar Fe chelate applications. Other research has shown that both foliar or soil applications of Fe chelates were beneficial in correcting Fe deficiency in calibrachoa and in fruit crops (Fisher et al., 2003; Swietlik and Faust, 1984). Chelate drench applications on *O. regnellii* have not been conducted to date.

A series of studies were conducted to investigate different potential factors affecting chlorosis in oxalis. The objectives for these studies were as follows. First, a screening of oxalis plants was conducted to assess the plausibility of a viral infection. Second we conducted a study to establish the relationship between total chlorophyll content with SPAD-502 meter readings in oxalis leaves, in order to non-destructively estimate chlorophyll content and qualify leaf greenness in further studies. A third study investigated the effects of different growing temperatures on chlorosis incidence. Finally, preliminary studies were conducted to assess micronutrient chelate applications and their effects re-greening chlorotic oxalis leaves.

Materials and Methods

Virus Screening

Tissue samples of putative virus infected plants (n=4), that were showing chlorosis and characteristic ringspots, and plants (n=26) suspected to be virus-free

(showing no symptoms as previously described) were examined. Tissues were tested for presence of virus by transmission electron microscopy (TEM) using partially purified extracts. Leaf tissue samples (1.5-2.0 grams) were powdered in liquid nitrogen in a mortar and then extracted with 17 ml extraction buffer (500 mM NaK-PO_4, pH 7.5, 1M urea, 5% w/v polyvinyl pyrrolidone, 0.5% (v/v) 2-mercaptoethanol). The mixture was filtered through Miracloth, the filtrate centrifuged at 27,000x g for 15 min and the pellet discarded. The supernatant was layered over 5 ml 30% (w/v) sucrose in 100 mM Na-PO_4 pH 7.0 and centrifuged at 148,000x g for 90 min at 10C. The pellet was resuspended in 100 μl 100 mM Na-PO_4, pH 7.0 and clarified by vortexing with an equal volume of chloroform and centrifugation in a micro-centrifuge for 10 min at 15,000 rpm. The upper aqueous phase constituted the partially purified extract, and was examined by TEM following negative staining with 2% sodium phosphotungstate, pH 7.0. Virus particles observed in positive samples were flexuous filamentous particles measuring 750-800 x 12 nm.

SPAD-502 Meter and Chlorophyll Correlation

Leaf samples used in this study were obtained from oxalis plants that were grown in the greenhouse at 21°C with 20-20-20 fertigation at the rate of 200 ppm N (JR Peters, Inc.; Allentown, PA). Leaves showing a range of chlorosis symptoms were selected. Three SPAD-502 meter readings were taken on each leaflet, to the right of the mid-vein. Three, 1 cm^2 leaf discs were taken from the same area as the SPAD reading and placed in test tubes containing 5 ml dimethylsulfoxide (DMSO) and incubated in the dark for 30 minutes in a 21°C water bath. After incubation, spectrophotometric measurements were obtained at 665 and 645 nm using a Shimadzu UV-1601 (Shimadzu Corp., Japan). Total chlorophyll *a* and *b* concentration of the leaves was calculated using the equations in Wellburn (1994).

Greenhouse Forcing Temperature

Oxalis rhizomes (and *O. triangularis)* supplied from The Netherlands were used for this experiment. Eight rhizomes of each species that had been stored for several months at 3° C were planted on 5 February, 2010. One rhizome was planted per 10 cm pot using a commercial greenhouse media substrate (LC1; Sun Gro Horticulture Ltd., Vancouver, Canada) and grown under four glasshouse temperature regimes; 21/16˚ C (Day/Night); 22˚ C constant; 22 to 16˚ C (plants were moved to 16˚ C when 50% of the plants were in first flower); and 13˚ C constant. Plants were fertilized at each irrigation with 250 mg $N \cdot L^{-1}$ 20N–2.2P–16.6K (Jack's Professional LX Water Soluble Fertilizer 21-5-20 All Purpose; J. R. Peter's Inc., Allentown, PA). The date of shoot emergence (DTE) was recorded when the first leaf was visible above the media surface. Flower data recorded included the number of flowers per plant and the number of days to flower (DTF), recorded when the first flower fully opened. After 10 weeks, plant height, SPAD meter readings (as above, *O. regnellii* only) and pH and electrical conductivity (EC) measurements using the pour through method were obtained. Plant tissues were oven dried at 70˚ C for at least 48 h then dry weight (DW) determined. Statistical analyses were conducted with Statistical Analysis System (SAS Version 9.1; SAS Institute, Cary, NC). One-way analysis of variance tests were conducted to identify differences in the measured parameters in response to temperature treatments and *Tukey's HSD* method was used to conduct pair wise comparisons for each species.

Foliar Chelate Applications

Five random leaves sampled from plants used for SPAD correlation (described above) exhibiting interveinal chlorosis symptoms were evenly hand-painted on both adaxial and abaxial surfaces with Fe-EDTA, Fe-EDDHA, or Mn-EDTA at concentrations of 0 (distilled water), 60, 120, or 240 $mg \cdot L^{-1}$ Fe or Mn. The solutions

contained a nonionic organosilicate surfactant, CapSil (CapSil 30; Aquatrols, Cherry Hill, N.J) that has been shown to assist the effectiveness of foliar chelate applications (Fisher et al., 2003). Chelate applications were made early in the day or late in the afternoon. SPAD meter readings (as above) of the five replicates were obtained immediately before chelate application (day 0) and three and nine days post application.

Media Drench Chelate Application

Oxalis plants exhibiting interveinal chlorosis due to Fe deficiency (high media pH; data from Chapter Four) and plants with no visible signs of Fe deficiency (controls) were selected. An Fe-EDDHA chelate (Sprint 138; Becker Underwood; Ames, IA) drench solution (45 $mg \cdot kg^{-1}$ Fe) was prepared and chlorotic plants were drenched until the solution leached through the pot (0.375 $g \cdot L^{-1}$). Control plants were drenched with RO water. SPAD readings were obtained immediately before chelate application and five days post application. Each SPAD reading was taken on the right side of the central vein of each leaflet. SPAD meter values were averages of six readings from two different leaves per pot. Statistical analyses were conducted with using JMP v. 8 (SAS Institute, Cary, NC). One-way analysis of variance tests were conducted and *Tukey's HSD* method was used to conduct pair wise comparisons.

Results and Discussion

Virus Screening

Virus symptoms and certain nutrient deficiency symptoms may be difficult to distinguish. The 26 plants thought to be virus free tested negative for any virus. Filamentous potyvirus-like particles were observed in three of four plants suspected to be virus infected. However, the viral particles were not conclusively identified as SCRV. With this information, all plants suspected with any virus-like symptoms were discarded.

Correlation of SPAD-502 Meter and Chlorophyll Concentration

Chlorophyll concentrations in oxalis leaves were significantly correlated with SPAD meter readings (r^2 =0.9784) (Fig. 2.2). In general, dark green *Oxalis regnellii* leaves typically had SPAD readings of 30 and higher, while leaves with readings of 15 to 17 were moderately yellow or chlorotic and leaves with readings less than 10 exhibited an extreme yellow or almost white appearance. The SPAD meter is an efficient and non-destructive method to assess the greenness of oxalis leaves and can allow for quicker and more frequent monitoring and assessment of the crop during production. Often by the time visual nutritional deficiencies occur in leaves, limited plant growth or irreversible damage may have already occurred. This correlation information will allow for the opportunity to investigate any possible correlation with Fe and Mn content, similar to SPAD-502 and nitrogen correlation work by Loh et al. (2002).

Foliar and Media Drench Chelate Applications

Foliar Fe-chelate applications were not effective in re-greening oxalis leaves and chlorophyll levels did not increase after micronutrient chelate applications. This contrast with the results of Fisher et al. (2003), where Fe-EDTA sprays significantly increased chlorophyll content in Fe deficient calibrachoa (*Calibrachoa xhybrida)* as the spray concentration increased. With oxalis, in the 9 days after chelate application, control leaves continued to yellow, losing greenness by slightly more than two SPAD units. The most effective chelate application was the 240 ppm Fe-EDDHA application, in which the average SPAD reading increased by three units. The hypothesis that foliar Fe EDDHA chelate applications would be most effective compared to other foliar Fe-EDTA treatments was not supported. Most other treatments showed little change (Table 2.1). Similarly, little difference was observed

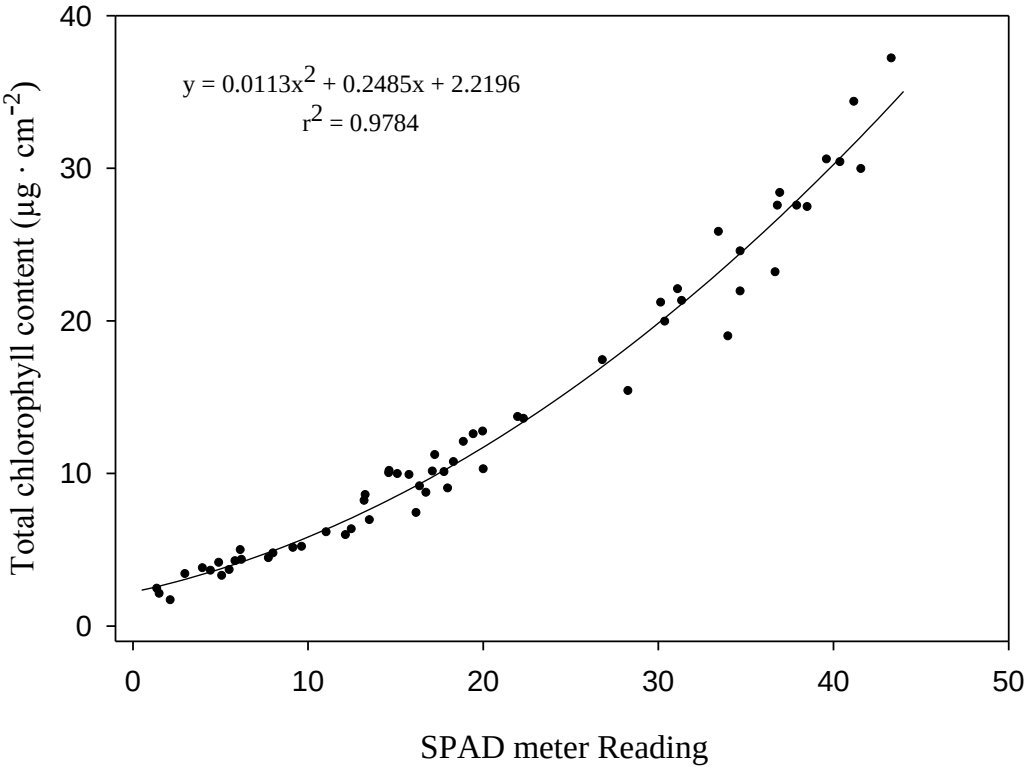

Figure 2.2. Relationship of SPAD 502 meter readings to total chlorophyll content in *Oxalis regnellii* leaves. Data points are averages of three measurements; one from each leaflet on the right hand side of the mid-vein.

Table 2.1 Effects of foliar iron (Fe) and manganese (Mn) micronutrient chelate applications on SPAD meter readings in *Oxalis regnellii*.

	SPAD Meter Reading (Days post application)								
	0	3	9	0	3	9	0	3	9
Concentration[z]	*Fe-EDTA*			*Fe-EDDHA*			*Mn-EDTA*		
0 (DI water)	17.3[z]	14.6	15.0	17.3	14.6	15.0	17.3	14.6	15.0
60 mg $\cdot L^{-1}$	15.7	14.0	16.0	16.0	16.2	17.0	15.1	14.3	14.6
120 mg $\cdot L^{-1}$	14.2	13.5	15.8	16.5	16.0	16.2	14.3	13.6	14.8
240 mg $\cdot L^{-1}$	14.7	14.7	15.7	16.1	17.3	19.2	14.4	14.3	14.2

[z]Concentration refers to Fe or Mn concentration.

[y]Each reading is an average of five replications (leaves) of three averaged SPAD readings.

with Mn-chelate applications. This is reasonable, as decreased manganese levels often have little effect on overall chlorophyll content. Swietlik and Faust (1984) reported limited metal mobility even with chelated micronutrient forms and this may be a concern with chelate applications in oxalis. Another possible explanation for a lack of foliar chelate effect on re-greening is that the degree or severity of interveinal chlorosis in the leaves was severe enough that the photochemistry components were already damaged and the leaves could not respond to the micronutrient chelate applications.

Unlike foliar sprays, data indicate that chelate drenches were very effective in re-greening of oxalis (Table 2.2). Figure 2.3 shows chlorotic oxalis plants and plants 5 days after drenching with Fe chelate. Drenched chlorotic plants had nearly double the chlorophyll content and SPAD measurements increased five days after chelate application, while chlorophyll content did not increase in control plants.

The data obtained in these studies provide evidence that Fe chelate drenches are effective in re-greening Fe deficient, chlorotic oxalis plants. The best way to correct Fe deficiency (or other nutrient problems) is prevention, by monitoring and maintaining proper pH and nutrition during production. However, problems are sometimes unavoidable and thus, corrective procedures need to be employed. Additional studies on different Fe chelate rates and chelate forms for both foliar sprays and drenches should be conducted.

Greenhouse Forcing Temperature

Temperature had a significant effect on growth parameters in both oxalis species (Table 2.3; Fig. 2.4). The warmest temperature (22˚ C) significantly decreased DTE for both species compared to the coldest temperature (13˚ C). Similarly, the coldest temperature significantly increased DTF for both species, with *O. triangularis* not flowering by the end of the experiment. Little temperature effect was observed for

Table 2.2. Effects of ferric ethylenediaminedi (*o* -hydroxyphenylacetic) acid (Fe-EDDHA) media drenches on regreening of iron (Fe) deficient *Oxalis regellii* after 5 days.

Treatment	Initial SPAD[z]	Ending SPAD[x]	SPAD difference
Fe Deficient	15.4 a	27.5 a	+12.1 a
Control	28.0 b	27.1 a	-0.9 b
Signficance	***[y]	NS	***

[z]SPAD meter values were averages of six readings from two different leaves per pot before chelate application. Means followed by different letters within each column are significantly different by Student's *t* test at $P \leq 0.05$.

[x]SPAD values 5 days after chelate drench.

[y]NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.001, or <0.0001

Figure 2.3. The effect of iron (Fe) chelate drenches 5 days after application on iron deficient *O. regnellii*. Representative plants selected for Fe-chelate applications are shown in the top row. Oxalis plants five days after drenching are shown in the bottom row.

Table 2.3. Temperature effects on several plant growth parameters in *Oxalis regnellii* and *O. triangularis.*

Temperature (˚C)	Days to emerge	Days to flower	No. of flowers	Height (cm)	Dry weight (g)	SPAD	pH	EC
	O. regnellii							
13	17 a [z]	54 a	2 a	3.1 a	0.18 a	36.6 a	4.61 a	10.5 a
21/16 [y]	17 a	44 b	5 a	6.6 b	0.79 b	31.9 b	5.74 b	5.6 b
22 to 16 [x]	15 a	42 b	8 ab	6.7 b	0.82 b	36.6 a	5.26 c	7.5 c
22	12 b	37 b	13 b	6.9 b	1.03 b	37.2 a	4.97 d	7.4 c
	O. triangularis							
13	19 a	dnf [w]	dnf	2.3 a	0.10 a	-	-	-
21/16	13 b	52 a	3 a	6.4 b	0.64 b	-	-	-
22 to 16	15 ab	48 a	3 a	5.8 b	0.48 b	-	-	-
22	13 b	49 a	4 a	6.9 b	0.66 b	-	-	-

[z] Day/Night temperature

[y] Plants were moved from 22 ˚C to 16 ˚C when 50% of plants were in flower.

[x] Letters after values in each column for each species represent mean separation using Tukey's honestly significant difference (HSD) at $P = 0.05$.

[w] Did not flower

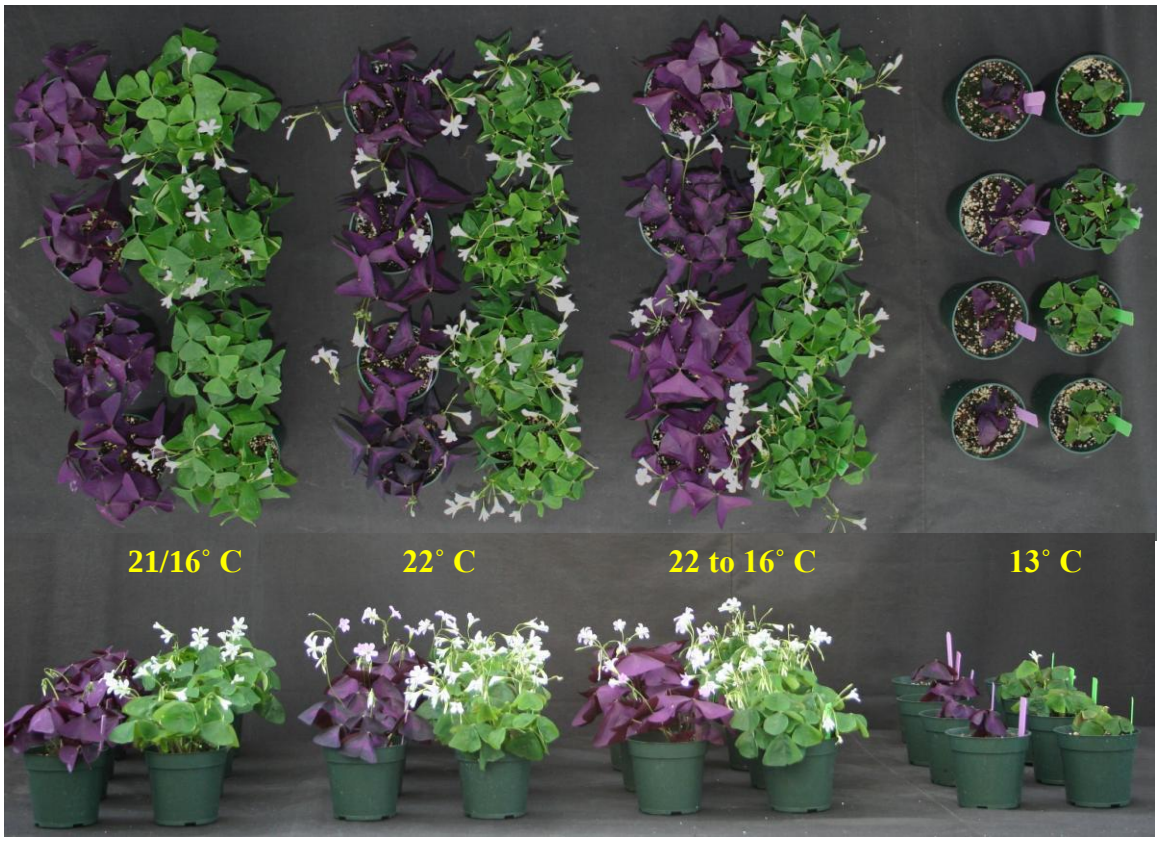

Figure 2.4. Effects of greenhouse forcing temperatures on growth and development of *Oxalis regnellii* (green leaved) and *O. triangularis* (purple leaved). L to R 21/16˚ C (D/N); 22˚ C constant; 22 to 16˚ C; and 13˚ C constant.

flower number for the plants that did flower in *O. triangularis*, however with *O. regnellii*, significantly more flowers were observed at the higher temperature. Plants were 50% and ~33% shorter for *O. regnellii* and *O. triangularis*, respectively, at 13˚ C compared to other temperature treatments. Correspondingly, when compared to other treatments, plant DW was significantly less in the coldest temperature treatment. SPAD readings were lowest for *O. regnellii* plants grown under the diurnal 21/16˚ C temperature treatment. The highest pH reading (5.74) and lowest EC reading (5.58) were obtained in the diurnal temperature treatment, while the lowest pH (4.61) and highest EC reading (10.46) were observed in the coldest temperature treatment.

It is well known that colder temperatures can significantly reduce plant growth and development. Plants grown at the other temperature regimes had similar growth and development. It has been often reported that *O. regnellii* often develops interveinal chlorosis before market, which has not been fully investigated. Cooler forcing temperatures did not significantly increase chlorosis levels in our study. Miller (1997) reported that interveinal chlorosis was more prevalent at lower growing temperatures and could be remedied with micronutrient applications, although no data were presented. There was little difference in greenness and chlorosis development among treatments; however, SPAD readings were significantly lower in the diurnal temperature treatment.

Based on this study, the current published literature is accurate. Greenhouse forcing temperatures should be at least 21˚ C constant. Increasing temperatures would most likely be beneficial for growth and development and further reduce the time to market stage. However, increasing temperatures could potentially increase greenhouse heating costs, unless other crop species already in production require similar warm temperatures. Further studies investigating greenhouse temperatures between 16˚ and 21˚ C could be beneficial.

Conclusions

The result obtained from this series of studies provides more information about the chlorosis phenomenon in *Oxalis regnellii*. The hypothesis that some of the foliar disorders observed in *O. regnellii* could be a direct result of a viral infection is plausible. Because *Oxalis* produces several daughter rhizomes and is vegetatively propagated, it is important that rhizome producers continually monitor and rogue any suspected virally infected plants, to reduce the spread of the virus. The SPAD meter is an acceptable, non destructive, cost effective tool that can assist in assessing chlorophyll levels (greenness) of oxalis. Our research also suggests that cooler forcing temperatures do not necessarily lead to increased chlorosis. However, other factors, such as media type and irrigation (quantity and method), coupled with cooler forcing temperatures could have a significant effect on growth and development of oxalis and chlorosis and need to be considered. Our investigations also showed that if leaf chlorosis occurs due to a high pH, iron chelate drench applications are more effective than foliar applications. Additional studies for both correction techniques would be beneficial. The chlorosis phenomenon is complex and does not appear to be attributed to any one specific cultural practice.

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CHAPTER 3

Nitrate:Ammonium ratio affects growth and development of *Oxalis regnellii* in hydroponic culture[1]

Abstract

Nitrogen (N) source ratio (NO_3^-:NH_4^+) effects on growth and development of oxalis have not been investigated previously. An experiment was conducted to establish an optimum NO_3^-:NH_4^+ ratio for oxalis growth in a hydroponic system. Five NO_3^--N:NH_4^+-N ratio treatments consisting of 100:0, 75:25, 50:50, 25:75, and 0:100 were tested. Increasing proportions of NO_3^--N increased total leaf, root, flower, and bottom shoot biomasses. New rhizome biomass increased with increasing NO_3^--N rates until 50 and 75%, after which, new rhizome biomass decreased. Plants that received increasing amounts of NO_3^--N were greener or had a greater chlorophyll index at the end of the experiment.

On a dry weight concentration basis, N, B, Fe and Al levels decreased as NO_3^--N levels increased, whereas K, Ca, and Mn levels decreased most likely due to N-ratio effects on solution pH. Tissue P, Mo, and Cu increased with increasing NO_3^--N levels up to 50% NO_3^--N supplied, and then decreased. No significant relationship was found with NO_3^-: NH_4^+ ratios for Mg, Zn, and Na.

Introduction

As global demand for flower bulbs increases, research into production and marketing of lesser known species will be increasingly important for the floriculture industry (Kamenetsky et al., 2005). One such group of species with potential market share is *Oxalis*.

[1] Modified version of Miller, C.T.; N.S. Mattson, and W.B. Miller. 2009. Nitrate:Ammonium ratio affects growth and development of *Oxalis regnellii* in hydroponic culture. Isr. J. Plant. Sci. 57(4): 389-396.

Ornamental *Oxalis* are typically marketed as indoor flowering potted plants or for use in landscapes and mixed containers. The potential for increased oxalis forcing exists because of the unusual plant characteristics, especially diverse leaf shapes and color and flower colors that include red, yellow, and orange, and pink. There are reported to be between 600 and 900 species of *Oxalis* (Family Oxalidaceae) (Bahattacharyya and Johnri, 1998; Bryan, 2002; De Azkue, 2000; Dole and Wilkins, 2005; Wilkins, 1985) of which, only a few species are cultivated for ornamental purposes**.** *Oxalis* are endemic to all continents, with two main centers of diversity: coastal South Africa and subtropical America (Argentina, Bolivia, Brazil, Paraguay, and Peru) (De Azkue, 2000; De Hertogh and Le Nard, 1993; Dole and Wilkins, 2005). Many *Oxalis* are pernicious landscape weeds, while others such as *O*. *deppei* and *O*. *tuberosa* (oca) produce edible roots or tubers (Wilkins, 1985) and are important crops in the Andes of South America (Roca et al., 2007).

"The Shamrock Plant", (*Oxalis regnellii* Miq.*)* (hereafter referred to as "oxalis") is a specialty potted plant grown and marketed primarily for the St. Patrick's Day holiday (March 17) because of its clover-like leaves (Dole and Wilkins, 2005; Miller, 1997). Oxalis are grown from underground scaly rhizomes and are commercially produced in The Netherlands and the United States (California and Oregon) (De Hertogh, 1996). Several foliar disorders have been observed in oxalis during greenhouse production including wrinkled leaves, leaf edge browning (necrosis), and most prevalent, interveinal chlorosis. It is hypothesized that the interveinal chlorosis is due to an iron deficiency; however this has never been substantiated.

Hydroponic systems are often used to investigate plant nutrition and its effect on plant growth and development. Nitrogen is the element required in greatest concentration for plant development. Plants can absorb both NO_3^- and NH_4^+ forms of

N. Nitrate-N must be reduced before it can be directly used in synthesis of plant organic compounds such as amino acids, while NH_4^+-N can be directly utilized (Bloom et al., 1989). The N form available to any given species can have an effect on plant growth and development, which can affect chlorophyll content, leaf area, plants size, and availability of other nutrients due to its effect on root-zone pH (Bar-Tal et al., 2001, Van Iersel et al., 1998a, 1998b). In many species a combination of N forms is most suitable for optimum growth and development (Gashaw and Mugwira, 1981; Salsac et al., 1987). The form of nitrogen used affects root-zone pH. Ammonium leads to a more acidic root-zone due to two process 1) NH_4^+uptake by roots which the release of protons by roots into the rhizosphere and 2) nitrification, or conversion of ammonium to nitrate, which results in a net release of two protons per molecule of ammonium converted to nitrate (Marschner, 1995). Excess nitrate can be absorbed and safely stored in vacuoles of plant cells; however ammonium/ammonia becomes toxic at low levels (Marschner, 1995). In greenhouse production NH_4^+toxicity occurs under high NH_4^+supply in combination with environmental conditions that hinder nitrification such as low temperatures, poor aerated media conditions, and low root-zone pH (Reed, 1996). High NH_4^+supply can arrest root growth and can cause leaf chlorosis; both resulting in poor plant growth due to reduced carbohydrate supplies.

There are no studies of hydroponic studies with oxalis. In order to develop a hydroponic system which could be used to further analyze plant nutrition effects on oxalis and foliar disorders, an experiment was conducted with the objective to evaluate different N-source ratios (NO_3^-: NH_4^+) in nutrient solutions.

Materials and Methods

Oxalis rhizomes, obtained from a Dutch supplier (Leo Berbee Bulb Co., Marysville, OH) were established in 5L plastic containers using full strength Hoagland solution (Hoagland and Arnon, 1950) and were aerated by pumping air through porous

stones placed in the solution. Containers were covered with aluminum foil to prevent light degradation of chelating agents (Albano and Miller, 2001) and algae growth. Oxalis were grown in a glass house at 21˚ C day/night temperature and grown at ambient photoperiod and light conditions (42 °N latitude). The experiment was initiated on 16 February 2009. Five treatments of NO_3^--N:NH_4^+-N ratios consisting of 100:0, 75:25, 50:50, 25:75, and 0:100 (molar basis) were supplied in a modified Hoagland nutrient solution using reverse osmosis water. Hereafter, NO_3^--N:NH_4^+-N ratios will be referred to as 100% NO_3^--N, 75% NO_3^--N, 50% NO_3^--N, 25% NO_3^--N, and 0% NO_3^--N, respectively. The composition of the various solutions is as follows: *(0:100 ratio)* 0.5 mM KH_2PO_4, 1 mM $MgSO_4 \cdot 7H_2O$, 4.5 mM $(NH_4)_2SO_4$, 2 mM $CaCl_2 \cdot 2H_2O$, 2.5 mM KCl; *(25:75 ratio*) 1 mM $MgSO_4 \cdot 7H_2O$, 0.5 mM KH_2PO_4, 1.125 mM KNO_3, 3.375 mM $(NH_4)_2SO_4$, 1.375 mM K_2SO_4, 2 mM $CaCl_2 \cdot 2H_2O$; *(50:50 ratio*) 1 mM $MgSO_4 \cdot 7H_2O$, 3 mM KH_2PO_4, 2.25 mM NH_4NO_3, 2 mM $CaCl_2 \cdot 2H_2O$; *(75:25 ratio*) 1 mM $MgSO_4 \cdot 7H_2O$, 1 mM KH_2PO_4, 3.375 mM KNO_3, 1.125 mM $(NH_4)_2SO_4$, 2 mM $CaCl_2 \cdot 2H_2O$; *(100:0 ratio*) 1 mM $MgSO_4 \cdot 7H_2O$, 05 mM KH_2PO_4, 2 mM $CaNO_3 \cdot 4H_2O$, 2.5 mM KNO_3. Micronutrients were supplied at the following levels in all treatments: 46µM H_3BO_3, 9.15 µM $MnCl_2 \cdot 4H_2O$, 0.08 µM $Na_2MoO_4 \cdot 2H_2O$, 0.32 µM $CuSO_4 \cdot 5H_2O$, 0.77 µM $ZnSO_4 \cdot 7H_2O$, 17.86 µM Fe-EDDHA per liter. There were four replicates of each treatment, with each hydroponic container of three oxalis plants comprising the experimental unit. The containers were organized in a complete randomized design. Nutrient solutions were changed weekly to maintain nutrient balance and reduce indirect pH effects. pH readings were taken daily.

Indirect chlorophyll measurements of leaf greenness were taken using a SPAD meter (Minolta Chlorophyll Meter SPAD-502; Spectrum Technologies, Plainfield, IL). Five randomly selected recently mature leaves in each container were measured and

averaged at experiment initiation and termination. Plants were destructively harvested 37 days after experiment initiation. Plants were partitioned into leaves (including petioles), roots, flower umbels, newly formed rhizomes and bottom shoots, defined as shoots that developed under the container lid that did not rise above the surface. Tissues were collected and dried at 70˚ C for at least 48 h after which dry weight was determined. Tissue samples were analyzed by Inductively Coupled Plasma-Atomic Emission Spectrophotometry at a commercial laboratory. Data were analyzed using JMP v. 7 (SAS Institute, Cary, NC). Analysis of variance and general linear or quadratic regression lines were applied as appropriate based on R^2 values.

Results

Figure 3.1 shows changes in pH levels for each N ratio for the first 23 days of the experiment. Over a one week period, pH increased for 100% NO_3^--N solutions, while all other solutions decreased. The 0% NO_3^--N solution decreased the first week to about pH 3 and each week after it decreased less. Leaf, root, flower, and bottom shoot biomass increased with increasing proportion of NO_3^--N in the nutrient solution (Figs. 3.2-3.4, 3.6). New rhizome biomass increased as NO_3^--N increased to 50 and 75% of total N, after which new rhizome biomass decreased (Fig. 3.5). Total plant biomass (leaf, root, flower, rhizome, and bottom shoot biomasses) increased significantly as NO_3^--N proportions increased (Fig. 3.7). Plants that received a greater proportion of NO_3^--N were greener and had greater SPAD measurements (an indirect measure of chlorophyll) at the end of the experiment (Fig. 3.8), which matched visual observations (Fig. 3.9). Qualitative shoot biomass differences can also be observed in Fig. 3.9. Little, if any abnormal or deformed leaves were observed.

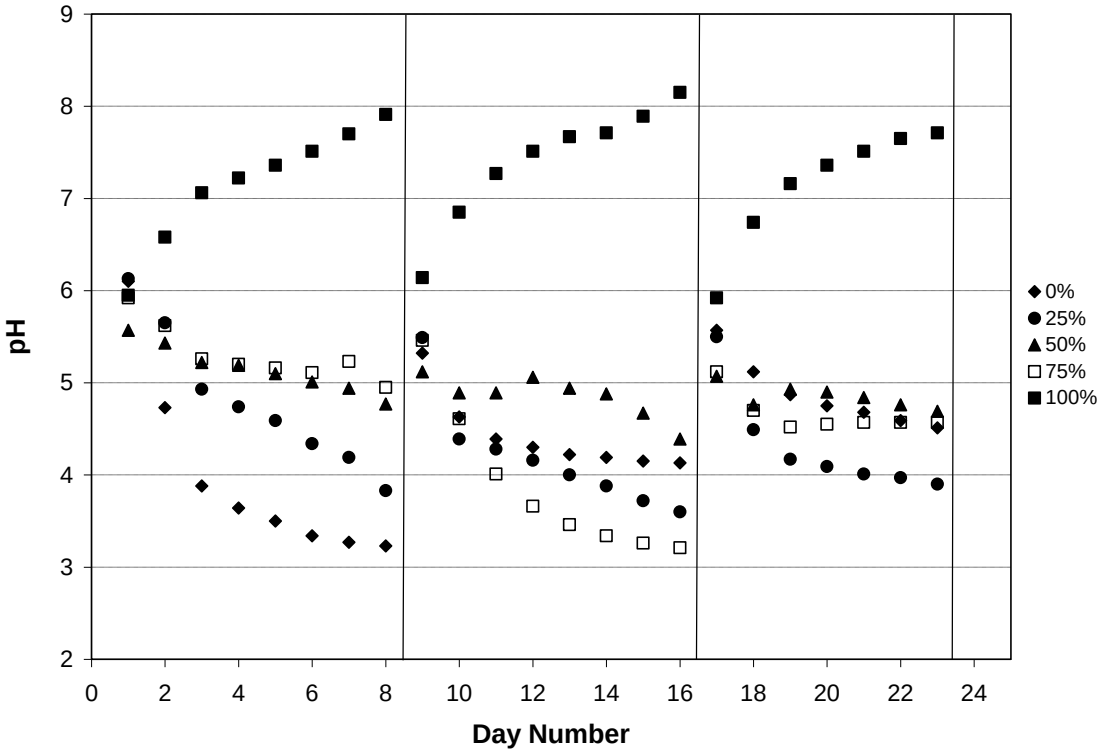


Figure 3.1. The effect of percent NO_3^--N on pH of hydroponically grown oxalis for the first 23 days of treatment. Vertical lines represent solution renewals.

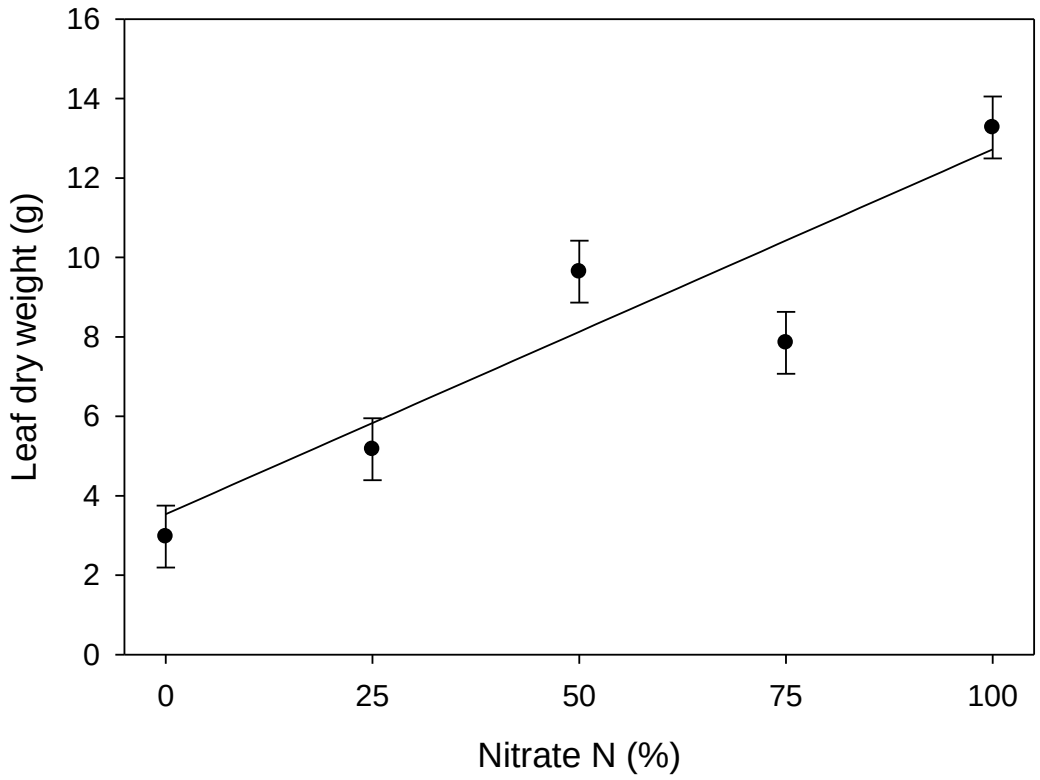


Figure 3.2. The effect of percent NO_3^--N on leaf dry weight of hydroponically grown oxalis. The regression equation is $y = 0.09185x + 3.535$, $r^2 = 0.77$.

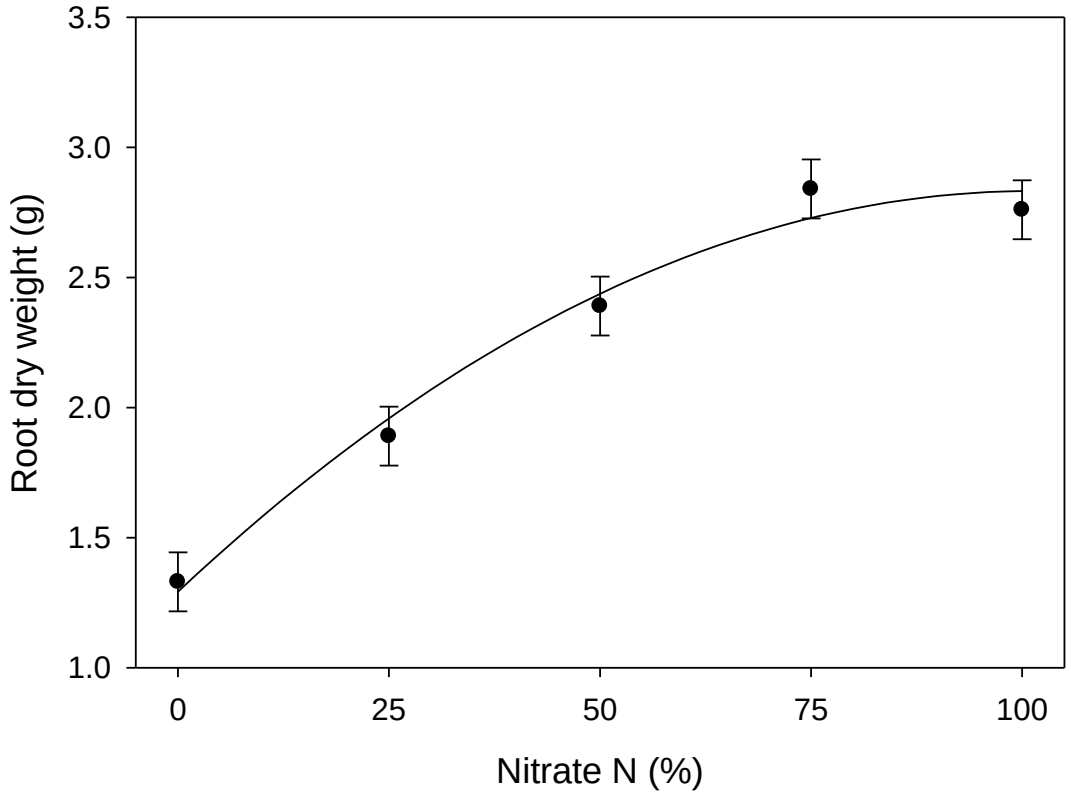


Figure 3.3. The effect of percent NO_3^--N on root dry weight of hydroponically grown oxalis. The regression equation is $y = - 0.00015x^2 + 0.0304x + 1.292$, $r^2 = 0.87$.

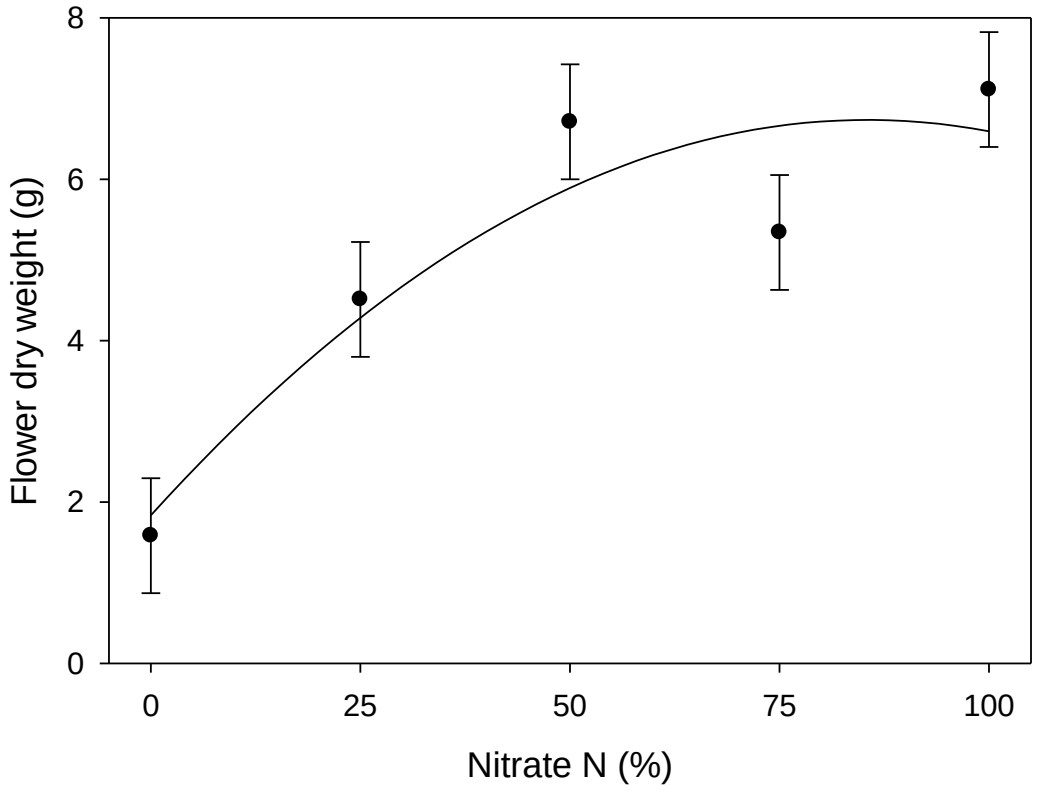


Figure 3.4. The effect of percent NO_3^--N on flower dry weight of hydroponically grown oxalis. The regression equation is $y = -0.00067x^2 + 0.1147x + 1.834$, $r^2 = 0.61$.

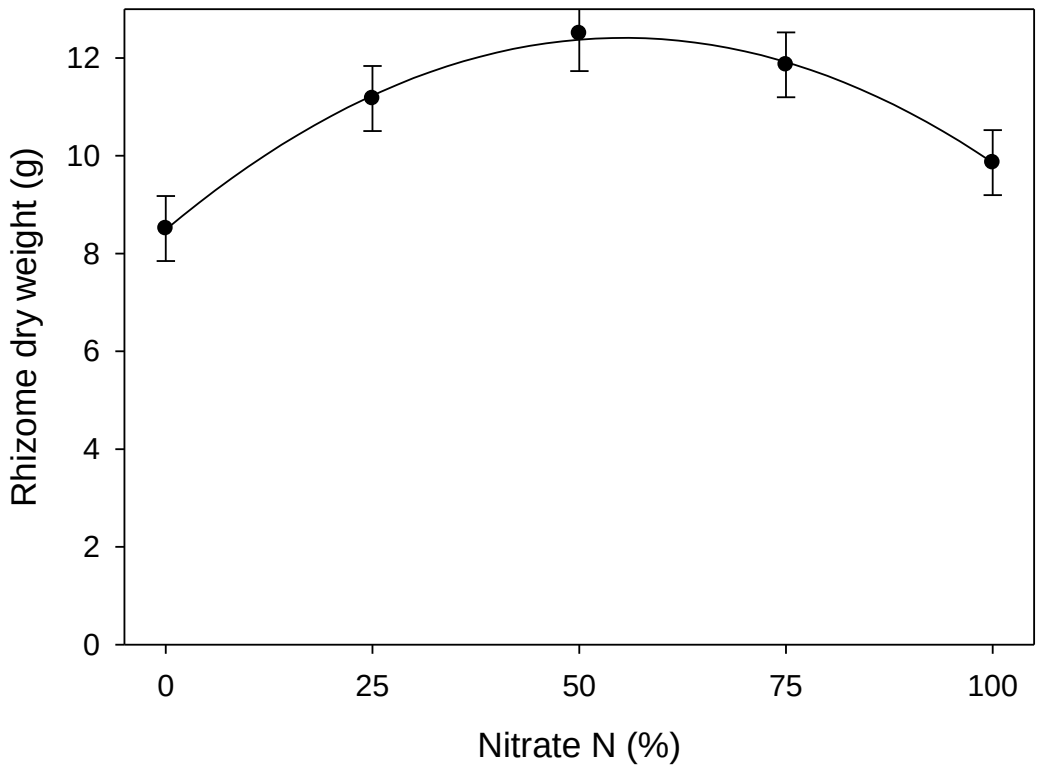


Figure 3.5. The effect of percent NO_3^--N on new rhizome dry weight of hydroponically grown oxalis. The regression equation is $y = -0.00128x^2 + 0.1417x + 8.49$, $r^2 = 0.60$.

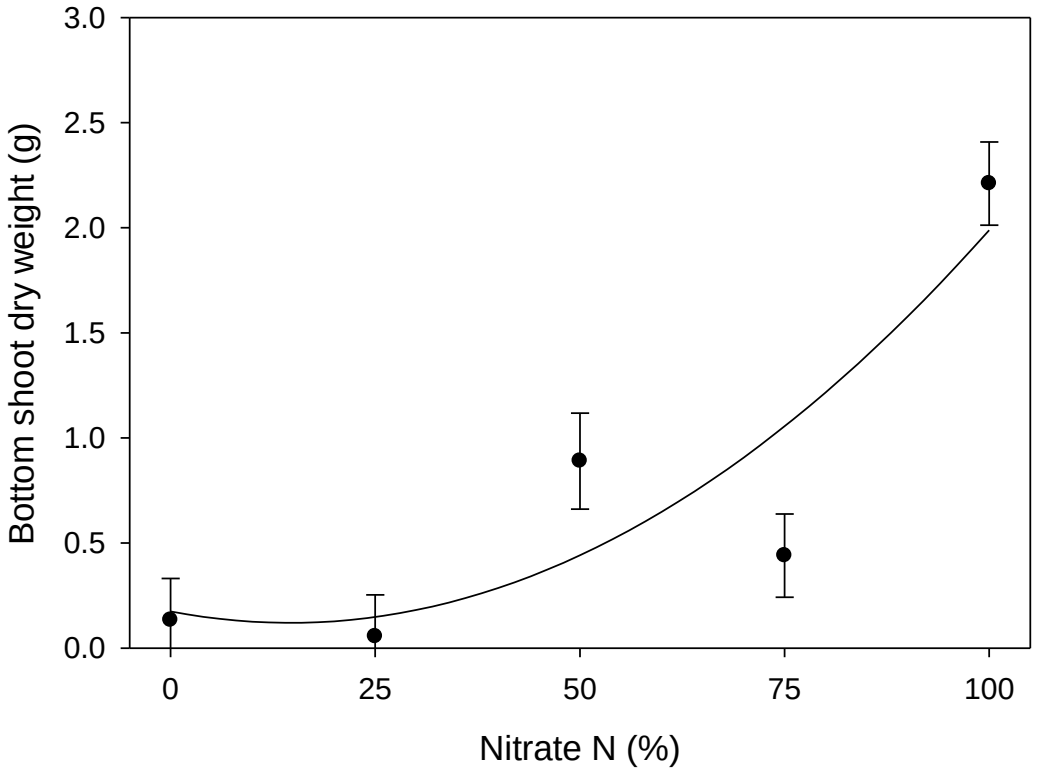


Figure 3.6. The effect of percent NO_3^--N on bottom shoot (defined as shoots that developed under the lid of the container) biomass of hydroponically grown oxalis. The regression equation is $y = 0.00026(x-50)^2+0.01814x-0.4655$, $r^2 = 0.65$

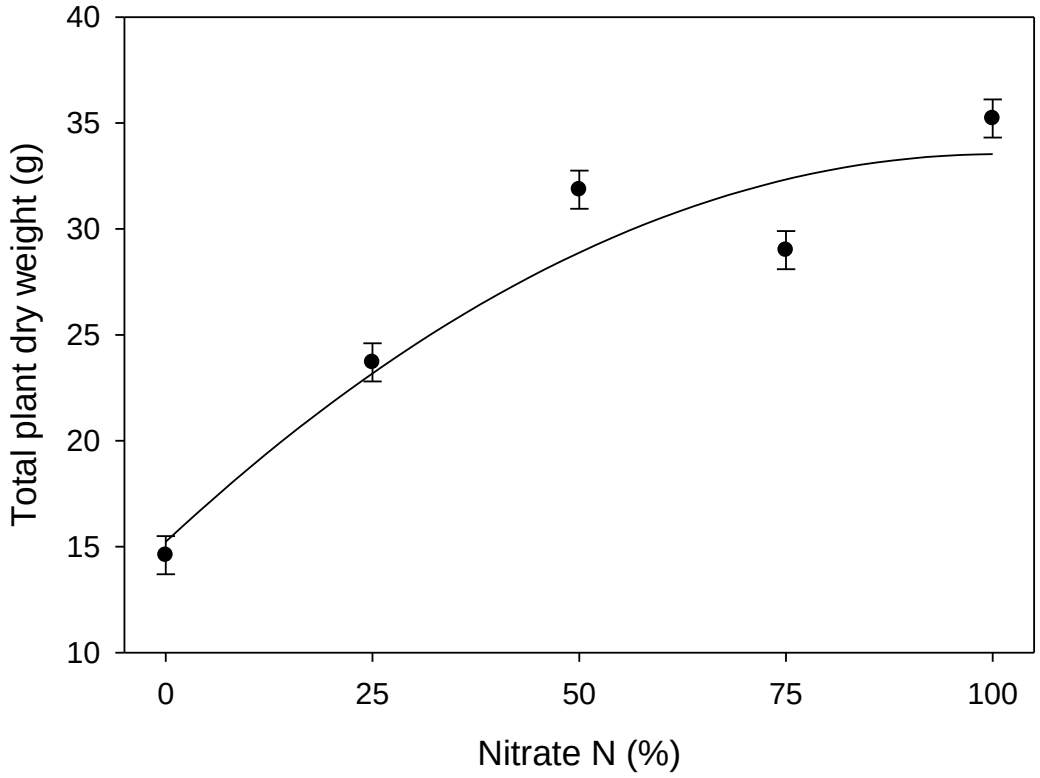


Figure 3.7. The effect of percent NO_3^--N on total plant dry weight of hydroponically grown oxalis. The regression equation is $y = -0.0018x^2 + 0.363x + 15.232$, $r^2 = 0.74$

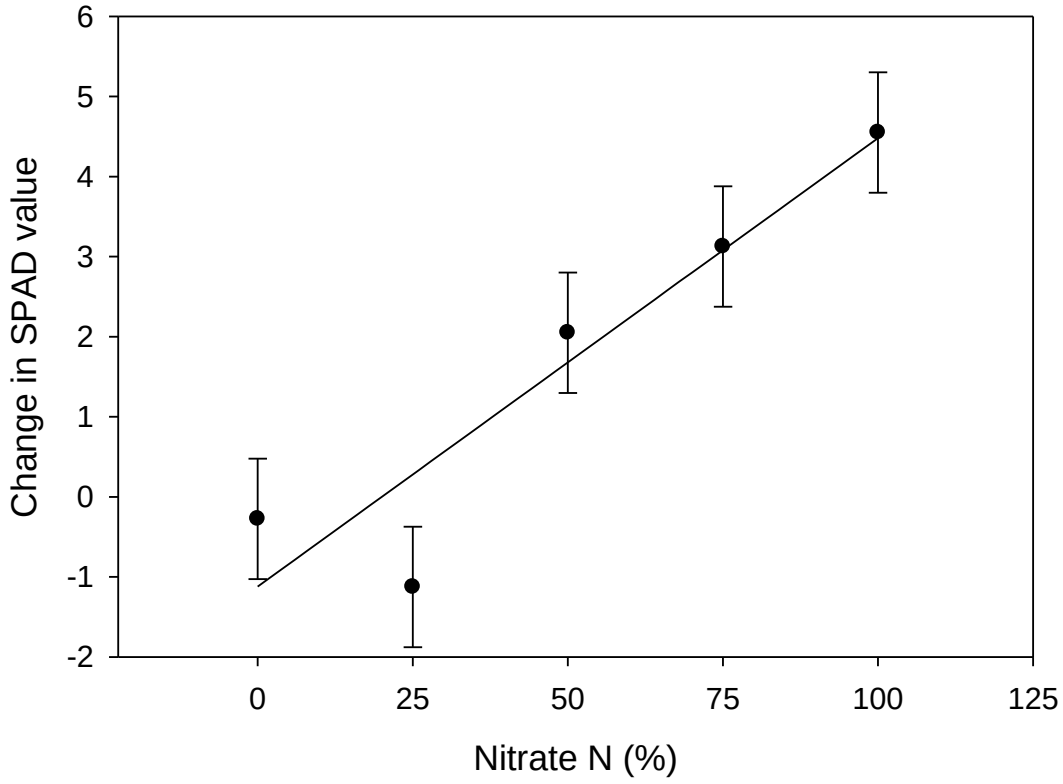


Figure 3.8. The effect of percent NO_3^--N on SPAD meter readings of hydroponically grown oxalis. Change in SPAD meter values were derived from five averaged values taken at experiment initiation and termination. The regression equation is y = 0.056x - 1.12), $r^2 = 0.63$.

Figure 3.9. The effect of percent NO_3^--N on hydroponically grown oxalis. Treatments are (L to R): 0, 25, 50, 75, and 100% NO_3^--N.

Figure 3.10. The effect of NO_3^--N on macronutrient concentrations in leaves of hydroponically grown oxalis plants. The regression equations, associated *P* values for the associated F statistic, and r^2 values are as follows; N: (n=18) y = - 0.0063963x + 4.06 (*P*=<0.0001)(r^2=0.63); P: y = -5.503e-5x^2 + 0.004x + 0.41 (*P*=0.0003)(r^2=0.61); K: y = -9.228e-5x^2 +0.0205x + 2.433 (*P*=<0.0001)(r^2=0.75); Ca: y = 0.006x + 1.278 (*P*=0.0055)(r^2=0.36); Mg: y = 0.000458x +0.39935 (*P*=0.1457)(r^2=0.11).

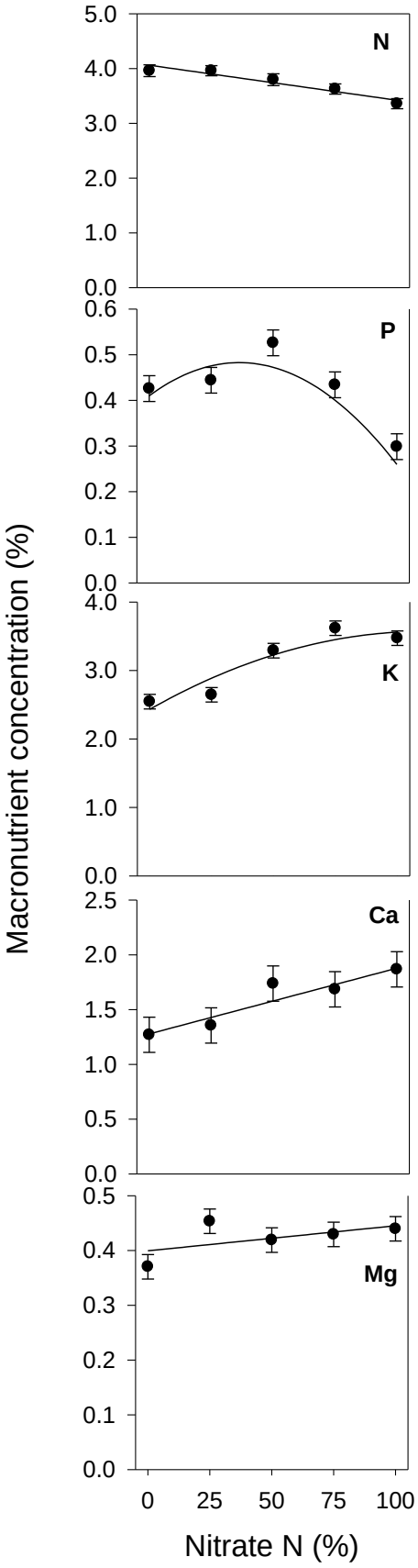
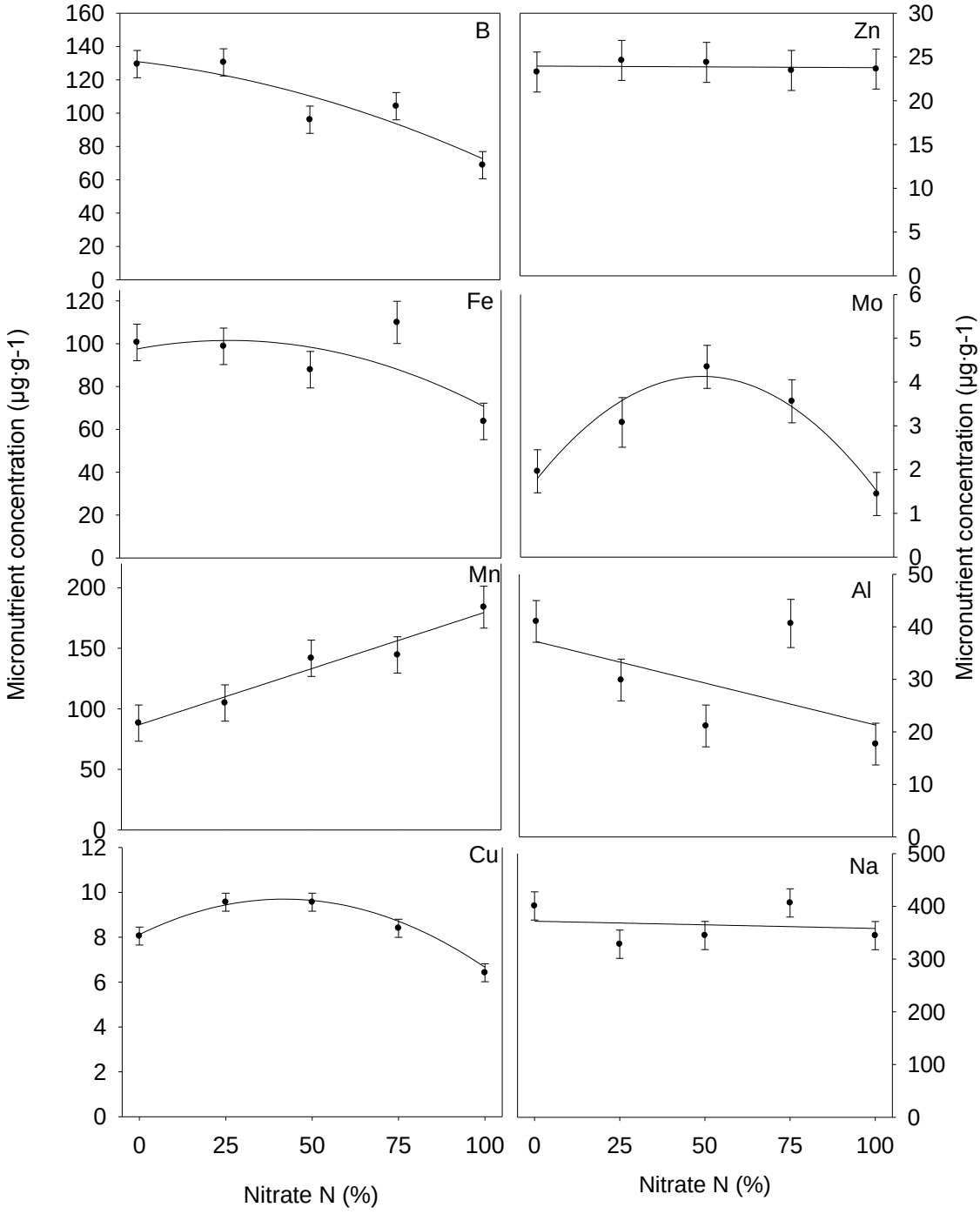

Macronutrient concentration (%)
N
P
K
Ca
Mg
Nitrate N (%)
0
25
50
75
100

Figure 3.11. The effect of NO_3^--N on micronutrient concentrations in leaves of hydroponically grown oxalis plants. The regression equations, associated *P* values for the associated F statistic, and r^2 values are as follows; B: $y = -0.0034x^2 - 0.243 + 130.97$ (*P*=0.0003)(r^2=0.61); Fe: (n=19) $y = -0.0057x^2 + 0.3017 + 97.54$ (*P*=0.06)(r^2=0.29); Mn: (n=19) $y = 0.927x + 86.73$ (*P*=0.0002)(r^2=0.57); Cu: $y = -0.0009x^2 + 0.0754x + 8.12$ (*P*=<0.0001)(r^2=0.73); Zn: $y = -0.0018x + 23.955$ (*P*=0.95)(r^2=0.00); Mo: (n=19) $y = -9.89e\text{-}4x^2 + 0.096 + 1.80$ (*P*=0.0008) (r^2=0.59); Al: (n=19) $y = -0.1590757x + 37.26$ (*P*=0.04)(r^2=0.23); Na: $y = -0.137x + 371.8$ (*P*=0.72) (r^2=0.01).


B
Zn
Fe
Mo
Mn
Al
Cu
Na
Micronutrient concentration (µg·g-1)
Micronutrient concentration (µg·g-1)
Nitrate N (%)
Nitrate N (%)
0
25
50
75
100

A significant relationship between NO_3^--N ratio and tissue nutrient concentration was found for most macronutrients, except Mg (Fig. 3.10). Nitrogen levels decreased from 4% in the 0% NO_3^--N solutions to 3.3% in the 100% NO_3^--N solutions. Potassium and Ca levels increased from 2.5 to 3.5 % and 1.2 to 1.9%, respectively as NO_3^--N levels increased from 0% to 100%. Maximum P levels were observed at 50% NO_3^--N levels.

Results for micronutrient concentrations are shown in Fig. 11. Manganese concentrations increased two-fold, from 88 $\mu g \cdot g^{-1}$ to 184 $\mu g \cdot g^{-1}$. Boron tissue concentration declined quadratically as NO_3^--N ratio increased, averaging 140 $\mu g \cdot g^{-1}$ at 0% NO_3^--N and declining to 70 ppm at 100% NO_3^--N. Similarly, Fe concentrations declined from 100 $\mu g \cdot g^{-1}$ to 63 $\mu g \cdot g^{-1}$, at 0% NO_3^--N to 100% NO_3^--N levels, respectfully. Aluminum concentrations decreased as NO_3^--N levels increased until 50% NO_3^--N levels, increased slightly at 75% NO_3^--N and decreased with 100% NO_3^--N solutions. Molybdenum and Cu reached maximum concentrations at 50% NO_3^--N levels, 4.3 and 9.6 $\mu g \cdot g^{-1}$ respectively. No significant relationship was found for Zn and Na.

Discussion

In our study, leaf, root, flower, bottom shoot and rhizome (up to 50% NO_3^--N) dry weights all increased with increasing proportion of NO_3^--N; this is similar to onion (*Allium cepa* L.) in which additional NO_3^--N alone or in combination with NH_4^+-N increased leaf and root dry weights when compared with a 100% NH_4^+-N fertilizer solution (Gamiely et al., 1991). Plant growth increased as the proportion of NO_3^--N increased in nutrient solutions for onions beyond seedling growth stages (Abbes, et al., 1995). Moreover, similar results were found in New Guinea impatiens (*Impatiens platypetala* Lindl.), wheat, cucumber, and bell pepper (Osoroio et al., 2003; Heuer, 1991, Weigle et al., 1982). Zhang, et al. (2005) found that spinach (*Spinacea oleracea* L.) plant biomass (leaves and petioles) reached a maximum at 50:50 NO_3^--N:NH_4^+-N

ratio. However, Palaniswamy, et al. (2002) found no significant differences in purslane (*Portulaca oleracea* L.) dry weight with different NO_3^--N:NH_4^+-N ratios. Romero et al. (2005) found that a lower NO_3^--N:NH_4^+-N ratio (1:3) maximized dry weight in impatiens (*Impatiens wallerana* Hook. F).

Plant species differ in preference for N-form and often this is simply related to the root-zone pH effect of the NO_3:NH_4 ratio (Marschner, 1995). It is well known that when NH_4^+-N is the sole source of N, rhizosphere acidification can occur resulting in reduced plant growth (Weir et al., 1972). In this experiment we cannot separate whether growth effects were due to pH or could be directly attributed to the N source (such as ammonium toxicity). With the results obtained in this study, a plausible conclusion would be that the differences in plant growth were indirect pH effects (high or low pH) on nutrient absorption, rather than direct effects of N-source. In this experiment, leaf, bottom shoot and flower dry weights decreased with increased NH_4^+-N levels, which we hypothesize, is due to NH_4^+ toxicity. This is supported by the fact that, in the 0% NO_3^--N treatment, even though pH decreased to nearly 3 in the first week, but in subsequent weeks the decrease in pH was not as substantial, which suggests reduced physiological root activity resulting in decreased rhizosphere acidification. This root damage may subsequently reduce nutrient and water absorption thereby decrease growth across the experimental period. Tissue nutrient analyses indicate sufficient levels for all N treatments, which would suggest root activity; however until the point of inactive or reduced activity, it is assumed nutrient acquisition was occurring. This hypothesis is further supported by the observed decrease in root and tissue dry weights, in addition to the dark brown, poor condition physical condition of the roots, and yellowing leaves (Fig. 3.9). Bar-Yosef, et al. (2009) concluded that ammonium toxicity reduced rose (*Rosa* L.) cut flower yield, as yield was significantly less with complete 100% NH_4^+-N solutions as compared to

50% NH_4^+-N solutions over a period of 80 days, with both solutions maintaining a pH 3, for the duration of the experiment.

Phosphorus uptake is enhanced by low pH (3 to 4). In our experiment, P levels were higher in nutrient solution treatments with a greater proportion of NH_4^+-N, which also had lower pH values. However they were only slightly different from the 100% NO_3^--N solution. In the case of P, the hydroponic solutions differed in P supply (due to our need to balance total molarity of N in each solution, in some cases using monoammoniumphosphate). The P tissue response is likely an interaction between P supply and pH.

Enhanced cation (Ca and Mg) uptake is reported to occur when NO_3^--N is used as opposed to ammonium (Deane-Drummond and Glass, 1983). Our results show no difference in Ca and Mg among the NO_3:NH_4 ratios for Ca and Mg. However, K concentrations for 50, 75, and 100% NO_3^--N solutions were slightly higher than 0 and 25% NO_3^--N solutions. High pH levels greatly influence nutrient uptake ability in most micronutrients. Few differences were detected between any of the NO_3:NH_4 treatments for micronutrient concentrations. Boron concentrations were greater in the 0 and 25% NO_3^--N solutions compared to 50 to 100% NO_3^--N solutions. Concentrations for both Cu and Mo were slightly less in the 25, 50, and 75% NO_3^--N solutions than 100% NO_3^--N solutions. Even though slight significant differences were found in some elements, the nutrient analyses for all treatments of all elements are acceptable nutrition levels for ornamental greenhouse potted plants (Reed, 1996).

Conclusions

The results observed in this study suggest that a 1:1 or higher ratio of NO_3:NH_4 is acceptable for oxalis in a hydroponic system, as leaf and root biomasses are greatest in those formulations. Moreover, SPAD meter readings and visual observations, based on greenness, the overall plant quality, is more acceptable at these ratios. With

this information, further research on the relationship between foliar disorders and nutrition will be possible. However, more information regarding optimum nutritional levels for oxalis is still needed, particular to extend our results to a substrate-based growing situation.

Horticultural researchers have long conducted experiments using hydroponic systems to determine the critical minimum and maximum levels and the interaction of nutrient levels needed for plant growth. The use of these systems is invaluable for characterizing nutrient toxicities/deficiencies and may be used to assist in establishing commercial greenhouse production fertilization recommendations. The research reported here is an initial step in examining and identifying causes of the various leaf abnormalities, interveinal chlorosis, in particular, that occurs in greenhouse oxalis production.

Acknowledgements

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CHAPTER 4

Characterizing iron and manganese deficiency in *Oxalis regnellii*

Abstract

Chlorosis in *Oxalis regnellii* has been hypothesized to be an iron (Fe) and/or manganese (Mn) deficiency. However these claims have not been substantiated. The following paper characterizes the effects of removing Fe and Mn in a hydroponic system and Fe deficiency due to elevated pH levels in a container media. Oxalis plants grown in Fe free (-Fe) solutions exhibited classic interveinal chlorosis symptoms on new growth at the apical meristem but plants grown in Mn free (-Mn) solutions did not show any signs of chlorosis or yellowing after seven weeks. Both control and –Mn plants had more than twice the amount of chlorophyll than –Fe treated plants based on SPAD meter readings. Minus Fe treatments had less than half as much total Fe, 30 $mg \cdot kg^{-1}$, compared to control and –Mn plants, at 74.9 and 73.2 $mg \cdot kg^{-1}$, respectively.

Media pH effects on oxalis growth and development were also investigated. Media pH was adjusted using lime rates 0, 2.5, 5, and 25 $lbs \cdot yd^{3}$ (0, 1.13, 2.27, and 11.34 34 $kg \cdot m^{-3}$). The highest lime rate, 11.34 $kg \cdot m^{-3}$, significantly decreased plant height and dry weight compared with the control (0 $kg \cdot m^{-3}$). After three weeks of growth, significant levels of interveinal chlorosis were observed at high the lime rates and continued until the end of the experiment. Tissue tests confirmed Fe levels significantly decreased as lime rates increased, while Mn levels increased.

Nutrient solution treatments affected oxalic acid and irradiance levels of hydroponically grown *O. regnellii* plants. Plants grown in Fe deficient conditions produced 101.0 $mg \cdot g^{-1}$ of oxalic acid compared to 81.4 and 82.7 $mg \cdot g^{-1}$ for control and –Mn plants, respectively. Maximum carbon assimilation rates for oxalis plants grown in –Fe solutions were about a third less at 3.21 $\mu mol \cdot m^{-2} \cdot s^{-1}$ than plants grown in –Mn

and complete solutions, at 9.63 and 9.66 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively (Fig. 4.10; Table 4.2).

Introduction

There are reported to be between 600 and 900 species of *Oxalis* (Bahattacharyya and Johnri, 1998; Bryan, 2002; De Azkue, 2000; Dole and Wilkins, 2005; Wilkins, 1985). Although many oxalis are considered weedy, some, like *O. regnellii,* are cultivated for ornamental purposes. Some species such as *O. deppei* and *O. tuberosa* (oca) produce edible roots or tubers (Roca et al., 2007; Wilkins, 1985). Several foliar disorders including wrinkled leaves, leaf edge burn, and of greatest interest, interveinal chlorosis, have been reported during greenhouse production of *O. regnellii* (De Hertogh, 1993; De Hertogh, 1996; Dole and Wilkins, 2005; Hammer, 2006). The interveinal chlorosis phenomenon has been hypothesized to be an Fe deficiency; however, no investigations of the hypothesis have been formally conducted. Another plausible hypothesis for leaf chlorosis is Mn deficiency, as deficiency symptoms are similar to Fe deficient plants (Marschner, 2003; Reed, 1996).

The oxalis name is derived from to the characteristic presence of oxalic acid and comes from the Greek meaning ‘sour’ or ‘acid’. Oxalic acid is an organic acid found in algae, fungi, lichens, higher plants, and animals (Oke, 1969). It exists both as a free acid and mineral crystals, which may include insoluble salts of calcium, magnesium, and iron, and soluble salts of potassium, sodium, and zinc, collectively known as “oxalates”. High oxalate content in plants consumed by humans can have adverse effects. Namely, excess oxalate levels reduce nutritional quality by restricting calcium and iron bioavailability and can lead to kidney stones (Libert and Fraceschi, 1987; Horner and Wagner, 1995; Massey, 2003; Franceshi and Nakata, 2005; Bataille and Fournier, 2001). Studies indicate that oxalates play various roles in plants including plant protection, calcium regulation, and ion balance (Liber and Franceshi,

1987; Franceshi and Nakata, 2005). Oxalates in buckwheat, taro and rice play a role in heavy metal (lead, aluminum, strontium, cadmium, and copper) detoxification (Ma et al., 1997; Ma and Miyasaka, 1998; Yang et al., 2000; Franceschi and Schueren, 1986; Choi et al., 2001; Mazen and Maghraby, 1997). Because, oxalic acid is able to oxidize and combine with various cations, namely Fe, to form less soluble salts, it is plausible that oxalic acid could be involved in the chlorosis phenomenon.

Iron deficiency is a common disorder that affects many plant species and Fe is often the first micronutrient that becomes limiting in greenhouse media (Nelson, 1994). Substrate pH affects nutrient solubility and the resulting uptake of nutrients into the plant (Nelson, 1998). As pH increases, the solubility of several elements, particularly, phosphorus, Fe, Mn, zinc (Zn), copper (Cu), and boron decreases, while molybdenum solubility increases (Bierenbaum and Argo, 1995; Lucas and Davis, 1961; Peterson, 1981; Wright and Niemiera, 1987). The optimum pH range for many floriculture crops grown in soil-less media is 5.6 to 6.2, and outside of that range deficiencies (higher pH) or toxicities (lower pH) may occur. Iron deficiency chlorosis has been reported in many species many ornamental and floriculture species such as crabapple (*Malus* sp.) (Wallace and Wallace, 1986), Ficus (*Ficus benjamina*) (Lang, H.J. et al., 1990), Piggy Back Plant (*Tolmiea menziesii*) (Smith, 1985), petunia (*Petunia xhybrida*) (Wallace and Wallace, 1986), marigold (*Tagetes erecta* L.) (Argo and Fisher, 2002), Holiday Cactus (*Schlumbergera* sp.) (Ramirez and Lang, 1997), calibrachoa (*Calibrachoa xhybrida*) (Argo and Fisher, 2002), and geranium (*Pelargonium x hortorum*) (Wallace and Wallace, 1986). The symptom most often observed in Fe deficient plants is interveinal yellowing or chlorosis of young leaves and reduced or stunted growth.

To date, no information has been published describing chlorosis in oxalis. A group of studies were conducted to characterize this phenomenon. The objectives of

these experiments were to; 1. characterize and induce Fe and Mn deficiencies in *O. regnellii* using a hydroponic system; 2. induce Fe deficiency in *O. regnellii* by altering (increasing) the pH of the growing media; 3. investigate oxalic acid levels and irradiance effects in Fe deficient (-Fe) and manganese deficient (-Mn) plants.

Materials and Methods

Hydroponic study. Established oxalis that had been growing in 5L plastic containers and aerated by pumping air through porous stones placed in the modified Hoagland's solution (Hoagland and Arnon, 1950) were used for this experiment. The half-strength Hoagland's solution was mixed using reverse osmosis (R.O.) water to give the following: 1 mM MgSO4·7H2O, 0.5 mM KH_2PO_4, 2 mM $CaNO_3 \cdot 4H_2O$, 2.5 mM KNO_3. Micronutrients were supplied at the following levels in all treatments: 46µM H_3BO_3, 9.15 µM $MnCl_2 \cdot 4H_2O$, 0.08 µM $Na_2MoO_4 \cdot 2H_2O$, 0.32 µM $CuSO_4 \cdot 5H_2O$, 0.77 µM $ZnSO_4 \cdot 7H_2O$, 53.58 µM ferric ethylenediaminedi(*o*-hydroxyphenylacetic) acid (Fe-EDDHA) per liter. Containers were covered with aluminum foil to prevent light degradation of chelating agents (Albano and Miller, 2001) and minimize algae growth. Oxalis were grown in a glass house at 21˚ C day/night temperature and grown at ambient photoperiod and light conditions (42 °N latitude). Treatments were initiated on 4 March 2010 and consisted of the half-strength Hoagland's solution (as described above); Fe deficient (-Fe), in which Fe-EDDHA was omitted from the solution; and Mn deficient (-Mn), where Mn was omitted from the micronutrient stock solution. There were seven replicates of each treatment, with each container of three oxalis plants comprising the experimental unit. The containers were organized in a complete randomized design. Nutrient solutions were renewed regularly (every ~2 days) to maintain nutrient balance and reduce indirect pH effects. The pH of fresh solution was (±SD) 5.86 ± 0.05, 5.83 ± 0.08, and 5.87 ± 0.06, for control, -Fe, and –Mn, respectively.

Indirect chlorophyll measurements of leaf greenness were taken using a SPAD meter (Minolta Chlorophyll Meter SPAD-502; Spectrum Technologies, Plainfield, IL). On weeks one and five, three readings were obtained for each of eight randomly selected recently mature leaves (RML) in each container and averaged for each replication. The experiment was conducted for five weeks.

After five weeks of treatment, tissue samples of RML from each replication were collected, washed in 0.1M HCl, triple rinsed in R.O. waer, and then dried at 70˚ C for at least 48 h after which dry weight was taken. Tissue samples were analyzed by Inductively Coupled Plasma-Atomic Emission Spectrophotometry at a commercial laboratory.

Media pH study. A custom media using peat:perlite (3:1 v/v) was prepared and four dolomitic lime treatments were incorporated, 0, 1.13, 2.27, and 11.34 kg $\cdot m^{-3}$ (0, 2.5, 5, and 25 lbs$\cdot yd^{3}$) . The media were allowed to incubate four weeks and on 23 February 2010, one oxalis rhizome was planted in a 10 cm pot. There were seven replications for each treatment. Pots were placed in the greenhouse at 21˚ C day/night temperature and grown at ambient photoperiod and light conditions (42 °N latitude). Plants were fertilized with 250 mg N$\cdot$ L^{-1} 20N–2.2P–16.6K (Jack's Professional LX Water Soluble Fertilizer 21-5-20 All Purpose; J. R. Peter's Inc., Allentown, PA). pH readings of five randomly selected plants from each treatment were obtained using the 2:1 pour thru method and averaged at weeks one, two, three and seven during the experiment.

Indirect chlorophyll measurements of leaf greenness were taken on weeks three and seven by a SPAD meter. Three readings were obtained at destructive harvest for each of three randomly selected RMLs per replication (n=15). Leaf chlorosis ratings were also obtained upon experiment termination. Ratings were from 0 to 5: 0 = no chlorosis; 1 = minimal chlorosis, slight yellowing; 2 = general leaf

yellowing; 3 = progressed leaf yellowing with initial stages of green veins; 4 = distinct interveinal chlorosis; 5 = severe chlorosis, often exhibiting bleaching (Fig 4.1).

After seven weeks, plants were harvested and final plant height (measured from pot rim to the top of the foliage canopy) was recorded. Leaf tissues were washed in 0.1M HCl, triple rinsed in R.O. water, and dried at 70˚ C for at least 48 h after which, dry weight was determined. Dried tissue samples were then analyzed by inductively coupled plasma-atomic emission spectrophotometry at a commercial laboratory to determine foliar nutrient levels. Tissue samples for the highest lime treatment were limited due to decreased growth. Therefore, to conduct tissue analysis, two tissue replications of equal sample weight (~0.25 g) were combined to make one sample sufficient for tissue analysis and another conglomerate sample was comprised of four different replicate tissue samples of differing weights. Thus for the 25 lbs/yd^{-3} treatment, tissue analysis sample size is n=2.

Oxalic acid analysis. Tissue samples from RMLs of hydroponically grown oxalis plants (see previous) were used for this experiment. Tissues were washed in 0.1M HCl, triple rinsed in reverse osmosis water and dried at 70˚ C for at least 48 h. After drying, tissue was ground with a mortar and pestle. A 0.01g leaf sample was vortexed in 5 ml deionized water for ~1 min, after which the sample was diluted with 5000 µl EDTA (10 mM, pH 7.6). Samples were centrifuged at 1,500 rcf for 5 mins. Oxalic acid concentration in the supernatant was measured using an oxalate kit (Procedure No. 591; Trinity Biotech, St. Louis, MO). Oxalate reagents were warmed to 37° C. 1 ml of oxalate reagent A (DMAB; (3-dimethylamino) benzoic acid + MBTH (3-methyl-2-benozothiazolinone hydrazone), pH=3.1) was added to each sample test tube, including blank and standard test tubes. Then, 50 µl sample (supernatant), de-ionized water, or oxalate standard was added to the sample, blank, and sample test tubes, respectively. Oxalate reagent B (oxalate oxidase and

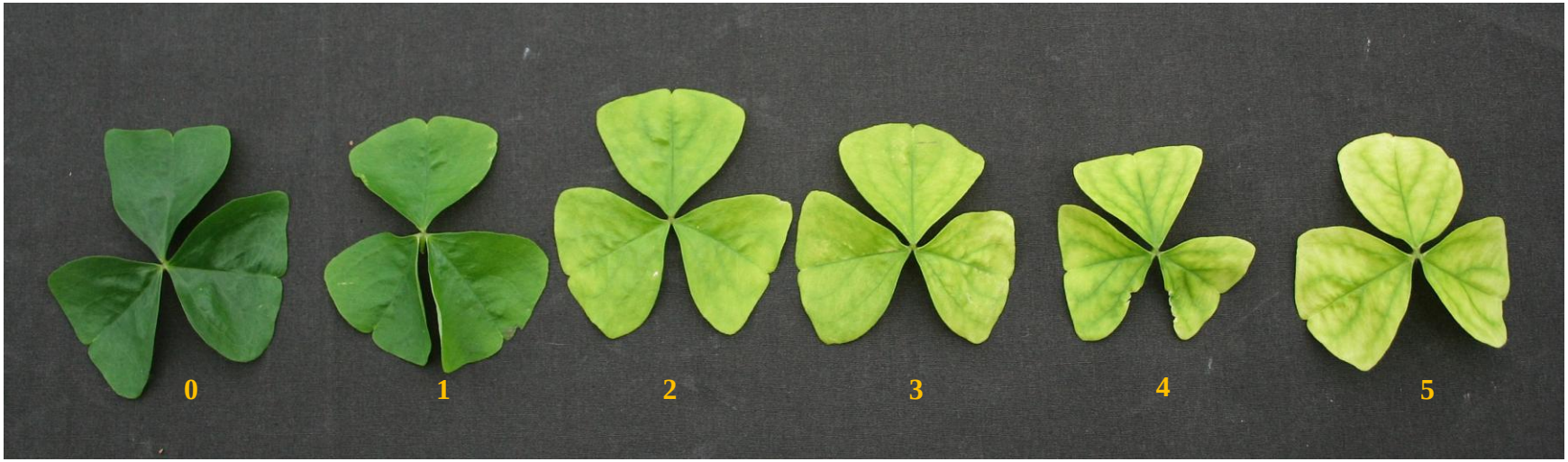


Figure 4.1. Leaf chlorosis ratings of *Oxalis regnellii*. Leaf chlorosis ratings were from 0 to 5: 0 = no chlorosis; 1 = minimal chlorosis, slight yellowing; 2 = general leaf yellowing; 3 = progressed leaf yellowing with initial stages of green veins; 4 = distinct interveinal chlorosis; 5 = severe chlorosis, often exhibiting bleaching.

peroxidase) (100 μl) was pipetted into each test tube and immediately mixed by gentle inversion. Tubes were incubated for 5 min at 37° C and absorbance determined at 590 nm using a Shimadzu UV-1601 spectrophotometer (Shimadzu Corp., Japan). Absorbances were measured twice to obtain consistent readings and averaged. Sample absorbance levels were determined by subtracting blank absorbance from standard and sample readings. Oxalate concentrations were determined on a dry weight basis per the Sigma analysis kit. An oxalate standard curve was generated ($y = 1.309x + 0.019$; $r^2 = 0.99$) using a multilevel oxalate standard set (Catalogue No. 591-11; Trinity Biotech, St. Lous, MO). Data were analyzed using JMP v. 7 (SAS Institute, Cary, NC). One-way analysis of variance tests were conducted and *Tukey's HSD* method was used to conduct pair wise comparisons.

Light Response Curve. Photosynthetic measurements ($\mu mol \cdot m^{-2} \cdot s^{-1}$) of hydroponically grown oxalis (see previous) were obtained from RMLs using a LI-COR 6400 (LICOR Biosciences, Lincoln NE). Three leaves were measured twice (except 0) at 0, 50, 100, 200, 400, 600, 800, 1200, and 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ starting with 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ decreasing to 0 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and then increasing back to 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Measurements were taken when stability was achieved, which typically occurred after two minutes. The LI-COR block temperature was set at 22 ˚C, relative humidity was maintained between 45 and 48% and reference CO_2 levels were set at 400 ppm. The three replications in each treatment were averaged.

Statistical Analysis.

Statistical analyses were conducted using JMP v. 8 (SAS Institute, Cary, NC). One-way analysis of variance tests were conducted to identify differences in the measured parameters in response to nutrient solution treatments and *Tukey's HSD* method was used to conduct pair wise comparisons. General linear or quadratic

regression lines were applied as appropriate based on r^2 values for limestone rate applications.

Results

Hydroponic study. After five weeks, oxalis plants grown in –Fe deficient solutions exhibited classic interveinal chlorosis symptoms on new growth at the apical meristem, while plants grown on –Mn solutions had lighter green leaves than the control, but no signs of chlorosis or yellowing even after seven weeks. Control plants were green with no signs of nutritional deficiencies (Fig. 4.2). SPAD readings indicated that by the end of the experiment, the control and –Mn plants had more than two times the amount of chlorophyll than the –Fe treated plants (Table 4.1). Tissue tests confirmed reduced levels of Fe and Mn in leaf tissue in their respective treatments. Plants grown in –Fe treatments had less than half as much total Fe, 30 $mg \cdot kg^{-1}$, compared to control and –Mn plants, 74.9 and 73.2 $mg \cdot kg^{-1}$, respectively. Manganese tissue concentrations for –Mn were a third less than that of the control plants, while Mn levels in –Fe treatments were 30% higher than control plants (Table 4.1.).

Media pH study. Media pH levels gradually decreased over 7 weeks of growth (Figure 4.3). Lime rates had an effect on growth of oxalis plants. The highest lime rate significantly decreased plant height from 6.4 cm in the control treatments (0 $kg \cdot m^{-3}$) to 2.0 cm for the highest lime rate (11.34 $kg \cdot m^{-3}$) (Fig. 4.4A). Corresponding dry weights also significantly decreased by 66% (Fig. 4.4B). Chlorosis leaf ratings and SPAD meter readings indicated the incidence of interveinal chlorosis was apparent at three weeks (data not shown) in the high lime rate and persisted until destructive harvest at seven weeks (Fig. 4.5A,B; 4.6).

Leaf tissue nutrient analysis indicated N levels were not different between any of the lime rates (Fig 4.7A). Iron levels were highest when no lime was incorporated

Figure 4.2. Hydroponically grown *Oxalis regnellii* plants two weeks after treatment initiation. Treatments (L to R) are Control (complete nutrient solution); -Mn (Mn removed from nutrient solution); -Fe (Fe removed from nutrient solution). Iron deficient treatments exhibit characteristic interveinal chlorosis in young foliage at the apical regions; whereas no interveinal chlorosis was observed in –Mn treatments.

Table 4.1 Effects of removing iron (Fe) and manganese (Mn) from a hydroponic solution on *Oxalis renellii* growth. Letters after values in each column represent mean separation using Tukey's honestly significant difference (*HSD*) at $P = 0.05$.

Treatment	SPAD[z] (week 1)	SPAD (week 5)	Total leaf Fe content ($mg \cdot kg^{-1}$)	Total leaf Mn content ($mg \cdot kg^{-1}$)
Control	40.2 a	32.4 a	74.9 a	61.4 a
-Fe	38.4 a	14.7 b	30.1 b	83.3 a
-Mn	37.1 a	30.7 a	73.2 a	27.2 b

[z] SPAD readings represent averages of three readings per leaf of eight randomly selected recently mature leaves in each container.

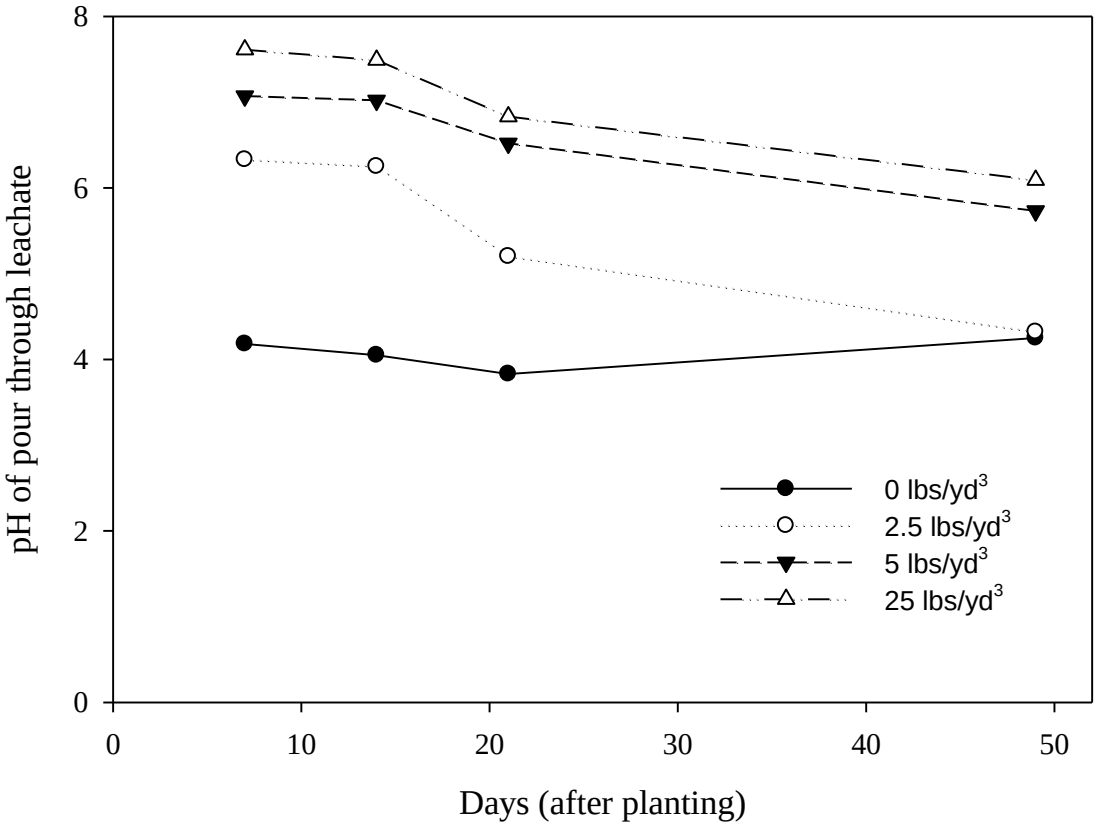


Figure 4.3. pH values for a peat:perlite (3:1 v/v) potting media with different lime rate treatments. Measurements were taken at 7, 14, 21, and 49 days using the saturated paste method. Individual data points are averages of five randomly selected plants.

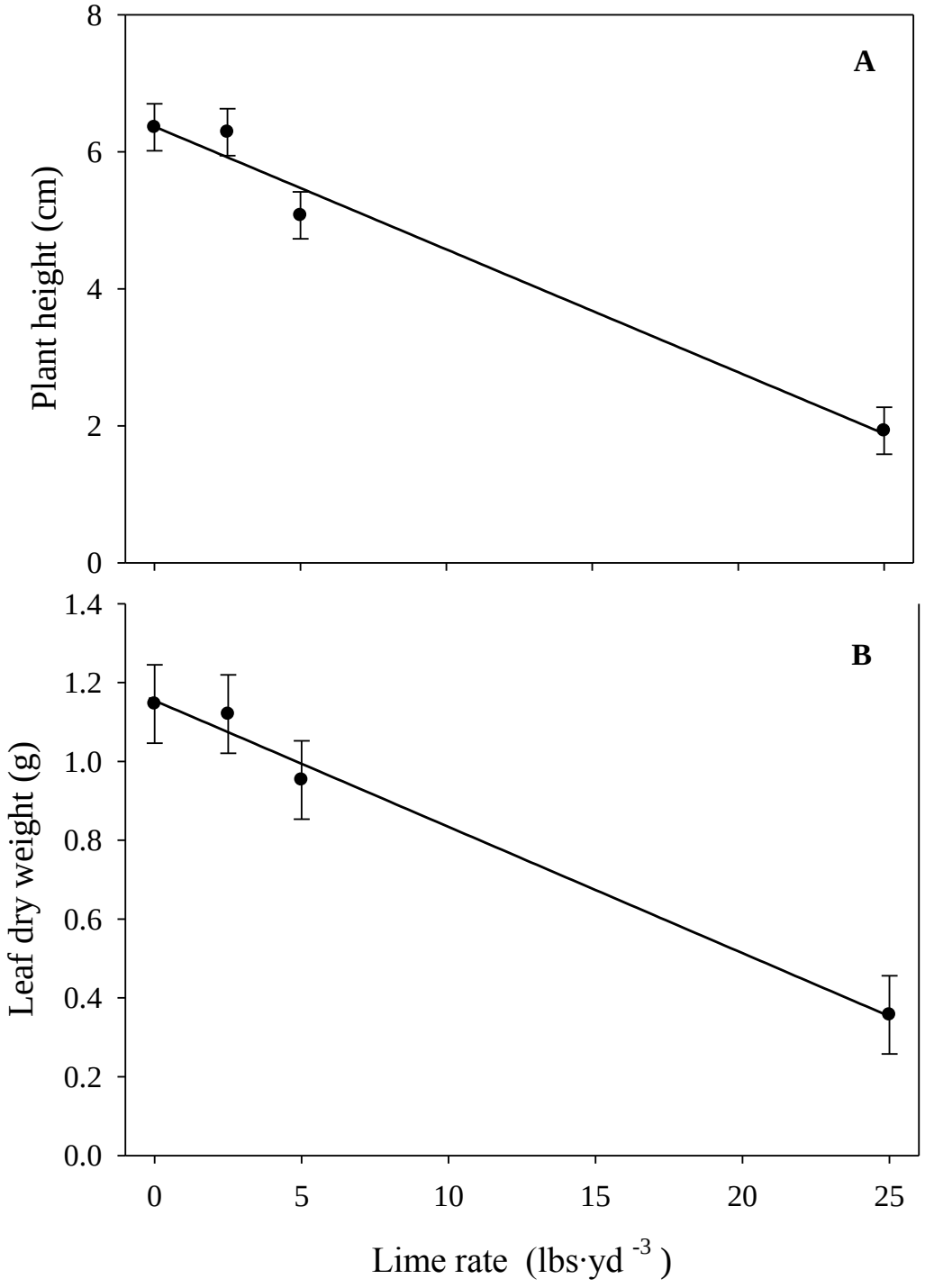


Figure 4.4. Lime rate effects on plant height and leaf dry weight of *Oxalis regnellii* after seven weeks. The regression equations, associated *P* values for the associated F statistic, and r^2 values are as follows; A. plant height: y = -0.1793x +6.367 (*P*=<0.0001) (r^2=0.80); B. leaf dry weight: y = - 0.0321x +1.154 (*P*=<0.0001) (r^2=0.63).

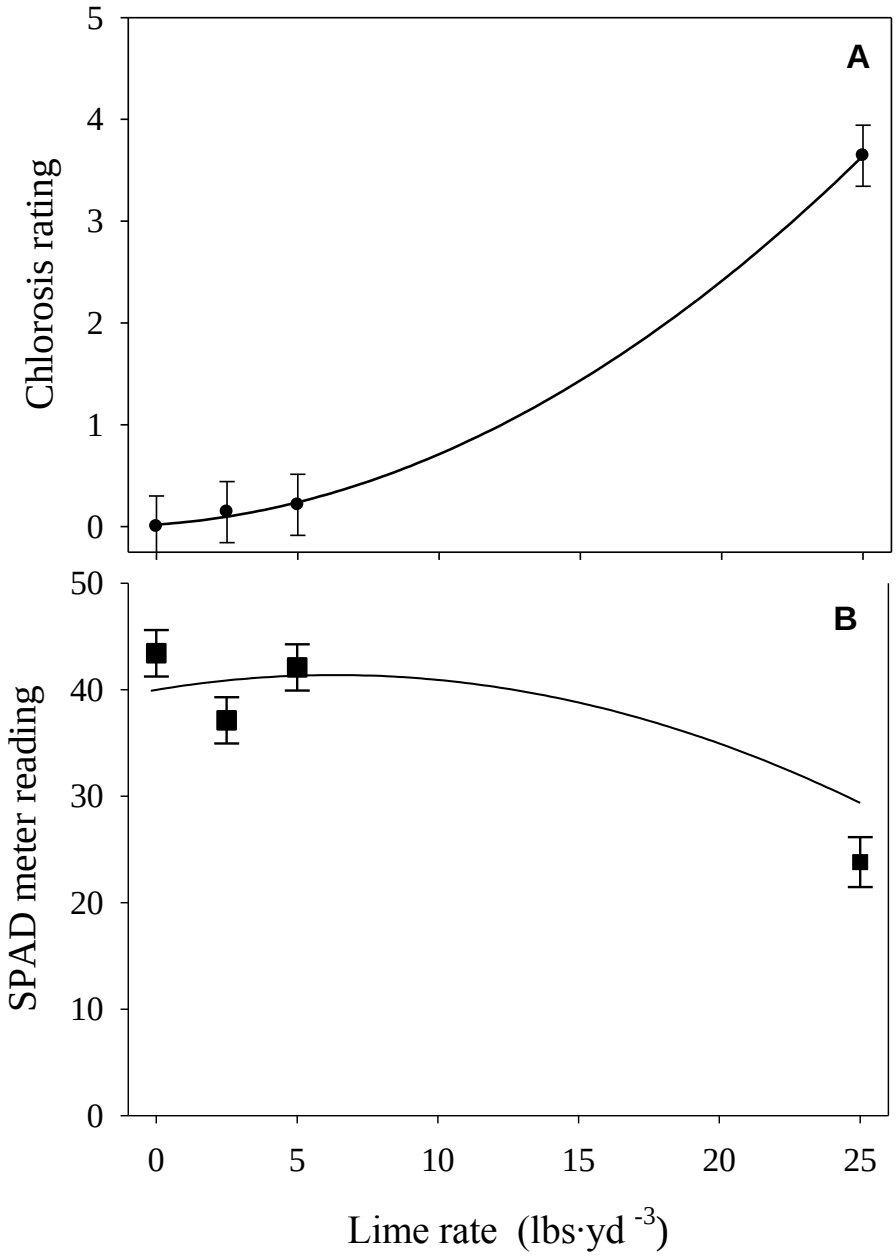
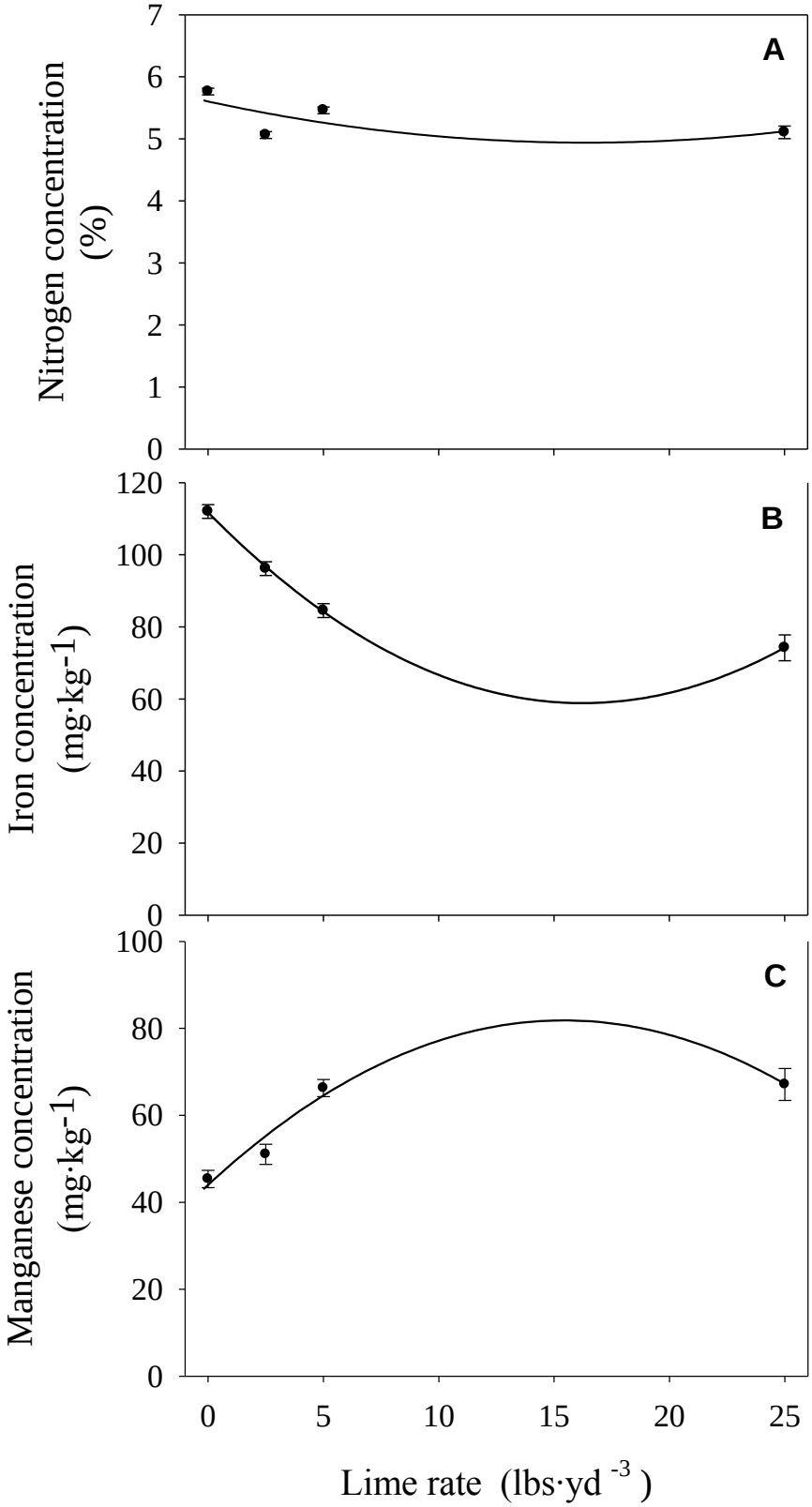


Figure 4.5. Lime rate effects on chlorosis and leaf greenness in *Oxalis regnellii* after seven weeks. Leaf chlorosis ratings were from 0 to 5: 0 = no chlorosis; 1 = minimal chlorosis, slight yellowing; 2 = general leaf yellowing; 3 = progressed leaf yellowing with initial stages of green veins; 4 = distinct interveinal chlorosis; 5 = severe chlorosis, often exhibiting bleaching (n=15). SPAD readings are averages of three readings obtained at destructive harvest three randomly selected recently matured leaves per replication (n=15). The regression equations, associated *P* values for the associated F statistic, and r^2 values are as follows; A. Chlorosis rating: $y = 0.0051(x-8.125)^2 + 0.1007x - 0.3134$ (*P*=<0.0001) (r^2=0.81); B. SPAD: $y = -0.0237(x-7.9)^2 - 0.5462x + 44.39$ (*P*=<0.0001) (r^2=0.74)

Figure 4.6. *Oxalis regnellii* plants grown for 7 weeks in a peat:perlite (3:1 v/v) potting media with different rates of dolomitic limestone. Application rates were (L to R) 0, 2, 5, and 25 lbs·yd^{3} (0, 1.13, 2.27, and 11.34 kg ·m^{-3}).

Figure 4.7. Lime rate effects on nitrogen, iron, and manganese leaf tissue nutrient concentration in *Oxalis regnellii* after seven weeks. The regression equations, associated *P* values for the associated F statistic, and r^2 values are as follows; A. Nitrogen: $y = -0.0025(x-4.456)^2 - 0.0589x + 5.555$ (*P*=0.0546) (r^2=0.25); B. Iron: $y = 0.2001(x-4.456)^2 – 4.728x + 107.77$ (*P*=<0.0001) (r^2=0.88); C. Manganese: $y = -0.1591(x-4.64286)^2 + 3.4300x + 47.427$ (*P*=<0.0001) (r^2=0.75).


A
B
C
Nitrogen concentration (%)
Iron concentration (mg·kg-1)
Manganese concentration (mg·kg-1)
Lime rate (lbs·yd -3)
0
1
2
3
4
5
6
7
20
40
60
80
100
120
0
5
10
15
20
25

and decreased significantly as lime rates increased (Fig 4.7B), while Mn levels were opposite, with the lowest concentrations at 0 and 2.5 lbs/yd^3 (Fig 4.7C).

Oxalic Acid Analysis. Oxalic acid levels were significantly greater in the –Fe deficient plants compared to control and –Mn plants (Fig. 4.8). Iron deficient plants produced 101.0 $mg \cdot g^{-1}$ of oxalic acid compared to 81.4 and 82.7 $mg \cdot g^{-1}$ for control and –Mn plants, respectively.

Light Response Curve. Maximum carbon assimilation rates for oxalis plants grown in –Fe solutions were about 3-fold less (3.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$) than plants grown in –Mn and complete solutions, (9.6 $\mu mol \cdot m^{-2} \cdot s^{-1}$), respectively (Fig. 4.9; Table 4.2). Quantum yields for –Mn and control plants were about 4 times greater (more efficient) than –Fe oxalis plants (Table 4.2). Typically with no irradiance, photosynthesis rates would be less than zero, as dark respiration occurs, however in control plants, this was not observed and is unexplainable (Fig. 4.9).

Discussion

It has long been hypothesized that the chlorosis is due to an Fe and/or a Mn deficiency; however no formal research has been conducted to characterize the chlorosis phenomenon and test these hypotheses. We were successful in characterizing Fe deficiency in oxalis, however clear and convincing Mn deficiencies were not observed. Iron deficiency symptoms in oxalis are very similar to many other plant species. Initial Fe deficiency symptoms begin as light chlorosis in young tissue, followed by the development of distinct interveinal chlorosis, and in severe cases, bleaching and reduced leaf size (Fig. 4.2). We also found the highest Fe concentrations in the –Mn treated plants, although they were not significantly different. This is plausible as it has been reported that there is an inverse relationship between the divalent cations Fe and Mn; increased Fe uptake can result in decreased Mn and vice versa (Bowen, 1969; Chinnery and Harding, 1980). On the contrary, in –

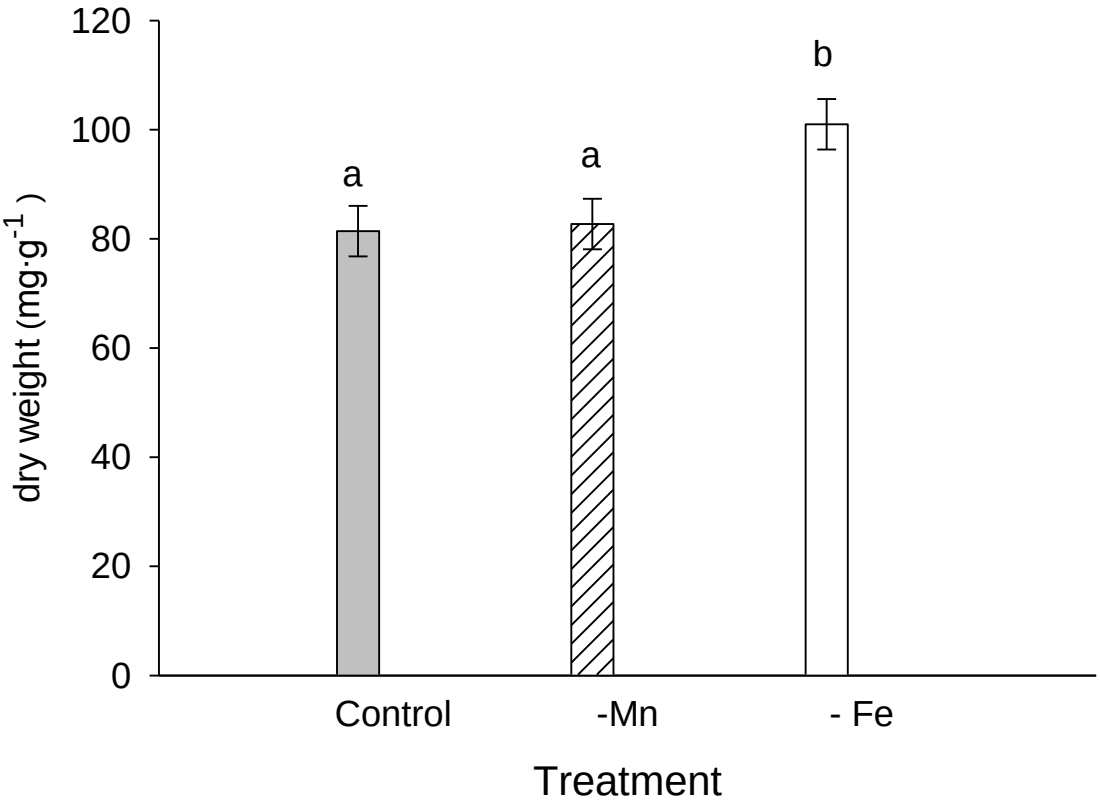


Figure 4.8. Effect of iron (-Fe) and manganese (-Mn) treatments on oxalic acid levels in hydroponically grown *Oxalis regnellii* plants. Treatments with a different letter represent mean separation using Tukey's honestly significant difference (HSD) at $P = 0.05$.

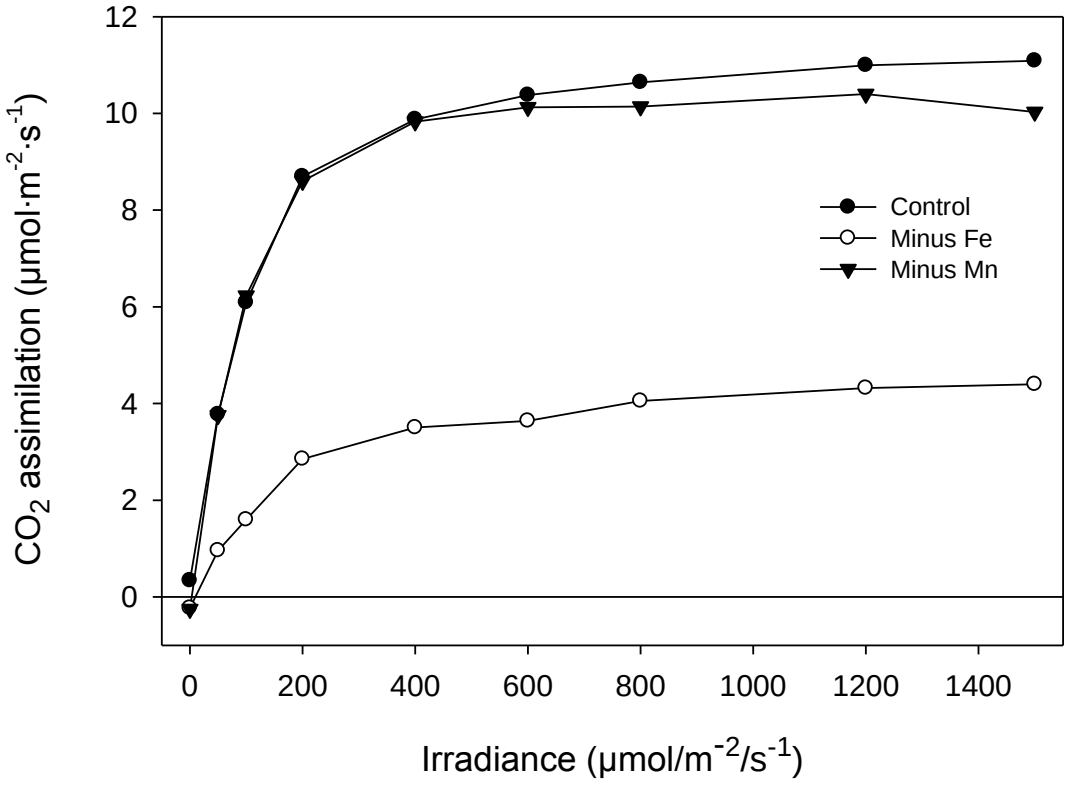


Figure 4.9. Photosynthetic responses of hydroponically grown *Oxalis regnellii* at different irradiance levels with iron (Fe) and manganese (Mn) removed from nutrient solutions.

Table 4.2 Regression coefficients for photosynthesis parameters of hydroponic *Oxalis regnellii* plants with iron (-Fe) and manganese (-Mn) removed from nutrient solutions.

	Treatment		
	Control	-Fe	-Mn
J_{max}	9.66	3.21	9.63
R^2	0.90	0.92	0.91
P value	0.0136	0.0096	0.048
Quantum yield	0.040	0.015	0.042
R^2	0.94	0.97	0.91
P value	0.028	0.013	0.044

Fe deficient treatments, no increased Mn uptake was observed compared to the control treatments (Table 4.2).

Manganese plant tissue concentrations range from 50 to 300 $mg \cdot kg^{-1}$ for general horticultural crops (Reed, 1996). Despite the fact that *O. regnellii* plants were grown in nutrient solutions without Mn for five weeks and tissue analysis revealed significantly lower levels of Mn than plants in the control or –Fe treatments, the plants did not exhibit any visual deficiency symptoms at a leaf tissue Mn level of 27 mg·kg-^{1}. Gibson et al. (2007) describe chlorosis patches on young and RML in early Mn deficiency; cupped edges and interveinal chlorosis in advanced stages; and light brown necrotic spotting in severe Mn deficiency in strawflower [(*Bracteantha bracteata* (Vent.) A. A. Anderberg]. The lack of visual symptoms in *O. regnellii* is plausible as it has been reported that Mn deficiency susceptibility can vary considerably by plant species and even cultivars within a species (Reuter et al., 1988). Our data suggest the critical Mn deficiency level is less than 27 $mg\ kg^{-1}$. Nutrient analysis indicates that oxalis rhizomes (n=9) contained 17.2 (SD ±4.05) $mg \cdot kg^{-1}$ Mn (unpublished data). Thus, potentially there were enough Mn reserves maintained in the rhizome to support the necessary growth and development during the treatment period. Ohki (1985) found the critical Mn deficiency level for significant photosynthesis reduction in wheat to be around 16.5 $mg \cdot kg^{-1}$. It is possible that there was enough residual Mn from existing tissue (leaf and/or rhizome) that Mn was not completely deficient and did not reach the critical minimum deficiency level.

Micronutrient solubility is considerably influenced by substrate pH (Peterson, 1981) and can affect nutrient uptake (Marschner, 2003). Nelson (1994) also reports other factors contributing to Fe deficiencies include high bicarbonates and carbonates; excessive lime applications, excessive phosphate fertilization levels due to Fe precipitation; excessive Mn, Cu, or Zn fertilization due to absorption competition; root

damage; and excessive watering. Plant species also differ in their ability to absorb Fe, as some are more efficient than others at a given pH range (Argo and Fisher, 2002).

As discussed, we successfully induced Fe deficiency in a hydroponic system. This system is an easy and common method to investigate plant nutrition, because nutrient solutions can easily be defined, modified and regulated to observe potential effect on plant growth and development. However, hydroponics may not directly relate to pot plant production and data obtained from using that type of system may not be fully characteristic of the typical greenhouse growing conditions of the species being evaluated. Thus, we found it useful to investigate practical growing conditions.

We conducted an experiment in order to further characterize Fe deficiency in a typical greenhouse substrate. Most substrate mixes used in the greenhouse industry are usually peat based and combined with other organic materials such as bark, coir (coconut byproduct), perlite, vermiculite, recycled tires, or other innovative 'recycled' materials. The individual characteristics of these media, namely the physical properties, nutrient exchange capacity, and water retention capabilities collectively have a significant impact on pH and nutrient availability. Dolomitic lime was applied at various rates to alter pH levels. pH levels for all lime rates gradually decreased over the seven week period due to the acidifying nature of the fertilizer (Fig. 4.1). Even with the gradual pH decrease over time, we were able to successfully induce interveinal chlorosis, especially at the highest lime rate (Fig 4.6).

We are confident that this chlorosis was due to Fe deficiency. This was evidenced by higher leaf chlorosis ratings, indicative of interveinal chlorosis and lower SPAD meter readings. Tissue tests directly support our conclusion as Fe levels were lowest at the two highest lime rates, which also had corresponding higher pH levels. Manganese concentrations were highest at the high lime rate thus we are confident it was not a Mn deficiency. Moreover, one might suggest the chlorosis

could potentially be N deficiency; however N levels were no different between the lime rates, in addition to the chlorosis was interveinal in nature rather than a general leaf yellowing. Despite the lack of tissue replications for the highest lime rate, we believe the limited data is not an artifact. This conclusion is further supported by the fact overall growth and development was poor in the highest lime treatment. This decrease in growth may likely be attributed to decreased leaf area resulting in decreased CO_2 uptake per unit of area (Briat et al., 1995) leading to overall decreased photosynthetic rates, which has been shown in several species (Larbi et. al, 2006) under Fe deficiency. Moreover, other data from Fe chelate drenches were successful in re-greening plants with identical interveinal chlorosis and high media pH reading.

Our data agree with published pH range recommendations (6.0-7.0) for oxalis (Dole and Wilkins, 2005) as plants grown in the 5 lbs/yd^3 treatment were exposed to pH levels between 6 and 7 for a majority of the experiment and showed no signs of chlorosis. Meanwhile, plants grown in the high lime rate (25 lbs/yd^3) had pH levels above 7 for the same period and were chlorotic. As time progressed, the pH in the high lime rate decreased to 6.09, which is an acceptable pH for many greenhouse crops. Plants in the high lime treatment began to grow out of the chlorosis; however, there was still a significant amount of yellow leaves and interveinal chlorosis.

As time progressed and pH decreased to an acceptable level (6.09) for more optimal plant growth and development, oxalis plants in the highest lime rate began to recover and grow out of the chlorosis, although there was still a significant level of yellow leaves and interveinal chlorosis. These data suggest the upper pH limit for *O. regnellii* is between 7.0 and 7.4 as incidence of interveinal chlorosis increases dramatically and dry matter accumulation dramatically decreases.

One mechanism by which plants are able to adapt and acquire nutrients in a deficiency situation is by exuding compounds through the root system. Ström (1997)

and Jones (1998) suggest that this mechanism plays an important role in nutrient acquisition, especially for plants growing in calcareous soils. Oxalic acid is one of several organic acids, which are often secreted as a chelating agent. It is possible that oxalic acid synthesis is up regulated in Fe deficient tissue, which then could be transported to the roots, subsequently secreted into the rhizosphere as a chelators to increase Fe acquisition. Ström et al. (1994), Tyler and Ström (1995), and Ström (1997) found that organic acids (oxalates and citrate) root secretions of calcicole plants were enhanced, compared to calcifuges plants. Okutani and Sugiyama (1992; 1994) found that oxalate concentration in spinach (*Spinacia olearacea* L.) was dependent on leaf position and younger leaves contained less oxalic acid than older leaves. We did not compare oxalic acid concentrations of different leaf positions or ages in this study.

It is known that oxidation of oxalates can lead to the formation of insoluble compounds. Excessive oxalate concentration in the human digestive system interferes with Ca adsorption (Libert and Franceschi, 1987). Rodenkirchen (1998a,b) described chlorosis in *O. acetosella* and concluded Ca played a role in the chlorosis, however oxalic acid concentrations were not investigated. There is the potential for insoluble Fe compound formation in *O. regnellii* that could result in Fe deficiency. Additional studies of oxalic acid and its relationship with different nutrients in *O. regnellii* would be beneficial.

Iron is an essential element in the photosynthetic process. Iron deficiency affects chlorophyll and carotenoid concentrations (Thoiron, et al., 1997), which in return, affect the chloroplast, altering photosystems I and II, and decreasing P700 and cytochromes f and b559 (Spiller and Terry, 1980). In addition chloroplast structure changes and the number of chloroplasts decrease (Marschner, 1985; Nishio et al., 1983; Stocking, 1975). The decrease in C fixation under Fe deficient conditions was

less efficient and would be expected as it has been reported that 80% of cellular Fe in photosynthetic cells is found in chloroplasts (Smith, 1984).

Removing Mn from nutrient solutions in our study did not adversely affect C fixation, as CO_2 assimilation (J_{max}) and quantum yield was nearly the same as control plants. Manganese deficiency can disrupt chlorophyll synthesis, resulting in chlorosis (Bottrill et al., 1970). Results obtained in our study do not support this, as no obvious Mn deficiency symptoms were observed. However, plants grown in –Mn solutions did appear lighter green in color than plants grown in complete nutrient solutions but SPAD readings were not significantly different.

Conclusions

Specific nutrient sufficiency ranges for many crops are unknown, including oxalis. Our data suggest oxalis is susceptible to Fe chlorosis and less susceptible to Mn deficiency. *Oxalis regnellii* exhibited characteristic interveinal chlorosis on new growth when Fe was removed nutrient solutions; while plants grown in -Mn solutions did not show any significant signs of chlorosis or yellowing after seven weeks. As predicted, a high media pH, due to a high incorporation of lime decreased plant height and dry weight and after three weeks of growth, significant levels of interveinal chlorosis were observed at the highest lime rate. Tissue tests confirmed Fe levels significantly decreased as lime rates increased, while Mn levels increased. Deficient levels of Fe cause an increase in oxalic acid concentrations; however it is not clear why this occurs. It is also clear that Fe deficiency will impact *O. regnellii* growth and development more than lower levels of Mn, as photosynthesis efficiency (CO_2 assimilation) decreases in Fe deficient conditions.

Greenhouse crop marketability is highly dependent on quality production practices, including fertilization practices. Poor understanding of fertilizers and media pH can severely affect plant quality, of which Fe is a major concern. Careful media

selection and attention to water quality and fertilization practices can reduce the risk of Fe deficiency.

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CHAPTER 5

Fertilization concentration, formulation and irrigation method affect growth and development of *Oxalis regnellii and Oxalis triangularis*

Abstract

Proper fertilizer and irrigation is important to efficiently produce high quality plants and ensure adequate nutrition levels and proper moisture levels. *Oxalis regnellii*, the Shamrock Plant, and *Oxalis triangularis* are niche greenhouse ornamental crops produced and marketed primarily for their foliage, thus it is imperative to produce the fullest, most colorful, and blemish free plants as possible. An experiment was conducted comparing irrigation methods, overhead (drip) irrigation and subirrigation, in addition to different 21-5-20 (20N–2.2P–16.6K) fertilizer concentrations; 50, 100, 200, 350, and 500 mg N· L^{-1}. For *O. regnellii*, overhead irrigation produced larger plants with increased root mass and high N fertilizer rates, above 350 mg N· L^{-1} and rates lower than 100 mg N· L^{-1} negatively affected growth. Plant height decreased as fertilizer concentration increased, although only at 500 mg N· L^{-1}. Chlorophyll content (based on SPAD readings) increased linearly and quadratically for subirrigated and overhead irrigated plants, respectively.

Little information exists regarding fertilizer recommendations for *O. regnellii* and *O. triangularis*. A study was initiated to analyze the effects of seven different fertilizers formulations. Growth of both species was significantly decreased by fertilizers that contained little or no phosphorus (P) and/or high NO_3^--N:NH_4^+-N ratios. Current fertilizer recommendations of 20-20-20 (21N-2.2P-16.6K) and 14-14-14 (14N-6.16P-11.62K) (Osmocote) produced acceptable, marketable plants; while the best *O. regnellii* and *O. triangularis* plants were produced using the 15-5-15 (15N-2.2P-12.45K) and 20-3-19 ((20N-1.32P-15.72K) formulations, likely due to the additional calcium (Ca), magnesium (Mg) and iron (Fe) in the mixtures.

Introduction

For any given species, greenhouse production of ornamentals requires careful attention to specific nutrition and moisture requirements produce the best quality, marketable product. It is beneficial to have hundreds of species, not to mention many cultivars of a given species for greenhouse producers to choose from. However, a major drawback to such a situation is that species and even cultivars do not all respond similarly to nutrition and irrigation rates and practices.

Overhead irrigation and subirrigation are two common irrigation methods used in the greenhouse industry. Overhead-irrigation provides more control for growers in controlling soluble salts, as excess water can be applied to leach the growing media. Major drawbacks to overhead-irrigation include labor and the potential for poor water use efficiency. Alternatively, subirrigation reduces labor costs and can improve water and fertilizer use efficiency (Uva et al., 1998). However soluble salts can accumulate more easily with subirrigation as leaching does not occur (Kang et al., 2004) and can reduce plant growth and development (Todd and Reed, 1998). Currently, with increasing social concerns regarding fertilizer use and runoff, along with concern regarding water use, it is imperative to conduct, at minimum, small scale trials to determine the best nutrition and irrigation practices for individual species.

Greenhouse fertilization is often applied using soluble formulations through irrigation water, using controlled-release formulations, or a combination of the two. Fertilizer selection for ornamental greenhouse production is important to efficiently produce high quality plants and ensure adequate nutrition levels. Moreover, proper fertilizer selection can reduce costs and excess nutrient leaching or runoff.

Oxalis regnellii, the Shamrock Plant, is a greenhouse ornamental niche crop produced primarily for the St. Patrick's Day holiday (De Hertogh and Le Nard, 1993; Dole and Wilkins, 2005). Fertilization recommendations for oxalis are limited. De

Hertogh and Le Nard (1993) recommend using 14-14-14 Osmocote after visible growth or a weekly 200 $mg \cdot L^{-1}$ N of 20-20-20 liquid feed. Leaf chlorosis has been reported during greenhouse production and has been hypothesized to be iron (Fe) deficiency (De Hertogh, 1993; De Hertogh, 1996; Dole and Wilkins, 2005; Hammer, 2006). Iron deficiency is a common disorder that affects many plant species and is often the first micronutrient that becomes limiting in greenhouse media as pH rises (Nelson, 1994). In addition to high pH levels, Nelson (1994) describes other potential Fe deficiency causes to include root damage and excessive watering.

Many oxalis species are native to the understory (Brickell and Zuk, 1996) and under high light conditions; leaves fold downward, a similar response to water stress. In *O. montana*, Comerro and Briggs (2000) suggest this leaf folding is a hydropassive response as the plant reorients its leaves due to lower water potential from increased transpiration. Thus, unknowingly, greenhouse producers could potentially overwater oxalis, causing root damage and subsequent induced nutrient deficiencies, including Fe. Therefore, a better understanding of a more efficient watering practice would be beneficial.

With little information regarding fertilizer recommendations and irrigation practices of greenhouse production of oxalis, two experiments were designed with the following objectives; 1. determine fertilizer and irrigation method influences on growth and 2. determine different fertilizer formulation effects on growth and development of *O. regnellii* and *O. triangularis*.

Materials and Methods

Expt. 1. Irrigation Method and Fertilization Concentration Study. One *Oxalis regnellii* rhizome (from Dutch stock) was planted per 10 cm plastic pot on 28 April 2009 using a commercial greenhouse media substrate (Metro Mix 360; Sun Gro Horticulture Ltd., Vancouver, Canada). Oxalis plants were grown in two adjoining

glasshouses; one providing subirrigation and one providing overhead-irrigation (drip irrigation) at 20˚ C set point temperature. Plants were irrigated daily with one of five fertilizer treatments; 50, 100, 200, 350, or 500 $mg \cdot L^{-1}$ of a commercial fertilizer mix that contained 20N–2.2P–16.6K (Jack's Professional LX Water Soluble Fertilizer 21-5-20 All Purpose; J. R. Peter's Inc., Allentown, PA). Supplemental magnesium ($MgSO_4 \cdot 7H_2O$) at the rate of 30 $mg \cdot L^{-1}$ was added due to low magnesium levels in the tap water. The municipal tap water had an EC of 0.4 $dS \cdot m^{-1}$ and alkalinity of 111 $mg \cdot L^{-1}$ $CaCO_3$. Fertilizer stocks were mixed were mixed at 100 times the applied concentration for each treatment. A calibrated injector (1:100 ratio) was used with the stock solution to fill corresponding sub-irrigation treatment reservoirs. Data collected at the end of the experiment included leaf fresh weight (FW) and dry weight (DW); root dry weight (RDW); total plant height and diameter, with diameter being calculated by two perpendicular measurements divided in half; and SPAD meter readings (Minolta Chlorophyll Meter SPAD-502; Spectrum Technologies, Plainfield, IL) of five randomly selected leaves per replication.

Expt. 2. Fertilizer Formulation Study. *Oxalis regnellii* and *O. triangularis* rhizomes supplied from The Netherlands were used for this experiment. Eight rhizomes of each species that had been stored at 3° C for several months were planted on 5 February, 2010. One rhizome was planted per 10 cm pot using a commercial greenhouse media substrate (LC1; Sun Gro Horticulture Ltd., Vancouver, Canada) and grown at 21˚ C set point temperature. Plants were typically fertilized twice a week, with one of seven fertilizers (Table 5.1) at 250 $mg \cdot L^{-1}$. The fertilizers chosen are typical greenhouse formulations representing a range of different nitrate:ammonium:urea percentages of total N and different calcium carbonate equivalents. Some fertilizers were specially formulated to provide additional macro and/or micro nutrients to enhance plant growth in a greenhouse situation. After 10

Table 5.1. Fertilizers used to evaluate growth and development of *Oxalis regnellii* and *Oxalis triangularis*

Fertilizer	Analysis (N-P-K)	CCE[z] (lbs)	Acidity/ Basicity	Percent of total NO_3^- : NH_4^+ : $(NH_2)_2CO$	Media pH
21-5-20 All Purpose[y]	21 - 2.2 - 16.6	407	A	13.08 : 7.92 : 0.00	6.92
20-20-20 General Purpose[x]	20 - 8.8 - 16.6	555	A	6.07 : 3.83 : 10.10	5.93
15-4-15 FeED + Ca +Mg[y]	15 - 1.76 - 12.45	77	B	12.12 : 2.88 : 0.00	6.84
15-0-15 Dark Weather Peat-Lite[x]	15 - 0 - 12.45	344	B	13.50 : 1.50 : 0.00	7
15-5-15 +Ca +Mg[x]	15 - 2.2 - 12.45	69	B	12.00 : 3.00 : 0.00	7.17
20-3-19 Petunia FeED +Mg[y]	20 - 1.32 - 15.72	420	A	11.96 : 8.04 : 0.00	6.65
14-14-14 Osmocote[x]	14 - 6.16 - 11.62	--	--	5.80 : 8.20 : 0.00	4.65

[z] Calcium carbonate equivalent (per ton)

[y] Products of Jr. Peters, Inc., Allentown, PA.

[z] Products of Scotts Company, LLC.; Marysville, OH.

weeks, plant height and width (calculated as described above), FWs and DWs, SPAD meter readings (as described in Expt. 1 Materials and Methods), and leaf chlorosis ratings that were determined from a rating scale from 0 to 5: 0 = no chlorosis; 1 = minimal chlorosis, slight yellowing; 2 = general leaf yellowing; 3 = progressed leaf yellowing with initial stages of green veins; 4 = distinct interveinal chlorosis; 5 = severe chlorosis, often exhibiting bleaching (Fig 4.2; Chapter 4). Dried tissue samples were analyzed at a commercial laboratory by inductively coupled plasma-atomic emission spectrophotometry to determine nutrition levels.

Statistical Analysis. Data were analyzed using JMP v. 8 (SAS Institute, Cary, NC). One-way analysis of variance tests were conducted to identify differences in the measured parameters and *Tukey's HSD* method was used to conduct pair wise comparisons. General linear or quadratic regression lines were applied as appropriate based on r^2 values.

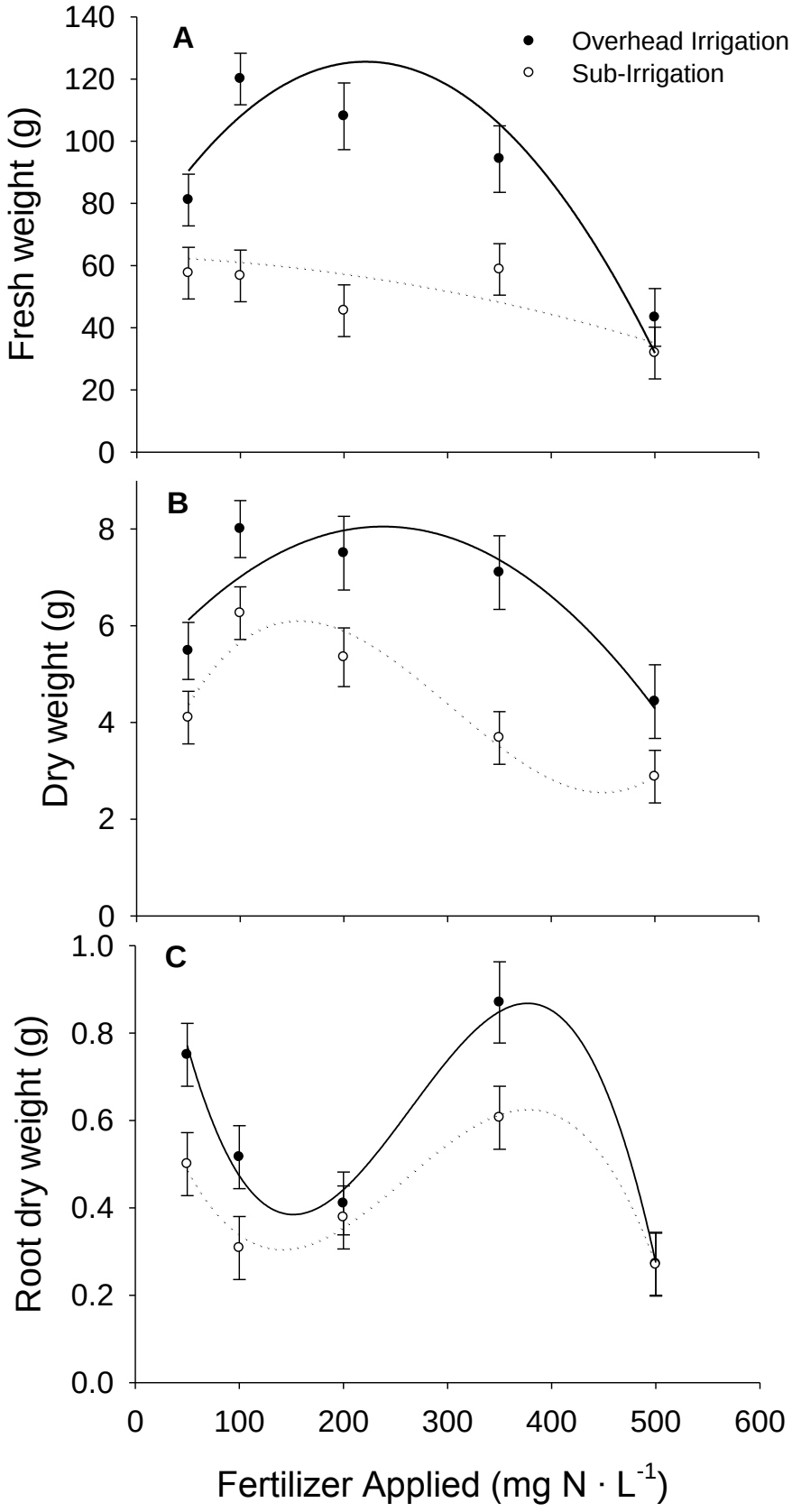
Results and Discussion

Expt. 1. Irrigation Method and Fertilization Concentration Study. The main effects of fertilizer concentration and irrigation type were significant for all growth parameters (Table 5.2) while nearly all interactions were not. The only significant interaction occurred in shoot FW. Greatest FW occurred at 100 $mg \cdot L^{-1}$ for overhead irrigation while at 300 $mg \cdot L^{-1}$ treatments were optimal for subirrigated plants. Fertilization rates at 500 $mg \cdot L^{-1}$ significantly decreased FW (Fig. 5.1A). Greatest DW occurred at 100 $mg \cdot L^{-1}$ with both irrigation methods. Dry weight decreased as concentration increased after 350 and 500 for subirrigation and overhead irrigation, respectively (Fig 5.1B). Frett et al. (1985) observed significant reduction of petunia shoot DW with higher N concentrations (400 $mg \cdot L^{-1}$). Greatest RDW were observed

Table 5.2. Two way analysis of variance for the effect of irrigation type (Irr; overhead and sub-irrigation), fertilizer concentration Conc; 50, 100, 200, 350, and 500 mg•L^{-1} N) and the interaction (Irr x Conc) on *Oxalis regnellii* growth parameters.

Main Effects and Interactions	df	MSE	p-value	df	MSE	p-value	df	MSE	p-value
Source	*Shoot FW*			*Shoot DW*			*Root DW*		
Irr	4	3412	<0.0001	4	16	0.0008	4	0.32	<0.0001
Conc	1	164485	<0.0001	1	43	0.0362	1	0.27	0.0027
Irr x Conc	4	1238	0.0148	4	1	0.646	4	0.04	0.2466
	SPAD			*Height*			*Diameter*		
Irr	4	220	<0.0001	4	34	0.0003	4	103	0.0003
Conc	1	176	0.0028	1	34	0.0134	1	238	0.0006
Irr x Conc	4	41	0.0696	4	7	0.2269	4	20	0.3197

Figure 5.1. Effects of irrigation type (overhead and sub-irrigation) and fertilizer concentration effects on growth parameters of *Oxalis regnellii*. The regression equations, associated *P* values for the associated F statistic, and r^2 values are as follows; A. *fresh weight*: overhead; $y = -0.0012026*(x-231.818)^2 -(0.0264029*x) + 131.54$ (*P*=<0.0001) (r^2=0.64); subirrigation; $y = -8.9264e^{-5}*(x-254.348)^2 - 0.0567637*x + 68.99$ (*P*=0.0135) (r^2=0.35); B. *dry weight*: overhead; $y = -5.4769e^{-5}*(x-205.263)^2 +0.0035645*x +7.2607295$ (*P*=0.0101) (r^2=0.44); sub irrigation; $y = 2.808e^{-7}*(x-241.667)^3 -5.134e^{-5}*(x-241.667)^2 -(0.0150027*x) +9.0027774$ (*P*=0.0012) (r^2=0.54); C. *root dry weight*: overhead; $y = -8.4858e^{-8}*(x-230.435)^3 +8.6248^{-6}*(x-230.435)^2 +0.002930*x -0.155$ (*P*=0.0005) (r^2=0.59); sub irrigation; y = - 4.907e-^{8}*(x-240)^3 +2.737e-6*(x-240)^2 +0.00199*x -0.049601 (*P*=0.0012) (r^2=0.52);


A
B
C
Overhead Irrigation
Sub-Irrigation
Fresh weight (g)
Dry weight (g)
Root dry weight (g)
Fertilizer Applied (mg N · L-1)
0
20
40
60
80
100
120
140
2
4
6
8
0.0
0.2
0.4
0.6
0.8
1.0
0
100
200
300
400
500
600

at 350 mg·L^{-1} for both irrigation methods. In both irrigation treatments, the highest fertilizer treatments reduced RDW by 75% (Fig. 5.1C). In both irrigation systems, a cubic response was observed for RDW, for which we do not have a clear explanation. A negative linear relationship occurred for plant height in both irrigation practices, although heights were only significantly decreased at the 500 mg·L^{-1} level for over head irrigation (Fig. 5.2A). Using subirrigation, Poole and Conover (1992) found increasing fertilizer rates did not significantly increase or decrease plant height in three different foliage plants. Similar results were observed for both irrigation methods in relation to plant diameter, as larger plants were obtained at rates of 100 mg·L^{-1} and the smallest plants at 500 mg·L^{-1} (Fig. 5.2B). The greenest plants, or those with the highest SPAD readings, were observed at 350 mg·L^{-1} for overhead irrigation and were significantly less green at 500 mg·L^{-1}. In subirrigated plants, the greenest plants were observed at higher N rates as SPAD readings increased linearly (Fig. 5.2C). Similarly, chlorophyll content in celosia, dianthus, gomphrena, stock, and zinnia increased as fertilizer solutions increased (Kang and van Iersel, 2002).

From these studies, overhead irrigation produces higher quality plants in terms of overall plant stature and increased root mass for *O. regnellii*. High N fertilizer rates, above 350 mg N· L^{-1} and rates lower than 100 mg N· L^{-1} should be avoided, as growth of *O. regnellii* is negatively affected. Similar to Kent and Reed (1996), Dole et al. (1994), Yelanich and Biernbaum (1993), optimal fertilization concentrations are slightly lower for subirrigation methods, as soluble salt accumulation may occur with less water leaching events.

Expt. 2. Fertilizer Formulation Study. Fertilizer type had significant effects on different growth parameters in *O. regnellii* and *O. triangularis* (Table 5.3). The greatest FW and DW occurred with 15-5-15 treatments in *O. regnellii* and 20-3-19 in *O. triangularis*; while the lowest FW was obtained using 15-0-15 fertilizer in both

Figure 5.2. Effects of irrigation type (overhead and sub-irrigation) and fertilizer concentration effects on growth parameters of *Oxalis regnellii*. SPAD readings are averages of five readings obtained at destructive harvest per replication. The regression equations, associated *P* values for the associated F statistic, and r^2 values are as follows; A. *plant height*: overhead; y = -0.0112*(x) +26.62 (*P*=0.0012) (r^2=0.42); sub irrigation; y = -0.0056*(x) +23.05 (*P*=0.0229) (r^2=0.21); B. *plant diameter*: overhead; y = $-8.9832e^{-5}*(x-219.048)^2$ -0.0035335x + 32.838127 (*P*= 0.018) (r^2=0.35); sub irrigation; y = -0.0131x +27.47 (*P*=0.006) (r^2=0.29); C. *SPAD reading*: overhead; y = $-0.0001254*(x-223.913)^2$ +0.0333212*x +28.18 (*P*=0.0011) (r^2=0.50); sub irrigation; y = 0.0314*(x) +28.72 (*P*=<0.0001) (r^2=0.67)

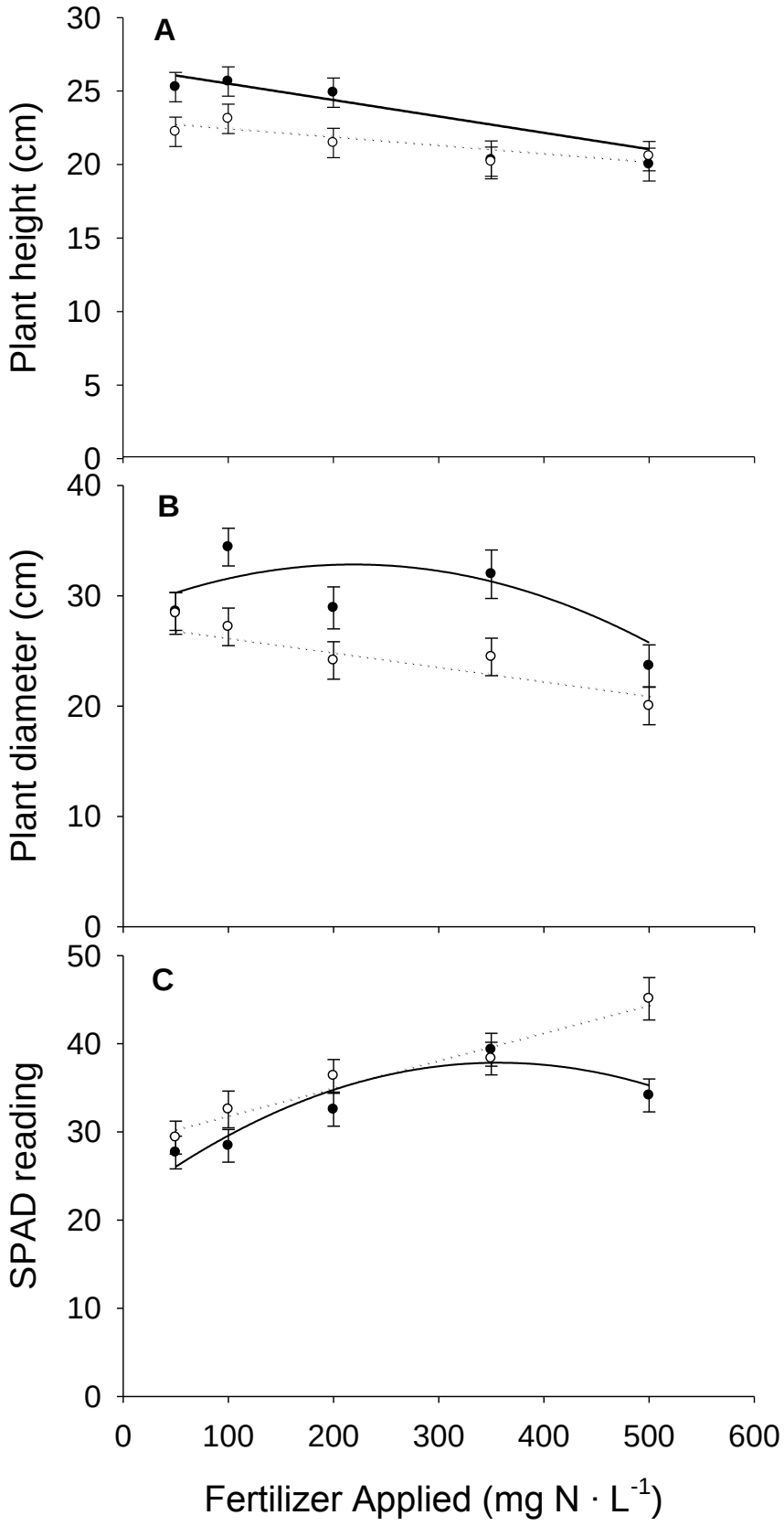

A
B
C
Plant height (cm)
Plant diameter (cm)
SPAD reading
Fertilizer Applied (mg N · L-1)
0
5
10
15
20
25
30
40
50
100
200
300
400
500
600

Table 5.3 Fertilizer effects on growth and development of *Oxalis regnellii* and *Oxalis*

Fertilizer Analysis (N-P_2O_5-K_2O)	Fresh weight (g)	Dry weight (g)	Height (cm)	Width (cm)	SPAD	Chlorosis rating[z]
			Oxalis regnellii			
21-5-20	36.5 ab[z]	2.58 ab	13.3 ab	21.7 ab	29.1 cd	1.20 ab
20-20-20	28.0 bc	1.94 b	11.4 bc	20.3 b	31.4 bc	1.13 ab
15-4-15	31.3 abc	2.24 ab	13.8 ab	21.8 ab	28.5 cd	1.63 a
15-0-15	18.8 c	1.78 b	9.38 c	21.7 ab	27.5 d	1.88 a
15-5-15	45.5 a	3.24 a	14.6 a	24.6 a	29.6 cd	1.00 abc
20-3-19	34.4 abc	2.50 ab	13.1 ab	24.0 ab	33.9 ab	0.08 c
14-14-14 (Osmocote)	28.3 bc	2.39 ab	13.3 ab	23.7 ab	35.8 a	0.50 bc
			Oxalis triangularis			
21-5-20	23.1 abc	1.67 ab	12.7 a	19.2 ab	34.5 ab	-
20-20-20	26.6 ab	1.81 ab	13.9 a	20.1 a	34.4 ab	-
15-4-15	22.0 abc	1.68 ab	14.1 a	20.7 a	33.1 b	-
15-0-15	12.2 c	1.03 b	7.9 b	16.0 b	35.4 ab	-
15-5-15	21.0 abc	1.49 ab	12.4 a	19.7 ab	35.8 ab	-
20-3-19	30.6 a	2.14 a	13.7 a	20.8 a	36.1 ab	-
14-14-14 (Osmocote)	18.5 bc	1.38 ab	13.5 a	17.9 ab	38.5 a	-

zLetters after values in each column for each species represent mean separation using Tukey's honestly significant difference (HSD) at P = 0.05.

triangularis. Plants were fertilized with 250 mg·L-1 with each watering.

species. Plant height and width in *O. regnellii* was greatest with 15-5-15 fertilizer formulation; while in *O. triangularis*, little differences were observed between the different fertilizer formulations, except for plants fertilized with 15-0-15 formulations, in which plant height and widths were significantly decreased by ~20 %. *Oxalis regnellii* plants with the highest SPAD reading (greenest color) were observed in the 20-3-19 and 14-14-14 formulations. Chlorosis symptoms for *O. regnellii* were most observed in plants fertilized with 15-0-15, which also had the lowest total Fe tissue concentration (Table 5.4). The least chlorosis was observed with plants given 20-3-19 fertilization, which correlated with the highest total Fe tissue concentrations.

Tissue nutrient analysis indicated that for most fertilizers and tissue concentrations for N, K, Mg, B, and Cu were in the sufficient range (per commercial lab guidelines). Phosphorus tissue levels were acceptable for all fertilizer treatments except for the fertilizer formulation with no P added, in which P levels were deficient. In both species, final plant height, a common deficiency symptom, was significantly shorter for P free fertilizer treatments. Calcium levels were generally acceptable, but marginally. Iron levels varied between different fertilizer treatments, as expected. Both 20-3-19 and 15-4-15 fertilizer formulations contain three Fe chelates to increase Fe availability at elevated pH levels. However, the 20-3-19 formulation provided more Fe, 85.0 $mg \cdot kg^{-1}$ compared to 60.2 $mg \cdot kg^{-1}$. This is likely due to the doubled amount of Fe (compared to most Fe fertilizer formulation additions) in the 20-3-19. Manganese (Mn) levels for several fertilizer treatments in *O. regnellii* had Mn levels near the lower sufficiency concentration, but should not be of concern. In a previous experiment (Chapter 4) we found that hydroponically grown oxalis plants grown in Mn free solutions were free from any Mn deficient symptoms at 27 $mg \cdot kg^{-1}$. Molybdenum (Mo) concentrations were significantly higher than the upper sufficiency range, especially for *O. triangularis*. Little information is known for Mo

Table 5.4. Fertilizer effects on leaf tissue nutrient concentrations after 10 weeks in *Oxalis regnellii* and *Oxalis triangularis*.

Fertilizer Analysis (N-P_2O_5-K_2O)	%					($mg \cdot kg^{-1}$)					
	N	P	K	Ca	Mg	B	Fe	Mn	Cu	Zn	Mo
	Oxalis regnellii										
21-5-20	4.35 a[z]	0.44 ab	4.28 a	0.85 bc	0.63 b	54.8 ab	65.5 bcd	57.8 c	13.3 ab	28.5 ab	7.91 b
20-20-20	4.16 a	0.43 ab	3.98 ab	0.81 c	0.66 ab	39.4 c	74.1 ab	63.6 bc	8.96 cd	22.7 b	3.55 cd
15-4-15	4.21 a	0.46 a	3.81 bc	1.00 a	0.73 a	36.0 c	60.2 de	82.0 ab	8.87 cd	28.7 ab	8.70 ab
15-0-15	3.52 b	0.10 d	2.88 d	0.80 c	0.50 c	48.5 b	49.5 e	67.0 bc	10.4 bc	27.7 ab	7.32 b
15-5-15	4.04 a	0.44 ab	3.96 b	0.94 ab	0.64 ab	54.3 b	62.7 cd	97.5 a	15.8 a	32.5 a	11.6 a
20-3-19	4.05 ab	0.27 c	3.93 b	0.88 abc	0.65 ab	57.6 ab	85.0 a	64.0 bc	12.7 ab	27.3 ab	6.31 bc
14-14-14 (Osmocote)	4.11 a	0.40 b	3.57 c	0.75 c	0.66 ab	63.6 a	72.1 bc	55.2 c	7.04 d	27.7 ab	1.81 d
	Oxalis triangularis										
21-5-20	4.13 a	0.41 a	3.54 a	0.86 ab	0.71 bc	71.5 abc	70.6 bc	70.7 c	10.3 bc	42.7 bc	11.9 b
20-20-20	3.94 ab	0.42 a	3.53 a	0.83 b	0.71 bc	61.4 bc	78.7 ab	84.1 abc	8.4 d	34.6 de	5.1 c
15-4-15	3.65 bc	0.39 a	3.15 b	0.92 ab	0.80 a	56.0 c	54.2 d	95.9 ab	9.50 cd	34.5 de	11.8 b
15-0-15	3.61 c	0.26 b	3.18 b	0.90 ab	0.68 c	83.4 a	67.0 c	97.1 ab	11.9 b	46.2 ab	20.8 a
15-5-15	4.25 a	0.43 a	3.58 a	0.95 a	0.74 abc	78.9 a	78.3 ab	101.8 a	15.7 a	49.8 a	18.3 a
20-3-19	3.98 ab	0.37 a	3.45 ab	0.82 b	0.69 bc	73.5 ab	80.3 ab	71.0 c	8.9 cd	38.2 cd	11.3 b
14-14-14 (Osmocote)	3.98 ab	0.40 a	3.30 ab	0.83 b	0.77 ab	67.8 abc	88.8 a	73.9 bc	8.3 d	31.0 e	2.77 c

[z]Letters after values in each column for each species represent mean separation using Tukey's honestly significant difference (HSD) at $P = 0.05$.

concentration requirements for many plants and Mo toxicities in greenhouse production are not common. Typical requirement levels are low Zinc (Zn) levels in *O. regnellii* for all fertilizer formulations were marginally deficient; however no deficiency symptoms were observed. Zinc deficiencies are uncommon in greenhouse crops (Reed, 1994).

A well developed, efficient fertilization program requires well-timed and adequate nutrition levels throughout crop production and in post production (consumers). A controlled release fertilizer (CRF) (ie. Osmocote, 14-14-14) formulation produced marketable plants in our study. An added benefit to CRF is greater control over nutrient leaching during greenhouse production. Further investigations of CRF application rates and formulations should be investigated.

Current 20-20-20 and 14-14-14 (Osmocote) fertilizer recommendations are acceptable. However, more commercially acceptable *O. regnellii* and *O. triangularis* plants were produced using the 15-5-15 and 20-3-19 fertilizer formulation in this study. Results from the nutrient analysis suggest Ca, Mg, Fe, and Mn have the greatest influence on quality growth and development of *O. regnellii*. This is supported by the fact the best performing fertilizers, 15-5-15 and 20-3-19 both had extra macro- and micronutrients. Fertilizers containing little or no P and/or high NO_3^--N:NH_4^+-N ratios should be avoided as oxalis leaf growth and plant size was drastically decreased.

Conclusions

Results obtained in these studies provide more information into irrigation and fertilization greenhouse forcing parameters for *O. regnellii* and *O. triangularis*. Because these oxalis plants are marketed mostly for their foliage, it is imperative to for oxalis growers to produce the fullest, greenest or reddest, and blemish free plants as possible.

Irrigation method, fertilizer concentration, and fertilizer formulation are not the only factors that affect crop production, including, but not limited to, temperature (Kang and van Iersel, 2001), light intensity (Masson et al., 1991), growing medium (Poole and Conover, 1992; James and van Iersel, 2001) and specific nutrient proportions (Haley and Reed, 2004). These other factors should be considered when selecting a specific fertilizer, concentration, and irrigation method to best fit a specific producers conditions

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CHAPTER 6

Overall Conclusions

Interveinal chlorosis in Shamrock Plant (*Oxalis regnellii*) has long been reported during production and has long been hypothesized to be due to micronutrient deficiencies, either iron (Fe) or manganese (Mn) or virus. In our research we characterized and confirmed that *Oxalis regnellii* is susceptible to Fe deficiency. We characterized Fe deficiency through a hydroponic system and attempted to characterize Mn deficiency. However, despite a low level (27 $mg \cdot kg^{-1}$) of Mn, the only visual deficiency symptom was very slight chlorosis, although chlorophyll levels based on SPAD reading were not different than control plants. We also were successful in inducing Fe deficiency in a greenhouse media situation, using different rates of limestone to alter (increase) the media pH. The highest lime rate, 11.34 $kg \cdot m^{-3}$, significantly decreased plant height and dry weight compared with the control (0 $kg \cdot m^{-3}$). Increased incidence of interveinal chlorosis was observed at high the lime rates after three weeks and continued until the end of the experiment. Tissue tests confirmed Fe levels were significantly less as lime rates increased, while Mn levels increased. Our data suggest the observed and hypothesized interveinal chlorosis in *O. regnellii*, is more likely to be that of an Fe deficiency, rather than Mn.

Oxalis behaves very similar to other greenhouse ornamental crops, in that, when pH rises above the generally accepted upper threshold of 6.2, Fe can become limiting resulting in interveinal chlorosis. The best advice for growers would be to monitor pH for any given crop and keep accurate records to prevent deficiencies before they occur, whether it be through altering fertilizer, adjusting media pH before planting (or after), or modifying irrigation water. However, if Fe deficiencies occur, we were able to show that Fe chelate (Fe-EDDHA) drenches are successful at 45 $mg \cdot kg^{-1}$ Fe. However, in our small study, only one media drench concentration of one

chelate type was applied, thus further research should be conducted to evaluate different concentrations and different Fe chelators. Our foliar chelate applications of Fe-EDTA, Fe-EDDHA, or Mn-EDTA at concentrations of 0 (distilled water), 60, 120, or 240 $mg \cdot L^{-1}$ Fe or Mn were not successful. This is not to say that further foliar chelate studies should not be considered, as it is highly likely that the treated chlorotic leaves were in severe stages of chlorosis and unresponsive to chelate treatments.

Another long running hypothesis suggests chlorosis can be attributed to a viral infection. During our studies we observed many plants with classic virus symptoms, such as impatiens necrotic spot virus (INSV) and tobacco mosaic virus (TMV), two very common greenhouse viruses. Several plants exhibiting virus symptoms tested negative for INSV and TMV. Shamrock chlorotic ringspot virus (SCRV) was first reported in *Oxalis regnellii* in 1981 (Coyier). Further testing of suspected virus plants during in our research was done and a virus was confirmed in 3 of 4 tested plants. However, it could not be positively identified as SCRV. With this information, it is imperative that oxalis rhizome producers conduct regular stock plant inspections and rogue any suspected virus infected plants as necessary, because a single oxalis rhizome can produce many propagules.

Little information exists on greenhouse production of oxalis, including forcing temperatures, irrigation method, and fertilization requirements. In our studies we found cooler temperatures reduced oxalis leaf and flower numbers. Current recommendations suggest that after oxalis plants begin to flower, the greenhouse temperature could be reduced. Although reducing the temperatures would save input costs in the northern latitudes, especially as most oxalis is produced during winter months, careful attention must be paid to irrigation, so as not to overwater. Oxalis roots are fine and very sensitive to overwatering and easily decay (Fig. C1) and can

ultimately lead to nutrient deficiencies and chlorosis, which may be similar to what growers have reported.

We also found that irrigation method (overhead vs. subirrigation) had an effect on oxalis growth and development. Overhead irrigation produced larger, more robust plants, likely due to increased root mass as subirrigated plants had reduced root mass. In addition, as previously mentioned, oxalis roots are sensitive to overwatering and in a subirrigation system, if water sits in the ebb and flow table too long root damage or death is more likely to occur.

It is highly likely that much of the observed chlorosis problems in oxalis are due to overwatering and induced nutrient deficiencies, which can include Fe, rather than an inherent physiological Fe deficiency problem. This conclusion is based on several factors. The first factor is that growers describe the chlorosis problem occurring in patches or areas of a greenhouse bench, while in other adjacent plants symptom-less. Oxalis are vegetatively propagated thus, theoretically each plant should behave similarly. If the chlorosis problem was a physiological Fe problem, one would expect full benches or greenhouse areas would uniformly become chlorotic, similar to calibrachoa. A second factor closely related to the first, was observed during greenhouse experiments. Oxalis produce many waxy, hydrophobic leaves per pot. Therefore, uneven watering may occur and potentially lead to over and/or under watering if the correct amount of water is not applied. A third and probably most important factor relates to a physiological response of *O. regnellii* to light. *Oxalis regnellii* leaves are nyctanastic, in that they fold downward during dark periods and reopen during in the daylight. Moreover, some oxalis species respond to high light conditions by folding their leaves downward, a very similar response to a water stress. Thus, unknowingly, greenhouse producers could mistake these responses for lack of

water and potentially overwater oxalis, causing root damage (Fig. C1) and subsequent induced nutrient deficiencies, including Fe.

Oxalis are not heavy feeders and will grow and develop to produce quality, marketable plants at 200 to 250 ppm N; similar to what is currently recommended. The literature has recommended fertilizers of 20-20-20 and 14-14-14, with the former not used as widely in the industry. With this in mind, we conducted a fertilizer experiment to investigate different fertilizer types, hypothesizing that chlorosis incidence would vary by fertilizer formulation. We found that the current recommended fertilizers are suitable; however fertilizer formulations with added calcium, magnesium, and Fe produced better oxalis plants. We conducted an experiment to establish an optimum NO_3^-:NH_4^+ ratio for oxalis growth in a hydroponic system using five NO_3^--N:NH_4^+-N ratio treatments (100:0, 75:25, 50:50, 25:75, and 0:100). Increasing proportions of NO_3^--N increased total leaf, root, flower, and bottom shoot biomasses and produced greener plants. We also found a fertilizer with no phosphorus and little ammoniacal nitrogen (blended especially for cold, dark months of winter) produced poor plants.

There is little information available regarding any type of dormancy in *O. regnellii*. Anecdotes suggest *O. regnellii* plants may maintain active growth for years; while others suggest that a 'dormant' period occurs, especially in unfavorable conditions. Moreover, oxalis rhizome producers harvest rhizomes from actively growing plants and store (sometimes 3 months or more) and ship as necessary. We conducted a study storing rhizomes for various durations. We found no 'dormant' period is necessary or apparent; rhizomes will grow leaves and flower readily. We found that growth and development was greater (total leaves and plant height) with longer cold storage periods. Further investigations on longer term storage (greater than 5 weeks) would be beneficial.

Future, more in-depth research on nutrition and interactions with soil moisture (irrigation) and media type would be very useful. Specifically, the role of calcium seems to be of interest, in light of Rodenkirchen (1998a,b) findings of chlorosis in *O. acetosella*. Moreover, further investigations of oxalic acid synthesis would be interesting. Specifically, oxalic acid and its potential role as a root exudate to acquire nutrients would assist in better understanding plant nutrient acquisition mechanisms. Also, the role of oxalic acid and potential oxidation and formation of insoluble Fe compounds would be of interest and provide more insight to chlorosis in oxalis.

Aside from its reputation as a weedy species, *Oxalis* has great potential as an ornamental pot plant. Only a few species are currently cultivated as ornamentals. Abundant diversity exists in leaf shape (Fig. C2) and flower color (Fig. C3). Future breeding work, interspecific hybridization, and trait introgression could yield interesting and novel oxalis. Successful breeding and careful selections have an opportunity to provide ornamental greenhouse producers with more options.

The difficulty with under used, little-known, niche crops in the floriculture industry is that little information exists on forcing these unique species in the greenhouse. Niche crops like *Oxalis regnellii* provide depth and diversity to an already diverse plant palette. Moreover, these crops provide ample research opportunities.

Figure 6.1. An over-watered *Oxalis regnellii* plant. Note the lack of white, healthy roots.

Figure 6.2. Diverse *Oxalis* species leaf morphologies. Photo courtesy of Ron Vanderhoff.

Figure 6.3. Diverse *Oxalis* species flower colors. Photo courtesy of Ron Vanderhoff.

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APPENDIX ONE

Table A1.1 Reagents used to produce a full strength Hoagland's Solution (Hoagland and Arnon, 1950).

Reagent	Reagent formula	Formula weight	Stock solution concentration	Grams reagent needed for 1 L stock soln	Amount stock solution to make 1 L of 1X nutrient solution ($ml \cdot L^{-1}$)
Macronutrients					
Magnesium Sulfate	$MgSO_4.7H_2O$	246.48	1 M	246.48	2
Calcium Nitrate	$CaNO_3 \cdot 4H_2O$	236.15	1 M	236.15	5
Potassium Nitrate	KNO_3	101.11	1 M	101.11	5
Potassium Phosphate Monobasic	KH_2PO_4	136.09	1 M	136.09	1
Micronutrients [z]					1
Boric Acid	H_3BO_3	61.83	.5 ppm	2.86	
Manganese Chloride	$MnCl_2$	197.91	.5 ppm	1.81	
Molybidic Acid	$Na_2\ MoO_4\ 2H_2O$	241.95	.01 ppm	0.02	
Copper Sulfate	$CuSO_4$	249.68	.02 ppm	0.08	
Zinc Sulfate	$ZnSO_4$	287.54	.05 ppm	0.22	
Iron [y]	FeNa-EDTA	367.1	1000 ppm	22.94	1
Iron	FeNa-EDDHA	434.2	1000 ppm	7.77	1

[z]Mix all micronutrients together to make a "micronutrient stock solution"

[y]Mix appropriate iron form and add desired volume to nutrient stock solution.

APPENDIX TWO

Length of rhizome storage affects growth of *Oxalis regnellii*

Introduction

Oxalis regnellii is asexually propagated through small rhizomes. Some geophytic species do not require a dormant period, whereas others require a dormant period for proper growth and development.

Observations and personal communication with oxalis producers suggest there is no defined storage period for harvested oxalis rhizomes. Commercially, rhizomes are harvested as early as August or September, but primarily in October. After lifting, rhizomes are separated, washed, graded, and then stored in a cool environment at 1-5° C until shipment in November or December (De Hertogh, 1993; De Hertogh, 1996; Dole and Wilkins, 2005; Mellano, personal communication). Dole and Wilkins (2005) report that rhizomes stored can be stored for 10+ months in moist peat, while Mellano (personal communication) suggested a much shorter storage period of 1 to 2 months. In our research, we received Dutch oxalis rhizomes that had been stored longer than 60 days and after planting, the oxalis plants had little to no visible negative effects from such handling. Because little information exists regarding oxalis rhizome storage and subsequent greenhouse forcing, we designed an experiment to investigate effects of different storage periods on growth and development of *O. regnellii* rhizomes.

Materials and Methods

O. regnellii rhizomes were harvested weekly, for 6 weeks, from actively growing oxalis plants in the greenhouse (21º C). Rhizomes were carefully washed then placed in plastic bags with moist vermiculite and stored in a dark cooler at 3º C. After the sixth week, rhizomes that had been previously harvested and stored in the cooler were removed. Ten uniform rhizomes (2.15 g ± 0.5) were selected and one

rhizome planted per 10 cm pot using a commercial greenhouse media substrate (LC1; Sun Gro Horticulture Ltd., Vancouver, Canada). Pots were placed in the greenhouse and grown at 21º C. Plants were fertilized at each watering with 250 mg $N \cdot L^{-1}$ 20N–2.2P–16.6K (Jack's Professional LX Water Soluble Fertilizer 21-5-20 All Purpose; J. R. Peter's Inc., Allentown, PA). Data collected included days to shoot emergence (DTE) which was recorded when the first leaf was visible above the media surface; the number of days to flower (DTF); recorded when the first flower fully opened and the number of flower cymes per pot. After six weeks, plant height was recorded. Then above ground tissues were harvested, oven dried at 70˚ C for at least 48 h after which dry weight was determined. One-way analysis of variance tests were conducted to identify differences in the measured parameters in response to storage periods and general linear or quadratic regression lines were applied as appropriate based on r^2 values.

Results and Discussion

Storage period had a significant effect on DTE (Fig. A4.1); as storage duration increased, DTE decreased. Rhizomes stored for five weeks emerged in about 10 days compared to 16 days for rhizomes given no storage. Similar results were observed by Armitage et al. (1996) when *O. adenophylla* was stored at 5º C (dry or wet) for at least six weeks. Storage period had no effect on DTF (Fig. A4.1). The number of leaves and flowers increased as storage period increased (Fig. A4.2.). Rhizomes stored for 5 weeks produced more than twice as many leaves than rhizomes stored for zero or one week (Fig. A4.4). Leaf dry weight also increased linearly as storage period increased, more than doubling from 0.33 g with zero storage weeks to 0.85 g after 5 storage weeks (Fig. A4.3).

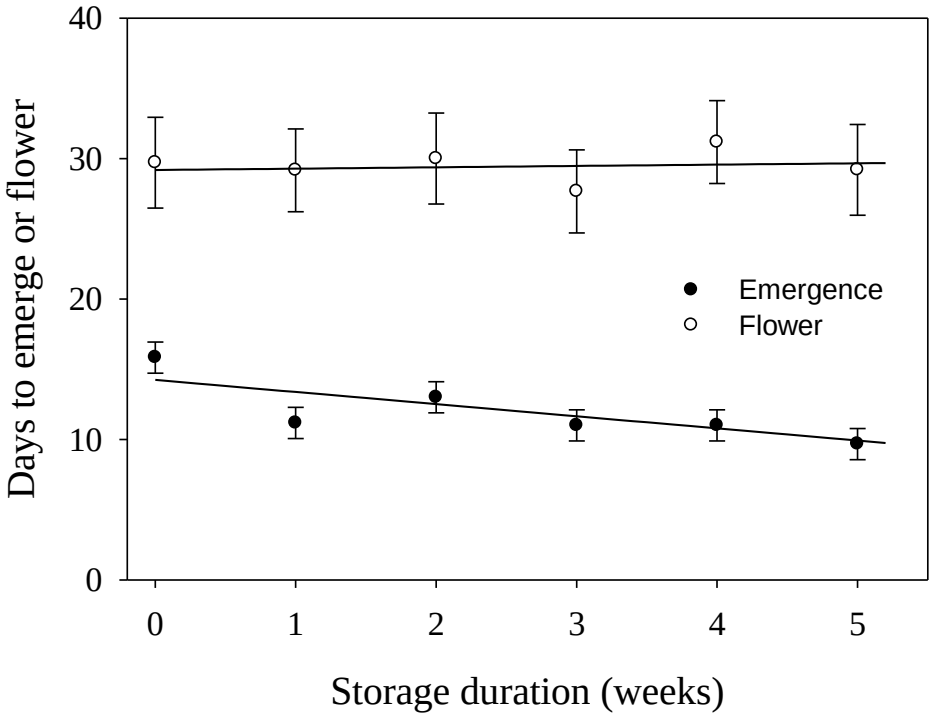

Figure A2.1. Effects of pre-plant storage at 3º C on days to shoot emergence and first flower in *Oxalis regnellii*. The regression equations, associated *P* values for the associated F statistic, and r^2 values are as follows; emergence: y = -0.8637x +14.2401 (*P*=0.0052) (r^2=0.21); flowering: y = 0.0952x +29.1846 (*P*=0.8944) (r^2=0.00).

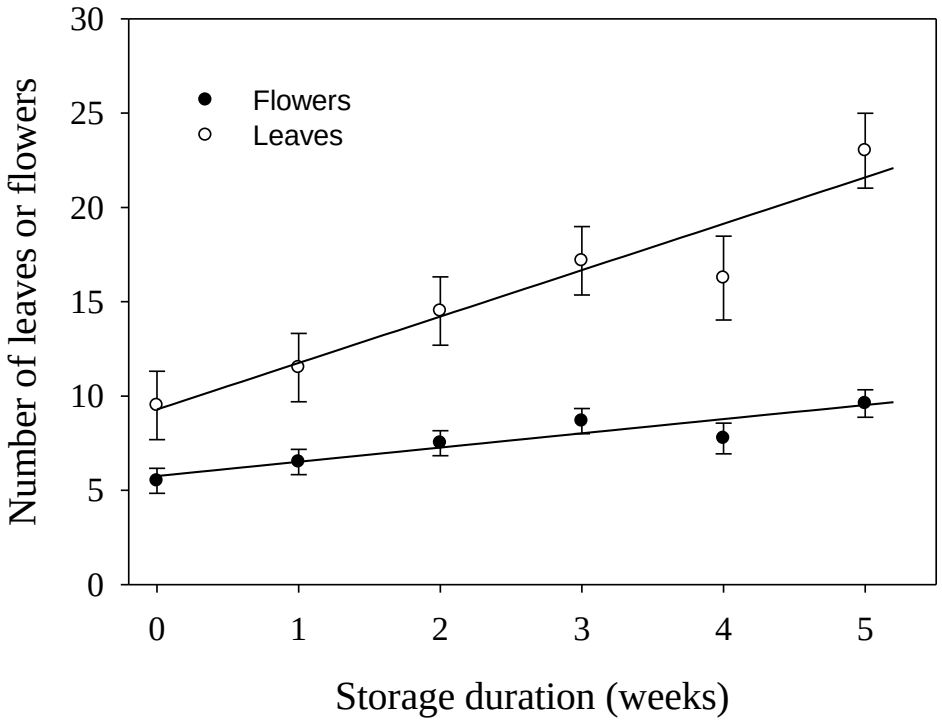

Figure A2.2. Effects of pre-plant storage at 3º C on flower and leaf numbers in *Oxalis regnellii*. The regression equations, associated *P* values for the associated F statistic, and r^2 values are as follows; flowers: y = 0.7536x +5.757 (*P*=<0.0001) (r^2=0..40); leaves: y = 2.4607x +9.289 (*P*=<0.0001) (r^2=0.49).

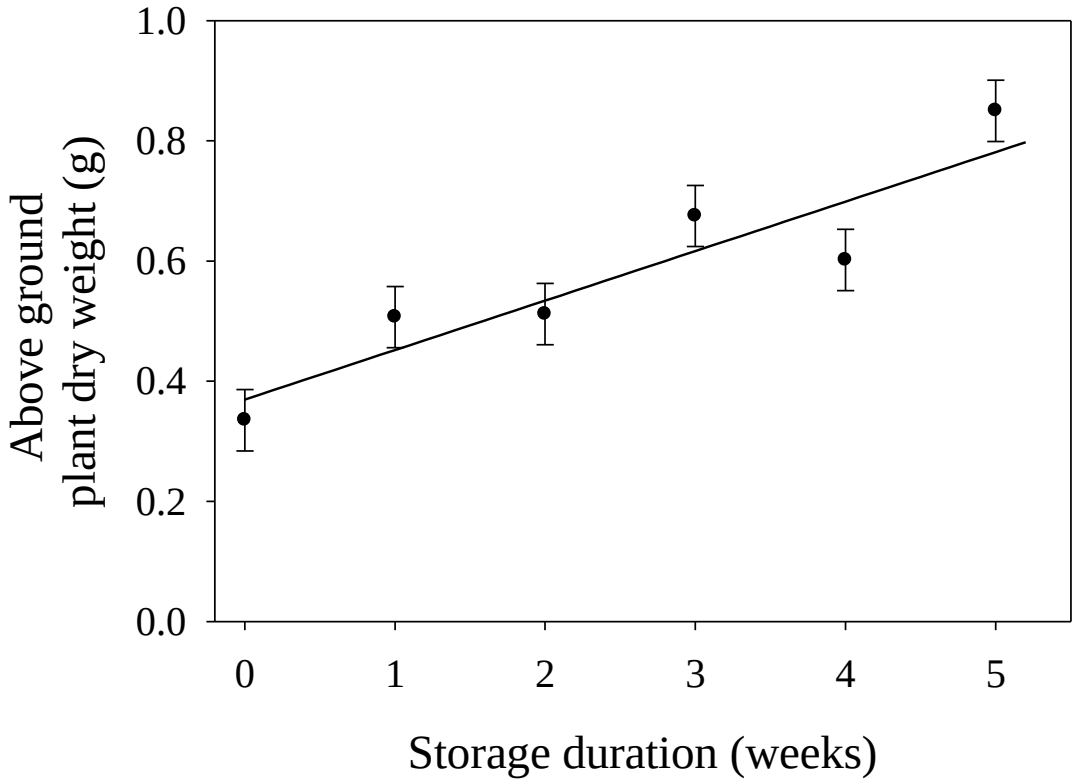

Figure A2.3. Effects of pre-plant storage at 3º C on leaf dry weight in *Oxalis regnellii*. The regression equation, associated *P* value for the associated F statistic, and r^2 value are as follows: y = 0.0824x +0.369 (*P*=<0.0001) (r^2=0.53)

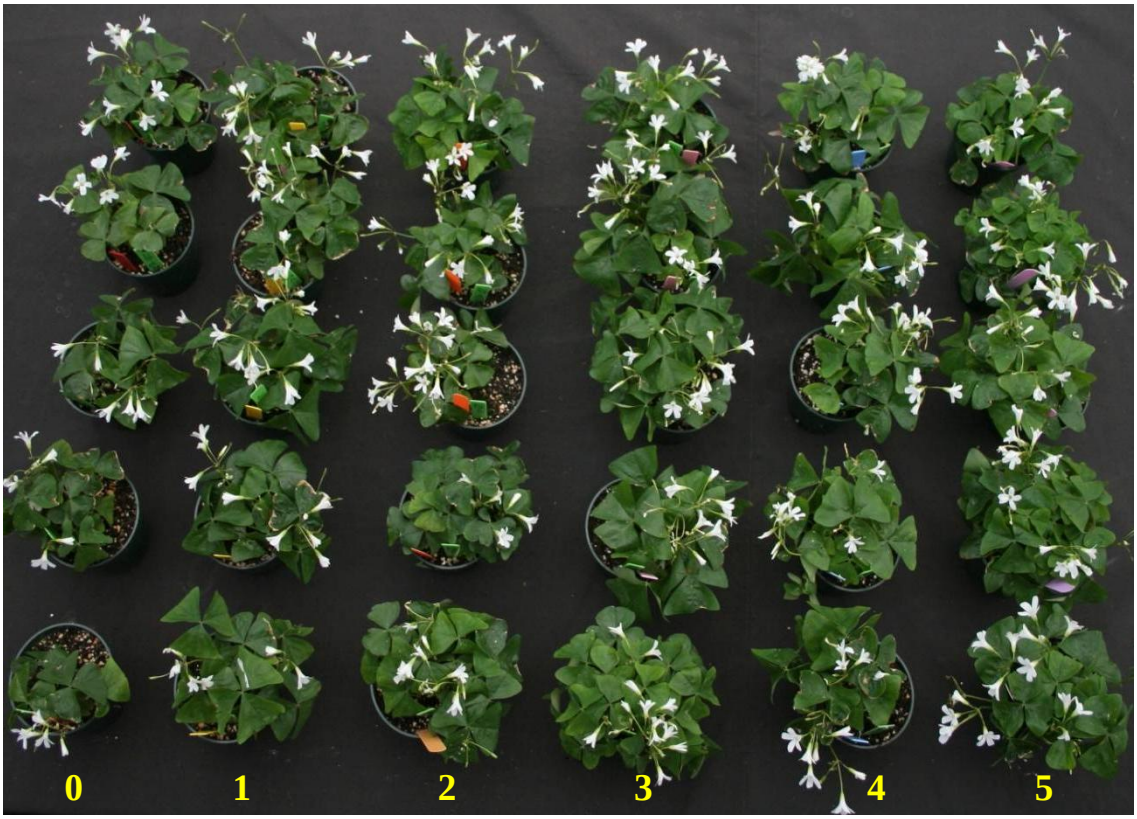

Figure A2.4. Effects of pre-plant storage at 3º C on growth and development of *O. regnellii* forced in the greenhouse at 21 ºC. L to R; 0, 1, 2, 3, 4, and 5 weeks of storage.

Conclusions

Pre-plant storage period had a positive effect on growth and development of oxalis, with more leaves and flowers produced as storage duration increased from 0 to 5 weeks. Because oxalis is primarily marketed as a foliage plant, more leaves are beneficial. The value of the flower is less important however more flowers have the potential to attract more customer interest. It is unclear how longer storage periods or storage at other temperatures may affect growth and development of *O. regnellii*, and should be investigated in future studies.

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