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Nutrient Management in Reblooming Iris 'Immortality'

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By

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For its fragrance, showy display and multi-colors, tall bearded (TB) iris (*Iris germanica* L.) has great potential as a specialty cut flower. This study was conducted to investigate the optimum nutrient management, especially nitrogen (N), of reblooming TB iris 'Immortality'. The objectives were to investigate the effects of N rate and form and phosphorus (P) rate on growth, flowering, and nutrient uptake, and to assess seasonal changes in the composition of nitrogenous compounds and carbohydrates. In general, greater N rates increased plant height, leaf SPAD reading, the number of inflorescence stems, plant dry weight, plant N content, and uptake of other nutrients. Spring flowering was more dependent on N stored from the previous year. Second bloom was largely influenced by N rate in the year of flowering. In spring, N uptake efficiency quadratically related with increasing N rate and was highest in the 10 mM N treatment. Percentage of tissue N derived from spring fertilizer decreased with increasing N rate applied from previous year. In comparison with N rates, P rates did not affect most of growth and flowering performances, but had slight influences on concentration of few nutrients (such as P, potassium, and boron). Considering N:P ratios in plant tissues in this study were low,

these results imply 5 mM P rate, the lowest P rate tested in this study, was sufficient for growth and development of reblooming TB iris. NH_4:NO_3 ratios did not affect plant height, flowering, dry weight, and N uptake, suggesting TB iris may not have preference for either ammonium or nitrate N. Higher NH_4:NO_3 ratios increased leachate pH, which might influence uptake of iron, manganese, and zinc. Nitrogen and carbon were predominately allocated to rhizomes in December and to leaves in May, suggesting a process of nutrient storage and remobilization happened in TB iris with seasonal changes. Concentration of starch, sucrose, glucose, and fructose showed seasonal changes, while concentration of free amino acids did not. Starch was the major form of storage carbohydrates in December. Glutamate, alanine, aspartate, serine, and tyrosine were main constituents among free amino acids.

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CHAPTER I

INTRODUCTION

In the cut flower industry, more and more new specialty cut flower species are being used to increase the profits of American growers and allow them to compete with imports. For its fragrance and showy display with multiple colors, tall bearded (TB) iris (*Iris germanica* L.) has great potential as a specialty cut flower. Remontant, or rebooming irises, a subclass of *I. germanica*, are capable of blooming more than once per growing season. Use of re-blooming iris for cut flower production has the potential to make iris cut flowers available over an extended season.

Previous research has been mainly focused on influences of temperature, vernalization, or plant growth regulators on TB iris, limited information is available on optimum nutrient management, which is critical to improve plant quality, increase yield, and reduce negative environmental impact.

Among all the nutrients required for plant growth and development, nitrogen (N) is one of the most important nutrients and is often required in the highest amount. There exists a discrepancy in the recommended optimal amount of N for growth and flowering of TB iris. In order to reduce susceptibility to disease caused by high N rates, most fertilizer recommendations for iris suggest using low N fertilizer rates. However, compared with once blooming iris, reblooming iris may need extra fertilizer to improve

second bloom. Thus, optimal N fertilizer rates to maximize economic production of this type of iris need to be determined.

Besides N rate, N form can also affect plant growth and uptake of other nutrients. Due to plant's preference for certain N form, NH_4:NO_3 ratios in fertilizer can influence N uptake efficiency, which could affect plant growth and development. In addition, in the process of taking up NO_3^- and NH_4^+, the rhizosphere pH changes correspondingly, such as when ammonium-fed plants accumulate more phosphate and sulfate due to acidification of the rhizosphere.

Phosphorus (P) is one of the most important nutrients, influencing root development and flower initiation, and reducing disease incidence. Crop productivity or quality might be affected by the balance between N and P. Phosphorus uptake is strongly influenced by N supply; on the other hand, N uptake can be increased by increasing P availability. Understanding the interaction between N and P is important in determining the optimum nutrient balance.

Iris has a special structure, the rhizome, which is a modified stem and works as a storage tissue for water and nutrients. Carbon and N storage increases in the fall, shows a stable trend during winter, and decreases at the beginning of spring. The stored carbon and nitrogen accumulated in storage organs are required to support the flowering and rapid early spring growth. Understanding the seasonal dynamics of carbohydrates and nitrogenous constituents plays an important role in improving flower quality and yield.

The objectives of this study were to investigate the effects of N rate on plant growth and flowering, to investigate the influence of both stored N and spring-applied N on spring growth and flowering, to investigate the responses of TB iris to different

NH_4:NO_3 ratios; to determine the influence of P rates and it's interaction with N on plant growth and uptake of essential nutrients, and to investigate composition of carbohydrates and nitrogenous constitutes and the season changes of these constituents in TB iris.

CHAPTER II

LITERATURE REVIEW

Specialty cut flowers

Floriculture crops include potted flowering plants, fresh cut flowers and cultivated greens, foliage plants, and bedding plants, which contribute largely to economy. In 2014, floriculture item sales at retail outlet were about $ 26.6 billion (U.S. Bureau of Economic Analysis, 2015).

About 64% of fresh cut flowers are imported from South America, mostly from Columbia (78%) and Ecuador (15%) (Huntrods, 2013). At present, in the face of fierce competition from low-cost foreign growers, American growers are focusing on specialty cut flowers which have proven to be profitable (Armitage, 1993) and allow domestic growers to compete with foreign growers.

Specialty cut flower crops generally refer to all species other than carnations, chrysanthemums, and roses. As a cut flower, iris wholesale value was $13 million in 2013 (USDA, 2014); however, the vast majority of iris cut flowers are Dutch iris (*Iris hollandica*). Compared with Dutch iris, tall bearded (TB) iris (*Iris germanica*) has fragrance, more colors and a showier display, thus having great potential as a specialty cut flower.

Reblooming iris

Tall bearded iris has a short season of availability to serve as a cutflower as most varieties only bloom in the spring, limiting cutflower production. A subclass of TB iris, the remoutant or reblooming iris, is capable of blooming more than once per growing season (Chapman, 2008; 2010a; 2010b). Using reblooming iris for cut flower production has the potential to make TB iris cut flowers available over an extended season.

As rebloomers have a genetic tendency to bloom a second time in late summer or fall, in mild winter climates, the reblooming may extend to November or even December (Chapman, 2010a). Under suitable culture, reblooming iris has potential for Thanksgiving, Christmas, and Valentine's Day sales with greater market value; thus, reblooming iris varieties are more valuable as cut flowers. Due to the state's relatively mild winter climate, rebloomers have great potential for extended production season as a specialty cut flower in Mississippi.

Why do they rebloom?

The reblooming iris cultivars are called rebloomer or remontant, which means plants can produce more than one growth of bloom stalks in a single growing season (Reblooming Iris Society, 2013). Usually iris only bloom in spring after the winter vernalization required for iris flowering. In rebloomers, the mother rhizome produces a flower stalk in spring and then matured axillary rhizomes produce a second growth and blooming in late summer or fall without vernalization (Chapman, 2010b).

The question is how can they rebloom? One hypothesis is that the rebloom trait of iris is controlled by a group of genes. When a homozygous recessive condition makes the

dominant gene controlling the requirement for vernalization inactive, the vernalization is not necessary to produce late summer or fall rebloom (Chapman, 2010a).

Another hypothesis is that, with the influence of environmental conditions and vigorous hybrid growth, plant hormones at genetically controlled levels are changed to allow certain irises to rebloom. For instance, if rebloomers are sheared, in which plant hormones levels are affected, in late summer they usually will not rebloom. A substance produced in the leaves could be the stimulus response for the initiation of reblooming (Reblooming Iris Sociaty, 2013).

Types of rebloomers

Based on the biological triggers of reblooming, rebloomers are classified as four types of rebloomer: direct rebloomers, fall cyclic rebloomers, extended season rebloomers, and whenever rebloomers (Chapman, 2010b). In whenever rebloomers, the new fans do not reset to a non-vernalized state when the main fan blooms and additional flowering happen whenever the new fan reaches a mature size (Chapman, 2010a). Thus, whenever rebloomers have the greatest potential to generate an everblooming iris.

According to recent research, rebloomers may be affected by multiple triggers to produce more than one bloom which includes vernalization, photoperiod, temperature and iris rhizome maturity (Chapman, 2008, 2010a, 2010b; Craver and Harkess, 2012; Harkess and Dhir, 2007).

Vernalization

In order to adapt to cold winters and protect the flower from freeze damage, some species require vernalization each year for plants to commence flowering. Most once

bloomer iris need vernalization to stimulate blooming. However, in rebloomers, vernalization did not improve the reblooming, and even inhibited flowering and reduced flower quality of reblooming iris (Harkess and Dhir, 2007).

Photoperiod

In many plant species, photoperiod is a trigger for plants to produce flowers. However, this environmental factor does not always appear to apply to rebloomers. If reblooming was controlled by photoperiod, the summer rebloomers should bloom in reverse order of their early spring blooming, but observation results show a different trend (Chapman, 2010a). In some cases, even the same cultivar in different climates showed different rebloom times. Day length may have influence on the fall cyclic rebloomers, but not on all reblooming types.

With the spring bloomer 'Royal Touch', the percentage of meristems initiating flowers increased with short photoperiod treatments (Pei, 2006); whereas, in the rebloomer 'White and Yellow', the percentage of meristems initiating flowers increased with long photoperiod treatments and floral meristem initiation occurred earlier with the 16/8 hour day/night photoperiod treatment.

Plant growth regulators (PGRs)

Flowering can be stimulated by PGRs, but the effects are specific to specific species. For example, ethylene promotes the flowering of bromeliads; however, it can also be an inhibitor to the flower formation in other species (Saltveit, 1999).

The research conducted by Leason and Harkess (2006) showed 100 or 200 mg/L benzylamino purine (BA, a cytokinins) induced more lateral branches that directly related

to the number of flower stalks when compared to the no-PGR control. This research also demonstrated blooming in iris was accelerated by the use of gibberellins (GA), but GA also inhibited further flowering. Flower stem length in BA treated plants was longer than under other treatments using GA or a combination of BA+GA. The combination of BA+GA promoted more inflorescences.

Low temperature

Vernalization treatments with temperatures lowered to 4 °C were proven to have no influence on reblooming of iris (Harkess and Dhir, 2007). Recent research suggested a series of days of low air temperatures below about 22 °C in a warm climate can stimulate reblooming on TB iris 'Immortality'. In contrast, in cold climates a period of a minimum nighttime air temperature of five days above 15 °C is required for reblooming (Chapman, 2010a). Ground temperature is the important trigger for reblooming, since the meristem is on the top of the rhizome where it is close to the soil surface. Air and soil temperatures are combining factors contributing to reblooming as the apical meristem is in the air and the rhizome itself underground.

Harkess et al. (2010) treated iris ‘Immortality’ with different numbers of night temperatures below 20 °C. The results implied the number of cold nights does not have an influence on floral development, but the increasing number of cool nights could increase the number of florets and stalk length, both an index of cut flower quality.

Maturity

For most iris rebloomers, maturity is an essential factor for reblooming as only mature rhizomes can produce flower stalks under suitable conditions (Chapman, 2008).

Chapman reported rhizome size is one of the ways to measure iris plant maturity. In the study of Craver and Harkess (2012), rhizomes with wide enough caliper have readiness for floral initiation, which implies maturity is related to rhizome size parameters. Rebloomers can carry a primed rhizome, a rhizome which has initiated a flower-stalk and is carried over the winter without damage (Chapman, 2011). If the primed rhizomes grow fast enough and get large enough, their vernalization status would not be reset by flowering of the mother rhizome and they can rebloom without vernalization to produce additional blooming in one year.

Nutrients

There exists a discrepancy in the recommended optimal amount of N for growth and flowering of *I. germanica*. Most fertilizer recommendations for iris suggest using low N fertilizer supply, such as 5-10-5, for growing spring bloomers since high N rates can increase susceptibility to disease (Morris, 2011). But the growth habit of rebloomers is different from spring bloomers, because rebloomers tend to initiate more new axillary rhizomes which require additional fertilizer to support growth and flowering. One study has shown high N fertilizer rates increase the number of flower stalks and stalk length of iris (Hanley et al., 2008). Lockatell and Spoon (2011) reported reblooming iris are heavy feeders and extra fertilizer during the summer season could improve fall blooming. In addition, increasing the N supply to a crop drives the production of a greater canopy biomass with the potential for higher photosynthesis and productivity (Wu et al., 2008). Appropriate nutrient management may accelerate the maturity rate of new rhizomes and increase cut flower stem production. Optimal N fertilizer rates to maximize economic production of iris need to be determined.

Mineral nutrients

Plants, like other living things, need nutrients for their growth and development. Sixteen elements known to be important to plant growth and survival are divided into two main groups: non-mineral, [hydrogen (H), oxygen (O), and carbon (C)] and mineral nutrients, [nitrogen (N), phosphorus (P), potassium (K). calcium (Ca), magnesium (Mg), sulfur (S), boron (B), copper (Cu), iron (Fe), chloride (Cl), manganese (Mn), molybdenum (Mo), and zinc (Zn)] (Marschner, 2012). There is not always enough of these nutrients in the soil for a plant to grow healthy, so farmers and gardeners often use fertilizers to add the nutrients to the soil. The following describes the functions and deficiency symptoms of N, P and K, which are usually the top three fertilizer expenses in crop production.

Nitrogen

In plants, N is combined with C, H, O, and S to create amino acids which are the building of blocks of protein; needed for all enzymatic reactions in a plant; and a major part of chlorophyll (Marschner, 2012). Nitrogen also increases the dry matter in leafy vegetables and protein in grain crops (Harper, 1987).

Deficiency of N will cause poor plant growth and pale green or yellow leaves, because leaves are unable to make sufficient chlorophyll. The yellow symptoms appear first on older leaves due to translocation of N from old leaves to young leaves (Marschner, 2012). Nitrogen deficiency lowers the protein content in plants and causes early maturity in some crops.

Phosphorus

Phosphorus is important in photosynthesis and respiration; plays a major role in energy storage and transfer as ATP, and is part of the RNA and DNA structures (Westheimer, 1987). In addition, phosphorus aids root development, flower initiation, seed and fruit development, and reduces disease incidence.

Deficiency of P causes poor growth, with leaves turning blue/green, but not yellow, with the oldest leaves affected first (Potash and Phosphate Institute, 1999). Under severe deficiency, purpling of leaves and stems may appear. Delayed maturity and poor seed and fruit development happen to those plants lacking P.

Potassium

Potassium is an enzyme activator that promotes metabolism; controls the opening and closing of leaf stomata to regulate exchange of carbon dioxide, water vapor, and oxygen with the atmosphere; maintains the balance of electrical charges at sites of ATP production in photosynthesis; improves disease resistance in plants; and improves size of grains and seeds (Potash and Phosphate Institute, 1998).

Plants lacking K will have slow and stunted growth (Marschner, 2012). Major symptoms are chlorosis along the edges of leaves (leaf margin scorching) which appear first in older leaves. Plant growth, root development and seed and fruit development are usually reduced in potassium-deficient plants (Potash and Phosphate Institute, 1998).

Nitrogen uptake from soil and plant assimilation

Nitrogen is a key nutrient in manipulating plant growth and is a main influential factor for plant growth and development. In the floral industry, large quantities of N

fertilizers are used to meet the needs of crops by most nursery producers (Chen et al., 2001). However, excess nitrogen use leads to N run-off and can cause environmental contamination. Nitrate is the dominant form of nitrogen pollution in surface and groundwater (Durand et al., 2011).

Each type of plant has a unique requirement of an optimum nutrient range (Bi et al., 2007; Huang et al., 2004; Ristvey et al., 2007). Even the same plant at each growth stage may have a different requirement for the amount of nutrients. In addition, plants often have preferences for either nitrate or ammonium, which are major N forms in fertililizer (Niu et al., 2011). Thus, N nutrition management should involve using rates and forms of N best suited for the plant species, stage of growth, time of year, and production objectives (Bi et al., 2007; Grindlay, 1997; Niu et al., 2011). The proper use of nitrogen can increase crop yields and quality and reduce environmental contamination.

Crop productivity relies heavily on N fertilization. The use of N by plants involves several steps, including N uptake, translocation, assimilation, and remobilization (Marschner, 2012). Plant growth is often limited by N availability to plant roots, except with plants capable of forming symbiosis with N_2-fixing microorganisms (Wagner, 2011). Nitrogen fertilizer is normally supplied to plants as nitrate (NO_3^-), ammonium (NH_4^+) (Niu et al., 2011).

The uptake of nitrate and ammonium into plant roots is mediated by transport proteins in root cells. There are two N transport systems in plants which are induced by different N availability to roots. High affinity transport system (HAT) works under low N availability conditions (<0.5 mM). If the external N concentration is greater than 0.5 mM,

the low affinity transport system (LAT) operates and allows large influxes of substrate (Glass et al., 2002).

Nitrate uptake from soil, transport and assimilation in plants.

The transporters involved in nitrate uptake by roots belong to NTR1 or NTR2 protein families (Tsay et al., 2007). Nitrate was transported by transporters across the plasma membrane in symport with protons (Forde, 2000). This process does not require metabolic energy, but in order to maintain the proton gradient over the plasma membrane, ATP is required by H^+-ATPase for proton extrusion (Marschner, 2012). Once nitrate is taken into the root system, it will be loaded from symplast into apoplast and transported to the shoot via the xylem transpiration stream. Through this transport pathway, nitrate is distributed throughout the plant and can be stored in vacuoles.

In the assimilation process, nitrate is first reduced to ammonium which is mediated by two enzymes: nitrate reductase (NR) and nitrite reductase (NIA) (Marschner, 2012). The NR reduces nitrate to nitrite in the cytosol of both roots and shoots and then NIA transforms nitrite to ammonium in the chloroplast. As nitrite is toxic to plant cells, the activity of NR is regulated by enzyme synthesis and degradation, concentration of substrate and products, light, sucrose, et al.

Ammonium uptake from soil, transport and assimilation in plants.

Ammonium uptake by roots is carried out by members of the ammonium transport family (AMT). Transporters in the AMT1 family constitute the major entry pathway for ammonium uptake (Loqué and von Wirén, 2004). In addition, NH_4^+ uptake can be through K^+ channels as NH_4^+ has similar ionic radius and size to K^+ (ten Hoopen

et al., 2010). Once ammonium is taken up by the roots, it can be assimilated or stored in vacuoles in roots or transported to aerial parts. Most of the ammonium can not be transported long-distance within plants; small amounts, in the milimolar range concentration, can be transported from roots to shoots (Yuan et al., 2007).

Ammonium assimilation processing includes two key enzymes, glutamine synthetase (GS) and glutamate synthase (GOGAT), both present in roots, in chloroplasts, and in N_2-fixing microorganisms (Marschner, 2012). In this process, ammonium is accepted by the amino acid glutamate forming the amide glutamine, then the amide group is transferred to oxoglutarate which is catalyzed by glutamate synthase. Glutamate or glutamine can be used for the synthesis of amino acids, amines, protein and nucleic acids.

Effects of N rates on plant growth and flowering

Determining optimal N application rates is important to optimize plant growth and flowering and to minimize N leaching and the potential for surface aquifer and ground water contamination (Bi et al., 2007; Chen et al., 2001). Several studies demonstrated N can enhance flowering. $Ca(NO_3)_2$ applied as an aqueous solution beginning 14 days after tulip planting decreased flower abortions and increased flower size and fresh weight (De Hertogh, 1978). Nutrition experiments also demonstrated fertilization is absolutely essential for tulip (*Tulip* L.) bulbs forced hydroponically in pea gravel (De Hertogh, 1987). Doss et al. (1980) found the only nutrients required for bulbous iris forcing were nitrogen, calcium, and boron.

The optimal N rate for maximal flower stem yield varies among plant species. The optimal N rates for anthurium (*Anthurium andraeanum* Linden ex André) producing maximum flowers is 7.5 to 11.3 mM N (Chang et al., 2012), a rate lower than this rate to

maximize flower stem production in Peruvian lily (*Alstroemeria* L.) (Smith et al., 1998). In tulip, more than 0.6% to 0.7% N concentration is required for floral differentiation (Baba and Ikarashi, 1967).

Nitrogen supply affects flower initiation, but not directly. These influences can be caused by phytohormone concentration or amount of photosynthates which are affected by N supply (Marschner, 2012). In apple (*Malus* mill.) trees, ammonium supply to the roots doubled the percentage of trees flowering which may be affected by the increase in stem arginine concentration induced by ammonium application (Rohozinski et al., 1986).

Healthy plants often contain 3% to 4% nitrogen in their aboveground tissues as N is needed to form key proteins in photosynthesis, RuBP carboxylase and thylakoid proteins, and photosynthesis capacity is thus influenced by N supply (Evans, 1989). In addition, CO_2 assimilation is affected by total rubisco activity. In N-deficient rice (Oryza sativa) plants, decrease of photosynthetic activity was caused by reduced carboxylation efficiency (Huang et al., 2004). Under light-saturation, net photosynthesis rate tends to increase linearly with increasing leaf N per unit leaf area (Anten et al., 1995).

The ability of plant to photosynthesize is not only affected by photosynthetic activity, but also by the photosynthetic area or leaf area. Insufficient N supply can reduce final leaf area which leads to low photosynthesis ability (Wu et al., 2008). Some plant species tend to reduce leaf growth while maximizing leaf N concentration, which may cause reduced leaf area and plant size. On the other hand, other plants tend to maximize leaf growth while reducing leaf N concentration (Grindlay, 1997). If the N supply limits leaf area, the photosynthesis capacity can also be reduced, which could affect flowering performance. In N-deficient plants, less sugar is used to assimilate N and support plant

growth where sugars are accumulated. The accumulation of sugar leads to suppression of photosynthetic rate (Paul and Driscoll, 1997).

Effects of N forms on plant growth and flowering

Both N rate and form of the N are important in a fertilization program (Bar-Yosef et al., 2009; Bernstein et al., 2005; Niu et al., 2011). The optimal NH_4^+:NO_3 ratio depends upon plant species, plant age, time of year, climate and location (Marschner, 2012). High ratio NH_4^+ in fertilizer with high N concentration may even have toxic effects on plants (Gerendás et al., 1997).

Usually, plants adapted to acid soils prefer NH_4^+, while plants adapted to high pH soils prefer NO_3^- (Marschner, 2012). As with the rate of N, the ratio of NH_4^+:NO_3^- can also affect plant growth and flowering. For example, a solution with 67:33 NH_4^+:NO_3^- ratio produced greater biomass than other ratios in mesquite (*Prosopis velutina*) (Hahne and Schuch, 2006). NH_4^+:NO_3^- ratios can also affect chlorophyll content which may be caused by low pH in the medium reducing the enzyme activity and cell growth (Mashayekhi-Nezamabadi, 2000) or ammonium accumulation increasing leaf sensitivity to ethylene which enhanced chlorophyll loss (Hsu, 2003).

When roots take up NO_3^- and NH_4^+, they typically release an identically charged molecule to maintain a balanced pH inside the plant cells. This process has strong impact on the uptake of other cations and anions and rhizosphere pH. For example, the assimilation process of one molecule of NH_4^+ produces one proton which is excreted into the external rhizosphere reducing rhizosphere pH (Marschner, 2012); whereas the process of NO_3^- uptake associates with uptake of protons from the rhizosphere and leads to an increase of pH (Hinsinger et al., 2003).

NH_4^+:NO_3^- ratios in fertilizer also influence the uptake of other nutrients. High levels of NH_4^+ can inhibit the uptake of cations and thus induce a deficiency of those elements in the crop (Siddiqi et al., 2002). However, ammonium-fed plants accumulate more phosphate and sulfate due to acidification of the rhizosphere.

Relations between N supply and uptake of other nutrients

When N availability limits plant growth, uptake of other nutrients is expected to decline accordingly. Insufficient N caused growth limitation and led to decreased uptake of P, K, S, Ca, and Mg in rhododendron (*Rhododendron* L.) (Ristvey et al., 2007). To optimize growth, increased N rates should accompany modified doses of other nutrients in a fertilizer formula. Phosphorous status can also influence the uptake of other nutrients. On the other hand, the availability of other nutrients can also affect uptake of N. Limiting P availability has negative effects on N and S absorption in eucalyptus (*Eucalyptus grandis*) (Graciano et al., 2006).

Nitrogen application timing

Throughout their lives, most plants require N from the soil. Both plant developmental stage and environmental factors influence plant N demands. Correct N application timing can optimize plant growth and N uptake efficiency. High N fertilizer application rates in late spring and early summer had greater effects on stimulating vegetative growth of fruit trees than applications in the spring or autumn (Sanchez et al., 1995). The allocation of N derived from fertilizer also varies depending on different application timing. Spring applied N tends to partition to shoot growth, whereas N supplied in late fall is stored more in roots (Bi et al., 2007; Cheng et al., 2001).

Storage N and carbohydrates

Tall bearded iris has a rhizome, a modified stem, that stores water and nutrients and connects the plant to the ground. Presence of stored compounds in underground storage is a major characteristic of geophytes (Khuankaew et al., 2010; Miller, 1992). Storage organs store food reserves, e.g. carbohydrates, proteins, lipids and nutrient elements, to maintain the viability of plants through unfavorable environmental periods. During early spring growth, the assimilation of exogenous carbon and nitrogen are always limited. The storage carbon and nitrogen accumulated in storage organs are required to support the rapid growth during these periods (Chapin et al. 1990; Miller, 1992).

Storage sites

Within geophytic plants' underground structures, such as roots, bulbs, or rhizomes, are mainly stored C and N. Rhizomes serve as storage organs for C and N in perennial plants with clonal growth (Suzuki and Stuefer, 1999). The predominant storage tissues are both roots and mother bulbs in tulip (Ohyama et al. 1988); both rhizomes and roots in Siam tulip (*Curcuma alismatifolia* Gagnep.) (Khuankaew et al., 2010); and both roots and stubble base in bushgrass (*Calamagrostis epigeios* L.) (Gloser, 2002). Tall bearded iris is a typical rhizomatous plant, but whether both rhizome and root of TB iris function as storage organs for N is unknown.

Storage nitrogen

Nitrogen in plants can be derived from external resources (fertilizer, microbial fixation of N_2) or internal resources (stored N). Especially in a perennial species, roots

and rhizomes are used for storage of nutrients to uncouple growth from the current nutrient supply and adjust growth to the nutrient availability integrated over several years (Chapin et al. 1990). Under sufficient N supply, part of the assimilated N will be used to synthesize proteins and enzymes and the remainder stored in plant tissues for future reuse. Storage N is defined as N resources in plants that can be remobilized from one tissue and used for the growth or maintenance of another (Millard, 1988).

The capacity for storing and reusing N has several advantages: (1) increasing the residence time of N in plants, (2) allowing plants to grow when external resources are limiting, and (3) allowing plants to accumulate more N than needed when the N supply exceeds demand for growth.

Nitrogen is a major element stored in storage organs of geophyte plants and is assimilated into free amino acids, proteins, and other nitrogenous compounds related to growth and development (Ruamrungsri et al. 2010). The storage N can be classed into three types in plants: free amino acids, their amides and proteins (Millard, 1988). Most of the proteins play metabolic and structural roles in plants. In addition to this, some of the proteins perform functions as storage forms of nitrogen.

In common nettle (*Urtica dioica* L.), the most important nitrogen stored in roots and rhizomes are free amino acids of which asparagine and arginine consisted up to 80% (Rosnitschek-Schimmel, 1985). In hydrilla (*Hydrilla verticillata*) turions, free amino acids constitute a large proportion of total N during overwintering (Ryan, 1994). Similarity with bushgrass, a rhizomatous grass, amino acids play a central role in N storage and roots and stubble base stores more N than rhizomes (Gloser, 2002; Gloser et al. 2007).

New shoot and leaf growth in early spring are influenced by storage N (Cheng and Fuchigami, 2002). The use of stored N for initial new growth increased with increasing N fertigation rates from the previous season (Bi et al., 2003). In many plant species, N used for initial growth depends more upon the reserved N rather than the uptake of N in spring, such as in boreal plant species where initial growth always happens before soil thaw (Chapin et al., 1990). The amount of storage N in previous years can also influence the uptake of N from soil the following spring. In a study with tulip, with increasing N concentration of the mother bulb, the subsequent nitrogen uptake, both from ammonia and nitrate, decreased (Amano, 1986).

Storage carbohydrates

Carbon constitutes about 50% of plant dry mass and provides a structural basis for plants (Agren, 2008). Carbon compounds provide both energy and the C-skeletons for amino acid assimilation. If C supply is insufficient, it will cause decreased N uptake and assimilation (Zhang, 2009). On the other hand, since N assimilation needs carbohydrates for carbon skeleton and energy supply, increasing N supply may decrease non-structural carbohydrates concentration (Cheng and Fuchigami, 2002).

Those carbohydrate resources are primarily from assimilation of CO_2 (photosynthesis). The remobilization of storage carbohydrates is determined by the balance between current photosynthesis and sink strength for new growth (Millard and Grelet, 2010), e.g. synthesis and breakdown of starch are tightly coupled to photosynthesis (Beck and Ziegler, 1989). Since carbon supply has a daily fluctuation, leaves of most plants store starch and/or vacuolar sucrose during the day and break starch down for export at night. With perennial plants, carbohydrate storage and reuse happens

seasonally (Chapin et al., 1990). Stored carbon is important for winter survival and can be used for maintenance of respiration or assimilation of nitrogen.

Resprouting of geophytes depends on reserves of carbohydrates, the most common storage carbohydrates being starch, fructans, sucrose and glucomannans (Chapin et al., 1990; Miller, 1992). In common hyacinth (*Hyacinthus* Tourn. ex L.), starch was the major storage carbohydrate (Addai and Scott, 2011). In snowdrop (*Galanthus nivalis* L.), the fructans and starch constituted the polysaccharide fraction of the bulbs and fructans were the major polysaccharides in the shoot, and the starch content was much lower (Orthen and Wehrmeyer, 2004). Not all storage carbohydrates serve as carbon and energy sources for sprouting, e.g. in Cape cowslip (*Lachenalia minima*), starch rather than fructan is used as the carbon and energy source for sprouting (Orthen, 2001).

Not all carbohydrate compounds can work as storage resources, as some of them cannot break down for reuse, such as lignin, condensed tannins, and terpene resins. These are included in sequestration which represents a metabolic dead-end. Millard and Grelet (2010) claim most non-structural carbohydrates in trees are sequestered. Spring growth in apple trees is mainly determined by reserved N rather than carbohydrates (Cheng and Fuchigami, 2002).

Seasonal changes of nitrogenous components and non-structural carbohydrates

Storage organs of geophytes permit plants to overcome unfavorable growth periods. Usually, those storage compounds show seasonal changes, and may rise in the fall and decline at beginning of spring to support spring shoot re-growth. In bluejoint reedgrass (*Calamagrostis canadensis*), total nonstructural carbohydrate (TNC) levels in

shoots decreased and TNC levels in rhizomes increased in fall. During the spring growth, TNC levels in rhizomes decreased (Hogg and Lieffers, 1991). Similar seasonal changes of sugars (fructose, glucose, sucrose) were observed in rhizomes of field bindweed (*Convolvulus arvensis* L.) and root buds of larger bindweed (*Calystegia sepium* L.) (Willeke et al., 2012). In bushgrass, content of amino acids increased in the fall, showed a stable trend during winter, and decreased at the beginning of spring (Gloser, 2002). Nitrogen remobilized from rhizomes provides about 60% of annual above-ground N requirement in American bistort (*Bistorta bistortoides*) (Monson et al., 2006).

References

Addai, I. K. and P. Scott. 2011. Regulation of carbohydrates partitioning and metabolism of the common hyacinth. Agr. Biol. J. North Amer. 2: 279-297.

Agren, G.I. 2008. Stoichiometry and nutrition of plant growth in natural communities. Annu. Rev. Ecol. Evol. Syst. 39:153-170.

Amano, M. 1986. Influence of mother bulb nitrogen on subsequent nitrogen uptake in tulips. Acta Hort. 177:423-43.

Anten, N.P.R., F. Schieving, and M.J.A. Werger. 1995. Patterns of light and nitrogen distribution in relation to whole canopy carbon gain in C3 and C4 mono- and dicotyledonous species. Oecologia 101:504-513.

Armitage, A.M. 1993. Specialty cut flowers: the production of annuals perennials, bulbs, and woody plants for fresh and dried cut flowers. Varsity Press, Portland Oregon.

Baba, A and T. Ikarashi. 1967. Mineral nutrition of tulip flowering phase. I. Shokubutu Seiri 6:47-55.

Bar-Yosef, B., N.S. Mattson, and H.J. Lieth. 2009. Effects of NH_4: NO_3: urea ratio on cut roses yield, leaf nutrients content and proton efflux by roots in closed hydroponic system. Sci. Hort. 122:610-619.

Beck, E., and P. Ziegler. 1989. Biosynthes and degradation of starch in higher plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 40:95-117.

Bernstein, N., M. Ioffe, M. Bruner, Y. Nishri, G. Luria, I. Dori, E. Matan, S. Philosoph-Hadas, N. Umiel, and A. Hagiladi. 2005. Effects of supplied nitrogen form and quantity on growth and postharvest quality of *Ranunculus asiaticus* flowers. HortScience 40:1879-1886.

Potash and Phosphate Institute. 1998. Functions of phosphorus in plants. Better Crops 82:4-5.

Potash and Phosphate Institute. 1999. Functions of potassium in plants. Better Crops 83:6-7.

Bi, G., C.F. Scagel, L. Cheng, S. Dong, and L.H. Fuchigami. 2003. Spring growth of almond nursery trees depends upon nitrogen from both plant reserves and spring fertilizer application. J. Hort. Sci. Biotechnol. 78:853-858.

Bi, G., C.F. Scagel, L.H. Fuchigami, and R.P. Regan. 2007. Rate of nitrogen application during the growing season alters the response of container-grown rhododendron and azalea to foliar application of urea in the autumn. J. Hort. Sci. Biotechnol. 82:753-763.

Chang, K.H., R.Y. Wu, G.P. Chang, T.F. Hsieh, and R.S. Chung. 2012. Effects of nitrogen concentration on growth and nutrient uptake of *Anthurium andraeanum* Lind. cultivated in coir under different seasonal conditions. HortScience 47:515-521.

Chapin, F.S., E.D. Schulze, and H.A. Mooney. 1990. The ecology and economics of storage in plants. Annu. Rev. Ecol. Syst. 21:423-447.

Chapman, C. 2008. Biology and genetics of rebloomer. The Iris Year Book 2008. British iris Soc.

Chapman, C. 2010a. Four genetic types of rebloomers. J. Reblooming Iris Soc. 75:22-24.

Chapman, C. 2010b. Plant maturity, temperature, and rebloom. Amer. Iris Soc. Bull. 92 (2):48-50.

Chen, J.Y. Huang, and R.D. Caldwell, 2001. Best management practices for minimizing nitrate leaching from container-grown nurseries. Sci.World J. 1:96-102.

Cheng, L. and L.H. Fuchigami. 2002. Growth of young apple trees in relation to the reserve nitrogen and carbohydrates. Tree Physiol. 22:1297-1303.

Cheng, L., S. Dong, S. Guak, and L.H. Fuchigami. 2001. Effects of nitrogen fertigation on reserve nitrogen and carbohydrate status and regrowth performance of pear nursery plants. Acta Hort. 564:51-62.

Craver, J.K. and R.L. Harkess. 2012. Determining rhizome maturity in reblooming iris. HortScience 47:S14.

De Hertogh, A.A., N. Blackely and J. Barrett. 1978. Fertilization of special precooled (5°C) tulips for cut-flower forcing. Sci. Hort. 9:167-174.

Doss, R.P., J.K. Christian, and J.L. Paul. 1980. Nutrient requirements for bulbous iris forcing. Acta Hort. 109:133-139.

Durand, P., L. Breuer. P. Johnes. G. Billen, A. Butturini, G. Pinay, and van H. Grinsven. 2011. Nitrogen processes in aquatic ecosystems. In The European Nitrogen Assessment. Cambridge University Press. p.126-146.

Evans, J.R. 1989. Photosynthesis and nitrogen relationships in leaves of C3 plants. Oecologia 78:9-19.

Forde, B.G. 2000. Nitrate transporters in plants: Structure, function and regulation. Biochim. Biophys. Acta. 1465: 219-235.

Gerendás, J., Z. Zhu, R. Bendixen, R.G. Ratcliffe, and B. Sattelmacher. 1997. Physiological and biochemical processes related to ammonium toxicity in higher plants. J. Plant Nutr. Soil Sci. 160:239-251.

Glass, A.D.M, D.T. Britto, B.N. Kaiser, J.R. Kinghorn, H.J. Kronzucker, A. Kumar, M. Okamoto. S. Rawat, M.Y. Siddiqi, S.E. Unkles, and J.J. Vidmar. 2002. The regulation of nitrate and ammonium transport systems in plants. J. Exp. Bot. 53:855-864.

Gloser, V. 2002. Seasonal changes of nitrogen storage com-pounds in rhizomatous grass *Calamagrostis epigejos*. Biol. Plant. 45:563-568.

Gloser, V., M. Košvancová and J. Gloser. 2007. Regrowth dynamics of *Calamagrostis epigejos* after defoliation as affected by nitrogen availability. Biol. Plant. 51:501-506.

Graciano, C., J.F. Goya, J.L. Frangi, and J.J. Guiamet. 2006. Fertilization with phosphorus increases soil nitrogen absorption in young plants of *Eucalyptus grandis*. For. Ecol. Mgt. 236:202-210.

Grindlay, D.J.C. 1997. Towards an explanation of crop nitrogen demand based on optimization of leaf nitrogen per unit leaf area. J. Agr. Sci., 128:377-396.

Hahne, K.S. and U.K. Schuch. 2006. Nitrogen form and concentration affect nitrogen leaching and seedling growth of *Prosopis velutina*. HortScience 41:239-243.

Hanley, N., R.L. Harkess, and M. Gu. 2008. Plant growth regulator and fertilizer effects on growth and flowering of re-blooming iris. HortScience 43:1176.

Harkess, R.L. and R. Dhir. 2007. Vernalization effect on growth and flowering of re-blooming iris. Proc. South. Nurs. Assoc. Res. Conf. 52:73-75.

Harkess, R.L., M. Zhang, and D. Cochran. 2010. Cool night temperature stimulate floral initiation in tall bearded iris. HortScience 45(8):S296.

Harper, J.E. 1987. Nitrogen metabolism. In J.R. Wilcox (ed.) Soybean: Improvement, production, and uses. ASA, CSSA, and SSSA, Madison, WI. p. 497-533.

Hinsinger, P., C. Plassard, C. Tang, and B. Jaillard. 2003. Origins of root-induced pH changes in the rhizosphere and their responses to environmental constraints: A review. Plant Soil 248:43-59.

Hogg, E.H., and V.J. Lieffers. 1991. The relationship between seasonal changes in rhizome carbohydrate reserves and recovery following disturbance in *Calamagrostis canadensis*. Can. J. Bot. 69:641-646.

Hsu, S.Y., Y.T. Hsu, and C.H. Kao. 2003. Ammonium ion, ethylene, and abscisic acid in polyethylene glycol-treated rice leaves. Biol. Plant. 46:239-242.

Huntrods, D. 2013. "Floriculture Profile." Agricultural Marketing Resource Center. 21 October 2015. <http://www.agmrc.org/commodities__products/specialty_crops/floriculture-profile/> .

Huang, Z., D. Jiang, Y. Yang, J. Sun, and S. Jin. 2004. Effects of nitrogen deficiency on gas exchange, chlorophyll fluorescence, and antioxidant enzymes in leaves of rice plants. Photosynthetica 42:357-364.

Khuankaew, T., S. Ruamrungsri, S. Ito, T. Sato, N. Ohtake, K. Sueyoshi, and T. Ohyama. 2010. Assimilation and translocation of nitrogen and carbon in *Curcuma alismatifolia* Gagnep. Plant Biol. 12:414-423.

Leason, T. and R.L. Harkess, 2006, Influence of cytokinins on lateral branching of *Iris germanica* rhizomes.Proc. South Nurs. Assoc. Res. Conf. 50: 300-301

Lockatell, M. and G. Don Spoon. 2011. Culturally Speaking: The secret of reblooming irises. Bul. Amer. Iris Soc. 92(3):32-33.

Loqué, D. and N. von Wirén, 2004. Regulatory levels for the transport of ammonium in plant roots. J. Exp. Bot. 55:1293-1305.

Marschner, P. 2012. Mineral nutrition of higher plants. 3rd ed. Academic Press, San Diego, CA.

Mashayekhi-Nezamabadi, K. 2000. The protein synthesis spectrum during the induction phase of somatic embryogenesis in carrot (*Daucus carota* L.) cultures and the role of nitrogen forms for embryo development. Justus Liebig University, Giessens, Dr. Sci. Thesis.

Millard, P. 1988. The accumulation and storage of nitrogen by herbaceous plants. Plant Cell Environ. 11:1-8.

Millard, P. and G.-A., Grelet. 2010. Nitrogen storage and remobilization by trees: ecophysiological relevance in a changing world. Tree Physiol. 30:1083-1095.

Miller, W.B. 1992. A review of carbohydrate metabolism in geophytes. Acta Hort. 325:239-246.

Monson, R.K., T.N. Rosenstiel. T.A. Forbis, D.A. Lipson, and C.H. Jaeger. 2006. Nitrogen and carbon storage in alpine plants. Integr. Compar. Biol. 46:35-48.

Morris, J. 2011. How to plant and grow bearded iris. 17 November 2014. <http://www.irises.org/About_Irises/Cultural%20Information/Grow_Bearded.html>

Niu, G., D. Rodriguez, and M. Gu. 2011. Response of *Sophora secundiflora* to nitrogen form and rate. HortScience 46:1303-1307.

Ohyama,T., T. Ikarashi, A. Obata, and A. Baba. 1988. Role of nitrogen accumulated in tulip roots during winter season. Soil Sci. Plant Nutr. 34:341-350.

Orthen, B. 2001. Sprouting of the fructan- and starch-storing geophyte *Lachenalia minima*: Effects on carbohydrate and water content within the bulbs. Physiol. Plant. 113:308-314.

Orthen, B. and A. Wehrmeyer. 2004 Seasonal dynamics of non-structural carbohydrates in bulbs and shoots of the geophyte *Galanthus nivalis*. Physiol. Plant 120:529-536.

Paul, M.J., and S.P. Driscoll. 1997. Sugar repression of photosynthesis: the role of carbohydrates in signaling nitrogen deficiency through source:sink imbalance. Plant Cell Environ. 20:110-116.

Pei H., 2006. Photosynthetic characteristic and effects of photoperiod on flower initiation in *Iris germanica*. Master Thesis. China Agriculture University, Beijing, China

Reblooming Iris Society. 2013. Reblooming iris culture. 1 June 2015. <http://www.rebloomingiris.com/Culture/>.

Ristvey, A.G., J.D. Lea-Cox, and D.S. Ross. 2007. Nitrogen and phosphorus uptake efficiency and partitioning of container-grown azalea during spring growth. J. Amer. Soc. Hort. Sci. 132:563-571.

Rohozinski, J., G.R. Edwards, and P. Hoskyns. 1986. Effects of brief exposure to nitrogenous compounds on floral initiation in apple trees. Physiol. Vég. 24:673-677.

Rosnitschek-Schimmel, I. 1985. Seasonal dynamics of nitrogenous compounds in a nitrophilic weed II. The role of free amino acids and proteins as nitrogen store in *Urtica dioica*. Plant Cell Physiol. 26:177-183.

Ruamrungsri, S., T. Kuankaew, N. Ohtake, K. Sueyoshi, and T. Ohyama. 2010. Nitrogen assimilation in flower bulbs, p. 319-328. In T. Ohyama and K. Sueyoshi, eds., Nitrogen Assimilation in Plants. Research Signpost, Kerala, India.

Ryan, F.J. 1994. Nitrogen and carbon concentrations, soluble proteins and free amino acids in subterranean turions of *Hydrilla* during overwintering. J. Aquat. Plant Manage. 32:67-70.

Saltveit, M.E. 1999. Effect of ethylene on quality of fresh fruits and vegetables. Postharvest Biol. Technol. 15:279-292.

Sanchez, E.E., H. Khemira, D. Sugar, and T.L. Righetti. 1995. Nitrogen management in orchards. In: P.E. Bacon (ed.). Nitrogen fertilization in the environment. Marcel Dekker, New York. p. 327-380.

Siddiqi, M.Y., B. Malhotra, X. Min, and A.D.M. Glass. 2002. Effects of ammonium and inorganic carbon enrichment on growth and yield of a hydroponic tomato crop. J. Plant Nutr. Soil Sci. 165:191-197

Smith, M.A., G.C. Elliott, and M.P. Bridgen. 1998. Calcium and nitrogen fertilization of *Alstroemeria* for cut flower production. HortScience 33:55-59.

Suzuki, J.I. and J. Stuefer. 1999. On the ecological and evolutionary significance of storage in clonal plants. Plant Spec. Biol. 14:11-17.

Ten Hoopen, F., T.A. Cuin, P. Pedas, J.N. Hegelund, S. Shabala, J.K. Schjoerring, and T.P. Jahn. 2010. Competition between uptake of ammonium and potassium in barley and Arabidopsis roots: molecular mechanisms and physiological consequences. J. Exp. Bot. 61:2303-2315.

Tsay, Y.F., C.C. Chiu, C.B. Tsai, C.H. Ho, and P.K. Hsu. 2007. Nitrate transporters and peptide transporters. FEBS Lett. 581:2290-2300.

U.S. Bureau of Economic Analysis. 2015. 1 October 2015. <http://www.aboutflowers.com/about-the-flower-industry/industry-overview.html>.

USDA. 2014. Floriculture crops 2013 summary. p. 50.

Wagner, S.C. 2011. Biological nitrogen fixation. Nature Education Knowledge 3:15.

Westheimer, F.H. 1987. Why nature chose phosphates. Science 235:1173-1178.

Willeke, L., H. Kraehmer, R.Gerhards, and W. Claupein. 2012. Seasonal variation of the sprouting ability of rhizome/root buds and concentrations of storage compounds in *Calystegia sepium* (L.) R. Br. and *Convolvulus arvensis* L. Julius-Kühn-Archiv. 434:694-701.

Wu, F., W. Bao, F. Li, and N. Wu. 2008. Effects of water stress and nitrogen supply on leaf gas exchange and fluorescence parameters of *Sophora davidii* seedlings. Photosynthetica 46:40-48.

Yuan, L., D. Loqué, S. Kojima, S. Rauch, K. Ishiyama, E. Inoue, H.Takahashi, and N. von Wirén. 2007. The organization of high-affinity ammonium uptake in *Arabidopsis* roots depends on the spatial arrangement and biochemical properties of AMT1-Type transporters. Plant Cell 19:2636-2652.

Zhang, Z. 2009. Review: Carbon and nitrogen nutrient balance signaling in plants. Plant Sig. Behav. 4:584-591.

CHAPTER III

NITROGEN FERTIGATION RATES AFFECT STORED NITROGEN, GROWTH AND BLOOMING IN *IRIS GERMANICA* 'IMMORTALITY'

Abstract

Tall bearded (TB) iris (*Iris germanica* L.) has great potential as a specialty cut flower due to its fragrance and showy, multicolor display; however, limited research has been reported on optimal nitrogen (N) nutrient management for TB iris. The objectives of this study were to investigate the effects of N fertilizer rate on plant growth and flowering of 'Immortality' iris and determine the influence of both stored N and spring-applied N fertilizer on spring growth and flowering. On 14 Mar. 2012, rhizomes of 'Immortality' iris were potted in a commercial substrate with no starter fertilizer. Plants were fertigated with 0, 5, 10, 15, or 20 mM N from NH_4NO_3 twice per week from 28 Mar. to 28 Sept. 2012. In 2013, half of the plants from each of the 2012 N rate were supplied with either 0 or 10 mM N from $^{15}NH_4{}^{15}NO_3$ twice per week from 25 Mar. to 7 May 2013. Growth and flowering data including plant height, leaf SPAD, number of fans and inflorescence stems, and length of inflorescence stem were collected during the growing season. Plants were harvested in Dec. 2012 and May 2013 to measure dry weight and N concentration in leaves, roots, and rhizomes. Result showed higher 2012 N rates increased plant height, leaf SPAD, and number of inflorescence stems at first and second blooming in 2012. Greater 2012 N rates also increased plant dry weight and N content in all structures, and

N concentration in roots and rhizomes. Rhizomes (58.8% to 66.3% of total N) were the dominant sink for N in Dec. 2012. Higher 2012 N rates increased plant height, number of fans, and the number of inflorescence stems at spring bloom in 2013. In May 2013, N in leaf tissue constituted the majority (51% to 64.3%) of the total plant N. Higher 2012 N rates increased total dry weight, N concentration, and N content in all 2013 ^{15}N rates; however, leaf dry weight in all plants was improved by 2013 ^{15}N rate. Percentage of tissue N derived from 2013 ^{15}N (NDFF) decreased with increasing 2012 N rate. New spring leaves were the dominant sink (56.8% to 72.2%) for 2013 applied ^{15}N. In summary, 'Immortality' iris is capable of a second blooming in a growing season, this second blooming being dependent on N fertilization rate. A relatively high N rate is recommended to produce a second bloom.

Introduction

Due to their showy, colorful flowers and sword-shaped leaves, tall bearded (TB) iris (*Iris germanica* L.) has potential as a specialty cut flower. Tall bearded iris plants are comprised of four parts: basal sword-shaped leaves (usually called fans) and inflorescence stems, rhizomes, and roots. Remontant, or rebooming irises (a subclass of *I. germanica*), are capable of blooming more than once per growing season. Use of reblooming iris for cut flower production has the potential to make TB iris cut flowers available over an extended season.

Nutrient management plays an important role in plant production. Nitrogen (N) is one of the key macronutrients required for plant growth and development. Nitrogen combines with other elements to form amino acids used in building enzymes, chlorophyll and other important compounds in plants (Marschner, 2012). Effective N management

can reduce inputs and minimize N losses to the environment, but requires a thorough understanding of plant nutrient demand in terms of amount and timing (Lea-Cox et al., 2001; Syvertsen and Smith, 1996).

Determining optimal N application rate is important for optimizing plant growth and flowering. The optimal amount of N varies among plant species. When receiving 7.5 or 11.3 mM N, anthurium (*Anthurium andraeanum* L.) produced more flowers than those receiving 5 or 15 mM N (Chang et al., 2012). The optimal N rate for maximal number of flower stems in Peruvian lily (*Alstroemeria* L.) was 28.5 mM (Smith et al., 1998) and low N supply has negative effects on vegetative and reproductive growth. In tulip (*Tulipa* L.), insufficient N application resulted in a marked decrease in N concentration in daughter bulbs and floral differentiation was delayed if N concentration of planted bulbs was less than 0.6% to 0.7% (Baba and Ikarashi, 1967).

There exists a discrepancy in the recommended optimal amount of N for growth and flowering of TB iris. Most fertilizer recommendations for iris suggest using low N fertilizer rates, probably because high N rates can increase susceptibility to disease (Morris, 2011); however, some research has shown high N fertilizer rates increase the number of flower stalks and stalk length of TB iris (Hanley et al., 2008). Lockatell and Spoon (2011) reported reblooming TB iris are heavy feeders and extra fertilizer during summer season could improve fall blooming; however, optimal N fertilizer rates to maximize economic production of this type of iris need to be determined.

Perennial species in general have the ability to build N reserves during progression to winter dormancy. In the rhizomatous plant Siam tulip (*Curcuma alismatifolia* Gagnep.), N is mainly stored in rhizomes (Khuankaew et al., 2010; Ohtake

et al., 2006). In a study by Ohyama et al. (1985), tulip plants stored nutrients in both scales and roots during the winter. Tall bearded iris has a thickened rhizome as storage tissue and spring growth and flowering production may be influenced by stored N from the previous year. In many plants, N reserves are remobilized during spring growth in support of early growth and development (Bi et al., 2003; Dong et al., 2004; Millard, 1995). In the woody tree pear (*Prunus communis* L.), both reserve and available soil N sources are important for spring growth (Cheng et al., 2001; Jordan et al., 2013).

A better understanding of how reblooming TB iris responds to fertilizer N rates and how plants utilize stored N in relation to spring applied N is needed to optimize growth and flowering and improve N fertilizer management. Therefore, the objectives of this study were to evaluate the effects of different N rates on plant growth and flowering of reblooming TB iris 'Immortality' and to determine the role of stored N on spring growth and uptake of spring-applied N fertilizer.

Materials and Methods

This study was conducted at Mississippi State University, Starkville, MS (latitude 33°46' N, longitude 88°82' W). On 14 Mar. 2012, rhizomes of 'Immortality' TB iris were field harvested, sorted for size (average caliper = 2.4 cm and length = 5.9 cm), and potted with one rhizome per pot into 3.78-L (23 cm diameter; 16 cm height) round plastic pots filled with a commercial substrate with no starter fertilizer (Fafard 2; Sun Gro Horticulture, Agawam, MA).

This experiment was a randomized complete block design with 5 blocks. In each block, 16 plant subsamples as a group were an experimental unit receiving one of five N rates. Fertigation was applied to plants twice per week from 28 Mar. to 28 Sept. 2012

with plants receiving 400 ml of modified Hoagland's solution (Hoagland and Arnon, 1950) containing one of five N rates (0, 5, 10, 15, or 20 mM N from NH_4NO_3). NH_4NO_3 (Sigma Aldrich, St. Louis, MO) was the only source of N. Other nutrients were from N-free fertilizer (1.06 $mg \cdot mL^{-1}$, Cornell No N Formula 0-6-27, Greencare Fertilizers, Kankakee, IL). On 8 Dec. 2012, five plants from each 2012 N rate were randomly selected and destructively harvested.

Beginning 25 Mar. 2013, half of the plants that had received each 2012 N rate were fertigated twice per week for 6 weeks with 250 ml modified Hoagland's solution containing 10 mM N from $^{15}NH_4{}^{15}NO_3$. ^{15}Nitrogen labeled fertilizer was used to distinguish between stored and applied N and to quantify allocation of N within the plant. The other half of the plants were fertigated with 250 ml N-free modified Hoagland's solution. The resulting treatment design was a factorial of five 2012 N rates and two 2013 ^{15}N rates. On 7 May 2013, five plants from each 2012 and 2013 fertigation combination were randomly selected and destructively harvested.

During the growing season, data for blooming (number of inflorescences and inflorescence stem length), plant height, and SPAD readings (SPAD-502, Minolta Camera Co., Japan) were collected. At harvest, plant height and number of fans were recorded. Each plant was divided into leaves, roots, and rhizomes. All samples were oven dried at 60 °C until constant weight, then dry weights were recorded by tissue type. All samples were ground to pass a 40-mesh sieve (Wiley Mill; Thomas Scientific, Swedesboro, NJ).

For plants harvested in Dec. 2012, the total N was determined by the Kjedahl method (Schuman et al., 1973) at the Soil Testing Lab of Mississippi State University.

For plants harvested in May 2013, N concentration was determined by an elemental C/N analyzer (Carlo Erba, Milan, Italy). Isotopic ^{15}N atom percent was determined by an elemental C/N analyzer coupled to an Isoprime mass spectrometer (Micromass, Beberly, MA). Nitrogen derived from the labeled fertilizer (NDFF) for each sampled plant tissue was calculated as follows:

$$NDFF\% = [(\%^{15}N \text{ sample} - \%^{15}N \text{ control})/(\%^{15}N \text{ fertilizer} - \%^{15}N \text{ natural abundance})]*100 \quad (3.1)$$

Natural abundance $\%^{15}N$ is considered equal to 0.3665 atom percent; $\%^{15}N$ sample = atom percent ^{15}N in plant sample; $\%^{15}N$ fertilizer = atom percent ^{15}N in fertilizer applied (2 atom percent); the mean abundance of $\%^{15}N$ in the control plants (0 mM ^{15}N rate in 2013) was 0.3700, 0.3753 and 0.3862 atom percent for leaves, roots, and rhizomes, respectively. The amount of fertilizer ^{15}N allocated to different tissue structures was calculated by multiplying NDFF% by the N content of leaves, roots, and rhizomes. The amount of fertilizer ^{15}N recovered by each plant was calculated as the sum of fertilizer ^{15}N allocated to leaves, roots, and rhizomes. The N content of each structure was calculated by multiplying the dry mass by its N concentration. Total plant N content was calculated as the sum of the content in leaves, roots, and rhizomes. For iris plants that did not receive fertilizer N in spring 2013, the total N content of the new leaves, roots, and rhizomes was considered the amount of reserve N remobilized from storage tissues to new growth. Nitrogen uptake efficiency during spring 2013 was calculated as N in plants derived from fertilizer divided by amount of ^{15}N fertilizer applied in 2013.

Data collected using 2012 N rate treatments were analyzed as a single factor and data collected using 2012 N rate and 2013 ^{15}N rate treatment combinations were analyzed

as a two-factor study. Continuous response data was analyzed using linear models with the GLM procedure of SAS (version 9.3: SAS Institute, Cary, NC) and count data were analyzed using generalized linear mixed models with the GLIMMIX procedure of SAS. Differences among 2012 N rate or main effects of 2012 N rate were compared using polynomial contrasts. Mean comparisons were made using Tukey's honestly significant difference.

Results and Discussions

Plant growth (May 2012)

In May 2012, greater 2012 N rates increased plant height and leaf SPAD readings (Table 3.1), which suggests higher N rates enhanced shoot growth. Since SPAD reading correlates with leaf N concentration (Gáborčík, 2003; Islam et al., 2009), greater N rates likely increased leaf N concentration as well.

Plant flowering in 2012

Greater 2012 N rates increased number of inflorescence stems at first bloom in 2012 (Table 3.2). All plants fertigated with N produced a inflorescence stem at first bloom; however, plants receiving 0 or 5 mM N did not produce any inflorescence stems at second bloom. Stem length was similar among plants receiving different 2012 N rates at both first and second bloom (Table 3.2).

Dry weight and dry weight allocation in 2012

After harvest in Dec. 2012, dry weight of all plant structures showed an increase with increasing 2012 N rate. Regardless of 2012 N rate, rhizomes had greater proportion of total plant dry weight than leaves or roots (Table 3.3).

Nitrogen concentration, content and allocation in Dec. 2012

Greater 2012 N rates increased N concentration and content in roots and rhizome, but increased only N content in leaves (Table 3.4). Leaf N concentration was not affected by 2012 N rate. Rhizome N concentration increased (about 3-fold) when N rate increased from 0 to 10 mM. In general, N concentration and content in rhizomes and roots was little affected by 2012 N rates greater than 5 mM. Allocation of N to leaves, roots and rhizomes was not affected by 2012 N rate (data not shown). Regardless of 2012 N rate, rhizomes were the primary sink for N (58.8% to 66.3%) in Dec. 2012.

Plant growth before 2013 ^{15}N fertigation

In March 2013, before spring fertigation, plant height and number of new fans increased with increasing 2012 N rate. Plants receiving 20 mM N in 2012 had almost 3-fold more fans than those receiving 0 mM N (Table 3.1).

Plant growth in May 2013

In May 2013, plant height was influenced by the interaction of 2012 N rate and 2013 ^{15}N rate. For plants not receiving any N in 2012, supplying these plants with 10 mM N in spring 2013 increased plant height. Plant size, as indicated by the number of fans, was only influenced by 2012 N rate, not 2013 ^{15}N application. Both greater 2012 N rates and 2013 ^{15}N rates increased leaf SPAD readings in May 2013 (Table 3.5).

Plant flowering in 2013

Number of inflorescence stems at first blooming in 2013 was influenced by the interaction of 2012 N rate and 2013 ^{15}N rate. In general, number of inflorescence stems showed an increasing trend with increasing 2012 N rate, while in those receiving the

same 2012 N rate there was no difference on number of inflorescence stems between 0 and 10 mM 2013 ^{15}N rates. Inflorescence stem length was not affected by 2012 N rate or 2013 ^{15}N rate (Table 3.5).

Dry weight and dry weight allocation in May 2013

In May 2013 (post-flowering), greater 2012 N rates had positive effects on dry weight of all tissues. 2013 ^{15}N application increased dry weight of leaves only (Table 3.5). Both increasing 2012 N rate and 2013 ^{15}N rate increased dry weight allocation to leaves. Dry weight allocation to rhizomes decreased with 2013 ^{15}N application, regardless of 2012 N rate (Table 3.5).

Nitrogen concentration, content and allocation in May 2013

Nitrogen concentration in leaves was similar among different 2012 N rate and 2013 ^{15}N rate treatment combinations (Table 3.6). Nitrogen concentration in rhizomes increased with increasing 2012 N rates and 2013 ^{15}N rates. Nitrogen concentration in roots increased with increasing 2012 N rate, but was not affected by 2013 ^{15}N application.

At 0 mM N in 2013, N content in leaves increased with increasing 2012 N rate. At 10 mM N rate in 2013, N content in leaves increased as 2012 N rates increased from 0 to 10 mM, then remained the same as N rate increased to 20 mM (Table 3.6). Both rhizome and total N content in plants increased with increasing 2012 N rate and 2013^{15}N rate. Nitrogen content in roots was positively affected by increasing N rates in 2012, regardless of 2013^{15}N rate (Table 3.6).

Allocation of N was greatest to the leaves, followed by rhizomes and roots. Leaves were the primary N sink in May 2013. Nitrogen allocation to leaves improved with 2013 ^{15}N regardless of 2012 N rate (Table 3.6). Allocation of N to roots decreased with increasing fertilizer N rates in 2012 using both 0 and 10 mM ^{15}N in 2013. Unlike roots, N allocation to rhizomes increased with increasing 2012 N rate, regardless of ^{15}N rate in 2013 (Table 3.6).

Amount and proportion of spring uptake of ^{15}N in plant tissues

In spring 2013, the amount of recovered ^{15}N in leaves and total plant tissue was similar across the 2012 N rates (data not shown); however, the proportion of ^{15}N in leaves, roots and rhizomes derived from $^{15}NH_4{}^{15}NO_3$ fertilizer (NDFF%) decreased as the 2012 N rates increased (Table 3.7). In addition, considering the same amount of ^{15}N (10 mM) was applied across the 2012 N rates and a similar amount of ^{15}N was taken up, N uptake efficiency (ratio of ^{15}N uptake to ^{15}N applied) in the spring growing season of 2013 was not affected by 2012 N rate.

With the various 2012 N and 2013 ^{15}N treatment combinations, ^{15}N allocation followed a similar pattern to N allocation with leaves as the primary sink followed by rhizomes and roots (data not shown). The allocation of ^{15}N to the leaves (57% to 72%) confirms new leaves were the dominant sink for N uptake in spring 2013.

Discussion

In May 2012, greater 2012 N rates led to more vigorous growth of 'Immortality' TB iris. These results are consistent with those reported for gladiolus (*Gladiolus* L.) (Khan et al., 2012) and dahlia (*Dahlia* Cav.) (Younis et al., 2009) of which the height of

plants was improved by higher N fertilizer rates. Increasing 2012 N rates increased leaf SPAD readings in May 2012, which indicates N concentration in leaves was increased by increasing 2012 N rates. In agricultural production, fertilizer N is a major input and plant tissue N concentrations have been closely correlated with leaf SPAD reading in various crops (Gáborčík, 2003; Islam et al., 2009; Yasumoto et al., 2011). Leaf SPAD reading can be used as a preliminary diagnostic tool for efficient N management based on plant N status (Ghosh et al., 2013; Netton et al., 2005).

Only plants receiving 10, 15, or 20 mM 2012 N produced a second bloom. Flowering and reproductive growth require additional energy and nutrients. In dendrobium (*Dendrobium nobile* Lindl.), greater N fertilizer rate increased the number of flowers (Bichsel et al., 2008). Thus, a relatively high N rate may be necessary to produce a second bloom in late summer or fall (Lockatell and Spoon, 2011).

Nitrogen is one of the primary factors affecting vegetative growth. In this study, dry weight in Dec. 2012 had a positive relation with 2012 N rate, which is consistent with studies on 'Casa Blanca' lily (*Lilium* L.) and the rhizomatous plants ginger (*Globba rosae* L.) and Siam tulip (Ruamrungsri et al., 2005, 2007; Zhu et al., 2012).

Plants receiving the 0 mM 2012 N rate had a lower proportion of dry weight allocated to roots than other 2012 N rates which is contrary to common belief that under insufficient N supply plants tend to develop a larger root system to take up enough nutrients (Bi et al., 2007). One explanation could be that plants receiving 0 mM N may not have received enough nutrients to support basic root growth.

Nitrogen concentration in leaves was not affected by 2012 N rate in Dec. 2012 and both 2012 N rate and 2013 ^{15}N rate in May 2013. Considering leaf dry weight of

plants decreased with decreasing N rates, 'Immortality' TB iris plants may control leaf growth while maintaining optimal leaf N concentration under low N rates. This interpretation is supported by Lemaire and Millard (1999) who reported there was a trade-off between leaf growth and leaf N concentration in plants under restricted N supply.

In this study, N content in plants was increased by N supply. This result is consistent with studies of other geophyte species. For example, in lily (*Lilium davidii* Duch. ex Elwes), N accumulation was increased with increasing amount of N fertilizer (Lin et al., 2011). In Cape cowslip (*Lachenalia* Jacq.), N supply increased N content in leaves and bulbs, and N content in leaves was higher than bulbs (Roodbol et al., 2002).

Regardless of 2012 N rate, in Dec. 2012 more than half of total N was allocated to rhizomes, which indicates rhizome is a major N storage organ in winter. The capacity for storing N in rhizomes could increase the residence time of N in plants as the leaves dieback in winter and allow plants to grow when external resources are limiting. Storage organs store nutrients to maintain the viability of plants through unfavorable environmental periods.

With increasing 2012 N rate, a greater amount of N stored in rhizomes also indicates capacity for storing N in rhizomes allows plants to accumulate more N than needed when the N supply exceeds demand for growth. This opinion is supported by other rhizomatous plant studies. In Siam tulip, the rhizome is the principal organ for N storage (Khuankaew et al., 2010; Ohtake et al., 2006). Tulips store nutrients in both scales and roots (Ohyama et al., 1985, 1988).

In March 2013, both plant height and number of fans were positively affected by 2012 N rate. Higher 2012 N rates produced larger iris plants in early spring 2013 which indicates early spring growth of TB iris relys on N application in the previous year. In plants receiving higher 2012 N rates, a greater amount of N was stored in the rhizome, which reserves the N requirement for early spring growth. Greater 2012 N rates led to an increased number of axillary rhizomes (each new fan develops an axillary rhizome) which may be beneficial for propagation; however, large numbers of axillary rhizomes may limit flower production due to reduced rhizome size.

In spring 2013, ^{15}N rate had only a slight influence on the number of inflorescence stems. The previous season's fertilization and plant growth is important for production of inflorescences the following spring. Spring fertilization did not stimulate new growth that matured soon enough to initiate flowers and bloom the same year. Applying 10 mM ^{15}N to plants receiving 0 mM N in 2012 resulted in some flowers. Plants receiving 0 mM N from 2012 fertigation may have initiated flower meristems in 2012 and nitrogen applied in 2013 may have supplied the necessary nitrogen nutrition to support flower development for blooming in 2013.

The number of inflorescence stems at first bloom in 2013 was less than that in 2012. In Mar. 2013, plants in each pot had more than 6 fans and the rhizomes may not have gained sufficient size or maturity to flower in spring 2013. This is supported by the rhizome maturity study of Craver and Harkess (2012) which showed floral initiation was related to rhizome caliper and larger rhizomes were more likely to initiate flowers. Similar results were observed with gladiolus (*Gladiolus grandiflorous* L.) which had the greatest flowering rate using largest corm size and highest N rate.

Compared to plant tissue N concentration in Dec. 2012, N concentration in May 2013 declined in all tissues. Increased dry matter production can cause a dilution in tissue N concentration. For rhizomes, the greatest N concentration in May 2013 was about 3-fold less than N concentration in Dec. 2012. Considering rhizomes serve as storage organs in TB iris, this reduction suggests translocation of N from N stored in the rhizomes to new growing tissues. In addition, a greater proportion of N was allocated to rhizomes, but in May 2013 it was allocated to leaves, with leaves being a stronger sink for N than rhizomes and roots. This result also supports N stored in rhizomes being remobilized to the leaves during spring growth.

In spring 2013, the proportion of ^{15}N in leaves, roots, and rhizomes derived from $^{15}NH_4{}^{15}NO_3$ fertilizer (NDFF%) decreased as the 2012 N application rate increased. Considering greater 2012 N rates increased reserve N (N content in Dec. 2012) and the amount of ^{15}N recovered in 2013 was similar across the 2012 N rate, the proportion of ^{15}N in plant tissues was reduced by increasing amount of reserve N from the previous year.

In this study, the amount of ^{15}N uptake in 2013 in leaves and total plant tissue was not affected by 2012 N rate. With tulip, greater N supplies increased N concentration of the mother bulb and decreased subsequent nitrogen uptake (Amano, 1986). Greater 2012 N rates led to larger plants in March 2013, which required a greater amount of N to support growth. Even with more reserve N from the previous year, those plants treated with greater N rates took up a similar amount of N as those plants treated with lower N rates in 2012.

Conclusion

Increasing 2012 N rate increased the number of inflorescence stems, plant dry weight, and plant N content of TB iris 'Immortality' in 2012. 2013 ^{15}N rate promoted leaf growth, and had only a slight influence on flowering in spring 2013. Nitrogen was predominantly allocated to rhizomes in Dec. 2012 and to leaves in May 2013. Amount of N uptake from 2013 ^{15}N was not affected by 2012 N rate. As N supply in the previous year increased, the proportion of N derived from 2013 ^{15}N decreased due to a dilution effect by greater amount of reserve N from the previous year.

'Immortality' TB iris is capable of repeat blooming in a growing season; however, the second bloom was largely influenced by N fertilization rate in the year of flowering. Thus, a relatively high N rate is needed to produce a second bloom. Flowering of plants in the spring was more dependent on N applied and stored from the previous year than N applied in the spring. Higher N rate in the previous year is recommended to improve production of inflorescence stems the following spring.

Table 3.1 Plant height (cm), leaf SPAD and number of fans of container-grown 'Immortality' TB iris.

2012 N rate (mM)	2012 May		2013 March	
	Plant height[z] (cm)	Leaf SPAD	Plant height (cm)	Fans/plant (No.)
0	41d[y]	57d	11c	6c
5	47c	64c	18b	11bc
10	52b	69b	21b	15ab
15	54ab	69b	23b	13ab
20	56a	73a	30a	17a
Contrasts[x]				
L	****	****	****	*****
Q	NS	**	*	NS

Plants were fertigated twice weekly with 0, 5, 10, 15, or 20 mM nitrogen (N) from Mar. to Sept. 2012 using a modified Hoagland's solution. Rhizomes were planted in Mar. 2012 and data was collected in May 2012 and Mar. 2013.

[z]Plant height was the average height of the three tallest fans.

[y]Means within a column followed by different letters denote significant differences (Tukey's honestly significant difference; $P \leq 0.05$).

[x]Significant linear (L) or quadratic (Q) trends at not significant or $P \leq 0.05$ (*), 0.01 (**), 0.001 (***), 0.0001 (****).

Table 3.2 Number and length (cm) of blooming stalks of container-grown 'Immortality' TB iris.

2012 N rate (mM)	2012, 1st blooming		2012, 2nd blooming	
	Inflorescence/plant (no.)	Stem length (cm)	Inflorescence/plant (no.)	Stem length (cm)
0	0.03c[z]	34.0b	0b	-
5	0.75b	37.4b	0b	-
10	0.95ab	39.0ab	0.06b	49.0
15	1.2a	40.4ab	0.11b	46.0
20	1.3a	45.1a	0.34a	46.0
Contrasts[y]				
L	****	NS	NS	NS
Q	****	NS	NS	NS

Plants fertigated twice weekly with 0, 5, 10, 15, or 20 mM nitrogen (N) from Mar. to Sept. 2012 using a modified Hoagland's solution. Rhizomes were planted in Mar. 2012 and data was collected during the first blooming in spring and second blooming in fall 2012.

[z]Means within a column followed by different letters denote significant differences (Tukey's honestly significant difference; $P \leq 0.05$).

[y]Significant linear (L) or quadratic (Q) trends at not significant or $P \leq 0.05$ (*), 0.01 (**), 0.001 (***), 0.0001 (****

Table 3.3 Dry weight and dry weight allocation in tissues of container-grown 'Immortality' TB iris.

2012 N rate (mM)	Dry weight (g)				Dry weight allocation (%)		
	Leaf	Rhizome	Root	Total	Leaf	Rhizome	Root
0	3.4c[z]	18.2c	3.0b	24.6b	13.9c	73.9a	12.2c
5	14.5b	28.7ab	15.8a	59.0a	24.6b	48.8b	26.6a
10	20.2ab	30.7a	14.4a	65.2a	30.9ab	47.1b	22.0ab
15	20.1ab	30.4a	14.8a	65.2a	31.1ab	46.2b	22.7ab
20	25.2a	32.1a	13.4a	70.7a	36.1a	44.7b	19.2b
Contrasts[y]							
L	****	*	****	****	****	****	**
Q	****	*	****	****	NS	****	****

Plants were fertigated twice weekly with 0, 5, 10, 15, or 20 mM nitrogen (N) from Mar. to Sept. 2012 using a modified Hoagland's solution. Rhizomes were planted in Mar. 2012 and plants were harvested in Dec. 2012.

[z]Means within a column followed by different letters denote significant differences (Tukey's honestly significant difference; $P \leq 0.05$).

[y]Significant linear (L) or quadratic (Q) trends at not significant or $P \leq 0.05$ (*), 0.01 (**), 0.001 (***), 0.0001 (****)

Table 3.4 Nitrogen (N) concentration and content of container-grown 'Immortality' TB iris.

2012 N rate (mM)	N concentration (%)			N content (g/plant)			
	Leaf	Rhizome	Root	Leaf	Rhizome	Root	Total
0	2.7a[z]	1.3b	0.71b	0.09c	0.24b	0.02b	0.35c
5	2.6a	2.3ab	0.7b	0.36b	0.60ab	0.11a	1.10b
10	2.6a	3.4a	0.8a	0.51b	1.10a	0.12a	1.70ab
15	2.6a	3.4a	1.1a	0.51b	1.00a	0.16a	1.70ab
20	2.8a	3.3a	1.1a	0.65a	1.10a	0.15a	1.90a
Contrasts[y]							
L	NS	*	NS	****	*	***	**
Q	NS	****	****	****	****	****	****

Plants were fertigated twice weekly with 0, 5, 10, 15, or 20 mM N from Mar. to Sept. 2012 using a modified Hoagland's solution. Rhizomes were planted in Mar. 2012 and plants were harvested in Dec. 2012.

[z]Means within a column followed by different letters denote significant differences (Tukey's honestly significant difference; $P \leq 0.05$).

[y]Significant linear (L) or quadratic (Q) trends at not significant or $P \leq 0.05$ (*), 0.01 (**), 0.001 (***), 0.0001 (****).

Table 3.5 Dry weight, dry weight allocation in tissues, growth, and flowering of container-grown 'Immortality' TB iris.

2013 ^{15}N rate (mM)	2012 N rate (mM)	Growth and flowering data in 2013				Dry weight (g)				Dry weight allocation (%)		
		Plant height (cm)	Fans/plant (no.)	SPAD	Inflorescence/plant (no.)	Leaf	Rhizome	Root	Total	Leaf	Rhizome	Root
0	0	41e[z]			0d							25.0a[y]
	5	58cd			0.20cd							16.4bc
	10	65ab			0.33bcd							13.6bc
	15	61bc			0.47a-d							11.6c
	20	68a			0.67abc							11.0c
	Contrasts[y]											
	L	****			****							**
	Q	***			NS							NS
10	0	52d			0.27bcd							18.6b
	5	61bc			0.13d							15.0bc
	10	63abc			0.73ab							12.1c
	15	65ab			0.27bcd							12.4c
	20	67ab			0.87a							11.8c
	Contrasts											
	L	****			***							**
	Q	NS			NS							**
Main effects of 2012 N rate												
	0		6.4b	58.6b		27.4d	30.5c	15.9ab	73.8c	36.3b		
	5		8.8b	58.8b		41.5c	44.5bc	15.7ab	101.7b	41.1ab		
	10		10.9ab	61.3ab		46.5bc	45.0b	13.9b	110.4b	41.9ab		

Table 3.5 (Continued)

15	10.8ab	60.1ab	52.7b	55.6ab	14.0b	122.2b	44.1a	
20	13.9a	63.0a	64.8a	72.8a	17.5a	155.2a	42.0ab	
Contrasts								
L	*		****	****	NS	****	*	
Q	NS		NS	NS	**	NS	NS	
Main effects of 2013 ^{15}N rate								
0		58.5b	41.5b				37.8b	46.7a
10		62.2a	51.6a				44.3a	41.7b

Plants were fertigated twice weekly with 0, 5, 10, 15, or 20 mM nitrogen (N) from Mar. to Sept. 2012 and twice weekly with 0 or 10 mM ^{15}N from Mar. to May 2013 using a modified Hoagland's solution. Rhizomes were planted in Mar. 2012, plants were harvested in May 2013, flowering data collected during first bloom season in 2013 and growth data was collected in May 2013.

[z]Means within a column followed by different letters denote significant differences (Tukey's honestly significant difference; $P \leq 0.05$).

[y]Significant linear (L) or quadratic (Q) trends at not significant or $P \leq 0.05$ (*), 0.01 (**), 0.001 (***), 0.0001 (****).

Table 3.6 Nitrogen (N) concentration, content and allocation in tissues of container-grown 'Immortality' TB iris..

2013 ^{15}N rate (mM)	2012 N rate (mM)	N concentration (%)			N content (g/plant)				N allocation (%)		
		Leaf	Rhizome	Root	Leaf	Rhizome	Root	Total	Leaf	Rhizome	Root
0	0	1.4bc[z]			0.26d						20a
	5	1.4bc			0.49dc						12b
	10	1.3c			0.53c						11bc
	15	1.4bc			0.65c						9bc
	20	1.6abc			0.99a						7bc
	Contrasts[y]										
	L	***			****						****
	Q	NS			NS						NS
10	0	1.6abc			0.58c						11bc
	5	1.5bc			0.7bc						10bc
	10	1.8a			0.92ab						8bc
	15	1.6abc			1.00a						7c
	20	1.6abc			1.10a						7c
	Contrasts										
	L	*			****						****
	Q	NS			NS						*
Main effects of 2012 N rate											
	0		0.56c	0.63c		0.17d	0.10b	0.7d		25c	
	5		0.67c	0.69bc		0.30cd	0.11b	1.0cd		29bc	
	10		0.93b	0.84a		0.47bc	0.11b	1.3bc		36ab	
	15		0.98ab	0.86a		0.58b	0.12ab	1.5b		38ab	
	20		1.20a	0.82ab		0.84a	0.14a	2.0a		41a	
	Contrasts										
	L		****	****		****	****	****		****	
	Q		NS	*		NS	NS	NS		NS	
Main effects of 2013 ^{15}N rate											
0			0.75b			0.41b		1.1b	54b		
10			0.97a			0.54a		1.5a	58a		

Plants were fertigated twice weekly with 0, 5, 10, 15, or 20 mM N from Mar. to Sept. 2012 and twice weekly with 0 or 10 mM ^{15}N from Mar. to May 2013 using a modified Hoagland's solution. Rhizomes were planted in Mar. 2012 and plants were harvested in May 2013

[z]Means within a column followed by different letters denote significant differences (Tukey's honestly significant difference; $P \leq 0.05$).

[y]Significant linear (L) or quadratic (Q) trends at not significant or $P \leq 0.05$ (*), 0.01 (**), 0.001 (***), 0.0001 (****).

Table 3.7 Percentage of nitrogen (N) derived from15NH415NO3 fertilizer (NDFF%) of container-grown 'Immortality' TB iris.

2012 N rate (mM)	NDFF%		
	Leaf	Rhizome	Root
0	55.4a[z]	36.4a	42.7a
5	37.9b	32.3ab	34.6ab
10	30.6bc	23.5abc	28.2bc
15	31.5bc	24.3bc	31.5bc
20	17.1c	14.6c	22.5c
Contrasts[y]			
L	****	****	****
Q	NS	NS	NS

Plants were fertigated twice weekly with 0, 5, 10, 15, or 20 mM N from Mar. to Sept. 2012 and twice weekly with 0 or 10 mM 15N from Mar. to May 2013 using a modified Hoagland's solution. Rhizomes were planted in Mar. 2012 and plants were harvested in May 2013.

[z]Means within a column followed by different letters denote significant differences (Tukey's honestly significant difference; $P \leq 0.05$).

[y]Significant linear (L) or quadratic (Q) trends at not significant or $P \leq 0.05$ (*), 0.01 (**), 0.001 (***), 0.0001 (****).

References

Amano, M. 1986. Influence of mother bulb nitrogen on subsequent nitrogen uptake in tulips. Acta Hort. 177:423-43.

Baba, A. and T. Ikarashi. 1967. Mineral nutrition of tulip flowering phase. I. Shokubutu Seiri 6:47-55.

Bi, G., C.F. Scagel, L. Cheng, S. Dong, and L.H. Fuchigami. 2003. Spring growth of almond nursery trees depends upon nitrogen from both plant reserves and spring fertilizer application. J. Hort. Sci. Biotechnol. 78:853-858.

Bi, G., C.F. Scagel, L.H. Fuchigami, and R.P. Regan. 2007. Rate of nitrogen application during the growing season alters the response of container-grown rhododendron and azalea to foliar application of urea in the autumn. J. Hort. Sci. Biotechnol. 82:753-763.

Bichsel, R.G., T.W. Starman, and Y.T. Wang. 2008. Nitrogen, phosphorus, and potassium requirements for optimizing growth and flowering of the nobile dendrobium as a potted orchid. HortScience 43:328-332.

Chang, K.H., R.Y. Wu, G.P. Chang, T.F. Hsieh, and R.S. Chung. 2012. Effects of nitrogen concentration on growth and nutrient uptake of *Anthurium andraeanum* Lind. cultivated in coir under different seasonal conditions. HortScience 47:515-521.

Cheng, L., S. Dong, S. Guak, and L.H. Fuchigami. 2001. Effects of nitrogen fertigation on reserve nitrogen and carbohydrate status and regrowth performance of pear nursery plants. Acta Hort. 564:51-62.

Craver, J.K. and R.L. Harkess. 2012. Determining rhizome maturity in reblooming iris. HortScience 47:S14.

Dong, S., L. Cheng, C.F. Scagel, and L.H. Fuchigami. 2004. Nitrogen mobilization, nitrogen uptake and growth of cuttings obtained from poplar stock plants grown in different N regimes and sprayed with urea in autumn. Tree Physiol. 24:355-359.

Gáborčík, N. 2003. Relationship between contents of chlorophyll (a+b) (SPAD values) and nitrogen of some temperate grasses. Photosynthetica 41:285-287.

Ghosh, M., D.K. Swain, M.K. Jha, and V.K. Tewari. 2013. Precision nitrogen management using chlorophyll meter for improving growth, productivity and N use efficiency of rice in subtropical climate. J. Agr. Sci. 5:253-266.

Hanley, N., R.L. Harkess, and M. Gu. 2008. Plant growth regulator and fertilizer effects on growth and flowering of re-blooming iris. HortScience 43:1176.

Hoagland, D.R. and D.I. Arnon. 1950. The water-culture method for growing plants without soil. Calif. Agr. Expt. Sta. Circ. 347:1-32.

Islam, M.Sh., M.S.U. Bhuiya, S. Rahmanand, and M.M. Hussain. 2009. Evaluation of SPAD and LCC based nitrogen management in rice (*Oryza sativa* L.). Bangladesh J. Agr. Res. 34:661-672.

Jordan, M.O., G. Vercambre, L. Gomez, and L. Pagès. 2013. The early spring N uptake of young peach trees (*Prunus persica*) is affected by past and current fertilizations and levels of C and N stores. Tree Physiol. 34:61-72.

Khan, F.N., M.M. Rahman, A.J.M.S. Karim, and K.M. Hossain. 2012. Effects of nitrogen and potassium on growth and yield of gladiolus corms. Bangladesh J. Agr. Res. 7:607-616.

Khuankaew, T., S. Ruamrungsri, S. Ito, T. Sato, N. Ohtake, K. Sueyoshi, and T. Ohyama. 2010. Assimilation and translocation of nitrogen and carbon in *Curcuma alismatifolia* Gagnep. Plant Biol. 12:414-423.

Lea-Cox, J.D., J.P. Syvertsen, and D.A. Graetz. 2001. Springtime ^{15}nitrogen uptake, partitioning, and losses from young bearing *Citrus* tree of differing nitrogen status. J. Amer. Soc. Hort. Sci. 126:242-251.

Lemaire, G. and P. Millard. 1999. An ecophysiological approach to modelling resource fluxes in competing plants. J. Exp. Bot. 50:15-28.

Lin, Y., F. Guo, J. Luo, J. Sun , and Y. Zhang. 2011. Effect of different N rates on nutrient accumulation and nitrogen use efficiency in rainfed land Lanzhou lily. Acta Prataculturae Sinica 20:101-108.

Lockatell, M. and G.D. Spoon. 2011. Culturally speaking: The secret of reblooming irises. Bul. Amer. Iris Soc. 92(3):32-33.

Marschner, P. 2012. Mineral nutrition of higher plants. 3rd ed. Academic Press, London. UK.

Millard, P. 1995. Internal cycling of nitrogen in trees. Acta Hort. 383:3-13.

Morris, J. 2011. How to plant and grow bearded iris. 17 November 2014. <http://www.irises.org/About_Irises/Cultural%20Information/Grow_Bearded.html>

Netton, A.T., E. Campostrini, J.G. Oliveira, and R.E. Bressan-Smith. 2005. Photosynthetic pigments, nitrogen, chlorophyll a fluorescence and SPAD-502 readings in coffee leaves. Sci. Hort. 104:199-209.

Ohtake, N., S. Ruamrungsri, S. Ito, K. Sueyoshi, T. Ohyama, and P. Apavatjrut. 2006 Effect of nitrogen supply on nitrogen and carbohydrate constituent accumulation in rhizomes and storage roots of *Curcuma alismatifolia* Gagnep. Soil Sci. Plant Nutr. 52:711-716.

Ohyama, T., T. Ikarashi, and A. Baba. 1985. Nitrogen accumulation of tulip (*Tulipa gesneriana*). Soil Sci. Plant Nutr. 31:581-588.

Ohyama, T., T. Ikarashi, A. Obata, and A. Baba. 1988. Role of nitrogen accumulated in tulip roots during winter season. Soil Sci. Plant Nutr. 34:341-350.

Retamales, J.B. and E.J. Hanson. 1989. Fate of ^{15}N-labeled urea applied to mature high bush blueberries. J. Amer. Soc. Hort. Sci. 114:920-923.

Roodbol, F., E. Louw, and J.G. Niederwieser. 2002. Effects of nutrient regime on bulb yield and plant quality of *Lachenalia* Jacq. (Hyacinthaceae). South African J. Plant Soil 19:23-26.

Ruamrungsri, S., N. Ohtake, K. Sueyoshi, C. Suwanthada, T. Ohyama, and P. Apavatjrut. 2005. Effect of nitrogen and potassium on growth and development of *Curcuma alismatifolia* Gagnep. Acta Hort. 673:443-448.

Ruamrungsri, S., W. Bumphenyoo, R. Sriwichai, and P. Apavatjrut 2007. Effects of nitrogen, phosphorus and potassium deficiencies on growth and development of *Globba rosae* Gagnep. Kasetsart J. Nat. Sci. 41:72-83.

Schuman, G.E., M.A. Stanley, and D. Knudsen. 1973. Automated total nitrogen analysis of soil and plant samples. Proc. Soil Sci. Soc. Amer. 37:480-481.

Smith, M.A., G.C. Elliott, and M.P. Bridgen. 1998. Calcium and nitrogen fertilization of *Alstroemeria* for cut flower production. HortScience 33:55-59.

Syvertsen, J.P. and M.L. Smith, Jr. 1996. Nitrogen uptake efficiency and leaching losses from lysimeter-grown *Citrus* trees fertilized at three nitrogen rates. J. Amer. Soc. Hort. Sci. 121:57-62.

Yasumoto, S., K. Suzuki, M. Matsuzaki, S. Hiradate, K. Oose, H. Hirokane, and K. Okada. 2011. Effects of plant residue, root exudate and juvenile plants of rapeseed (*Brassica napus* L.) on the germination, growth, yield, and quality of subsequent crops in successive and rotational cropping systems. Plant Prod. Sci. 14:339-348.

Younis, A., M.A.P. Khan, and A. Riaz. 2009. Effect of different levels of nitrogen, phosphorus, and potash fertilizers on growth of *Dahlia coccinea* cv. decorative. Caderno de Pesquisa série Biologia 18:8-14.

Zhu, Q., Y. Pan, and L. Zhao. 2012. The effects of N, P, K, and Ca on plant growth and nutrient content of lily leaves. Acta Prataculturae Sinica 21:274-284.

CHAPTER IV

SPRING NITROGEN UPTAKE, USE EFFICIENCY, AND PARTITIONING FOR GROWTH IN *IRIS GERMANICA* 'IMMORTALITY'

Abstract

This study investigated how spring nitrogen (N) application affects N uptake and growth performance in tall bearded (TB) iris 'Immortality' (*Iris germanica* L.). Container-grown iris plants were treated with 0, 5, 10, 15, or 20 mM N from $^{15}NH_4{}^{15}NO_3$ through fertigation using a modified Hoagland's solution twice a week for six weeks in spring 2013. Greater N rates increased plant height, leaf SPAD reading, total plant dry weight, and N concentration in leaves and rhizomes. Both N and carbon (C) content were closely related to total plant dry weight. The allocation of N and C to different tissues followed a similar trend as the allocation of dry weight. The C/N ratio in leaves, roots, and rhizomes decreased with increasing N rates. In leaves, roots, and rhizomes, the amount of N derived from fertilizer increased with increasing N rate. Leaves were the major sink for N derived from fertilizer. As N supply increased, dry weight accumulation in leaves increased, whereas dry weight accumulation in roots and rhizomes was unchanged. This indicates increasing N rate contributed more to leaf growth in spring. Nitrogen uptake efficiency had a quadratic relation with increasing N rate and was highest in the 10 mM N treatment which suggests the 10 mM is optimal N rate for improving N uptake efficiency.

Introduction

Tall bearded (TB) iris 'Immortality' (*Iris germanica*) are perennial plants belonging to the family *Iridaceae*. Hundreds of TB iris hybrids exist representing every color from jet black to sparkling white. It is a popular garden plant with potential as a cut-flower crop. In spring, TB iris produce great amounts of shoot growth which requires sufficient nutrient supply from both internal and external sources. Usually, fertilization in early spring and after spring flowering is recommended for growing TB iris (Lockatell and Spoon, 2011). However, limited information is available revealing how N rate affects spring N uptake and use efficiency in TB iris.

Nitrogen (N) plays an important role in plant growth and development. Insufficient N supply restricts plant growth. Increasing N application rate influences plant growth (Bi et al., 2007), leaf CO_2 assimilation (Cheng and Xia, 2004), and uptake and allocation of other nutrients (Scagel et al., 2008; 2012). However, excessive N fertilizer application results in higher root zone electrical conductivity (EC) which causes lower gas exchange rates, shoot dry weight, and SPAD readings (Niu et al., 2011). Increasing N supply may decrease nitrogen uptake efficiency (NupE) and lead to more N run-off to the environment (Syvertsen and Smith, 1996). Understanding a plant's N requirement and the way N affects production and quality of plants is important to both the environment and crop production (Bi et al., 2008; Dong et al., 2004; Lea-Cox et al., 2001; Scagel et al., 2012).

Nitrogen use efficiency (NUE) is the ability of the plant to use N to produce biomass or grain yield (Marschner, 2012). Nitrogen use efficiency integrates two components: use efficiency of absorbed N (N_aUE) by the plant (Benincasa et al., 2011)

and plant N uptake efficiency (NupE). Nitrogen use efficiency is estimated as the amount of dry matter fixed in plant biomass (N use) per unit of N applied. Nitrogen use efficiency reveals plant responses to nutrient availability gradients and is used to estimate a plant's N use capacity as a limit to growth. Nitrogen uptake efficiency is the ability of the plant to uptake applied N. Considering mean residence time of N in plant tissue dampened NupE responses to increasing N availability, NupE showed a more dynamic response to N availability from applied N (Iversen et al., 2010).

Carbon (C) to N ratio of biomass (C/N ratio) may indicate relative availability of C and N sources (Herms and Mattson, 1992). Carbon constitutes about 50 % of plant dry mass and provides the structural basis for plants (Agren, 2008) and carbon compounds provide both energy and the C-skeletons for amino acid assimilation. If C supply is insufficient, it will cause decreased N uptake and assimilation (Zhang, 2009). On the other hand, insufficient N supply reduces photosynthetic output, various carbohydrates (Coruzzi and Zhou, 2001). By controlling N application, C/N ratios can be adjusted in crops to enhance yield and quality.

The objectives of this study were to investigate influences of N rate on plant growth and N and C concentration, content, allocation, and ratio, and to evaluate the effects of increasing N rate on N uptake and NUE during the spring growth period.

Materials and Methods

This study was conducted under natural conditions in Starkville, MS (latitude 33°46' N, longitude 88°82' W). In Aug. 2012, rhizomes (average caliper = 4.7 cm and length = 5.8 cm) of TB iris 'Immortality' (Schreiner's Iris Gardens, Salem, OR) were potted one rhizome per pot into 3.78-L (23 cm diameter; 16 cm height) round plastic pots

filled with commercial substrate with no starter fertilizer (Fafard growing mix 2; Sun Gro Horticulture, Agawam, MA). Fertigation was applied to plants twice per week from 28 Aug. to 28 Sept. in 2012 with plants receiving 400 ml of modified Hoagland's solution (Hoagland and Arnon, 1950) containing 10 mM N from NH_4NO_3 to provide basic nutrient supply for fall growth.

On 25 Mar. 2013, before the start of spring N treatments, five plants were harvested for background biomass and nutrient composition. Plants were fertigated twice per week from 25 Mar. to 3 May 2013 with 250 ml of modified Hoagland's solution containing one of five N concentrations (0, 5, 10, 15, or 20 mM N) from $^{15}NH_4{}^{15}NO_3$. The experiment was arranged as a randomized complete block design with 5 blocks. In each block, 4 plants in one group was an experimental unit receiving one of five N rates. Five plants from each N rate were randomly selected and destructively harvested on 7 May 2013 and the remaining of plants were continually treated with the same N rate treatments from NH_4NO_3 until Sept. 2013.

During the 2013 growing season, number of inflorescences, inflorescence stem length, plant height, and SPAD readings (SPAD-502, Minolta Camera Co., Japan) data were collected. During harvesting on 7 May 2013, plant height and number of fans data were recorded. Each plant was divided into leaves, roots and rhizomes. All samples were oven dried at 60 °C until constant weight and dry weights were recorded by tissue type. All samples were ground to pass a 40-mesh sieve in a Wiley Mill (Thomas Scientific, Swedesboro, NJ).

Total N was determined using an elemental C/N analyzer (Carlo Erba, Milan, Italy). Isotopic ^{15}N atom percent was determined using an elemental C/N analyzer

coupled to an Isoprime mass spectrometer (Micromass, Beberly, MA). Nitrogen derived from the labeled fertilizer (NDFF) for each sampled plant tissue was calculated as follows:

$$NDFF\% = [(\%^{15}N \text{ sample} - \%^{15}N \text{ control})/(\%^{15}N \text{ fertilizer} - \%^{15}N \text{ natural abundance})]*100 \quad (4.1)$$

Natural abundance $\%^{15}N$ is considered equal to 0.3665 atom percent; $\%^{15}N$ sample = atom percent ^{15}N in plant sample; $\%^{15}N$ fertilizer = atom percent ^{15}N in fertilizer applied (2 atom percent); the mean abundance of $\%^{15}N$ in the control sample (0 mM ^{15}N rate in 2013) is 0.3752, 0.3786 and 0.3742 atom percent for leaves, roots and, rhizomes, respectively.

The N content of each structure was calculated by multiplying the dry mass by its N concentration. Total plant N and C content were calculated as the sum of the content in leaves, roots, and rhizomes. Plant N and C allocation were calculated by dividing the N content in different tissues by total plant N content. C/N ratio was calculated by dividing C concentration by N concentration. The amount of ^{15}N in different tissue structures was calculated by multiplying NDFF% by the N content of leaves, roots, and rhizomes. The amount of ^{15}N by each plant was calculated as the sum of ^{15}N in leaves, roots, and rhizomes, which was used as net N uptake from 25 Mar. to 7 May in 2013. Dry weight accumulation was estimated by subtracting the average total dry weight on 25 Mar. from dry weight on 7 May in 2013. Nitrogen uptake efficiency between 25 Mar. and 7 May was calculated by dividing the net N uptake from fertilizer by the total amount of N applied. Absorbed nitrogen use efficiency between 25 Mar. and 7 May was calculated by dividing the net dry weight accumulation in by the net N uptake from fertilizer. Nitrogen

use efficiency between 25 Mar. and 7 May was calculated by dividing dry weight accumulation by the total amount of N applied.

Data were analyzed as a single factor treatment design. Continuous response data using linear models with the GLM procedure of SAS (version 9.3: SAS Institute, Cary, NC) and count data were analyzed using generalized linear mixed model with the GLIMMIX procedure of SAS.

Differences in plant height, number of fans, dry weight, and dry weight allocation in tissues, N and C concentration, content, and allocation among various rates of 2012 N application were compared using polynomial contrasts at α=0.05. Effects of N rates on NupE, N_aUE, and NUE, and relation between dry weight and total N and C content were determined through linear regression analysis. Nitrogen and C concentration in tissues were considered as covariates in an analysis of C/N ratio to evaluate the contribution of N and C concentrations to variance in C/N ratio. Eta-squared [$\acute{\eta}^2 = (SS_{effect}/SS_{total})$] was used to assess the proportion of total variance attributable to covariates. Mean comparisons were made using Tukey's honestly significant difference. All analyses were performed using SAS 9.3.

Results and Discussions

Plant height, leaf SPAD reading, and flowering

Plant height increased as the season progressed (Table 4.1). From March to April, plant height increased about 40 centimeter (cm) and there was no difference in plant height among N rate treatments. However, starting from May, plants receiving higher N rates had greater plant height than those receiving lower N rates. From April to July, plant leaf SPAD readings showed a declining trend irrespective of N rate. However,

starting from June, plants receiving higher N rates had higher SPAD readings than those receiving lower N rates.

In many species, leaf SPAD reading has a strong correlation with leaf chlorophyll content (Islam et al., 2009; Wang et al., 2004, 2005). In a previous study with 'Immortality' iris, chlorophyll content decreased during high temperatures in summer and increased after August (Pei, 2006). This might explain the declining trend in leaf SPAD readings in our study. The declining trend of chlorophyll content caused by high temperatures also been noticed with other plants. For example, in creeping bentgrass (*Agrostis stolonifera* L.), chlorophyll content decreased when soil temperature was high (Liu and Huang, 2004).

Spring flowering of 'Immortality' iris occurred from late April to middle May. Flowering performance, including number of inflorescence stems and inflorescence stem length, were not affected by N rate (data not shown). In May, number of rhizomes increased with increasing N rate, but diameter and length of rhizomes was not affected by N rate (data not shown).

Dry weight and dry weight allocation

Greater N rates increased leaf and total plant dry weight (DW), but did not affect root and rhizome dry weight (Table 4.2). With increasing N rate, the DW allocation to leaves increased but allocation to roots and rhizomes decreased. Plants receiving lower N rates had a higher proportion of total plant DW allocated to roots and rhizomes. This is consistent with other research that plants tend to allocate more biomass to the root system to maximize nutrient uptake when limited nutrients are available (Bi et al., 2007; Dong et al., 2004; Scagel et al., 2011). Plants receiving lower N rates allocated the greatest

proportion of plant dry weight to rhizomes. However, plants receiving higher N rates allocated the greatest proportion of plant dry weight to leaves. These results indicate increasing N fertilization rates had more effect on promoting leaf growth than root and rhizome growth. Between March and May 2013, dry weight accumulation in leaves increased 6-fold as N increased from 5 mM to 15 mM (Table 4.2).

Nitrogen concentration, content and allocation in different tissues

Increasing N rate increased N concentration in leaves and rhizomes, but did not affect N concentration in roots (Table 4.3). The N content in leaves, roots and total N was quadratically related to N rate, whereas a linear relationship best explained N content in rhizomes. With increasing N rate, the N allocated to leaves increased and N allocated to roots and rhizomes decreased. Photosynthesis capacity is influenced by N content, as N is needed to form key proteins in photosynthesis, RuBP carboxylase and Thylakoid proteins (Evans, 1989). The increasing N content in leaves indicates more photosynthates were produced in higher N rate treatments.

Nitrogen allocation trend is similar to the dry weight allocation to different tissues. The close correlation between N content and dry weight (Fig. 4.1A) demonstrates the increasing N content is related to increasing DW. Nitrogen allocation to leaves and rhizomes was linearly related to N rate, whereas, allocation to roots was quadratically related to the N rate. A greater portion of N was allocated to leaves across the different N rates, indicating leaves were the major N sink in May.

Carbon concentration, content and allocation in different tissues

Carbon concentration was affected by N rate, but there was not a clear trend (Table 4.4). Carbon content in leaves and total plant C increased with increasing N rate. Carbon content in roots and rhizomes were not affected by N rate. With increasing N rate, the C allocated to leaves increased and allocation to roots and rhizomes decreased. There was a closely positive correlation between dry weight and C content (r^2= 0.998, Fig. 4.1B).

C/N ratio

In general, C/N ratios in all tissues decreased with increasing N rate (Table 4.4). C/N ratios ranked in the order of root > rhizome > leaf, which is contrary to the order of N concentration. Nitrogen concentration in leaves, roots and rhizomes increased 1.3, 1.5, and 1.7 fold, respectively, as N rate increased from 0 to 20 mM, while C concentration was much less affected by increasing N rate. The decline in C/N ratio was more affected by increasing N concentration which was related to N rate. Nitrogen rates explained 79%, 83% and 66% of variation in C/N ratio in leaves, roots and rhizomes, respectively (data not shown), Eta-squared [$\acute{\eta}^2 = (SS_{effect}/SS_{total})$] was used to assess the proportion of total variance attributable to covariates.

Nitrogen derived from fertilizer

The amount of NDFF in the leaves, roots, rhizomes and total plant increased with increasing N rate (Fig. 4.2A). These results are consistent with many previous studies which also found applying more N increased the amount of N derived from fertilizer (Andersen et al., 1999; Bi et al., 2007; Righetti et al., 2007). The relationship between N

rate and amount of total N uptake was best described using a quadratic model. The increment of total N uptake from fertilizer declined with increasing N rates which could lead to more N run-off into the environment.

A greater portion of N up taken from fertilizer was allocated to leaves (data not shown) which suggests leaves were the major sink of spring N uptake. This is consistent with the results of previous research, which also demonstrated N up taken in spring preferentially allocated to leaves (Dong et al., 2004; Salaün et al., 2005). In leaves, roots and rhizomes, the percentage of N derived from fertilizer increased with increasing N rate. Percentage of N derived from fertilizer in leaves was higher than in roots and rhizomes (Fig. 4.2B). Daily uptake of N increased from about 5 to 20 mg/d with increasing N rate from 5 to 15mM, and plants receiving 20 mM N had similar daily uptake of N as those receiving 15 mM N (Fig. 4.2C). This daily N uptake amount is helpful to estimate suitable spring N fertigation rate for growing TB iris.

Nitrogen uptake efficiency (NupE)

In this study, the relationship between NupE and N rate was best described using a quadratic model (Fig. 4.3A). When N rate increased from 5 mM to 10 mM, the NupE increased from 17.1% to 33.7% and then decreased to 26.8% as N rate increased from 10 to 20 mM. The NupE was highest in plants in the 10 mM N treatment (about 33.7%). This indicates N uptake did not increase commensurate with increased N availability (Iversen et al., 2010). To increase NupE and reduce N run-off to the environment, 10 mM (140 ppm) N fertilizer may be considered most appropriate for spring fertilization in TB iris.

Absorbed nitrogen use efficiency (N_aUE)

Absorbed nitrogen use efficiency demonstrates the ability of a plant to use the absorbed N to produce dry biomass. In this study, N_aUE is linearly related to N rate. Absorbed nitrogen use efficiency was highest in plants receiving 5 mM N and decreased as N rate increased from 5 to 20 mM (Fig. 4.3B). The decreasing trend of N_aUE indicates the amount of dry mass produced by a certain amount of absorbed N decreased with increasing N rate. Considering N concentration in rhizomes was significantly increased by increasing N rates, the extra N may be stored in the rhizomes instead of being used to produce biomass which could lead to a decrease in N_aUE.

Nitrogen use efficiency (NUE)

Nitrogen use efficiency is defined as the amount of dry matter fixed in plant biomass per unit of N applied from external sources. In this study, NUE showed a declining trend as N rate increased, but was not statistically significant (Fig. 4.3C). NUE was not only influenced by the amount of biomass produced by per unit N, but also affected by the mean residence time of N in the plant. Thus, NupE can better indicate plant responses to nutrient availability gradients than NUE (Iversen et al., 2010).

Conclusion

In summary, plant height, leaf SPAD reading, dry weight, and amount of N derived from fertilizer increased with increasing N application rate. The C/N ratio of leaves, roots, and rhizomes decreased with increasing N rate as a result of the influence of N rate on N concentration in plant tissues. In leaves, roots, and rhizomes, the amount of N derived from fertilizer increased with increasing N rate. Leaves were the major sink

for N derived from fertilizer. As N supply increased, dry weight accumulation in leaves increased, whereas dry weight accumulation in roots and rhizomes was unchanged. Nitrogen use efficiency was not affected by N rate; N_aUE decreased with increasing N rate. Nitrogen uptake efficiency was related to N rate in a quadratic manner and was highest at the 10 mM N rate, suggesting 10 mM N is optimal for improving NupE.

Table 4.1 Plant height, leaf SPAD reading and number of rhizomes (with diameter >1cm) of container-grown 'Immortality' TB iris.

Nitrogen rates (mM)	Plant height (cm)					SPAD reading				Rhizomes (no.)
	March	April	May	June	July	April	May	June	July	May
0	6.1	48.3	48.4	50.1	51	64.2	55.3	49.1	42.6	6.2
5	6.1	49.8	55.6	53.7	56.1	63.5	59.7	49.1	45.7	8.2
10	6.1	53.1	62.7	62.3	60.2	70.5	57.8	52.7	51.3	9.4
15	6.1	55.0	63.8	58.9	60	69.5	59.1	53.7	51.9	10.6
20	6.1	57.8	66.3	68.5	64.9	70.2	61.7	55.4	55.7	10.2
HSD[z]	-	10	7.9	10.2	6.8	10.0	7.4	6.9	8.1	3.5
Significance[y]	NS	NS	****	***	***	NS	NS	*	**	*
Contrasts[x]	NS	NS	L****	L***	L***	NS	NS	L*	L***	L**

Plants were fertigated with 0, 5, 10, 15, or 20 mM N from NH4NO3 using a modified Hoagland's solution from Mar. to Sept. 2013. Rhizomes were planted in Aug. 2012, plant height data were collected from Mar. to July 2013, leaf SPAD reading data were collected from Apr. to July 2013, and number of rhizomes data were collected after harvest in May 2013.

[z]Tukey's honestly significant difference (α = 0.05, n = 5) for N rates.

[y]NS, *, **, ***, ****: means Not significant or significant at $P \leq 0.05$, 0.01, 0.001, 0.0001, respectively.

[x]Significant linear (L) or quadratic (Q) contrasts at $P \leq 0.05$ (*), 0.01 (**), 0.001 (***), 0.0001 (****) across different N rates.

Table 4.2 Plant dry weight (DW), DW allocation, and DW accumulation from March to May 2013 of container-grown 'Immortality' TB iris

Nitrogen rates (mM)	Dry weight (g)				Dry weight allocation (%)			Dry weight accumulation (g)			
	leaves	roots	rhizomes	Total	leaves	roots	rhizomes	leaves	roots	rhizomes	Total
0	13.9	7.9	29.5	51.4	26.9	15.6	57.5	5.2	0.6	6.0	11.8
5	20.2	8.8	27.8	56.8	35.6	15.7	48.7	11.4	1.5	4.3	17.2
10	34.2	9.1	32.7	76.0	45.1	11.9	43.0	25.5	1.8	9.2	36.4
15	45.1	10.3	35.1	90.5	49.7	11.7	38.6	36.4	3.0	11.6	51.0
20	40.4	9.7	36.9	87.1	46.6	11.3	42.2	31.7	2.4	13.4	47.5
HSD[z]	11.3	2.6	14	24.4	6.2	4.3	7.1	11.3	2.60	14.0	24.4
Significance[y]	****	NS	NS	***	***	**	****	****	NS	NS	***
Contrasts[x]											
L	****	NS	NS	****	****	****	****	****	NS	NS	****
Q	*	NS	NS	NS	****	NS	****	*	NS	NS	NS

Plants were fertigated with 0, 5, 10, 15, or 20 mM ^{15}N from NH_4NO_3 using a modified Hoagland's solution from Mar. to May 2013. Rhizomes were planted in Aug. 2012 and plants were harvested in May 2013.

[z]Tukey's honestly significant difference ($\alpha = 0.05$, n = 5) for N rates.

[y]NS, *, **, ***, ****: means Not significant or significant at $P \leq 0.05$, 0.01, 0.001, 0.0001, respectively.

[x]Significant linear (L) or quadratic (Q) contrasts at $P \leq 0.05$ (*), 0.01 (**), 0.001 (***), 0.0001 (****) across different N rates.

Table 4.3 Nitrogen (N) concentration, content and allocation in tissues of container-grown 'Immortality' TB iris.

Nitrogen rates (mM)	Concentration (%)			Content (g)				Allocation (%)		
	Leaf	Root	Rhizome	Leaf	Root	Rhizome	Total	Leaf	Root	Rhizome
0	1.32	0.48	0.57	0.21	0.04	0.19	0.45	48	9.5	42.6
5	1.44	0.64	0.71	0.34	0.06	0.21	0.61	56.1	9.8	34.1
10	1.60	0.51	0.81	0.67	0.06	0.27	1	67.2	6	26.8
15	1.64	0.66	0.89	0.81	0.07	0.34	1.22	66.8	5.7	27.5
20	1.76	0.71	0.97	0.78	0.07	0.4	1.25	62.7	5.5	31.8
HSD[z]	0.18	0.27	0.24	0.19	0.02	0.15	0.26	9.7	3.6	11
Significance[y]	****	NS	**	****	****	**	****	**	**	****
Contrasts[x]										
L	****	NS	****	*	*	**	**	****	***	****
Q	NS	NS	NS	****	**	NS	****	***	NS	***

Plants were fertigated with 0, 5, 10, 15, or 20 mM N from NH_4NO_3 using a modified Hoagland's solution from Mar. to May 2013. Rhizomes were planted in Aug. 2012 and plants were harvested in May 2013.

[z]Tukey's honestly significant difference ($\alpha = 0.05$, n = 5) for N rates.

[y]NS, *, **, ***, ****: means Not significant or significant at $P \leq 0.05$, 0.01, 0.001, 0.0001, respectively.

[x]Significant linear (L) or quadratic (Q) contrasts at $P \leq 0.05$ (*), 0.01 (**), 0.001 (***), 0.0001 (****) across different N rates.

Table 4.4 Carbon (C) concentration, content and allocation in tissues of container-grown 'Immortality' TB iris.

Nitrogen rates (mM)	Concentration (%)			Content (g)				Allocation (%)			C/N ratio		
	Leaf	Root	Rhizome	Leaf	Root	Rhizome	Total	Leaf	Root	Rhizome	Leaf	Root	Rhizome
0	45.3	49.0	47.5	6.3	3.9	14.0	24.2	25.9	16.2	57.9	34.5	103.7	86.9
5	45.0	49.0	48.5	9.1	4.3	13.4	26.8	33.8	16.3	49.9	31.2	76.8	69.1
10	46.1	47.9	47.9	15.8	4.3	15.7	35.8	44.2	12.1	43.7	28.8	74.6	59.6
15	45.1	47.6	46.7	20.3	4.9	16.4	41.6	48.7	12.1	39.2	27.5	72.5	53.6
20	45.5	49.6	48.3	18.4	4.8	17.8	41.1	45.0	11.9	43.1	25.9	70.3	51.0
HSD[z]	0.69	0.78	1.2	5.2	1.24	6.5	11.3	6.2	4.3	7.0	3.4	11.9	19.7
Significance[y]	**	****	**	****	NS	NS	***	****	****	****	****	****	***
Contrasts[x]													
L	NS	NS	NS	*	*	*	NS	****	***	**	****	****	****
Q	NS	****	NS	****	NS	NS	****	****	NS	**	NS	***	NS

Plants were fertigated with 0, 5, 10, 15, or 20 mM N from NH_4NO_3 using a modified Hoagland's solution from Mar. to May 2013. Rhizomes were planted in Aug. 2012 and plants were harvested in May 2013.

[z]Tukey's honestly significant difference (α = 0.05, n = 5) for N rates.

[y]NS, *, **, ***, ****: means Not significant or significant at $P \leq 0.05$, 0.01, 0.001, 0.0001, respectively.

[x]Significant linear (L) or quadratic (Q) contrasts at $P \leq 0.05$ (*), 0.01 (**), 0.001 (***), 0.0001 (****) across different N rates.

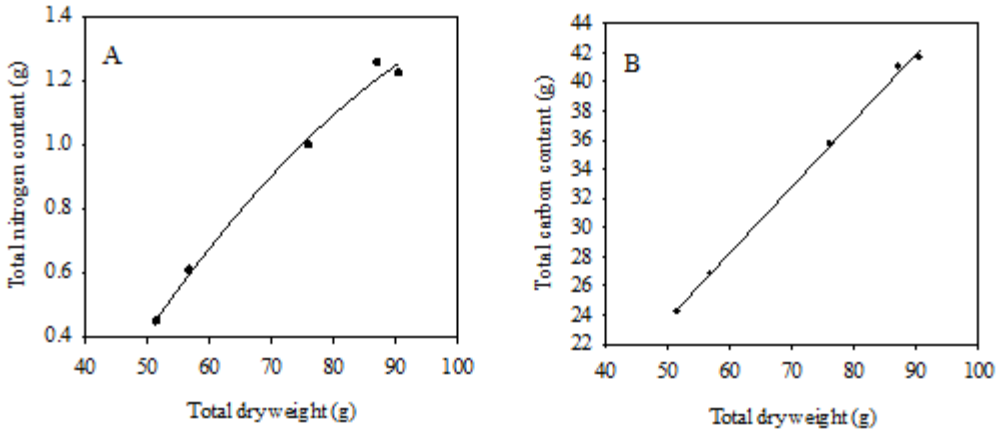

Figure 4.1 (A) Total nitrogen content and (B) total carbon content in relation to total dry weight of container-grown 'Immortality' TB iris.

Plants were fertigated with 0, 5, 10, 15, or 20 mM N from NH_4NO_3 using a modified Hoagland's solution from Mar. to May 2013. Rhizomes were planted in Aug. 2012 and plants were harvested in May 2013. Each value is the mean of five replicates. Regression equations of total nitrogen content and total dry weight: $y=0.019x-0.0002x^2$, $r^2=0.9$. Regression equations of total nitrogen content and total dry weight: $y=0.46x$, $r^2= 0.998$.

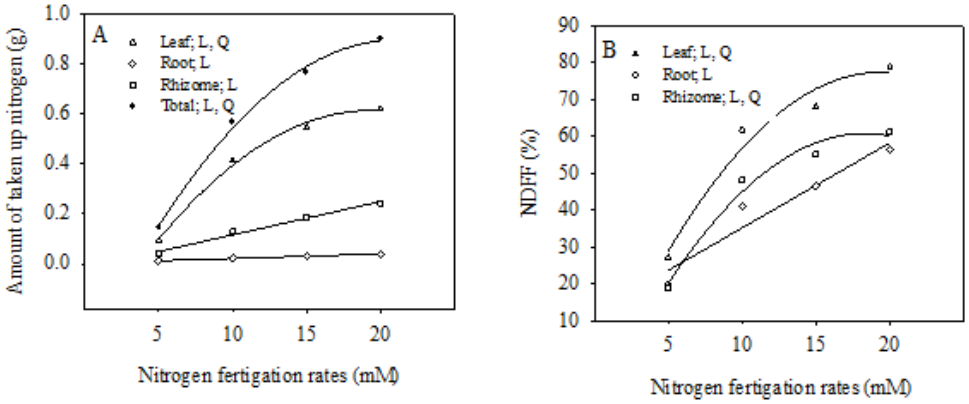

Figure 4.2 (A) Amount of taken up nitrogen and (B) proportion of NDFF in relation to N fertigation rates of container-grown 'Immortality' TB iris.

Plants were fertigated with 0, 5, 10, 15, or 20 mM N from NH_4NO_3 using a modified Hoagland's solution from Mar. to May 2013. Rhizomes were planted in Aug. 2012 and plants were harvested in May 2013. Each value is the mean of five replicates. Regression equations of amount of taken up N and N rates: (Leaf) $y=0.077+0.034x-0.0025x^2$, $r^2=0.93$; (Root) $y=0.00033x$, $r^2= 0.62$; (Rhizome) $y=0.0016x$, $r^2= 0.79$; (Total) $y=0.09+0.049x-0.0063x^2$, $r^2=0.95$. Linear (L); Quadratic (Q). Regression equations of NDFF% and N rates: (Leaf) $y= 0.0740+0.03225x-0.0024x^2$, $r^2= 0.91$; (Root) $y= 0.022x$, $r^2= 0.68$; (Rhizome) $y=0.072+0.027x-0.0023x^2$, $r^2=0.93$. Linear (L); Quadratic (Q).

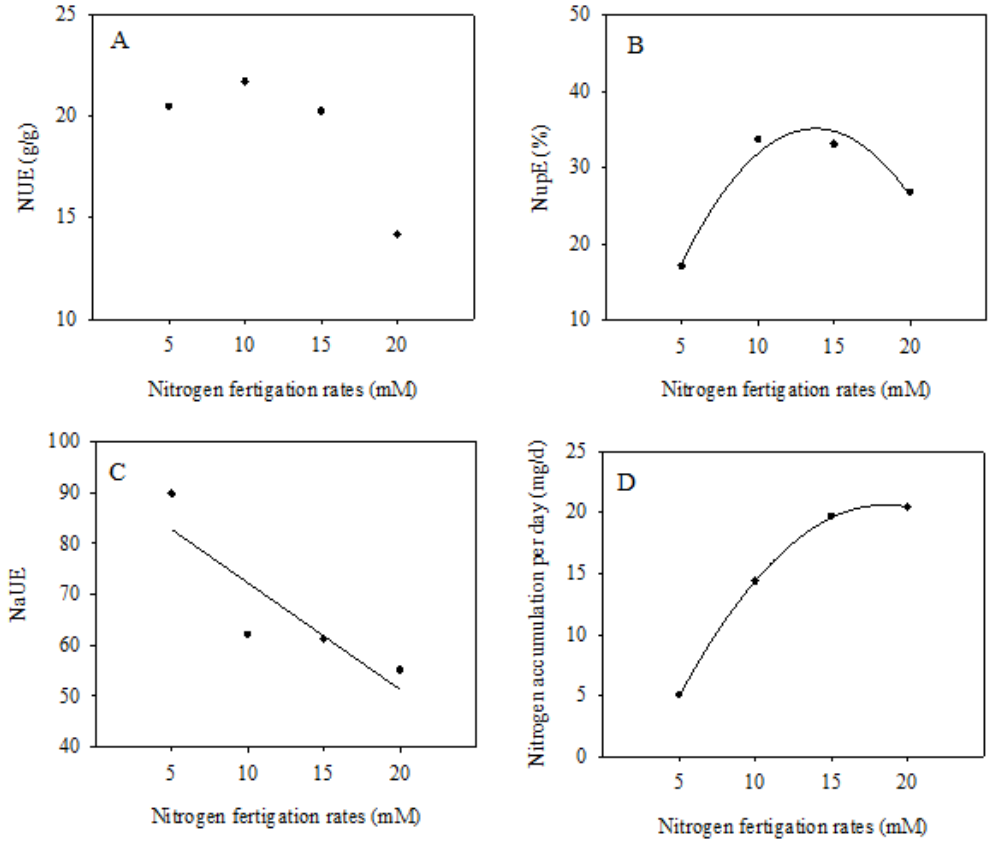

Figure 4.3 (A) Nitrogen use efficiency (NUE), (B) nitrogen uptake efficiency (NupE), (C) absorbed nitrogen use efficiency (N_aUE) and (D) nitrogen accumulation per day per plants in relation to nitrogen fertigation rates of container-grown 'Immortality' TB iris.

Plants were fertigated with 0, 5, 10, 15, or 20 mM N from NH_4NO_3 using a modified Hoagland's solution from Mar. to May 2013. Rhizomes were planted in Aug. 2012 and plants were harvested in May 2013. Regression equation: (NupE) $y= 0.063+0.00518x-0.002x^2$, $r^2= 0.68$; (N_aUE) $y=-0.00518x$, $r^2= 0.35$; (nitrogen accumulation per day) $y=2.67+ 1.03x-0.0855x^2$, $r^2= 0.8$.

References

Agren, G.I. 2008. Stoichiometry and nutrition of plant growth in natural communities. Annu. Rev. Ecol. Evol. Syst. 39:153-170.

Andersen, P.C., F.M. Rhoads, S.M. Olsen, and K.D. Hill. 1999. Carbon and nitrogen budgets in spring and fall tomato crops. HortScience 34:648-652.

Benincasa, P, M. Guiducci, and F. Tei. 2011. The nitrogen use efficiency: Meaning and sources of variation-case studies on three vegetable crops in central Italy. HortTechnology 21:266-273.

Bi, G., C.F. Scagel, L.H. Fuchigami, and R.P. Regan. 2007. Rate of nitrogen application during the growing season alters the response of container-grown rhododendron and azalea to foliar application of urea in the autumn. J. Hort. Sci. Biotechnol. 82:753-763.

Bi, G., C.F. Scagel, and R.L. Harkess. 2008. Rate of nitrogen fertigation during vegetative growth and spray applications of urea in the fall alters growth and flowering of florists' hydrangeas. HortScience 43:472-477.

Cheng, L. and G. Xia. 2004. Growth and fruiting of young 'Concord' grapevines in relation to reserve nitrogen and carbohydrates. J. Amer. Hort. Sci. 129:660-666.

Coruzzi, G.M. and L. Zhou. 2001. Carbon and nitrogen sensing and signaling in plants:emerging ‘matrix effects’. Curr. Opin. Plant. Biol. 4:247-253.

Dong, S., L. Cheng, C.F. Scagel, and L.H. Fuchigami. 2004. Nitrogen mobilization, nitrogen uptake and growth of cuttings obtained from poplar stock plants grown in different N regimes and sprayed with urea in autumn. Tree Physiol. 24: 355-359.

Evans, J.R. 1989. Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia 78:9-19.

Herms, D.A. and W.J. Mattson. 1992. The dilemma of plants:to grow or defend. Q. Rev. Biol. 67:283-335.

Hoagland, D.R. and D.I. Arnon. 1950. The water-culture method for growing plants without soil. Calif. Agr. Expt. Sta. Circ. 347:1-32.

Islam, M.S., M.S.U. Bhuiya, S. Rahman, and M.M. Hussain. 2009. Evaluation of SPAD and LCC based nitrogen management in rice (*Oryza sativa* L.). Bangladesh J. Agril. Res. 34:661-672.

Iversen, C.M., S.D. Bridgham, and L.E. Kellogg. 2010. Scaling plant nitrogen use and uptake efficiencies in response to nutrient addition in peatlands. Ecology 91:693-707.

Justes, E., B. Mary, J.M. Meynard, J.M. Machet, and L. Thelier-Huche. 1994. Determination of a critical nitrogen dilution curve for winter wheat crops. Ann. Bot. 74:397-407.

Lea-Cox, J.D., J.P. Syvertsen, and D.A. Graetz. 2001 Springtime ^{15}nitrogen uptake, partitioning, and leaching losses from young bearing citrus trees of differing nitrogen status. J. Amer. Soc. Hort. Sci. 126:242-251.

Lockatell, M. and G. Don Spoon. 2011. Culturally speaking: The secret of reblooming irises. Bul. Amer. Iris Soc. 92(3):32-33.

Liu, X. and B. Huang. 2004. Changes in fatty acid composition and saturation in leaves and roots of creeping bentgrass exposed to high soil temperature. J. Amer. Soc. Hort. Sci. 129:795-801.

Marschner, P. 2012. Mineral nutrition of higher plants. 3rd ed. Academic Press, San Diego, CA.

Niu, G., D. Rodriguez, and M. Gu. 2011. Response of *Sophora secundiflora* to nitrogen form and rate. HortScience 46:1303-1307.

Pei H., 2006. Photosynthetic characteristic and effects of photoperiod on flower initiation in *Iris germanica*. Master Thesis. China Agriculture University, Beijing China.

Righetti, T., D.R. Sandrock, B.Strik, and A. Azarenko, 2007. Appropriate analysis and interpretation approaches to determine fertilizer-derived nitrogen in plant tissues. HortScience 132:429-436.

Salaün, M., V. Guérin, L. Huché-Thélier, S. Charpentier, and F.L. Dily. 2005. Nitrogen storage and mobilization for spring growth in *Ligustrum* cultivated in container. Sci. Hort. 103:461-471.

Scagel, C.F., G. Bi, L.H. Fuchigami, and R.P. Regan. 2008. Nitrogen availability alters mineral nutrient uptake and demand in container-grown deciduous and evergreen *Rhododendron*. J. Environ. Hort. 26:177-187.

Scagel, C.F., G. Bi, L.H. Fuchigami, and R.P. Regan. 2011. Effects of irrigation frequency and nitrogen fertilizer rate on water stress, nitrogen uptake, and plant growth of container-grown *Rhododendron*. HortScience 46:1598-1603.

Scagel, C.F., G. Bi, L.H. Fuchigami, and R.P. Regan. 2012. Irrigation frequency alters nutrient uptake in container-grown *Rhododendron* with different rates of nitrogen. HortScience 47:189-197.

Smith, M.A., G.C. Elliott, and M.P. Bridgen. 1998. Calcium and nitrogen fertilization of *Alstroemeria* for cut flower production. HortScience 33:55-59.

Syvertsen, J.P. and M.L. Smith. 1996. Nitrogen uptake efficiency and leaching losses from lysimeter-grown *Citrus* trees fertilized at three nitrogen rates. J. Amer. Hort. Sci. 121:57-62.

Wang, Q., J. Chen, and Y. Li. 2004. Nondestructive and rapid estimation of leaf chlorophyll and nitrogen status of peace lily using a chlorophyll meter. J. Plant Nutr. 27:557-569.

Wang, Q., J. Chen, R.H. Stamps, and Y. Li. 2005. Correlation of visual quality grading and SPAD reading of green-leaved foliage plants. J. Plant Nutr. 28:1215-1225.

Zhang, Z. 2009. Review: Carbon and nitrogen nutrient balance signaling in plants. Plant Sig. Beha. 4:584-591.

CHAPTER V

EFFECTS OF DIFFERENT NH_4:NO_3 RATIOS ON GROWTH, NUTRITIONAL STATUS IN *IRIS GERMANICA* 'IMMORTALITY'

Abstract

The form of nitrogen (N) in fertilizer can influence plant growth, nutrient uptake and physiological process in the plant. However, few studies have been conducted on the effects of N form on growing tall bearded (TB) iris (*Iris germanica* L.). In this study, five NH_4:NO_3 ratios (0:100, 25:75, 50:50, 75:25, 100:0) were applied to investigate the response of TB iris to different N form ratios. NH_4:NO_3 ratios in fertilizer did not affect the leaf, root and rhizome dry weight, or total dry weight. Plant height and SPAD reading were affected by NH_4:NO_3 ratios in some months, but not over the whole growing season. Neither spring nor fall flowering were influenced by NH_4:NO_3 ratios. Across the whole growing season, leachate pH was increased by higher NH_4:NO_3 ratios. In December, concentration of phosphorous (P), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu) in leaf; concentration of calcium (Ca), magnesium (Mg), Mn, boron (B) in root and concentration of N, P, Mg, Fe, Mn, Zn in rhizome tissues was affected by NH_4:NO_3 ratios. Greater NH_4:NO_3 ratios increased the uptake of Fe, Mn and Zn. The net uptake of N was not affected by NH_4:NO_3 ratios which indicates TB iris may not have a preference for either ammonium or nitrate N.

Introduction

Nitrogen (N) is an important macronutrient needed by plants and often required in the highest amount of all the mineral elements. N fertilization is unique in that both the rate and the form of N fertilizer can influence plant growth and must be managed appropriately to maximize plant growth and development (Bar-Yosef et al., 2009; Bernstein et al., 2005; Niu et al., 2011).

Nitrogen fertilizer is normally supplied as nitrate (NO_3^-), ammonium (NH_4^+) or urea ($CO(NH_2)_2$). Usually a large proportion of urea would be converted to ammonium in the substrate and then absorbed by plants. In other words, urea may be considered to be the same as ammonium during the uptake process. Thus, the two major N forms taken up by plants are NO_3^- and NH_4^+. Many fertilizers provide nitrogen in one or both of these forms. The optimal NH_4:NO_3 ratio depends upon plant species, age of the plant, application timing, climate, and location (Marschner, 2012). Great ratio NH_4^+ in high N concentration fertilizer may even have toxic effects on plants (Gerendás et al., 1997).

The responses of plant growth to N forms or NH_4:NO_3 ratio are different for many crops (Bar-Yosef et al., 2009; Bernstein et al., 2005; Hewins and Hyatt, 2010; Mendoza-Villarreal et al., 2015; Niu et al., 2011). Usually, plants adapted to acid soils prefer NH_4^+, whereas plants adapted to high pH soils prefer NO_3^- (Marschner, 2012). Solutions with 67:33 NH_4:NO_3 ratio produced greater biomass than other ratios in mesquite (*Prosopis vekutina*) (Hahne and Schuch, 2006). Seventy-five percent of NO_3^- in total N is preferable for improving growth and flowering in hybrid phalaenopsis orchid (*Phalaenopsis*) (Wang, 2008). In some plants, N form has no significant effects on plant growth, for

instance, the dry weight of shoot and root, and root to shoot ratio in Texas mountain laurel (*Sophora secundiflora*) were not affected by NH_4:NO_3 ratio (Niu et al. 2011).

In endive (*Chicorium endivia* L. var. *crispum*), chlorophyll content increased with increasing NH_4:NO_3 ratios due to its tolerance to ammonium nutrition (Bonasia et al. 2008). In apple (*Malus domestica*) sole ammonium nutrition led to the lowest chlorophyll content (Sotiropoulos et al., 2005). This negative effect of high ammonium ratio on chlorophyll content could be caused by low pH in the medium reducing the enzyme activity and cell growth (Mashayekhi-Nezamabadi, 2000) or ammonium accumulation increasing leaf sensitivity to ethylene which enhanced chlorophyll loss (Hsu, 2003). But, in some plants, NH_4:NO_3 ratio had no effect on chlorophyll content or SPAD reading, such as in garlic mustard (*Alliaria petiolata*) (Hewins and Hyatt, 2010).

When roots take up NO_3^-, which has a negative charge, and NH_4^+, which has a positive charge, they typically release an identically charged molecule to maintain a balanced pH inside the plant cells. This process has a strong impact on the uptake of other cations and anions and rhizosphere pH. For example, the assimilation process of one molecule of NH_4^+ produces one proton which will be excreted into the external rhizosphere, reducing rhizosphere pH (Marschner, 2012). Since NO_3^- has a negative charge, the process of NO_3^- uptake is associated with an uptake of protons from the rhizosphere that leads to increasing pH (Hinsinger et al., 2003).

High levels of NH_4^+ can also inhibit the uptake of cations such as calcium and magnesium from the substrate and thus induce a deficiency of those elements in the crop (Adams, 1966; Siddiqi et al., 2002). This decreased uptake of essential cations could cause more problems for plant growth and metabolism. For instance, calcium deficiency

led to 'toppling' disorder in tulip (*Tulipa* L.) (Nelson and Niedziela, 1998.). However, ammonium-fed plants accumulate more phosphate and sulfate due to acidification of the rhizosphere, while nitrate depresses the uptake of those essential anions (Marschner, 2012). Ammonium applications can also reduce the incidence of ion (Fe) deficiency in calcareous soils (Mills and Jones, 1997). Thus, most of the time, supplying both NO_3^- and NH_4^+ results in the highest growth rates and plant yields (Kafkafi, 1990; Santamaria and Elia, 1997).

The objective of this study was to investigate the effects of NH_4:NO_3 ratios on plant growth, flowering and uptake of nutrients in TB iris 'Immortality'.

Materials and Methods

This study was conducted under natural conditions in Starkville, MS (latitude 33°46' N, longitude 88°82' W). Rhizomes (average caliper = 4.7 cm and length = 5.8 cm) of 'Immortality' TB iris (Schreiner's Iris Gardens, Salem, OR) were potted in Aug. 2012 into 3.78-L (23 cm diameter; 16 cm height) round plastic pots (one rhizome per pot) containing a commercial substrate with no starter fertilizer (Fafard growing mix 2; Sun Gro Horticulture, Agawam, MA). From 28 Aug. to 28 Sept. 2012 plants were supplied twice per week with 400 ml of modified Hoagland's solution (Hoagland and Arnon, 1950) containing 10 mM N from NH_4NO_3 to provide basic nutrients for fall growth.

On 5 Apr. 2013, before the start of the NH_4:NO_3 ratio treatments, five plants were harvested for background dry weight and nutrient composition. The experiment was a completely randomized design with five treatments and 16 replications in each treatment. Five treatments of NH_4:NO_3 at 0:100, 25:75, 50:50, 75:25, 100:0 and having the same concentration of N (12 mM), K^+ (10 mM) and PO_4^{3-} (5 mM) were used. The nutrient

solutions were prepared by adding analytical grade chemicals KNO_3, NH_4NO_3, $Ca(NO_3)_2$, $CaCl_2$, $(NH_4)_2SO_4$, Na_2SO_4, K_2SO_4, KH_2PO_4, KCl, and $MgSO_4$ to tap water with the composition shown in Table 5.1. Other micronutrients, including Fe (0.1mM), Mn (0.01mM), Zn (10^{-3} mM), Cu (10^{-3} mM), and B (0.05mM), were also added to all nutrient solutions. Plants were supplied with 400 ml solution containing one of five NH_4:NO_3 ratio twice per week from 8 Apr. 2013 to 17 Sept. 2013.

Throughout the experiment, plant height, leaf SPAD readings (SPAD-502, Minolta Camera Co., Japan), and pH and electrical conductivity (EC) in the leachate (using the pour through extraction method) were measured weekly. At the end of the growing season, four plants from each treatment were randomly selected and destructively harvested on 5 Dec. 2013. Each plant was divided into leaves, roots and rhizomes. All samples were oven dried at 60 °C until constant weight and dry weights were recorded by tissue type. All samples were ground to pass a 40-mesh sieve in a Wiley Mill (Thomas Scientific, Swedesboro, NJ) for tissue nutrients analyses.

Data were analyzed by using the five NH_4:NO_3 ratios as a one-factor study. Continuous response data were analyzed using linear models with GLM procedure of SAS 9.3 (SAS Institute, Cary, NC) and count data were analyzed using generalized linear mixed models with the GLIMMIX procedure of SAS. To distinguish the differences among NH_4:NO_3 ratios, mean comparisons were made by Tukey's honestly significant difference (HSD) test.

Results and Discussions

Plant height and SPAD reading

From April to August, plant height was not affected by N forms, except in June in which plant height with 100:0 NH_4:NO_3 ratio was significantly shorter than the others (Figure 5.1A). The effects of N form on plant height may vary among different species; for example, in pepper (*Capsicum annuum*), decreasing NH_4:NO_3 ratio led to shorter and more compacted plants (Bar-Tal et al., 2001), whereas in tomato (*Lycopersicon esculentum*) NH_4:NO_3 ratio had no effect on plant height (Sandoval-Villa et al., 2001).

It is well known that SPAD readings are highly linearly related to chlorophyll content (Wang et al., 2005). Other research also indicated SPAD readings may be used to indicate N status in plant leaves (Ghosh et al., 2013; Islam et al., 2009). In June, SPAD readings of plants receiving 75:25 NH_4:NO_3 was greater than other treatments. In August, SPAD readings of plants receiving 75:25, 50:50 and 25:75 NH_4:NO_3 were greater than those receiving sole ammonium or nitrate form fertilizer. In November, SPAD readings of plants fertigated with 100:0, 75:25, and 50:50 NH_4:NO_3 were greater than those with 25:75 and 0:100 NH_4:NO_3 ratios. During other months, there was no significant difference on SPAD reading of plants receiving different N form (Figure 5.1B).

In general, SPAD readings were higher in those treatments with both ammonium and nitrate. NH_4:NO_3 ratio may influence leaf chlorophyll content or SPAD reading differently depending on plant species due to a plant's preference of N form (Bonasia et al., 2008; Hewins and Hyatt, 2010; Sotiropoulos et al., 2005). The results of SPAD readings in this study indicated fertilizer with both ammonium and nitrate may benefit the growth of TB iris .

Regardless of NH_4:NO_3 ratio, SPAD reading trended to decrease from April to July and then increase from July to October (Figure 5.1B). This trend is consistent with Pei's study in which chlorophyll content in 'Immortality' TB iris plants decreased during high temperatures of summer, and increased again in August (Pei, 2006). This declining trend of SPAD reading may be influenced by high temperatures in the summer when iris plants go dormant. A declining trend of chlorophyll content caused by high temperatures also happens in other plants. Chlorophyll content decreased when soil temperature was high in creeping bentgrass (*Agrostis stolonifera*) (Liu and Huang, 2004).

Flowering

Neither spring nor fall flowering (including number of inflorescences per plant and inflorescence stem length) were influenced by N form (data not shown). In cup butter (*Ranunculus asiaticus*), when percentage of ammonium increased from 10% to 30%, number of flowers was affected (Bernstein et al, 2005). When NH_4:NO_3 ratios were greater than 60:40, cut rose (Rosa) yield declined due to calcium and potassium deficiency in leaves induced by ammonium in a closed hydroponic system (Bar-Yosef et al., 2009). Flower production of gerbera (*Gerbera jamesonii*) was highest at the substrate NH_4:NO_3 ratios 33:67 in one experiment and 25:75 or 50:50 in another experiment (Guba, 1994).

Leachate electrical conductivity (EC) and pH

In general, leachate EC was higher in substrate treated with higher NH_4^+ ratio (Table 5.2, Figure 5.2B). A possible explanation could be higher NH_4^+ ratio led to low pH which increased solubilization of salt elements from fertilizer.

Throughout the growing season, pH of the leachate ranged from 6.2 to 7.5 and decreased with higher NH_4^+ ratios (Table 5.2, Figure 5.2A). According to growing practice, the suitable pH for growing TB iris is 6.8 (slightly acidic) (Morris, 2011). The pH in treatments with 25:75 and 50:50 NH_4:NO_3 ratios are closer to this suggested pH. In the study of cone bush 'Safari Sunset' (*Leucadendron*), rhizosphere pH decreased below pH 5.0 at high NH_4^+ application, while, the pH rose above 7.0 at low NH_4^+ application (Silber et al., 2001). In a closed hydroponic system, low pH with greater percentage of NH_4^+ in solution caused Ca and K deficiency in leaves. High pH with a greater percentage of nitrate led to Ca and Mn precipitation and reduced the availability of these nutrients (Bar-Yosef et al. 2009).

Dry weight

The dry weight of leaf, root, rhizome, total plant, and shoot to root ratio (sum of leaf and rhizome dry weight divided by root dry weight) were not affected by the NH_4:NO_3 ratios (data not shown). The impact of NH_4:NO_3 ratios on the accumulation of biomass varied among plant species. In both Texas mountain laurel and garlic mustard N form had no significant effects on biomass of both leaf and root (Hewins and Hyatt, 2010; Niu et al., 2011). In prairie gentian (*Eustoma grandiflorum*), the dry weight of leaf, stem and shoot increased linearly with increasing NH_4:NO_3 ratios (Mendoza-Villarreal et al., 2015). In addition, if available N form was not the one preferred by plants, then it may cause N deficiency symptoms, such as lower dry biomass and larger root to shoot ratio (Garbin and Dillenburg, 2008). Thus, dry weight may be used as an indicator of N form preference.

Tissue nutrient concentrations

NH_4:NO_3 ratios in fertilizer significantly affected concentration of P, Fe, Mn, Zn, and Cu in leaves; concentration of Ca, Mg, Mn, and B in roots; and concentration of N P, Mg, Fe, Mn, and Zn in rhizomes (Table 5.3). In general, N concentration in leaves, roots, and rhizomes was not affected by NH_4:NO_3 ratios. There was a decreasing trend in Ca and Mg concentration in leaves, roots, and rhizomes with higher NH_4:NO_3 ratios. Due to the antagonism between NH_4^+ and Ca^{2+} in the process of uptake, greater NH_4^+ ratio in fertilizer can cause a decrease in Ca concentration in plant tissues (Siddiqi et al., 2002).

Uptake of nutrients

The net uptake of N was not affected by NH_4:NO_3 ratios. The uptake of Fe, Mn and Zn was significantly increased by higher NH_4:NO_3 ratios (Table 5.4). Higher NH_4:NO_3 ratios induced low pH in the rhizosphere, which increased Fe^{2+}, Mn^{2+} and Zn^{2+} availability and uptake (Marschner, 2012).

Conclusion

In summary, NH_4:NO_3 ratios affected substrate leachate EC and pH, but had no influence on plant height, flowering, dry weight accumulation or net uptake of N and some other nutrients. The uptake of Fe, Mn and Zn was affected by NH_4:NO_3 ratio, which could be related to the changes in pH in the rhizosphere. In conclusion, TB iris 'Immortality' may not have a preference for either ammonium or nitrate N form.

Table 5.1 Chemical and nutrient composition of fertigation solution of five NH_4:NO_3 ratios at a constant total N concentration of 12 mM.

NH_4:NO_3 ratios	Chemical composition (mM)									
	KNO_3	NH_4NO_3	$Ca(NO_3)_2$	$CaCl_2$	$(NH4)_2SO_4$	Na_2SO_4	K_2SO_4	KH_2PO_4	KCl	$MgSO_4$
0:100	5	0	3.5	0	0	2.5	0	5	0	1
25:75	0	3	3	0	0	0	2.5	5	0	1
50:50	0	6	0	3	0	0	2.5	5	0	1
75:25	0	3	0	3	3	0	0	5	5	1
100:0	0	0	0	3	6	0	0	5	5	1
	Nutrient composition (mM)									
	N	NH_4^+	NO_3^-	PO_4^{3-}	K^+	Ca^{2+}	Mg^{2+}	Na^+	SO_4^{2-}	Cl^-
0:100	12	0	12	5	10	3.5	1	5	2.5	0
25:75	12	3	9	5	10	3	1	0	2.5	0
50:50	12	6	6	5	10	3	1	0	2.5	6
75:25	12	9	3	5	10	3	1	0	3	11
100:0	12	12	0	5	10	3	1	0	6	11

Table 5.2 Leachate pH and electrical conductivity (EC) of container-grown 'Immortality' TB iris.

NH_4:NO_3 ratios	May		June		July		August		September		October		November	
	pH	EC	pH	EC	pH	EC	pH	EC	pH	EC	pH	EC	pH	EC
0:100	7.31a[z]	2.1b	7.47a	3.6b	7.29a	6.9bc	7.22a	3.2b	7.36a	6.7ab	7.81a	4.4	7.50a	3.9
25:75	7.22ab	2.7b	7.42a	3.5b	6.94ab	10.9a	7.01b	2.9b	7.18ab	4.6b	7.34b	3.9	7.18ab	3.9
50:50	7.08c	3.1b	7.18b	4.3ab	6.80b	4.0c	7.15ab	3.1b	6.85bc	5.5b	7.15bc	4.0	7.17ab	4.2
75:25	6.96c	3.2b	6.95c	4.3ab	6.25c	8.3ab	6.67b	3.1b	6.34d	7.7a	6.92cd	4.9	6.79bc	5.7
100:0	6.56d	5.7a	6.67d	5.3a	6.19c	8.4ab	6.24c	5.0a	6.51cd	7.0ab	6.67d	4.9	6.37c	4.3

Plants were treated with different NH_4:NO_3 ratios from Apr. to Sept. 2013. Rhizomes were planted in Aug. 2012 and plants were harvested in Dec. 2013.

[z]Means within a column followed by different letters denotes significant differences between N treatments (Tukey's honestly significant difference, $P \leq 0.05$.)

Table 5.3 Concentration of nutrients in leaves, roots, and rhizomes of container-grown 'Immortality' TB iris.

NH_4:NO_3 ratios	N[z] (%)	C (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	B (ppm)
					Leaf						
0:100	2.4	45.9	0.54a[y]	4.7	1.5	0.48	39.0c	8.5c	22bc	3.5c	30.5
25:75	2.1	46.0	0.53a	4.2	1.5	0.47	36.8c	11.8c	20.3c	6.8bc	36.8
50:50	2.1	46.7	0.51a	4.1	1.4	0.44	36.5c	10.8c	22.3bc	8.5ab	37.5
75:25	2.5	47.2	0.38b	4.2	1.3	0.38	74.0b	30.8b	29.0b	11.0b	32.0
100:0	2.5	47.3	0.42b	4.2	1.3	0.38	112.8a	49.5a	40.8a	12.0a	34.3
					Root						
0:100	0.87	49.5	0.24	3.0	0.45a	0.17a	71.5	15.8c	14.5	5.0	13.5ab
25:75	0.92	49.1	0.34	3.3	0.43a	0.18a	77.8	19.0c	18.8	9.3	14.0a
50:50	0.87	48.6	0.32	3.9	0.35b	0.18a	62.5	17.3c	15.3	6.8	13.8ab
75:25	0.88	49.5	0.27	3.5	0.32b	0.15b	78.8	25.0b	18.5	6.8	12.5bc
100:0	0.79	48.5	0.29	4.0	0.32b	0.15b	65.8	30.8a	21.3	7.5	12.0c
					Rhizome						
0:100	2.7ab	48.2	0.46a	1.64	0.56	0.29a	46.3b	6.3d	14.5c	7.8	9.8
25:75	2.5bc	48.4	0.38bc	1.51	0.56	0.28a	45.3b	6.5cd	20.0bc	10.3	9.8
50:50	2.0c	47.9	0.33c	1.43	0.52	0.21b	49.8b	8.0b	14.3c	8.3	9.3
75:25	3.1a	48.9	0.40ab	1.49	0.48	0.23b	62.8a	14.0b	28.3ab	11.0	10.0
100:0	3.ab	48.4	0.41ab	1.62	0.45	0.23b	65.0a	16.0a	37.0a	13.3	9.5

Plants were treated with different NH_4:NO_3 ratios from Apr. to Sept. 2013. Rhizomes were planted in Aug. 2012 and plants were harvested in Dec. 2013.

[z]Nitrogen (N); Carbon (C); Phosphorous (P); Potassium (K); Calcium (Ca); Magnesium (Mg); Iron (Fe); Manganese (Mn); Zinc (Zn); Copper (Cu); Boron (B).

[y]Means within a column followed by different letters denotes significant differences between N treatments (Tukey's honestly significant difference, $P \leq 0.05$).

Table 5.4 Net nutrient uptake of container-grown 'Immortality' TB iris.

NH_4:NO_3 ratios	N[z] (g)	P (g)	K (g)	Ca (g)	Mg (g)	Fe (mg)	Mn (mg)	Zn (mg)	Cu (mg)	B (mg)
0:100	1.31	0.23	0.57	0.18	0.11	33.7c[y]	5.6b	10.3b	0.07	0.24
25:75	1.00	0.18	0.49	0.16	0.07	30.5c	6.2b	11.8b	0.17	0.30
50:50	0.90	0.17	0.49	0.17	0.05	34.8bc	6.9b	10.6b	0.14	0.30
75:25	1.55	0.21	0.80	0.23	0.07	51.7a	14.7a	19.9a	0.19	0.24
100:0	1.24	0.18	0.68	0.13	0.04	48.9ab	16.6a	22.1a	0.23	0.26

Plants were treated with different NH_4:NO_3 ratios from Apr. to Sept. 2013. Rhizomes were planted in Aug. 2012 and plants were harvested in Dec. 2013.
[z]Nitrogen (N); Phosphorous (P); Potassium (K); Calcium (Ca); Magnesium (Mg); Iron (Fe); Manganese (Mn); Zinc (Zn); Copper (Cu); Boron (B).
[y]Means within a column followed by different letters denotes significant differences between N treatments (Tukey's honestly significant difference, $P \leq 0.05$).

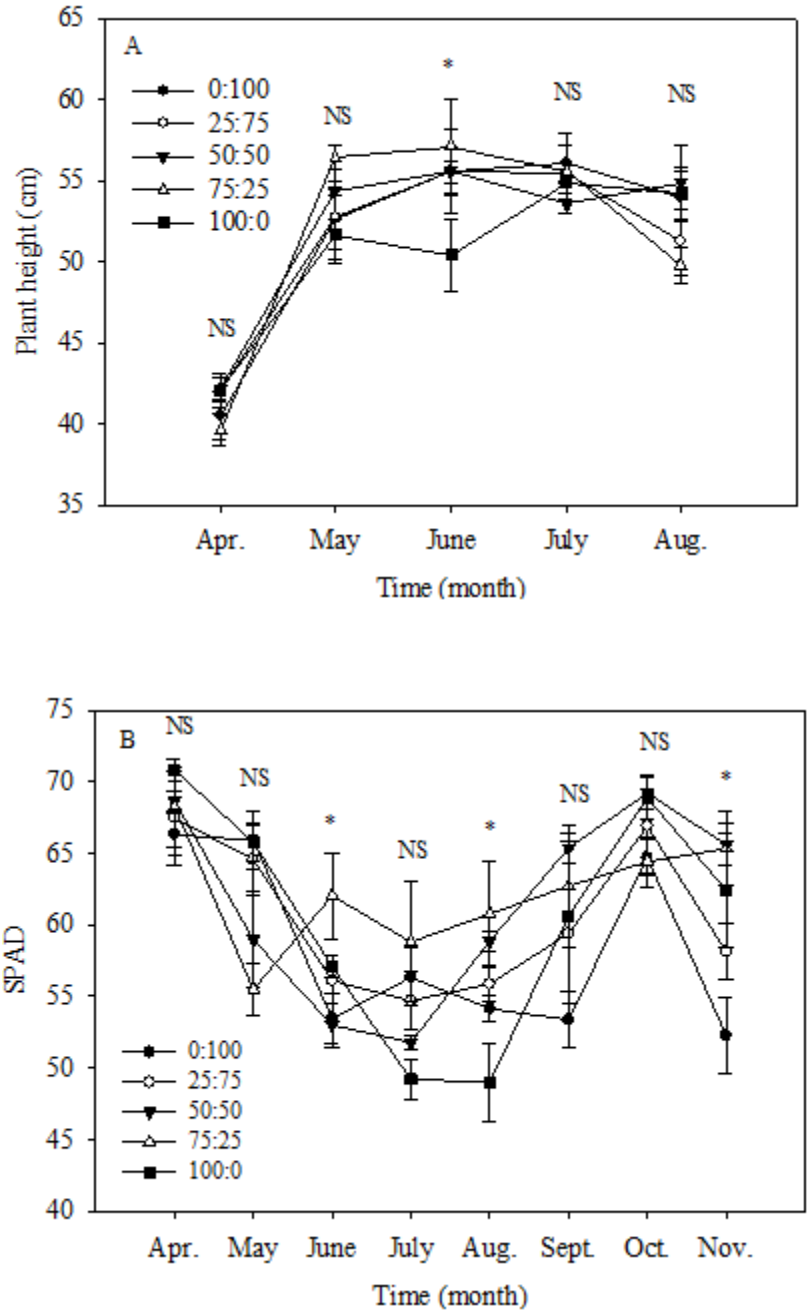

Figure 5.1 (A) Plant height and (B) leaf SPAD reading of container-grown 'Immortality' TB iris.

Plants were treated with different NH_4:NO_3 ratios from Apr. to Sept. 2013. Rhizomes were planted in Aug. 2012 and data were collected monthly in 2013. NS,* non-significant or significant at $P \leq 0.05$ respectively.

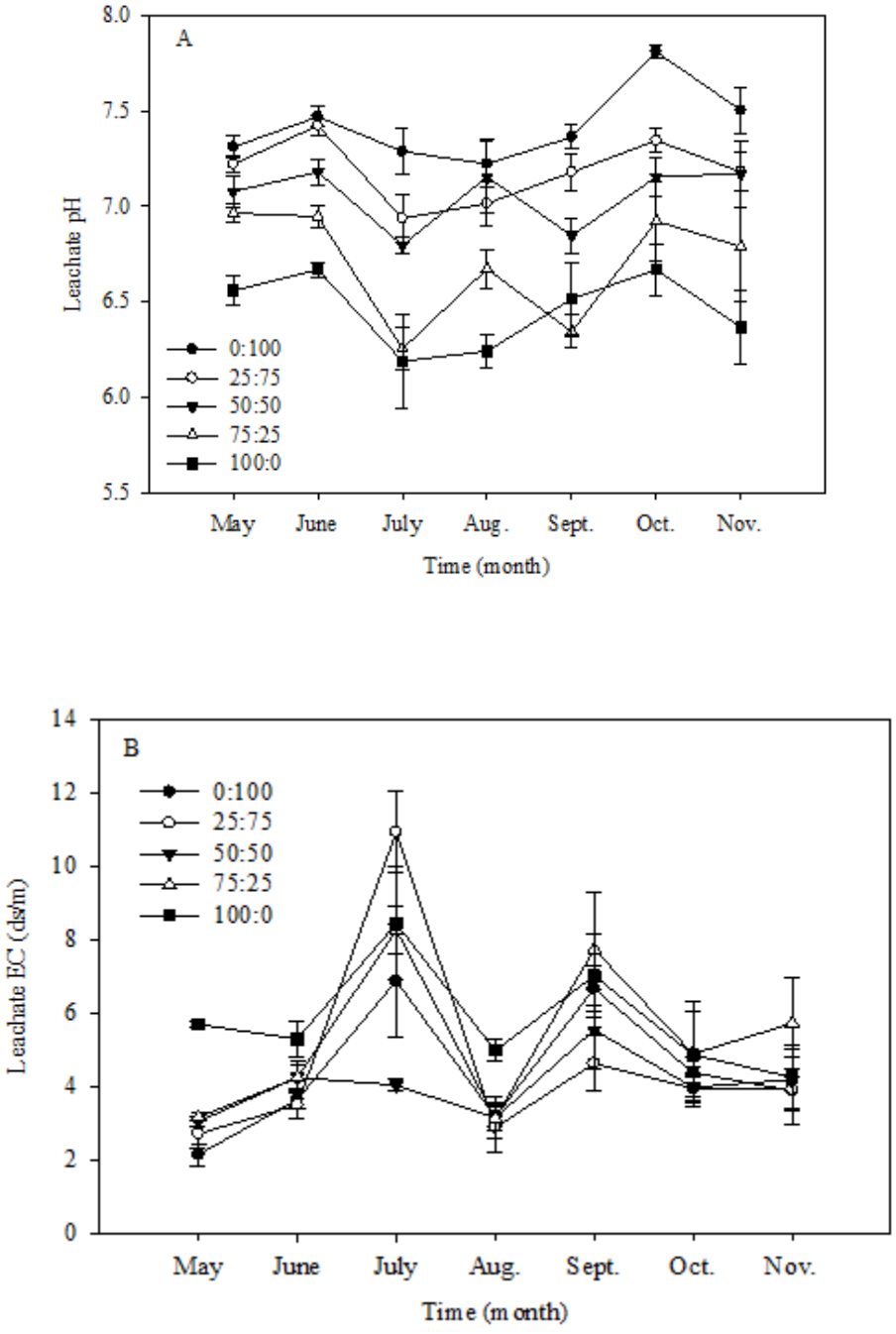

Figure 5.2 Substrate leachate (A) pH and (B) electrical conductivity (EC) of container-grown 'Immortality' TB iris.

Plants were treated with different NH_4:NO_3 ratios from Apr. to Sept. 2013. Rhizomes were planted in Aug. 2012 and data were collected monthly in 2013.

References

Adams, F. 1966. Calcium deficiency as a causal agent of ammonium phosphate injury to cotton seedlings. Soil Sci. Soc. Amer. J. 30:485-488.

Bar-Tal, A., B. Aloni, L. Karni, and R. Rosenberg. 2001. Nitrogen nutrition of greenhouse pepper. II. Effects of nitrogen concentration and NO_3:NH_4 ratio on growth, transpiration, and nutrient uptake. HortScience 36:1252-1259.

Bar-Yosef, B., N.S. Mattson, and H.J. Lieth. 2009. Effects of NH_4:NO_3: urea ratio on cut roses yield, leaf nutrients content and proton efflux by roots in closed hydroponic system. Sci. Hort. 122:610-619.

Bernstein, N., M. Ioffe, M. Bruner, Y. Nishri, G. Luria, I. Dori, E. Matan, S. Philosoph-Hadas, N. Umiel, and A. Hagiladi. 2005. Effects of supplied nitrogen form and quantity on growth and postharvest quality of *Ranunculus asiaticus* flowers. HortScience 40:1879-1886.

Bonasia, A., G. Conversa, M. Gonnella, F. Serio, and P. Santamaria. 2008. Effects of ammonium and nitrate nutrition on yield and quality in endive. J. Hort. Sci. Biotechnol. 83:64-70.

Garbin, M.L. and L.R. Dillenburg. 2008. Effects of different nitrogen sources o n growth, chlorophyll concentration, nitrate reductase activity and carbon and nitrogen distribution in *Auracaria angustifolia*. Brazilian Soc. Plant Physiol. 20: 295-303

Gerendás, J., Z.Zhu, R. Bendixen, R.G. Ratcliffe, and B. Sattelmacher. 1997. Physiological and biochemical processes related to ammonium toxicity in higher plants. J. Plant Nutr. Soil Sci. 160:239-251.

Ghosh, M., D.K. Swain, M.K. Jha, and V.K. Tewari. 2013. Precision nitrogen management using chlorophyll meter for improving growth, productivity and N use efficiency of rice in subtropical climate. J. Agric. Sci. 5:253-266.

Guba, W. 1994. The effect of different NH_4/NO_3 ratios on the production and quality of gerbera grown in rockwool. J. Fruit Ornam. Plant Res. 2:143-155.

Hahne, K.S. and U.K. Schuch. 2006. Nitrogen form and concentration affect nitrogen leaching and seedling growth of *Prosopis velutina*. HortScience 41:239-243.

Hewins, D.B. and L.A., Hyatt. 2010. Flexible N uptake and assimilation mechanisms may assist biological invasion by *Alliaria petiolata*. Biol. Invasions 12:2639-2647.

Hinsinger, P., C. Plassard, C. Tang, and B. Jaillard. 2003. Origins of root-induced pH changes in the rhizosphere and their responses to environmental constraints: A review. Plant Soil 248:43-59.

Hoagland, D.R. and D.I. Arnon. 1950. The water-culture method for growing plants without soil. Calif. Agr. Expt. Sta. Circ. 347:1-32.

Hsu, S.Y., Y.T. Hsu, and C.H Kao. 2003. Ammonium ion, ethylene, and abscisic acid in polyethylene glycol-treated rice leaves. Biol. Plant. 46:239-242.

Islam, M.S., M.S.U. Bhuiya, S. Rahman, and M.M. Hussain. 2009. Evaluation of SPAD and LCC based nitrogen management in rice (*Oryza sativa* L.). Bangladesh J. Agri. Res. 34:661-672.

Kafkafi, U. 1990. Root temperature, concentration and the ratio NO_3/NH_4 effect on plant development. J. Plant Nutr. 13:1291-1306.

Liu, X. and B. Huang. 2004. Changes in fatty acid composition and saturation in leaves and roots of creeping bentgrass exposed to high soil temperature. J. Amer. Soc. Hort. Sci. 129:795-801.

Marschner, P. 2012. Mineral nutrition of higher plants. 3rd Ed. Academic Press, London. UK.

Mashayekhi-Nezamabadi, K. 2000. The protein synthesis spectrum during the induction phase of somatic embryogenesis in carrot (*Daucus carota* L.) cultures and the role of nitrogen forms for embryo development. Dr. Sci. Thesis. Justus Liebig University, Giessen, Germany.

Mendoza-Villarreal, R., L.A. Valdez-Aguilar, A. Sandoval-Rangel, V. Robledo-Torres, and A. Benavides-Mendoza. 2015. Tolerance of Lisianthus to high ammonium levels in rockwool culture. J. Plant Nutr. 38:73-82.

Mills, H.A. and J.B. Jr. Jones. 1997. Plant analysis handbook II: a practical sampling, preparation, analysis, and interpretation guide. 2nd Ed. MicroMacro Publishing, GA. USA.

Morris, J. 2011. How to plant and grow bearded iris. 5 May 2015. http://www.irises.org/About_Irises/Cultural Information/Grow_Bearded.html.

Nelson, P.V. and C.E. Niedziela Jr.. 1998. Effects of calcium source and temperature regime on calcium deficiency during hydroponic forcing of tulip. Sci. Hort. 73:137-150.

Niu, G., D. Rodriguez, and M. Gu. 2011. Response of *Sophora secundiflora* to nitrogen form and rate. HortScience 46:1303-1307.

Pei, H. 2006. Photosynthetic characteristic and effects of photoperiod on flower initiation in *Iris germanica*. Master Thesis. China Agriculture University, Beijing, China.

Sandoval-Villa, M., E.A. Guertal, and C.W. Wood. 2001. Greenhouse tomato response to low ammonium-nitrogen concentrations and duration of ammonium-nitrogen supply. J. Plant Nutr. 24:1787-1798.

Santamaria, P. and A. Elia. 1997. Producing nitrate-free endive heads: Effect of nitrogen form on growth, yield, and ion composition of endive. J. Amer. Soc. Hort. Sci. 122:140-145.

Schuman, G.E., M.A. Stanley, and D. Knudsen. 1973. Automated total nitrogen analysis of soil and plant samples. Proc. Soil Sci. Soc. Amer. 37:480-481.

Siddiqi, M.Y., B. Malhotra, X. Min, and A.D.M. Glass. 2002. Effects of ammonium and inorganic carbon enrichment on growth and yield of a hydroponic tomato crop. J. Plant Nutr. Soil Sci. 165:191-197

Silber, A, B., Mitchnick, J.J. Ben, and R.A. Criley. 2001. Phos-phorus nutrition and the rhizosphere pH in *Leucadendron* 'Safari Sunset'. Acta Hort. 545:135-143.

Sotiropoulos, T.E., G.N. Mouhtaridou, T. Thomidis, V. Tsirakoglou, K.N. Dimassi, and I.N. Therios. 2005. Effects of different N-sources on growth, nutritional status, chlorophyll content, and photosynthetic parameters of shoots of the apple rootstock MM 106 cultured *in vitro*. Biol. Plant. 49:297-299.

Wang, Q., J. Chen, R.H. Stamps, and Y. Li. 2005. Correlation of visual quality grading and SPAD reading of green-leaved foliage plants. J. Plant Nutr. 28:1215-1225.

Wang, Y. 2008. High NO_3-N to NH_4-N ratios promote growth and flowering of a hybrid *Phalaenopsis* grown in two root substrates. HortScience 43:350-353

CHAPTER VI

NITROGEN AND PHOSPHORUS RATES INFLUENCE GROWTH, FLOWERING, NUTRIENT UPTAKE AND ALLOCATION IN IRIS GERMANICA 'IMMORTALITY'

Abstract.

The influence of nitrogen (N) and phosphorus (P) rates on plant growth and uptake of essential nutrients was evaluated in container-grown tall bearded (TB) iris (*Iris germanica* L.) 'Immortality'. Factorial combinations of three N (5, 10, or 15 mM) rates and three P (5, 10, or 15 mM) rates were applied to plants twice per week from March to September 2013. Plant height and leaf SPAD data were collected during the growing season. Plants were harvested in December 2013 to measure dry weight (DW) and analyze essential mineral elements concentration. Greater N rates increased plant height, leaf SPAD reading, tissue DW, and uptake of many essential elements, such as potassium (K), calcium (Ca) and iron (Fe). P rates did not affect plant height or DW and only increased leaf SPAD reading in October. Greater P rates increased concentration of P in leaves and roots and decreased boron (B) concentration in the leaves, but did not influence net uptake of other nutrients, except copper (Cu). The average N:P ratio ranged from 4.7 to 7.5, 2.4 to 4.0 and 6.0 to 8.7 in leaves, roots and rhizomes, respectively. Compared to the commonly recommended threshold N:P ratio of 16:1, plants in this study may be N limited; however, P supply was sufficient, even at 5 mM P application.

Introduction

Plant growth is often limited by nitrogen (N) and phosphorus (P) availability (Iversen et al., 2010; Vitousek et al., 2002). Nitrogen is the most commonly used mineral nutrient in plants; about 1-5% of total plant dry matter consists of N. It plays pivotal roles as a constituent of proteins, nucleic acids, chlorophyll and other compounds (Marschner, 2012). Phosphorus is an essential nutrient which works as a structural element in nucleic acids, phospholipids and plays an important role in energy transfer. Regulation of resource allocation between vegetative and reproductive development may be affected by leaf P concentration (Fitter et al., 1998).

Greater N fertilization rates can significantly promote shoot growth (Bi et al., 2007; Dong et al., 2004; Scagel et al., 2011; Wang, 1996), and P is associated with root growth (Graciano et al., 2006; Ristvey et al., 2007; Zhang et al., 2002). Hanley et al. (2008) showed high N rates improved inflorescences and inflorescence stem length of TB iris, but limited information is available on the effects of N and P rates and their interactions on growth and development in TB iris.

Understanding the interactions between nutrients are important in determining the optimum nutrient balance and rates for plant growth (Dighton et al., 1993; Graciano et al., 2006; Hasanuzzaman et al., 2012). Crop productivity or quality can be affected by the balance between nutrients (Ingestad, 1991). Phosphorus uptake is strongly influenced by N supply and no effects of P fertilization may be expected when soil N availability is very low (Herbert, 1990). On the other hand, N uptake efficiency was increased by increasing P availability (Iversen et al., 2010). This indicates the interaction of P and N availability plays an important role in growth related processes (Cornelissen et al., 1997).

In agriculture and forestry, nutrient limitations can be analyzed using N/P ratios (Fenn et al., 1998; Koerselman and Meuleman, 1996; Tessier and Raynal, 2003; Williams et al., 1996). The most common threshold of N:P ratio is 16:1. N:P ratio >16 means P is limiting and a N:P <16 ratio means N is limiting. A N:P ratio between 14 and 16 indicates plant growth is limited by either N or P, or N and P together. However, this threshold may not be applicable to all plant species (Li et al., 2001).

When N availability limits plant growth, uptake of other nutrients is expected to decline accordingly (Marschner, 1995). For example, insufficient N supply caused growth limitation and led to decreased uptake of P, K, S, Ca and Mg in azalea (*Rhododendron* L. 'Karen') (Ristvey et al., 2007). Nitrogen application can improve uptake of other nutrients (Scagel et al., 2011). To optimize growth, increased N rate should accompany modified doses of other nutrients in a fertilizer formulation (Scagel et al., 2008a). Phosphorus status can also influence the uptake of other nutrients. In eucalyptus (*Eucalyptus grandis*), limiting P-availability had negative effects on the uptake of N and sulfate (S) which reduced plant growth (Graciano et al., 2006).

The rock phosphate used to make most phosphate fertilizers is a non-renewable resource and current global reserves may be depleted in 50-100 years (Cordell et al., 2009; Dawson and Hilton, 2011). In many crops the need for high P rates may have been overemphasized (Ristvey et al., 2007; Wang and Konow, 2002), besides, the run-off of N fertilizer also causes many environmental problems. Thus, understanding a plant's N and P requirement and how the interaction between N and P affects plant growth and quality is important to both the environment and crop production.

The objective of this research is to investigate the effects of N and P rates and their interactions on plant growth, flowering and uptake of other nutrients in TB iris 'Immortality'.

Materials and Method

This study was conducted under natural conditions in Starkville, MS (latitude 33°46' N, longitude 88°82' W). In Aug. 2012, rhizomes (average caliper = 4.7 cm and length = 5.8 cm) of 'Immortality' TB iris (Schreiner's Iris Gardens, Salem, OR) were potted one rhizome per pot into 3.78-L (23 cm diameter; 16 cm height) round plastic pots filled with commercial substrate with no starter fertilizer (Fafard 2; Sun Gro Horticulture, Agawam, MA). Two weeks after transplanting, plants were fertigated (400 ml each) with 10 mM N from NH_4NO_3 (Sigma Aldrich, St. Louis, MO) plus N-free fertilizer (1.06 $mg \cdot mL^{-1}$, Cornell No N Formula 0-6-27, Greencare Fertilizers, Kankakee, IL) twice per week from August through September in 2012 to provide basic nutrients for fall growth.

In April 2013, five plants were harvested before fertigation treatment for background biomass and nutrient composition. From April to Sept. 2013, nine N and P rate combinations using a 3 by 3 factorial treatment design in a completely randomized experimental design (Table 6.1) were applied twice per week to plants. The three rates of N and P were 5, 10, or 15 mM and each treatment was designed to allow for only N or P rate to change while all other nutrients were held constant, except for chlorine (Cl^-). Other micronutrients, Fe (0.1 mM); Mn (0.01 mM); zinc (Zn, 10^{-3} mM); Cu (10^{-3} mM); B (0.05 mM), were also added to nutrient solutions. Analytical grade chemicals were used to add nutrients to fertilizer solutions.

During the growing season, data for flowering (number of inflorescences and inflorescence stem length), plant height (average height of top three fans), and leaf SPAD readings were collected. The SPAD readings were taken using a chlorophyll meter (SPAD-502, Minolta Camera Co., Japan).

In December 2013, four plants from each treatment were randomly selected and destructively harvested. During harvesting, rhizome size, number of rhizomes, and number of floral meristems (visible to the naked eye) were measured or counted. Each plant was divided into leaves, roots and rhizomes. All samples were dried in a 60°C oven until constant weight, then dry weights were recorded by tissue types. All samples were ground to pass a 40-mesh sieve in a Wiley Mill (Thomas Scientific, Swedesboro, NJ) for nutrient analysis.

Total N was determined by the Kjedahl method (Schuman et al., 1973) and concentrations of other macronutrients and micronutrients in the samples were obtained using inductively coupled plasma optical emission spectroscopy (ICP-OES) at the Soil Testing Lab of Mississippi State University. Nutrient content of each tissue was calculated by multiplying dry weight by concentration. Total plant content of each nutrient was calculated from the sum of the content in leaves, roots and rhizomes. Nutrient uptake was estimated by subtracting the average total nutrient in Dec. 2013 from the average total nutrient in Apr. 2013.

In the following spring (2014), the remaining five plants from each treatment combination were grown outdoors under natural conditions but without any fertilizer supply. During the 2013 growing season and spring 2014, data on flowering (number of

inflorescences and inflorescence stem length), plant height (average height of top three fans), and leaf SPAD reading were collected.

All data were analyzed as a 3 × 3 (N rate × P rate) complete factorial design. Continuous response data were analyzed using linear models with the GLM procedure of SAS (version 9.3; SAS Institute, Cary, N.C.) and count data were analyzed using generalized linear mixed model with the GLIMMIX procedure of SAS. If the interaction was term not significant, main effects are reported and discussed; if interaction was significant, simple effects (the effect of a variable at each level of the other variable) are reported and discussed. Mean comparisons were made using Tukey's honest significant difference (HSD).

Results and Discussions

Plant height and leaf SPAD reading in 2013

During the 2013 growing season, N rates had positive effects on plant height (Table 6.2) which is consistent with previous studies (Bi et al., 2007; Ruamrungsri and Apavatjrut, 2003). Phosphorus rates did not affect plant height. This is different from findings on gloriosa lily (*Gloriosa rothschildiana*) for which N did not affect plant height, but low P rate reduced plant height (Ruamrungsri et al., 2011); however, the P rate in their study was 50 and 100 mg/L, and the P rates in our study were 5 mM (155 mg/L), 10 mM (310 mg/L) and 15 mM (465 mg/L), suggesting that a 5mM P rate is likely high enough to satisfy the P requirement for the growth of TB iris 'Immortality'. Thus, plant height in our study was not affected by P rates. Leaf SPAD reading in June 2013 was only affected by N rate (Table 6.2).

Plant height and leaf SPAD reading in March 2014

Greater N rates in 2013 increased plant height in March 2014 (Table 6.2). SPAD readings were affected by the interactions of N and P rates in March (Table 6.2). In the plants receiving the same N rates in 2013, SPAD readings were decreased by P rates. In plants receiving 5 and 10 mM P, SPAD readings were increased by N rates, but in plants receiving 15 mM P, higher N rates had a negative effect on SPAD readings.

Number of fans and floral meristems and size of top three largest rhizomes in Dec. 2013

In Dec. 2013, number of fans, number of floral meristems (visible to the naked eye), and size of rhizomes were only affected by N rate (Table 6.2); P rate had no effect. Greater N rates also increased diameter of the second and third largest axillary rhizome (#2 and #3 rhizome) and length of the second largest rhizomes (data not shown). This is supported by research with Siam tulip (*Curcuma alismatifolia*), in which increasing N rates also increased rhizomes size (Ruamrungsri and Apavatjrut, 2003).

The diameter and length of the largest rhizome, which was the mother rhizome planted in 2012 and flowered in spring 2013 (#1 rhizome), were not affected by N or P levels in 2013 (data not shown). The second largest rhizome has great potential to produce flowering in the next spring. Considering number of floral meristems was also increased by higher N rates, floral initiation may be related to rhizomes diameter or size (Craver and Harkess, 2012).

Flowering in 2013 and 2014

Neither N nor P rates affected number of flower inflorescences and inflorescence stem length in spring 2013 (data not shown). A possible explanation for this result could

be the most flowers bloomed in late April or early May with fertigation having started less than one month earlier, so inflorescence yield and quality in 2013 were not affected by N and P treatments.

Flowering data in 2014 spring showed greater N rates in 2013 significantly increased inflorescence length. Neither N nor P rates affected number of inflorescences (data not shown). In orchids, low N and high P fertilizer reduced flower stem yield (Wang, 2000). The plant tissue N:P ratio in this study was low (2.41-8.67 in different tissues), which indicated N supply may have been limited. We suspect N in spring 2014 was not sufficient to support flowering, even though floral meristems initiated in fall 2013.

Dry weight in Dec. 2013

In Dec. 2013, total plant dry weight (DW) and in rhizomes increased with increasing N rates (Table 6.2). The DW in leaves and roots was not affected by the N rate. Phosphorus rate had no influence on DW, which is consistent with previous research with azalea (*Rhododendron* L. 'Karen') (Ristvey et al., 2007).

In Dec. 2013, a greater proportion of DW was allocated to rhizomes (66%-79%). At that time, most of the leaves had died back and the rhizome acted as a storage organ which accounted for most DW of the whole plant. The proportion of DW allocated to different tissues was not affected by N or P rates (data not shown).

Nutrient concentration in leaves, roots and rhizomes in Dec. 2013

Greater N rates increased leaf N, P, Mg, and Cu concentrations (Table 6.3), and had no influence on concentration of other nutrients. Higher P rates resulted in higher P

concentration and lower B concentration in plants receiving 15 mM P (Table 6.3). Phosphorus rate had no effect on concentration of other nutrients in leaves. In ear-leaf of maize, higher rates of P application increased leaf P, Mg, Mn and Fe concentrations, but K, Ca, Zn and Cu concentrations were significantly decreased (Banaj et al., 2006). The influence of N rates on concentration of elements in leaves likely various among plant species. Other research also has showed N altered mineral concentration in leaves and these effects even vary in the same species grown at different sites (McKenzie, 2002).

Concentration of K in roots tissues was decreased with both higher N and P rates (Table 6.4). Higher P rates increased P concentrations in roots (Table 6.4). The concentration of Mg, Zn and Cu was affected by the interaction of P and N rates (Table 6.4).

In rhizomes, greater N rates increased N, P, and Mg concentrations and Fe concentration was highest in plants receiving 10 mM N (Table 6.5). Concentration of Cu was affected by the interaction of N and P rates. When N rate was 10 mM, P had positive effects on concentration of Cu, and when N rate was 15 mM P, had a negative effect on Cu (Table 6.5). Interestingly, P rate had no effect on concentration of P in rhizomes.

In summary, higher P fertilizer rates increased P concentration in leaves and roots, but had no effect on P concentration in rhizomes. In leaves and rhizomes, P concentration was increased by N rates. Usually, N concentration decreases with P limitation (De Groot et al., 2003; Jeschke et al., 1996); however, in this study P rate did not affect N concentration. Thus, P was not a limiting factor of plant growth in this study.

N:P ratio in Dec. 2013

Liebig's law of the minimum stated that growth is controlled not by the total amount of resources available, but by the limiting factor. In this study, N may be the limiting factor to growth which may explain why most growth related data was only affected by N rate. In this study, N:P ratio varied from 4.7 to 7.5, 2.4 to 4 and 6 to 8.7 in leaves, roots and rhizomes, respectively, under different treatments (Tables 6.3, 6.4, and 6.5). In leaves, N:P ratio was lowest in plants receiving 15mM P. In roots, N:P ratio decreased with increasing P rates (Table 6.4), but increasing N rate had no influence on N:P ratio.

Crop productivity or quality may be limited by the balance between different nutrients (Ingestad, 1991), N:P ratio has often been used in agriculture and forestry to analyze nutrient limitations (Fenn et al., 1998; Tessier and Raynal, 2003). Usually, a N:P ratio less than 16:1 indicates N in plants is limited. In one-year-old rhododendron, N:P ratio was greater than 14:1 without N limitation and N:P ratio was less than 9:1 when plants were N-deficient (Scagel et al., 2008b).

The threshold N:P ratio may vary among different species. In plants grown on semi-arid sandy grassland, N:P ratio was 5.6 in control and 7.5 under N fertilization treatment. Li et al. stated that, in the context of semi-arid sandy grassland, the threshold of N:P ratio (14 to 16) was not applicable as a test for nutrient limitations (Li et al., 2001). In the same way, the common N:P ratio threshold 16:1 is not a precise test for nutrient limitations in our study; however, the N:P ratio in our study was low, which still suggests N was limiting.

Nutrient content in Dec. 2013

Greater N rates increased total content of many nutrients, except for K, Ca and Fe (Table 6.7). Higher N rates improved dry weight of plants which could lead to increased content of many nutrients. Phosphorus rates had no effect on any nutrient content.

Nutrients uptake in 2013

Greater N rates increased N, P, Mg, Mn, Zn, Cu and B uptake (Table 6.6). Uptake of K, Ca and Fe was not affected by either N or P rate. With higher N rates, plants accumulated greater dry weight (Table 6.2) which could increase demands for other nutrients. There is a spread wide belief that growth rate is one of the primary factors affecting nutrient uptake (Marschner, 1995). Phosphorus uptake is strongly influenced by N supply which affect plant growth (Herbert, 1990). In research with azalea, P uptake was influenced by both P fertilization rate and plant growth which was affected by N rate (Ristvey et al., 2007). In wheat (*Triticum aestivum*), differences in growth rate is the only reason for differences in nitrate uptake rate among cultivars (Rodgers and Barneix, 1988).

In this study, greater N rates increased both dry weight and content of many mineral nutrients. This indicates N altered the uptake ability and demands of the plant for P, Mg, Mn, Zn, Cu and B mineral nutrients. So, to optimize growth, increased N rates should accompany modified doses of other nutrients in a fertilizer formulation (Scagel et al., 2008a).

Phosphorus rates had no influence on net uptake of other nutrients. Interestingly, P rate did not even influence P uptake, although P concentration was improved by increasing P rate in leaves and roots. One explanation could be the amount of P uptake was more related to DW which was affected by N rate. In addition, the N:P ratio

indicated plants in our study were under N limitation. That also suggests plants in this study had a relatively high concentration of P and even 5 mM P rate satisfied plant demand. In previous research, amount of P in μM units was sufficient for maximum growth of some species (First and Edwards, 1987; Hansen and Lynch, 1998; Lynch et al. 1991). Thus, in this study, plants receiving 5 mM P fertilization may have sufficient P to support optimal growth and other plant activities.

Nutrient allocation in Dec. 2013

In general, the greatest proportions of most nutrients were allocated to rhizomes from 60% to 89% depending on nutrient. This pattern of allocation of most nutrients is consistent with the DW allocation to different tissues.

In leaves, allocation of Zn and Cu was affected by N rate and allocation of N and B was affected by P rate (Tables 6.8). The proportion of Cu in leaves increased with N rate which might be caused by increasing Cu concentration in leaves. The greatest proportion of Zn in leaves occurred with the 10 mM N rate. 10 mM P rate resulted in the greatest proportion of N allocated to leaves. The proportion of B was greatest in plants receiving 10 mM P rate. The allocation of other nutrients was similar with different N and P treatments.

In roots, allocation of most nutrients was not affected by N or P rates, except for Cu which increased with increasing N rate (Table 6.9). Copper concentration increased with N rate, but DW did not. The greater proportion of Cu allocated to roots was more related with increasing Cu concentration. In rhizomes, allocation of Cu decreased with N rate and Zn was lowest with N 10 mM rate (Table 6.10). Allocation of B was lowest in plants receiving 10 mM P (Table 6.10).

Conclusion

In summary, higher N rates improved DW, plant height, leaf SPAD readings and uptake of other nutrients in TB iris. Changing P rates had no effect on DW, plant height, or nutrient uptake. Phosphorus rate only had influences on concentration of a few nutrients. Considering N:P ratios in plant tissues in this experiment were low, this indicates 5 mM P rate was sufficient for growth and development, while 15 mM N rate may not have been sufficient to support optimal growth.

Table 6.1 Chemicals used (in mM) to prepare fertilizer solutions with various N:P ratios.

Chemicals composition (mM)	N:P ratios in fertilizer								
	5:5	5:10	5:15	10:5	10:10	10:15	15:5	15:10	15:15
NH_4NO_3	2.5	2.5	2.5	5	5	5	7.5	7.5	7.5
KH_2PO_4	5	10	15	5	10	15	5	10	15
KCl	10	5	0	10	5	0	10	5	0

Table 6.2 Plant height, SPAD readings, number of fans and floral meristems, dry weight in container-grown 'Immortality' TB iris.

Treatments (mM)	Plant height (cm)		SPAD reading		Dry weight (g) in Dec. 2013				Fans/plant (no.) in Dec. 2013	Floral meristems/plant (no.) in Dec. 2013
	June 2013	March 2014	June 2013	March 2014	Leaves	Roots	Rhizomes	Total plant		
5N:5P	56	21	45.2	73.0bc	3.9	9.1	48.7	61.7	16	3.0
5N:10P	49	25	50.0	74.6abc	6.4	8.3	43.3	58.0	14	2.5
5N:15P	53	26	46.4	73.9abc	3.8	11.3	45.6	60.7	19	2.8
10N:5P	55	26	50.4	73.1bc	5.6	14.4	55.2	75.2	22	3.3
10N:10P	57	28	51.0	71.8c	5.3	13.8	35.7	54.8	18	5.3
10N:15P	57	25	54.9	77.4a	6.8	14.1	49.0	69.9	21	4.3
15N:5P	61	27	53.2	77.2a	6.3	12.0	56.4	74.7	24	5.3
15N:10P	59	28	52.8	75.6ab	6.9	11.8	60.5	79.2	20	4.3
15N:15P	61	26	52.8	73.0bc	6.2	14.6	62.1	82.8	25	4.0
Main effects of N rates (mM)										
5	53c[z]	24b	47.2b				46.2b	60.2b	16b	2.8b
10	57b	27a	52.1a				47.3b	67.3b	20a	4.3a
15	60a	27a	52.9a				60.3a	79.2a	23a	4.5a

Plants were treated with N (0, 10, or 15 mM) and phosphorus (0, 10, or 15 mM) combinations from Mar. to Sept. 2013. Rhizomes were planted in Aug. 2012, data were collected during growing season 2013 and Mar. 2014, and after harvest in Dec. 2013.
[z]Means within a column followed by different lower case letters denotes significant differences (Tukey's honestly significant difference, $P \leq 0.05$).

Table 6.3 Mineral nutrient concentration in leaves in container-grown 'Immortality' TB iris.

Treatments (mM)	N[z] (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	B(ppm)	N:P
5N:5P	1.85	0.34[y]	5.05	0.94	0.58	40.5	12.5	29.3ab	3.5	36.8	5.8
5N:10P	1.78	0.24	4.41	1.04	0.56	36.5	8.8	20.5c	2.3	47.5	7.5
5N:15P	2.23	0.41	3.72	1.18	0.66	39.5	12.8	30.3a	6.0	33.3	5.6
10N:5P	2.11	0.44	4.47	1.25	0.68	37.5	11.3	30.0a	5.5	31.3	4.8
10N:10P	2.38	0.41	4.52	1.06	0.58	47.8	13.3	33.3a	5.3	45.8	5.9
10N:15P	2.25	0.45	4.65	0.98	0.59	47.0	14.8	32.8a	5.0	34.8	5.1
15N:5P	2.42	0.36	4.28	0.85	0.50	49.7	15.5	32.5a	7.5	39.3	7.2
15N:10P	2.26	0.35	4.13	1.08	0.55	39.8	15.5	26.8abc	7.5	38.0	6.5
15N:15P	2.08	0.45	3.88	1.02	0.50	48.3	12.5	21.8bc	9.0	19.8	4.7
Main effects of N rate (mM)											
5	1.95b[y]	0.33b			0.60a				3.9b		
10	2.24a	0.43a			0.62a				5.3b		
15	2.25a	0.39ab			0.52b				8.0a		
Main effects of P rate (mM)											
5		0.38ab								36.8ab	6.0ab
10		0.34b								44.8a	6.6a
15		0.43a								29.3b	5.1b

Plants were treated with N (0, 10, or 15 mM) and P (0, 10, or 15 mM) combinations from Mar. to Sept. 2013. Rhizomes were planted in Aug. 2012 and plants were harvested in Dec. 2013.

[z]Nitrogen (N); Phosphorous (P); Potassium (K); Calcium (Ca); Magnesium (Mg); Iron (Fe); Manganese (Mn); Zinc (Zn); Copper (Cu); Boron (B); N:P concentration (N:P).

[y]Means within a column followed by different lower case letters denotes significant differences (Tukey's honestly significant difference, $P \leq 0.05$).

Table 6.4 Mineral nutrient concentration in roots in container-grown 'Immortality' TB iris

Treatments (mM)	N[z] (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	B(ppm)	N:P
5N:5P	0.75	0.21	4.09	0.29	0.19b	49.3	15.5	18.8ab	3.0d	15.0	
5N:10P	0.76	0.24	3.73	0.31	0.20b	62.3	15.5	14.0bc	2.8d	14.0	
5N:15P	0.73	0.30	3.41	0.32	0.20ab	58.0	14.3	14.5bc	4.3cd	13.3	
10N:5P	0.75	0.19	3.25	0.29	0.18bc	63.3	14.8	13.5c	4.0cd	14.0	
10N:10P	0.79	0.32	3.79	0.34	0.22a	70.5	16.3	19.5a	7.3ab	15.3	
10N:15P	0.77	0.31	2.61	0.32	0.20ab	65.3	16.3	19.8a	6.3bc	13.3	
15N:5P	0.79	0.20	3.54	0.31	0.19b	65.7	21.3	20.5a	9.8a	14.3	
15N:10P	0.92	0.27	3.23	0.32	0.18bc	72.0	18.5	18.3abc	8.5ab	13.3	
15N:15P	0.82	0.29	3.12	0.28	0.16c	48.0	13.0	20.3a	6.0bc	16.5	
Main effects of N rate (mM)											
5			3.7a[y]								
10			3.3b								
15			3.2b								
Main effects of P rate (mM)											
5		0.20b	3.63a								3.9a
10		0.27a	3.58a								3.1b
15		0.30a	3.05b								2.6c

Plants were treated with N (0, 10, or 15 mM) and P (0, 10, or 15 mM) combinations from Mar. to Sept. 2013. Rhizomes were planted in Aug. 2012 and plants were harvested in Dec. 2013.

[z]Nitrogen (N); Phosphorous (P); Potassium (K); Calcium (Ca); Magnesium (Mg); Iron (Fe); Manganese (Mn); Zinc (Zn); Copper (Cu); Boron (B);N:P concentration (N:P).

[y]Means within a column followed by different lower case letters denotes significant differences (Tukey's honestly significant difference, $P \leq 0.05$).

Table 6.5 Mineral nutrient concentration in rhizomes in container-grown 'Immortality' TB iris.

Treatments (mM)	N[z] (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)	B(ppm)	N:P
5N:5P	1.59	0.20	1.71	0.46	52.00	52.0	9.0	21.0	7.0bc	10.0	
5N:10P	1.52	0.20	1.78	0.46	62.59	62.6	9.8	18.8	5.5c	9.8	
5N:15P	1.61	0.24	1.53	0.47	44.25	44.3	7.3	17.8	6.0bc	9.5	
10N:5P	2.15	0.32	1.72	0.48	51.25	51.3	7.8	16.0	5.0c	9.8	
10N:10P	2.20	0.37	1.65	0.57	68.00	68.0	9.0	23.8	11.3a	11.5	
10N:15P	2.18	0.36	1.93	0.47	64.00	64.0	13.0	22.5	8.0abc	11.5	
15N:5P	2.51	0.33	1.54	0.45	55.00	55.0	12.0	21.8	10.0ab	10.5	
15N:10P	2.30	0.32	1.48	0.39	41.75	41.8	8.0	17.8	8.0abc	9.5	
15N:15P	2.46	0.36	1.42	0.39	39.00	39.0	11.3	18.5	4.3c	15.0	
Main effects of N rate (mM)											
5	1.57b[y]	0.21b			0.22b	54.0ab					
10	2.18a	0.35a			0.28a	61.1a					
15	2.42a	0.34a			0.25a	45.3b					

Plants were treated with N (0, 10, or 15 mM) and P (0, 10, or 15 mM) combinations from Mar. to Sept. 2013. Rhizomes were planted in Aug. 2012 and plants were harvested in Dec. 2013.

[z]Nitrogen (N); Phosphorous (P); Potassium (K); Calcium (Ca); Magnesium (Mg); Iron (Fe); Manganese (Mn); Zinc (Zn); Copper (Cu); Boron (B);N:P concentration (N:P). [y]Means within a column followed by different lower case letters denotes significant differences (Tukey's honestly significant difference, $P \leq 0.05$).

Table 6.6 Net nutrient uptake in container-grown 'Immortality' TB iris.

Treatments (mM)	N[z] (g)	P (g)	K (g)	Ca (g)	Mg (g)	Fe (mg)	Mn (mg)	Zn (mg)	Cu (mg)	B (mg)
5N:5P	0.49	0.06	0.35	0.05	0.03	1.60	0.41	0.90	0.27cd	0.47
5N:10P	0.44	0.05	0.29	0.05	0.03	1.99	0.39	0.68	0.19d	0.54
5N:15P	0.49	0.09	0.18	0.05	0.03	1.33	0.33	0.69	0.24cd	0.41
10N:5P	1.01	0.16	0.63	0.13	0.10	2.47	0.49	0.86	0.26cd	0.62
10N:10P	0.60	0.12	0.30	0.06	0.05	2.10	0.40	0.84	0.39bc	0.56
10N:15P	0.93	0.17	0.58	0.09	0.08	3.04	0.78	1.15	0.41bc	0.68
15N:5P	1.25	0.16	0.49	0.10	0.09	2.70	0.84	1.30	0.63a	0.71
15N:10P	1.20	0.18	0.54	0.10	0.09	2.09	0.57	1.06	0.51ab	0.68
15N:15P	1.38	0.22	0.53	0.10	0.09	1.94	0.72	1.14	0.30cd	0.91
Main effects of N rate (mM)										
5	0.47c[y]	0.06b			0.03b		0.03b	0.75b		0.47c
10	0.84b	0.15a			0.07a		0.07a	0.95b		0.62b
15	1.28a	0.18a			0.09a		0.09a	1.17a		0.77a

Plants were treated with N (0, 10, or 15 mM) and P (0, 10, or 15 mM) combinations from Mar. to Sept. 2013. Rhizomes were planted in Aug. 2012 and plants were harvested in Dec. 2013.

[y]Means within a column followed by different lower case letters denotes significant differences (Tukey's honestly significant difference, $P \leq 0.05$).

[z]Nitrogen (N); Phosphorous (P); Potassium (K); Calcium (Ca); Magnesium (Mg); Iron (Fe); Manganese (Mn); Zinc (Zn); Copper (Cu); Boron (B).

Table 6.7 Nutrient content of container-grown 'Immortality' TB iris.

Treatments (mM)	N[z] (g)	P (g)	K (g)	Ca (g)	Mg (g)	Fe (mg)	Mn (mg)	Zn (mg)	Cu (mg)	B (mg)
5N:5P	0.89	0.13	1.40	0.29	0.14	3.08	0.62	1.27	0.37	0.77
5N:10P	0.84	0.12	1.34	0.29	0.15	3.47	0.60	1.05	0.28	0.84
5N:15P	0.88	0.16	1.22	0.30	0.15	2.81	0.54	1.06	0.33	0.71
10N:5P	1.40	0.23	1.67	0.38	0.21	3.95	0.70	1.23	0.36	0.92
10N:10P	0.99	0.19	1.35	0.30	0.17	3.58	0.61	1.22	0.49	0.86
10N:15P	1.32	0.25	1.62	0.33	0.20	4.52	0.99	1.53	0.50	0.98
15N:5P	1.65	0.23	1.54	0.34	0.20	4.18	1.05	1.67	0.73	1.01
15N:10P	1.60	0.25	1.59	0.34	0.21	3.57	0.78	1.43	0.60	0.98
15N:15P	1.78	0.29	1.58	0.34	0.21	3.42	0.93	1.51	0.40	1.21
Main effects of N rate (mM)										
5	0.87c[y]	0.14b			0.15b		0.56b	1.13b	0.33b	0.77c
10	1.24b	0.22a			0.19a		0.77ab	1.32ab	0.45ab	0.92b
15	1.67a	0.26a			0.21a		0.92a	1.54a	0.57a	1.07a

Plants were treated with N (0, 10, or 15 mM) and P (0, 10, or 15 mM) combinations from Mar. to Sept. 2013. Rhizomes were planted in Aug. 2012 and plants were harvested in Dec. 2013.

[z]Nitrogen (N); Phosphorous (P); Potassium (K); Calcium (Ca); Magnesium (Mg); Iron (Fe); Manganese (Mn); Zinc (Zn); Copper (Cu); Boron (B).

[y]Means within a column followed by different lower case letters denotes significant differences (Tukey's honestly significant difference, $P \leq 0.05$).

Table 6.8 Nutrient allocation to leaves of container-grown 'Immortality' TB iris.

Treatments (mM)	N[z] (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (%)	Mn (%)	Zn (%)	Cu (%)	B (%)
5N:5P	8.23	10.16	14.44	14.14	14.14	5.14	7.49	8.76	3.42	19.68
5N:10P	13.74	12.59	20.48	22.71	22.71	7.07	9.85	12.31	5.81	35.70
5N:15P	9.05	9.57	11.31	15.50	15.50	5.23	8.80	10.20	6.76	17.92
10N:5P	8.56	10.98	15.49	19.26	19.26	5.60	8.93	13.32	8.67	19.38
10N:10P	13.18	11.34	19.39	18.07	18.07	7.26	12.09	14.44	5.66	28.86
10N:15P	11.99	12.81	21.60	20.68	20.68	8.05	11.26	14.48	6.46	25.08
15N:5P	9.11	9.40	16.89	15.52	15.52	7.67	9.56	12.22	7.28	23.99
15N:10P	9.59	9.68	18.91	20.77	20.77	7.62	12.67	12.47	8.41	26.39
15N:15P	7.66	10.02	16.43	18.25	18.25	10.24	8.94	8.46	14.36	10.42
Main effects of N rate (mM)										
5								10.43b	5.33b	
10								14.08a	6.93ab	
15								11.05b	10.02a	
Main effects of P rate (mM)										
5	8.63b[y]									21.01b
10	12.17a									30.32a
15	9.57ab									18.81b

Plants were treated with N (0, 10, or 15 mM) and P (0, 10, or 15 mM) combinations from Mar. to Sept. 2013. Rhizomes were planted in Aug. 2012 and plants were harvested in Dec. 2013.

[y]Means within a column followed by different lower case letters denotes significant differences (Tukey's honestly significant difference, $P \leq 0.05$).

[z]Nitrogen (N); Phosphorous (P); Potassium (K); Calcium (Ca); Magnesium (Mg); Iron (Fe); Manganese (Mn); Zinc (Zn); Copper (Cu); Boron (B).

Table 6.9 Nutrient allocation to roots of container-grown 'Immortality' TB iris.

Treatments (mM)	N[z] (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (%)	Mn (%)	Zn (%)	Cu (%)	B (%)
5N:5P	7.54	15.09	26.47	9.34	9.34	14.63	22.96	13.67	6.95	17.70
5N:10P	7.66	16.41	23.02	8.92	8.92	15.29	22.13	11.18	8.55	14.02
5N:15P	9.34	22.29	31.96	12.42	12.42	23.51	30.37	15.43	14.75	21.26
10N:5P	7.41	11.34	27.54	11.01	11.01	23.14	30.01	15.74	16.18	21.69
10N:10P	10.51	21.26	34.44	15.95	15.95	25.60	32.88	19.28	17.23	22.15
10N:15P	7.50	16.65	20.80	14.55	14.55	20.18	23.56	17.19	17.12	17.71
15N:5P	5.64	10.65	27.10	10.72	10.72	18.43	25.11	15.00	16.62	16.85
15N:10P	6.33	12.61	23.69	10.72	10.72	22.71	26.66	14.40	15.82	15.51
15N:15P	6.51	14.26	28.12	11.70	11.70	20.48	21.64	18.77	20.79	19.97

Main effects of N rates (mM)

5	10.08b[y]
10	17.84a
15	18.74a

Plants were treated with N (0, 10, or 15 mM) and P (0, 10, or 15 mM) combinations from Mar. to Sept. 2013. Rhizomes were planted in Aug. 2012 and plants were harvested in Dec. 2013.

[z]Nitrogen (N); Phosphorous (P); Potassium (K); Calcium (Ca); Magnesium (Mg); Iron (Fe); Manganese (Mn); Zinc (Zn); Copper (Cu); Boron (B).

[y]Means within a column followed by different lower case letters denotes significant differences (Tukey's honestly significant difference, $P \leq 0.05$).

Table 6.10 Nutrient allocation to rhizomes of container-grown 'Immortality' TB iris.

Treatments (mM)	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (%)	Mn (%)	Zn (%)	Cu (%)	B (%)
5N:5P	84.23	74.75	59.09	76.52	76.52	80.24	69.56	77.57a	89.63a	62.63ab
5N:10P	78.61	71.00	56.51	68.38	68.38	77.64	68.02	76.50a	85.64a	50.28b
5N:15P	81.61	68.15	56.74	72.09	72.09	71.27	60.83	74.37a	78.49a	60.82a
10N:5P	84.04	77.68	56.97	69.73	69.73	71.26	61.06	70.94b	75.15b	58.93ab
10N:10P	76.32	67.41	46.18	65.98	65.98	67.14	55.03	66.28b	77.11b	48.98b
10N:15P	80.51	70.55	57.60	64.78	64.78	71.77	65.19	68.34b	76.42b	57.21a
15N:5P	85.25	79.95	56.02	73.76	73.76	73.90	65.33	72.78ab	76.11b	59.17ab
15N:10P	84.09	77.71	57.41	68.52	68.52	69.67	60.68	73.13ab	75.77b	58.11b
15N:15P	85.83	75.73	55.46	70.04	70.04	69.29	69.43	72.76ab	64.85b	69.61a
Main effects of N rates (mM)										
5								76.15a	85.59a	
10								69.52b	76.23b	
15								73.89ab	72.24b	
Main effects of P rates (mM)										
5										60.24ab
10										52.46b
15										63.54a

Plants were treated with N (0, 10, or 15 mM) and P (0, 10, or 15 mM) combinations from Mar. to Sept. 2013. Rhizomes were planted in Aug. 2012 and plants were harvested in Dec. 2013.

[z]Nitrogen (N); Phosphorous (P); Potassium (K); Calcium (Ca); Magnesium (Mg); Iron (Fe); Manganese (Mn); Zinc (Zn); Copper (Cu); Boron (B).

[y]Means within a column followed by different lower case letters denotes significant differences (Tukey's honestly significant difference, $P \leq 0.05$).

References

Balasubramanian, V., A.C. Morales, R.T. Cruz, and S. Abdulrachman. 1998. On farm adaptation of knowledge-intensive nitrogen management technologies for rice systems. Nutr. Cycl. Agroecosyst. 53:59-69.

Banaj, D., V. Kovacevic, D. Simic, M. Seput, and B. Stojic. 2006. Phosphorus impacts on yield and nutritional status of maize. Cereal Res. Commun. 34:393-396.

Bi, G., C.F. Scagel, L.H. Fuchigami, and R.P. Regan. 2007. Rate of nitrogen application during the growing season alters the response of container-grown rhododendron and azalea to foliar application of urea in the autumn. J. Hort. Sci. Biotechnol. 82:753-763.

Cordell, D., J. Drangert, and S. White. 2009. The story of phosphorus: Global food security and food for thought. Global Environ. Chang. 19:292-305.

Cornelissen, J.H.C., M.J.A. Werger, P. Castro-Díez, J.W.A. van Rheenen, and A.P. Rowland. 1997. Foliar nutrients in relation to growth, allocation and leaf traits in seedlings of a wide range of woody plant species and types. Oecologia 111:460-469.

Craver, J.K. and R.L. Harkess. 2012. Determining rhizome maturity in reblooming iris. HortScience 47(9):S14.

Dawson, C.J. and J. Hilton. 2011. Fertilizer availability in a resource-limited world: Production and recycling of nitrogen and phosphorus. Food Policy 36:14-22.

de Groot, C.C., L.F.M. Marcelis, R. van den Boogaard, W.M. Kaiser, and H. Lambers. 2003. Interaction of nitrogen and phosphorus nutrition in determining growth. Plant Soil 248:257-268.

Dighton, J., H.E. Jones, and J.M. Poskitt. 1993. The use of nutrient bioassays to assess the response of *Eucalyptus grandis* to fertilizer application. 1: Interaction between nitrogen, phosphorus and potassium in seedling nutrition. Can. J. Forest Res. 23:1-6.

Dong, S., L. Cheng, C.F. Scagel, and L.H. Fuchigami. 2004. Nitrogen mobilization, nitrogen uptake and growth of cuttings obtained from poplar stock plants grown in different N regimes and sprayed with urea in autumn. Tree Physiol. 24:355-359.

Fenn, M.E., M.A. Poth, J.D. Aber, J.S. Baron, B.T. Bormann, D.W. Johnson, A.D. Lemly, S.G. McNulty, D.F. Ryan, and R. Stottlemyer.1998. Nitrogen excess in North American ecosystems: Predisposing factors, ecosystem responses, and management strategies. Ecol. Appl. 8:706-733.

First, A.J. and D.G. Edwards. 1987. External phosphorus requirements of five tropical grain legumes grown in flowing-solution culture. Plant Soil 99:75-84.

Fitter, A.H., W.J. Wright, L. Williamson, M. Belshaw, J. Fairclough, and A.A. Meharg. 1999. The phosphorus nutrition of wild plants and the paradox of arsenate tolerance: Does leaf phosphate concentration control flowering? In: Lynch JP, Deikman J, eds. Phosphorus in plant biology: regulatory roles in molecular, cellular, organismic and ecosystem processes. Rockville. USA. Amer. Soc. Plant Biol. 39-51.

Graciano, C., J.F. Goya, J.L. Frangi, and J.J. Guiamet. 2006. Fertilization with phosphorus increases soil nitrogen absorption in young plants of *Eucalyptus grandis*. For. Ecol. Mgt. 236:202-210.

Hanley, N., R.L. Harkess, and M. Gu. 2008. Plant growth regulator and fertilizer effects on growth and flowering of re-blooming iris. HortScience 43:1176.

Hansen, C.W. and J. Lynch. 1998. Response to phosphorus availability during vegetative and reproductive growth of Chrysanthemum: II. biomass and phosphorus dyamics. J. Amer. Soc. Hort. Sci. 123:223-229.

Hasanuzzaman, M., M.H. Ali, M.F.Karim, S.M. Masum, and J.A.Mahmud. 2012. Response of hybrid rice to different levels of nitrogen and phosphorus. Intl. Res. J. Appl. Basic Sci. 3:2522-2528.

Herbert, M.A. 1990. Fertilizer/site interactions on the growth and foliar nutrient levels of *Eucalyptus grandis*. For. Ecol. Mgt. 30:247-257.

Ingestad, T. 1991. The influence of plant nutrition on biomass allocation. Ecol. Appl. 1:168-174.

Inoue, K., H. Yokota, and Y. Yamada. 1988. Effect of Ca in the medium on root growth under low pH conditions. Soil Sci. Plant Nutr. 34:359-374.

Islam, M.S., M.S.U. Bhuiya, S. Rahman, and M.M. Hussain. 2009. Evaluation of SPAD and LCC based nitrogen management in rice (*Oryza sativa* L.). Bangladesh J. Agr. Res. 34:661-672.

Iversen, C.M., S.D. Bridgham, and L.E. Kellogg. 2010. Scaling plant nitrogen use and uptake efficiencies in response to nutrient addition in peatlands. Ecology 91:693-707.

Jeschke, W.D., A.D. Peuke, E.A. Kirkby, J.S. Pate, and W. Hartung. 1996. Effects of P deficiency on the uptake, flows and utilization of C, N and H_2O within intact plants of *Ricinus communis* L. J. Exp.Bot. 47:173-1754.

Koerselman, W. and A.F.M. Meuleman. 1996. The vegetation N:P ratio: A new tool to detect the as an indicator of nutrient limitation and nitrogen saturation. J. Appl. Ecol. 40:52-534.

Li, L., D. Zeng, Z. Yu, Z. Fan, R. Mao, and P.L. Peri. 2001. Foliar N/P ratio and nutrient limitation to vegetation growth on Keerqin sandy grassland of North-east China. Grass Forage Sci. 66:237-242.

Lynch, J., A. Läuchli, and E. Epstein. 1991. Vegetative growth of the common bean in response to phosphorus nutrition. Crop Sci. 31:380-387.

Marschner, H. 1995. Mineral nutrition of higher plants. 2nd Ed. Academic Press, San Diego, CA.

Marschner, P. 2012. Mineral nutrition of higher plants. 3rd Ed. Academic Press, San Diego, CA.

McKenzie, F.R. and J.L. Jacobs. 2002. Effects of application of nitrogen fertilizer on concentrations of P, K, S, Ca, Mg, Na, Cl, Mn, Fe, Cu and Zn in perennial ryegrass/white clover pastures in south-western Victoria, Australia. Grass Forage Sci. 57:48-53.

Ristvey, A.G., J.D. Lea-Cox, and D.S. Ross. 2007. Nitrogen and phosphorus uptake efficiency and partitioning of container-grown azalea during spring growth. J. Amer. Soc. Hort. Sci. 132:563-571.

Rodgers, C.O. and A.J. Barneix. 1988. Cultivar differences in the rate of nitrate uptake by intact wheat plants as related to growth rate. Physiol. Plant. 72:121-126.

Ruamrungsri, S., W. Bundithya, N. Potapohn, N. Ohtake, K. Sueyoshi, and T. Ohyama. 2011. Effect of NPK levels on growth and bulb quality of some geophytes in substrate culture. Acta Hort. 886:213-218.

Ruamrungsri, S. and P. Apavatjrut. 2003. Effect of nutrient deficiency on the growth and development of *Curcuma alismatifolia* Gagnep. Proc. of 3rd Symposium on the Family Zingiberaceae. Khon Kaen, Thailand. 7-12 July, 2002.

Scagel, C.F., G. Bi, L.H. Fuchigami, and R.P. Regan. 2008a. Nitrogen availability alters mineral nutrient uptake and demand in container-grown deciduous and evergreen *Rhododendron*. J. Environ. Hort. 26:177-187.

Scagel, C.F., G. Bi, L.H. Fuchigami, and R.P. Regan. 2008b. Rate of nitrogen application during the growing season and spraying plants with urea in the autumn alters uptake of other nutrients by deciduous and evergreen container-grown *Rhododendron* cultivars. HortScience 43:1569-1579.

Scagel, C.F., G. Bi and L.H. Fuchigami, and R.P. Regan. 2011. Nutrient uptake and loss by container grown deciduous and evergreen rhododendron nursery plants. HortScience 46:296-305.

Schuman, G.E., M.A.Stanley, and D. Knudsen. 1973. Automated total nitrogen analysis of soil and plant samples. Proc. Soil Sci. Soc. Amer. 37:480-481.

Tessier, J.T. and D.Y. Raynal. 2003. Use of nitrogen and phosphorus ratios in plant tissue nature of nutrient limitation. J. Appl. Ecol. 33:1441-1450.

Vitousek, P.M., S. Hattenschwiler, L. Olander, and S. Allison. 2002. Nitrogen and nature. Ambio 31:97-101.

Wang, Y.T. 1996. Effect of six fertilizers on vegetative growth and flowering of phalaenopsis orchids. Sci. Hort. 65:191-197.

Wang, Y.T. 2000. Impact of a high phosphorus fertilizer and timing of termination of fertilization of on flowering of a hybrid moth orchid. HortScience 35:60-62.

Wang, Y.T. and E.A. Konow. 2002. Fertilizer source and medium composition affect vegetative growth and mineral nutrition of a hybrid moth orchid. J. Amer. Soc. Hort. Sci. 127:442-447.

Williams, M.W., J.S. Baron, N. Caine, R. Sommerfeld, and R. Jr Sanford. 1996. Nitrogen saturation in the Rocky Mountains. Environ. Sci. Technol. 30:640-646.
Zhang, Y.J., L. Kuhns, J.P. Lynch, and K.M. Brown. 2002. Buffered phosphorus

fertilizer improves growth and drought tolerance of woody landscape plants. J. Environ.

Hort. 20:214-219.

CHAPTER VII

SEASONAL CHANGES OF NITROGEN AND CARBOHYDRATE CONSTITUENTS IN *IRIS GERMANICA* 'IMMORTALITY'

Abstract

Storage organs of geophytes allow plants to survive adverse environmental conditions. In Expt. 1, the seasonal changes in composition of nitrogenous compounds and carbohydrates were investigated in tall bearded (TB) iris 'Immortality' (*Iris germanica*). In Expt. 2, the main objective was to investigate the effects of late fall nitrogen (N) supply on changes in nitrogenous compounds and carbohydrates in TB iris 'Immortality'. The results showed N concentration and content in rhizomes continually declined from December to April in both Expt. 1 and 2, indicating rhizomes likely function as the main storage tissue for N. Concentration of starch, sucrose, glucose, and fructose showed seasonal changes in all tissues except for concentration of starch in leaf and concentration of glucose in roots. Starch was the major form of storage carbohydrate in December. Glutamate, alanine, aspartate, serine, and tyrosine were the main free amino acids in all tissues. Concentration of total free amino acids did not fluctuate with seasonal changes. Nitrogen applied in late fall influenced N concentration in all overwintering tissues. Nitrogen application influenced carbohydrates concentration, but there was no clear increasing or decreasing trend.

Introduction

Many geophyte species use belowground structures for storage of nutrients. Storage organs of geophytes allow plants to overcome periods when weather conditions are unfavorable or the external mineral nutrient supply is less than the demand of the plants. Usually, those storage compounds increase in the fall and decrease at the beginning of spring to support spring shoot growth. However, limited information is available about seasonal changes of various metabolites (such as sugars and amino acids) in TB iris.

Storage carbon (C) can be used for maintenance of respiration and assimilation of mineral nutrients. Storage nitrogen (N) is important for increasing residence time of N in plants and allowing plant growth when external sources are limiting. For example, N remobilized from rhizomes provides about 60% of annual aboveground N requirement in American bistort (*Bistorta bistortoides*) (Monson et al., 2006). Thus, the nitrogenous storage compounds and carbohydrates are important for the energy requirements for plant growth. In addition, there exists an interdependence of carbon and nitrogen metabolisms (Foyer et al., 2001).

Within geophytic plants, underground structures, such as roots, bulbs, or rhizomes, are the main storage sites for stored C and N (Gloser, 2002; Khuankaew et al., 2010; Ohyama et al., 1988). Nitrogen compounds can be withdrawn from leaves before leaf senescence in the fall and stored in storage organs during the winter. In TB iris, both rhizomes and roots can survive the winter, but little study demonstrates which organ functions as the storage structure.

Free amino acids, amides and proteins are three major types of N compounds stored in plants (Millard, 1988), while N compounds vary depending on plant species. In hydrilla (*Hydrilla verticillata*) turions, free amino acids constitute a large proportion of total N during overwintering (Ryan, 1994). In bushgrass (*Calamagrostis epigejos*), both free amino acids and soluble protein were the main storage compounds supporting shoot re-growth (Gloser et al., 2007).

The most common storage carbohydrates in plants include starch, fructans, and sucrose (Chapin et al., 1990). According to Miller (1992), starch is the major storage carbohydrate in most plants and is nearly ubiquitous throughout the plant kingdom. Starch was the dominant storage carbohydrate in hyacinth (*Hyacinthus*) (Addai and Scott, 2011). In snowdrop (*Galanthus nivalis*), fructans were the major polysaccharides in the shoot and starch content was much lower (Orthen and Wehrmeyer, 2004).

Dynamics of nitrogen compounds are always related with N supply. N supplied later in the season can increase the amount of storage N which is important for regrowth the next spring (Bi et al., 2004; Cheng et al., 2001; Invers et al., 2004; Ohyama, 1991; Quartieri et al., 2002). In tulip (*Tulipa* L.), the major portion of N in free amino acids in winter was derived from fertilizer N. In addition, in plants receiving N treatments glutamine was a major form of N during the winter, while in cases without N supply, 4-methyleneglutamine was a predominant form of amino acid (Ohyama, 1991).

Nitrogen application might interfere with C metabolism and decrease plant survival rate during overwintering (Invers et al., 2004). Nitrogen applications decreased carbon reserves due to N assimilation requiring energy and C skeletons. In Siam tulip (*Curcuma alismatifolia*), free amino acid concentration was increased by higher levels of

N, but starch concentration was higher in with no N treatments (Ohtake et al., 2006). Similar results were also observed in rhizomatous calamus (*Acorus calamus*) (Vojtišková et al., 2006).

The responses of total non-structural carbohydrate (TNC) to availability of N supply vary among different species. For example, in lesser bulrush (*Typha angustifolia*) TNC was increased by eutrophic treatment of rhizomes (Steinbachová-Vojtišková et al., 2006), while in neptune grass (*Posidonia oceanica*) TNC in rhizomes was decreased by N additions (Invers et al., 2004). However, limited information is available about the effects of N supply on changes in various metabolites (such as carbohydrates and amino acids) in TB iris.

The objectives of this study were to: (1) investigate which tissues act as repositories for stored N and C; (2) determine seasonal dynamics in plant biomass (dry weight), concentration and distribution of carbohydrates and nitrogenous compounds in December, February. and April; and (3) investigate the impact of fall N fertilization on carbon and nitrogen constituent concentrations and tissue distribution during overwintering.

Materials and Methods

Expt. 1.

This study was conducted under natural conditions in Starkville, MS (latitude 33°46' N, longitude 88°82' W). On July 18, 2013, 'Immortality' TB iris rhizomes were potted one rhizome per pot into 3.78-L (23 cm diameter; 16 cm height) round plastic pots filled with commercial substrate with no starter fertilizer (Fafard growing mix 2; Sun Gro Horticulture, Agawam, MA). Two weeks later, from Aug. 4, 2013, plants were fertigated

twice per week with 400 ml of modified Hoagland's solution containing 10 mM N from NH_4NO_3 for 4 weeks to provide a basic nutrient supply for fall growth. Four plants were randomly selected and destructively harvested on Dec. 4, 2014, Feb. 4, 2015, and Apr. 4, 2015 for carbohydrate and N content analysis.

Expt. 2.

On 18 Aug. 2014, 'Immortality' TB iris rhizomes were potted one rhizome per pot into 3.78 L (23 cm diameter; 16 cm height) round plastic pots filled with commercial substrate with no starter fertilizer (Fafard growing mix 2; Sun Gro Horticulture, Agawam, MA). Starting two weeks later, 2 Sept. 2014, plants were fertigated once per week with 400 ml of modified Hoagland's solution containing one of three N concentrations (0, 10, or 20 mM N) from $^{15}NH_4{}^{15}NO_3$ for three weeks. The experiment was arranged in a randomized complete design with 20 replications in each treatment. Three plants from each N rate were randomly selected and destructively harvested on Dec. 3, 2014, Feb. 3, 2015 and Apr. 3, 2015.

For both experiment, each plant was divided into leaves, roots and rhizomes. Half of each sample was first frozen in -80 °C, and then lyophilized until constant weight. The other half of each sample was oven dried at 60 °C until constant weight. Dry weights were recorded by tissue type. All samples were ground to pass a 40 mesh sieve in a Wiley Mill (Thomas Scientific, Swedesboro, NJ). Total N was determined using an elemental C/N analyzer (Carlo Erba, Milan, Italy). The composition and concentration of sugars and amino acids were determined using high performance liquid chromatography (1260 Infinity series HPLC, Agilent Technologies, Santa Clara, CA, USA).

Analysis of sugars

Ground tissue samples (0.1 g) were weighed and placed in glass culture tubes (16 x 100mm), then 1 ml of double distilled water was added and shaken horizontally for 15 min at 200 rpm. The extract was centrifuged at 14000 rpm for 10 min and 500 µl supernatant was transferred to 2 ml micro tubes. 0.7 ml of acetonitrile was added, mixed by inversion, and kept at room temperature for 30 min. The suspensions were centrifuged at 14000 rpm for 10 min and 500 µl supernatant was transferred into a new glass tube. Samples were dried at room temperature. Dried samples were dissolved in 500 µl 75% acetonitrile: 25% water solution and filtered through a 0.2 µm syringe into HPLC glass vials. The concentration of glucose, fructose, and sucrose was quantified using high performance liquid chromatography.

Analysis of starch

Ground tissue samples (0.1 g) were placed in 2.0 ml microcentrifuge tubes, extracted with 1.5 mL of 80% methanol and placed in a water bathed at 70 °C for 30 min. The extract was centrifuged at 14000 rpm for 10 min and supernatant was carefully poured off. The extraction procedure was repeated 3 times and the pellet was retained for evaluating starch content. After the residue was digested overnight with 30 units of amyloglucosidase at pH 4.5 to convert starch to glucose, 0.8 ml supernatant was filtered through a 0.2 µm syringe into a HPLC glass vial. The concentration of glucose was quantified using high performance liquid chromatography.

Analysis of free amino acids (FAA)

Ground tissue samples (0.1 g) were placed in 2.0 ml microcentrifuge tubes, extracted with cold 20 mM HCl and shaken for 10 min at room temperature. 60 µl of 250 ppm norleucine was added as an internal quantitative standard. The extract was centrifuged at 14000 rpm for 10 min. The supernatant was collected and filtered through a 0.2 µm syringe into HPLC glass vial. The concentration of amino acids was quantified using high performance liquid chromatography.

Statistical analysis

A one-factor (time) analysis of variance using the GLM procedure of SAS (version 9.3; SAS Institute, Cary, NC) was performed for Expt. 1. For Expt. 2, N rate and time combinations were analyzed as a two-factor study using SAS 9.3.

Results and Discussions

Seasonal changes of temperatures

In both Expt. 1 and 2, temperature decreased from December to February and then increased from February to April (Figs. 7.1A and B). This period covered winter to early spring in Mississippi. In most geophytes, phenological rhythms, such as shoot growth, flowering and dormancy, are mainly controlled by changes in temperature (Halevy, 1990; Le Nard and Hertogh, 1993; Rees, 1992).

Dry weight, N and C allocation, content and concentration in Expt. 1

From December to February, dry weight of leaves decreased due to dieback of leaves (Fig. 7.2) which indicates the plants were in their overwintering state. In early April, production of new leaves increased the contribution of leaves to total dry weight

(33%). Nitrogen and C showed a similar allocation trends to dry weight (Figs. 7.3A and B). Plants regulate distribution of resources by allocation between growth and storage.

Nitrogen concentration and content in rhizomes continually declined from December to April, while in roots N concentration and content were relatively stable. In addition, N concentration and dry weight in roots were much lower than those in rhizomes. These results indicate rhizomes likely function as the main storage tissue for N (Figs. 7.4A and 7.5A).

Carbon concentration in rhizomes which was lowest in February was affected by seasonal change (Table 7.1), suggesting carbon may be depleted at this time or supplied for winter respiration and spring regrowth. In Apr. 2014, C concentration increased in rhizomes and roots, which indicates the photosynthates produced in spring was replenished to those tissues N (Figs. 7.4B and 7.5B).

Concentration of starch, sucrose, glucose, and fructose in Expt. 1

Concentration of starch, sucrose, glucose, and fructose was affected by season changes in all tissues, except for concentration of starch in leaves and concentration of glucose in roots (Fig. 7.6A, B, C and D). In rhizomes, concentration of starch was higher than other carbohydrates, which suggests starch was the predominant carbohydrate. These results are consistent with previous research in which starch was the major storage carbohydrate in plants (Miller, 1992; Orthen, 2001). The concentration of starch declined dramatically from December to February and the decrease was slower from February to April (Figure 7.6A). Sucrose showed a declining during the period from December to February, but the concentration of sucrose was quite low compared to starch.

The depletion of starch in rhizomes suggests starch was used for carbon and energy supply for winter respiration and spring regrowth. In tulip, starch was decomposed, while sucrose and fractosylsucrose increased in winter (Ohyama, 1991). In response to low temperature acclimatization, a decrease in starch has been found in Easter lily (*Lilium longiflorum*) (Miller and Langhans, 1992) and Cornish lily (*Nerine bowdenii*) (Theron and Jacobs, 1996).

In roots, starch concentration first increased in Feb and then decreased in April, while sucrose concentration continuously declined from December to April (Figs. 7.6A and B), which indicates sucrose could also be degraded during overwintering. In lachenalia (*Lachenalia* cv. Ronina), concentration of sucrose negatively related with starch concentration in roots due to degrading of starch to sucrose (Toit et al., 2004).

The concentration of glucose in leaves increased from December to April, suggesting storage carbohydrates may be degraded to glucose in response to seasonal change (Fig. 7.6C). In bulbs, an increase of fructose, glucose and sucrose is a characteristic of the transition from the resting stage to growth (Orthen and Wehrmeyer, 2004).

Free amino acids in Expt. 1

Concentration of total free amino acids did not fluctuate with seasonal changes (Table 7.2), suggesting free amino acids might not function as storage N for overwintering TB iris. Glutamate, alanine, aspartate, serine, and tyrosine were the main constituent free amino acids in all tissues and this composition did not fluctuate with seasonal changes (Figs. 7.7A, B and C). In the study of Nordin and Näsholm (1997), free amino acids had the major role of N storage and most species had arginine functioning as

a major form of free amino acids, while in wavy hairgrass (*Deschampsia flexuosa*), and solidago (*Solidago virgaurea*), arginine and asparagine together dominated the pool of free amino acids.

Concentration of N and C in Expt. 2

Higher N rates in late fall increased N concentration in all tissues in December Only C concentration in leaves was affected by the interaction of time and N rates (Table 7.3, Figs. 7.8A, B and C , Figs. 7.9A, B and C). Plants receiving 20 mM N showed higher N concentration in roots and rhizomes in Dec. 2014 than those receiving 0 or 10 mM N. Regardless of N rate, both C and N concentrations in rhizomes showed a decreasing trend from Dec. 2014 to Apr. 2015 (Figs. 7.8B and C). Nitrogen concentration in roots also decreased with season change; however, the amount of the decline (less than 0.5%) was relatively smaller than of rhizomes.

Amount and allocation of ^{15}N derived from fertilizer.

The amount of ^{15}N in leaves, roots, and rhizomes was increased by higher N rates (Figs. 7.10A, B and C), which suggests more N was taken up from fertilizer. Allocation of ^{15}N to leaves was affected by both time and N rate, but allocation of ^{15}N to roots and rhizomes was only affected by time. In February, a great amount of ^{15}N was allocated to rhizomes, which was transferred to leaves in April (Figs. 7.11A, B and C).

Concentration of starch, sucrose, glucose and fructose in Expt. 2

Sucrose, glucose, and fructose concentrations, except fructose concentration in roots, showed no response to N rate. Starch concentration in leaves and rhizomes only responded to seasonal change, whereas the interaction of seasonal change and N rate

affected starch concentration in roots (Table 7.4). In rhizomes, the general trends of starch, sucrose, glucose, and fructose concentration in Expt. 2 (Figs. 7.12C, 7.13C, 7.14C, and 7.15C) were similar to those in Expt. 1.

In many studies, reserved C decreased due to N application, since N assimilation requires energy and C skeletons (Invers et al., 2004; Ohtake et al., 2006; Vojtišková et al., 2006). However, in this study N application influenced some carbohydrates concentrations, but there was no clear trend of decreasing carbohydrate concentration with increasing N application.

Conclusion

Nitrogen concentration in rhizomes continually declined from December to April in both Expt. 1 and 2 indicating the rhizome likely functions as the main storage tissue of remobilized N. Concentration of starch, sucrose, glucose, and fructose showed seasonal changes in all tissues, except for concentration of starch in leaves and concentration of glucose in roots. Starch was the major form of carbohydrates in December Total free amino acids in all tissues did not fluctuate with seasonal changes. Glutamate, alanine, aspartate, serine, and tyrosine contributed more than 90% of the total free amino acids in all tissues. Late fall N application increased N concentration in all tissues. Only carbon concentration in leaves was affected by the interaction of season change and N rate. Nitrogen application influences carbohydrate concentration, but there was no clearly increasing or decreasing trend.

Table 7.1 Results of one factor ANOVA of dry weight, nitrogen, and carbon in leaves (L), roots (R), and rhizomes (RZ), and total of 'Immortality' TB iris plants in Dec. 2013, Feb. 2014, and Apr. 2014.

Level of significance is indicated by asterisks: $P \leq 0.05$ (*), 0.01 (**), 0.001 (***), 0.0001 (****).

	Dry weight				Nitrogen			Carbon		
	L	R	RZ	Total	L	R	RZ	L	R	RZ
Time	****	**	NS	**	NS	*	**	NS	NS	***

Table 7.2 Results of one factor ANOVA of concentration of starch, sucrose, glucose, fructose, and total free amino acids (FAA) in leaves (L), roots (R), and rhizomes (RZ) of 'Immortality' TB iris plants in Dec. 2013, Feb. 2014, and Apr. 2014.

	Starch			Sucrose			Glucose			Fructose			Total FAA		
	L	R	RZ	L	R	RZ	L	R	RZ	L	R	RZ	L	R	RZ
Time	NS	****	****	***	**	*	***	NS	***	****	***	****	NS	NS	NS

Level of significance is indicated by asterisks: $P \leq 0.05$ (*), 0.01 (**), 0.001 (***), 0.0001 (****).

Table 7.3 Results of two-factor ANOVA of concentration of nitrogen, carbon, amount of N derived from ^{15}N, and allocation of ^{15}N from fertilizer in leaves (L), roots (R), and rhizomes (RZ) of 'Immortality' TB iris plants in Dec. 2014, Feb. 2015, and Apr. 2015.

	Nitrogen			Carbon			Amount of ^{15}N			Allocation of ^{15}N		
	L	R	RZ	L	R	RZ	L	R	RZ	L	R	RZ
Time	NS	NS	**	NS	NS	****	*	*	*	****	*	**
Nitrogen rates	*	*	*	NS	NS	NS	****	*	*	*	NS	NS
T*N	NS	NS	NS	**	NS	NS	NS	NS	NS	NS	NS	NS

Level of significance is indicated by asterisks: $P \leq 0.05$ (*), 0.01 (**), 0.001 (***), 0.0001 (****). T×N is abbreviation of Time×Nitrogen rates.

Table 7.4 Results of two-factor ANOVA of concentration of starch, sucrose, glucose, and fructose in leaves (L), roots (R), and rhizomes (RZ) of 'Immortality' TB iris plants in Dec. 2014, Feb. 2015, and Apr. 2015.

	Starch			Sucrose			Glucose			Fructose		
	L	R	RZ	L	R	RZ	L	R	RZ	L	R	RZ
Time	****	**	****	***	****	**	****	NS	****	NS	****	***
Nitrogen rates	NS	****	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
T×N	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	*	NS

Level of significance is indicated by asterisks: $P \leq 0.05$ (*), 0.01 (**), 0.001 (***), 0.0001 (****). T×N is abbreviation of Time×Nitrogen rates.

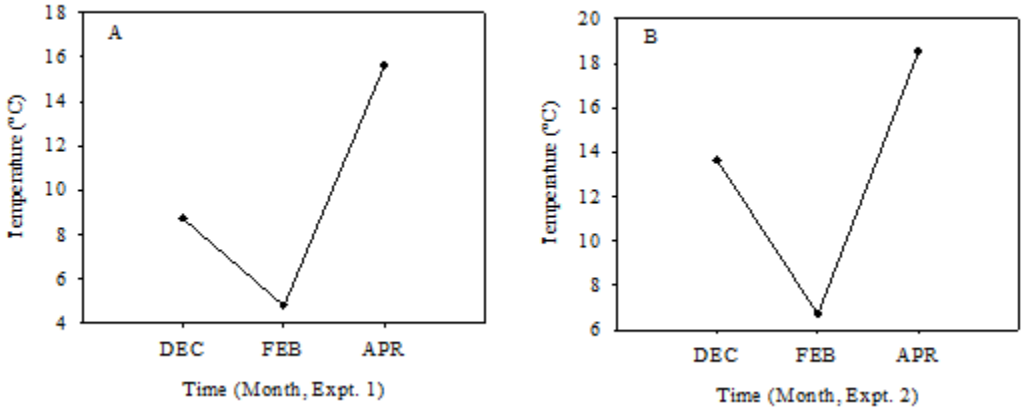

Figure 7.1 Changes of average temperature in Dec. 2013, Feb. 2014, and Apr. 2014 (A, Expt. 1) and in Dec. 2014, Feb. 2015, and Apr. 2015 (B, Expt. 2) at Mississippi State University, Starkville, MS (latitude 33°46' N, longitude 88°82' W).

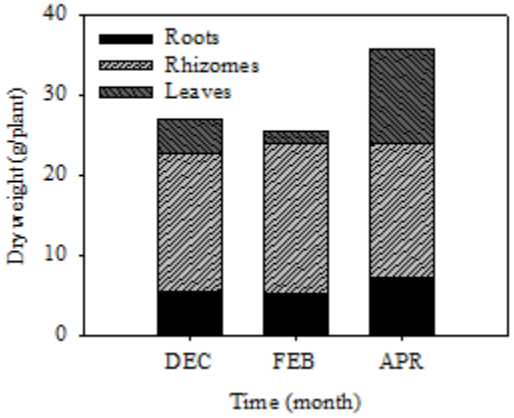

Figure 7.2 Changes of dry weight of 'Immortality' TB iris plants in Dec. 2013, Feb. 2014, and Apr. 2014 (Expt. 1).

Rhizomes were planted in Aug. 2013 and plants were harvested in Dec. 2013, Feb. 2014, and Apr. 2014.

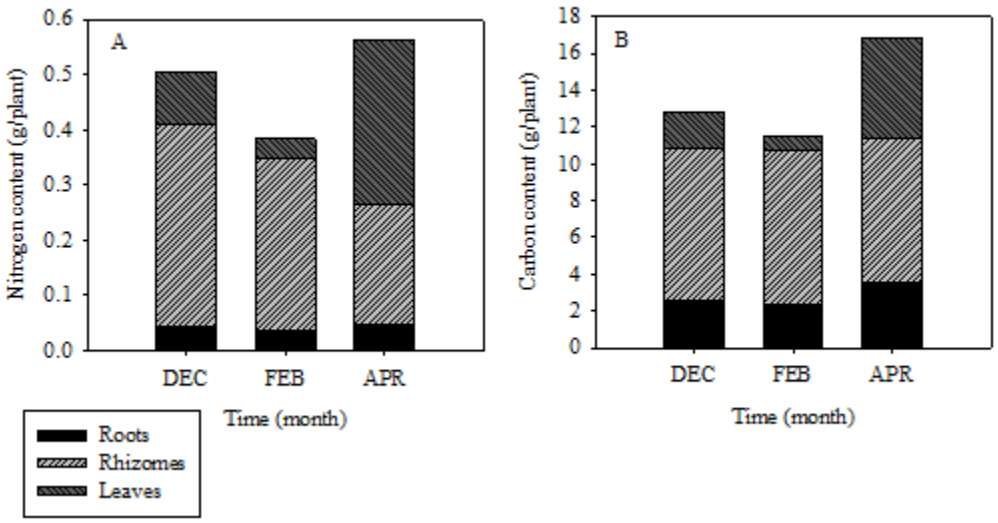

Figure 7.3 Changes of nitrogen (A) and carbon (B) content in leaves, roots, and rhizomes of 'Immortality' TB iris plants in Dec. 2013, Feb. 2014, and Apr. 2014 (Expt. 1).

Rhizomes were planted in Aug. 2013 and plants were harvested in Dec. 2013, Feb. 2014, and Apr. 2014.

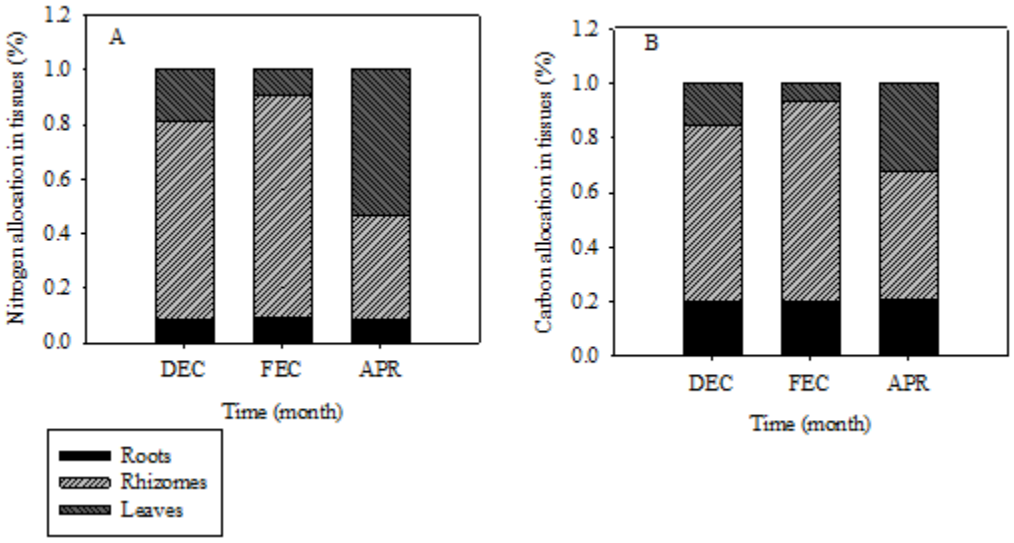

Figure 7.4 Changes of nitrogen (A) and carbon (B) allocation to leaves, roots, and rhizomes of TB iris plants in Dec. 2013, Feb. 2014, and Apr. 2014 (Expt. 1).

Rhizomes were planted in Aug. 2013 and plants were harvested in Dec. 2013, Feb. 2014, and Apr. 2014.

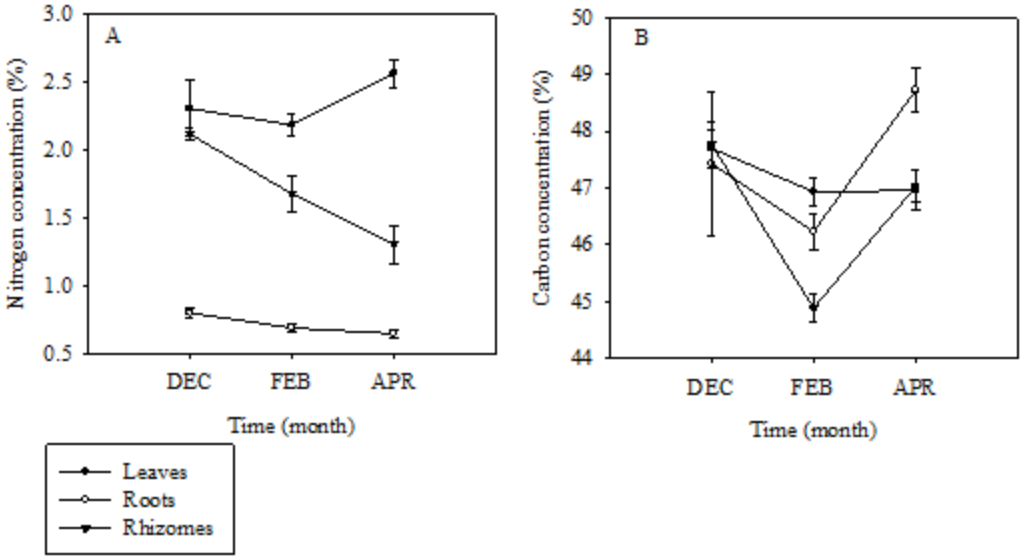

Figure 7.5 Changes of nitrogen (A) and carbon (B) concentration in leaves, roots, and rhizomes of 'Immortality' TB iris plants in Dec. 2013, Feb. 2014, and Apr. 2014 (Expt. 1).

Rhizomes were planted in Aug. 2013 and plants were harvested in Dec. 2013, Feb. 2014, and Apr. 2014. Data points present the means, vertical bars represent ± SE, n=4. SE Bars are not shown when smaller than point markers.

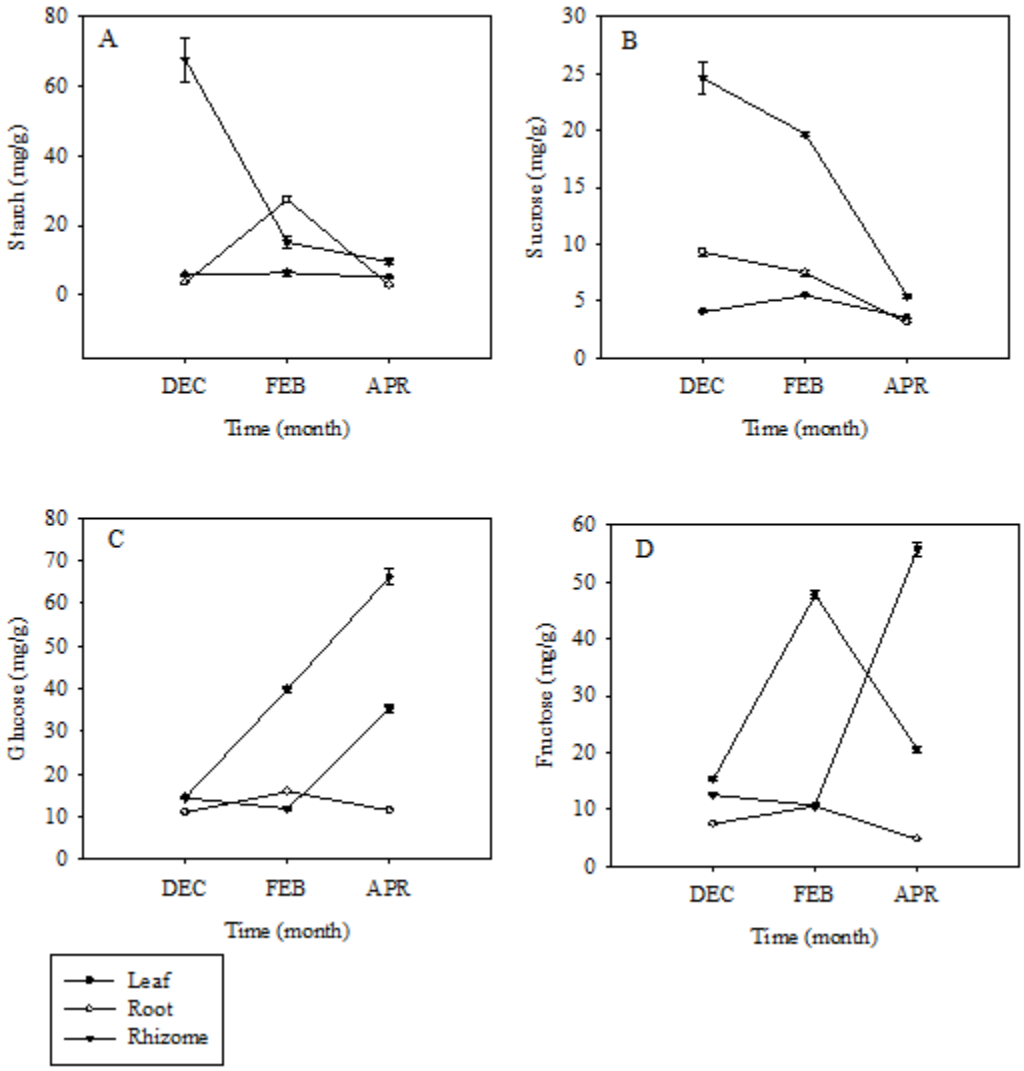

Figure 7.6 Changes of starch (A), sucrose (B), glucose(C), and fructose (D) concentration in leaves, roots, and rhizomes of 'Immortality' TB iris plants in Dec. 2013, Feb. 2014, and Apr. 2014 (Expt. 1).

Rhizomes were planted in Aug. 2013 and plants were harvested in Dec. 2013, Feb. 2014, and Apr. 2014. Data points present the means, vertical bars represent ± SE, n=4. SE Bars are not shown when smaller than point markers.

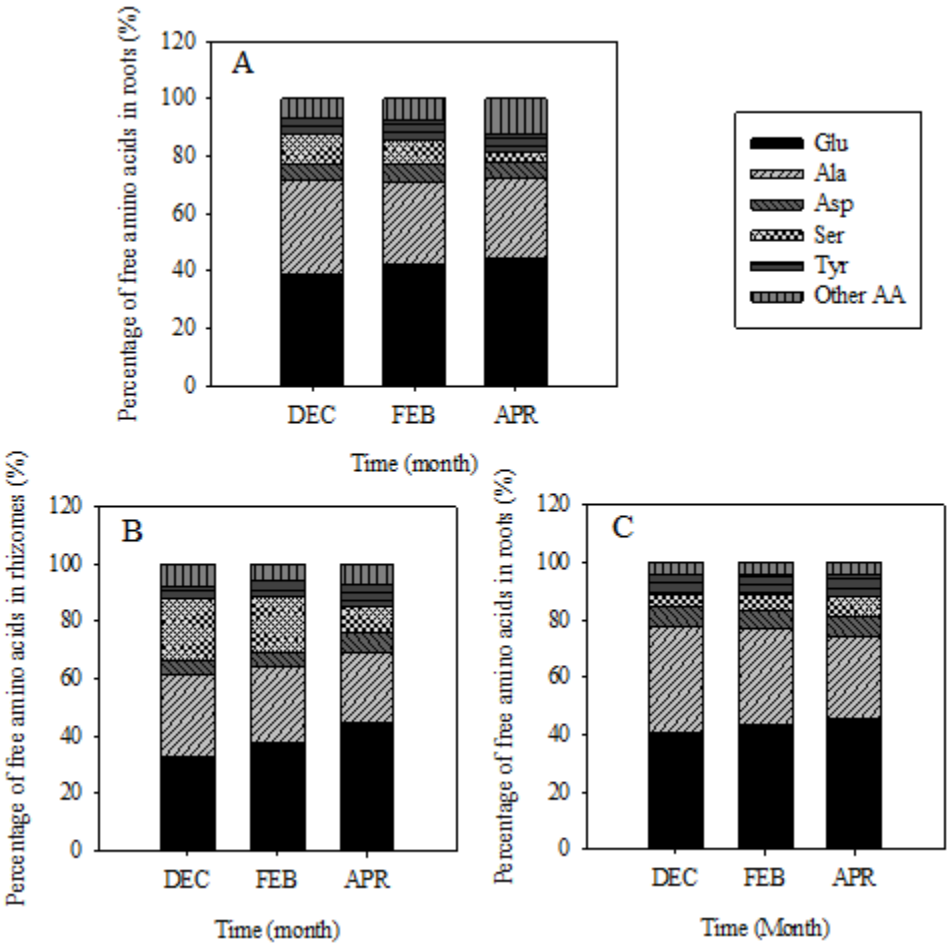

Figure 7.7 Percentage of major free amino acids in leaves (A), rhizomes (B), and roots (C) of 'Immortality' TB iris plants in Dec. 2013, Feb. 2014, and Apr. 2014.

Rhizomes were planted in Aug. 2013 and plants were harvested in Dec. 2013, Feb. 2014, and Apr. 2014.

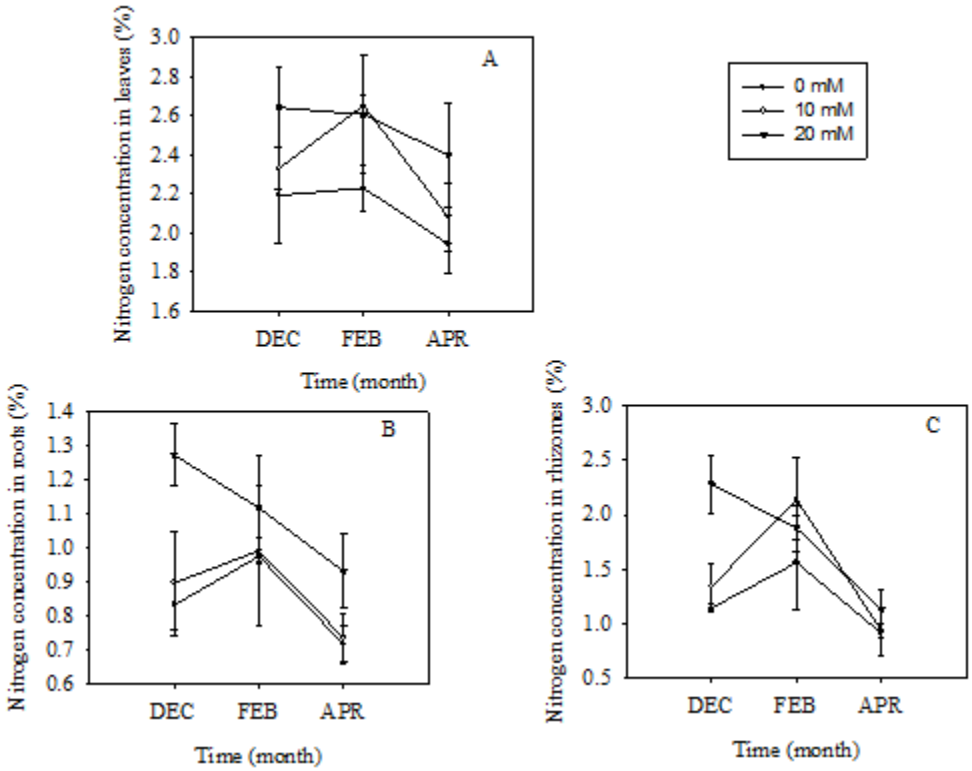

Figure 7.8 Nitrogen concentration of in Dec. 2014, Feb. 2015, and Apr. 2015 in leaves (A), roots (B), and rhizomes (C) 'Immortality' TB iris plants (Expt. 2).

Rhizomes were planted in Aug. 2014 and plants were harvested in Dec. 2014, Feb. 2015, and Apr. 2015. Data points present the means, vertical bars represent ± SE, n=3. SE Bars are not shown when smaller than point markers.

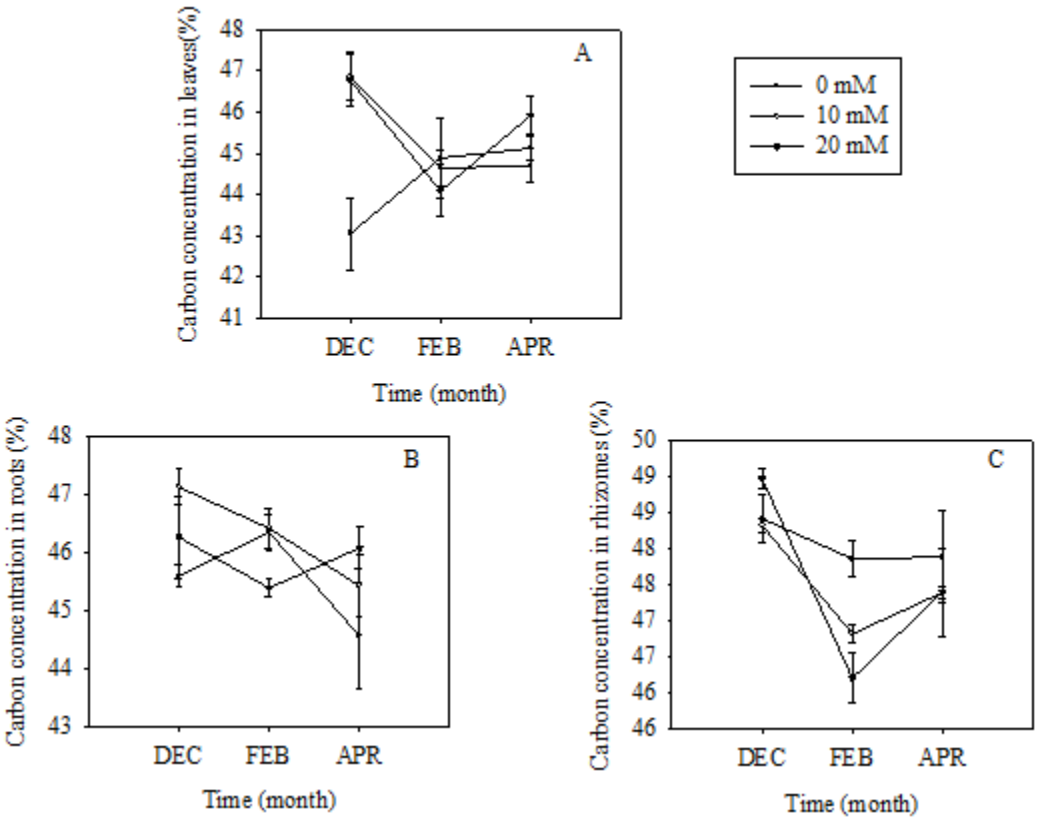

Figure 7.9 Carbon concentration in Dec. 2014, Feb. 2015, and Apr. 2015 in leaves (A), roots (B), and rhizomes (C) of 'Immortality' TB iris plants (Expt. 2).

Rhizomes were planted in Aug. 2014 and plants were harvested in Dec. 2014, Feb. 2015, and Apr. 2015. Data points present the means, vertical bars represent ± SE, n=3. SE Bars are not shown when smaller than point markers.

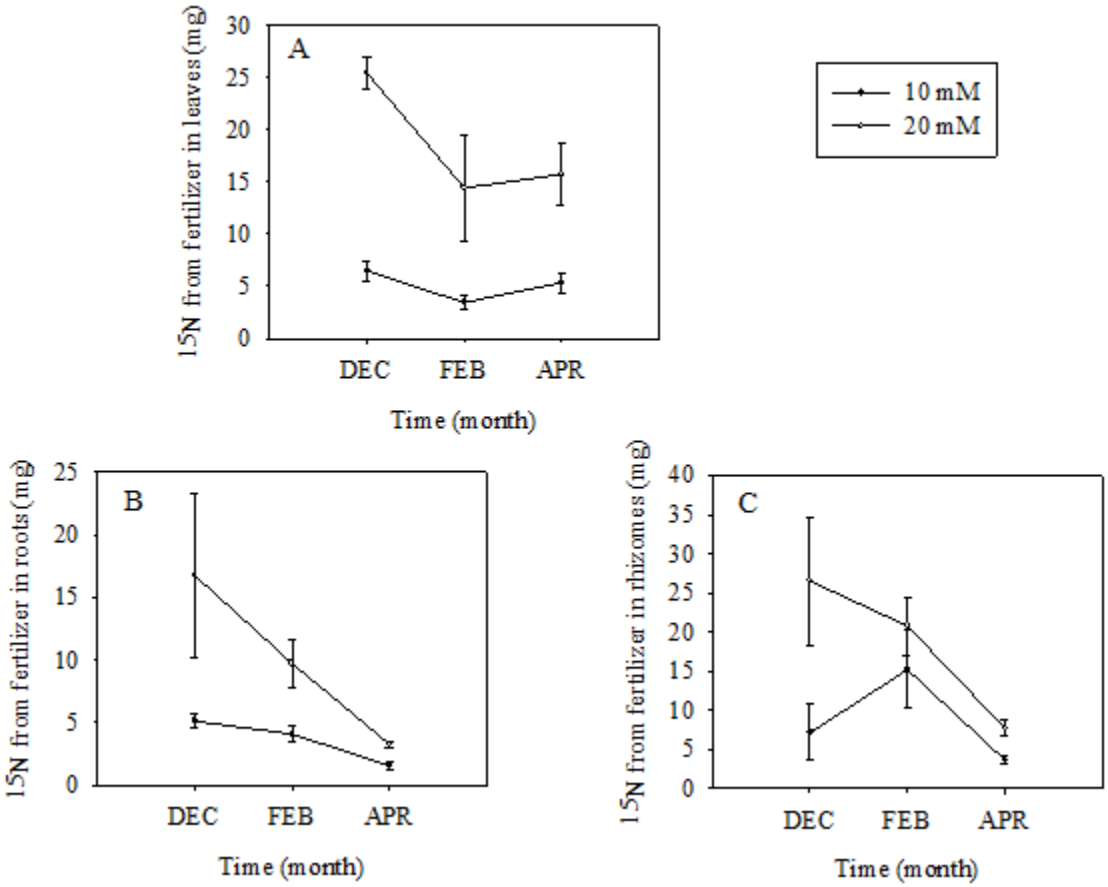

Figure 7.10 Amount of nitrogen derived from fertilizer in Dec. 2014, Feb. 2015, and Apr. 2015 in leaves (A), roots (B), and rhizomes (C) of 'Immortality' TB iris plants (Expt. 2).

Rhizomes were planted in Aug. 2014 and plants were harvested in Dec. 2014, Feb. 2015, and Apr. 2015. Data points present the means, vertical bars represent ± SE, n=3. SE Bars are not shown when smaller than point markers.

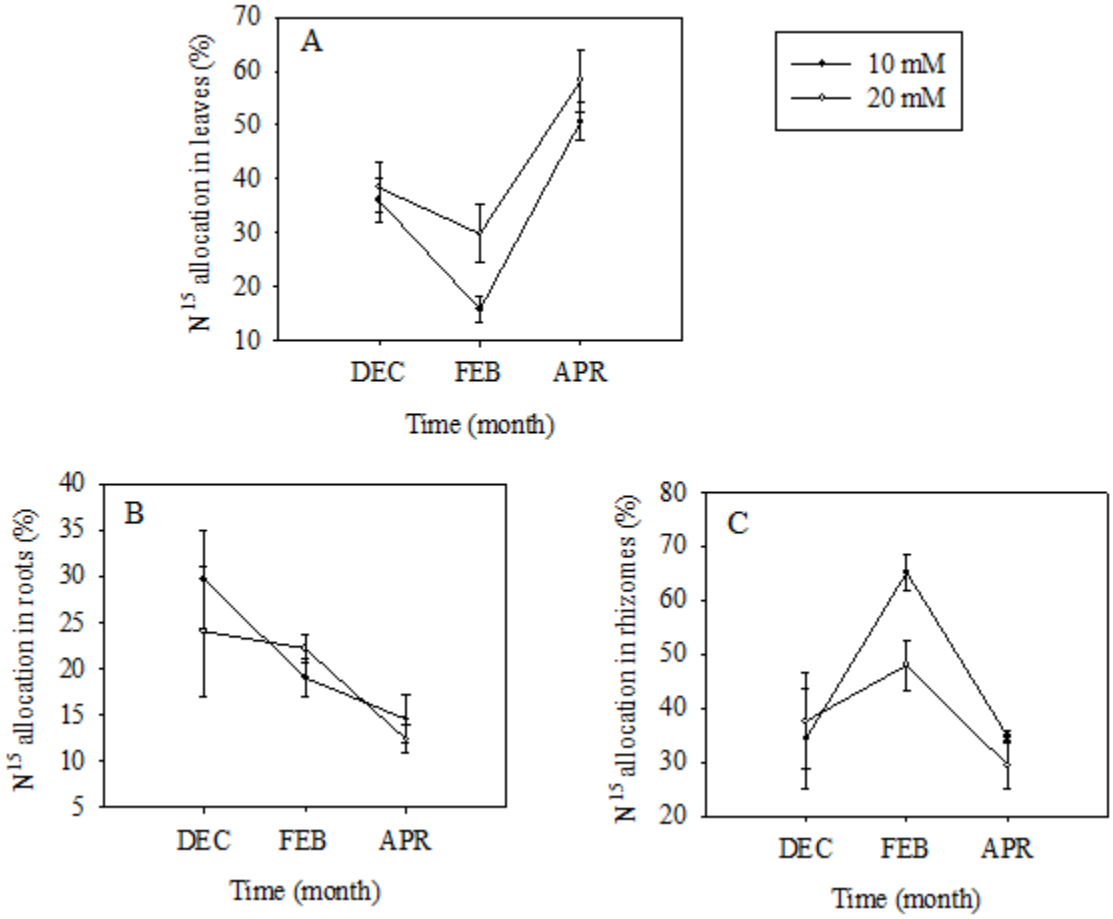

Figure 7.11 Allocation of nitrogen derived from fertilizer in Dec. 2014, Feb. 2015, and Apr. 2015 in leaves (A), roots (B), and rhizomes (C) of 'Immortality' TB iris plants (Expt. 2).

Rhizomes were planted in Aug. 2014 and plants were harvested in Dec. 2014, Feb. 2015, and Apr. 2015. Data points present the means, vertical bars represent ± SE, n=3. SE Bars are not shown when smaller than point markers.

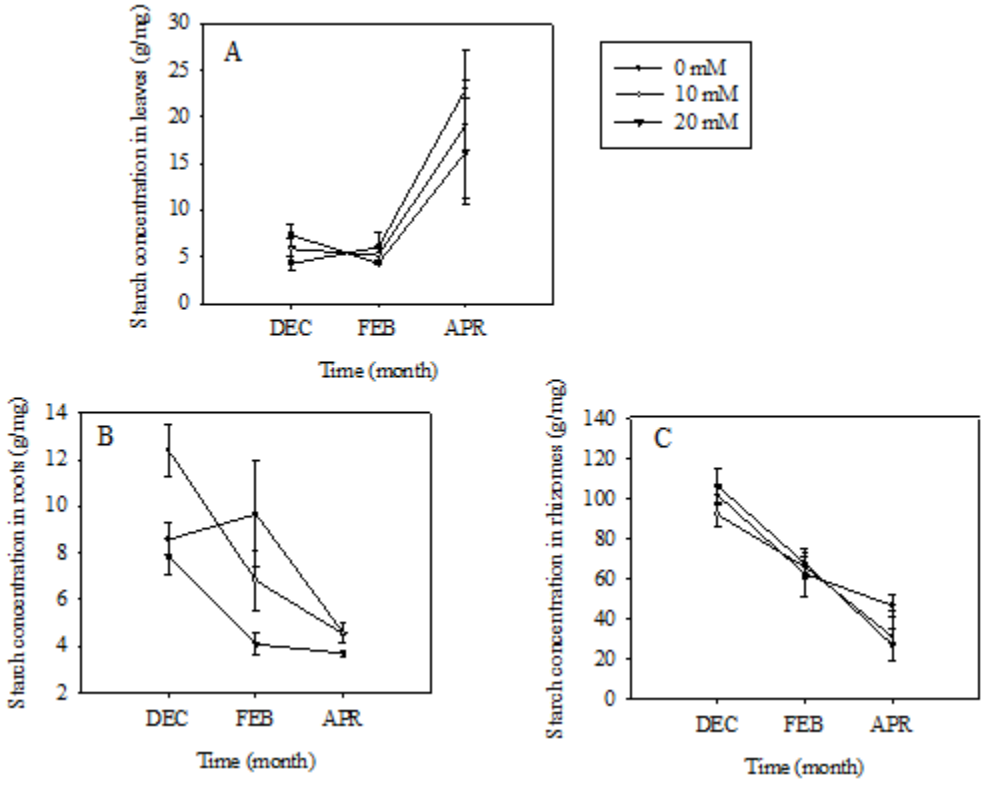

Figure 7.12 Starch concentration in Dec. 2014, Feb. 2015, and Apr. 2015 in leaves (A), roots (B), and rhizomes (C) of TB iris plants (Expt. 2).

Rhizomes were planted in Aug. 2014 and plants were harvested in Dec. 2014, Feb. 2015, and Apr. 2015. Data points present the means, vertical bars represent ± SE, n=3. SE Bars are not shown when smaller than point markers.

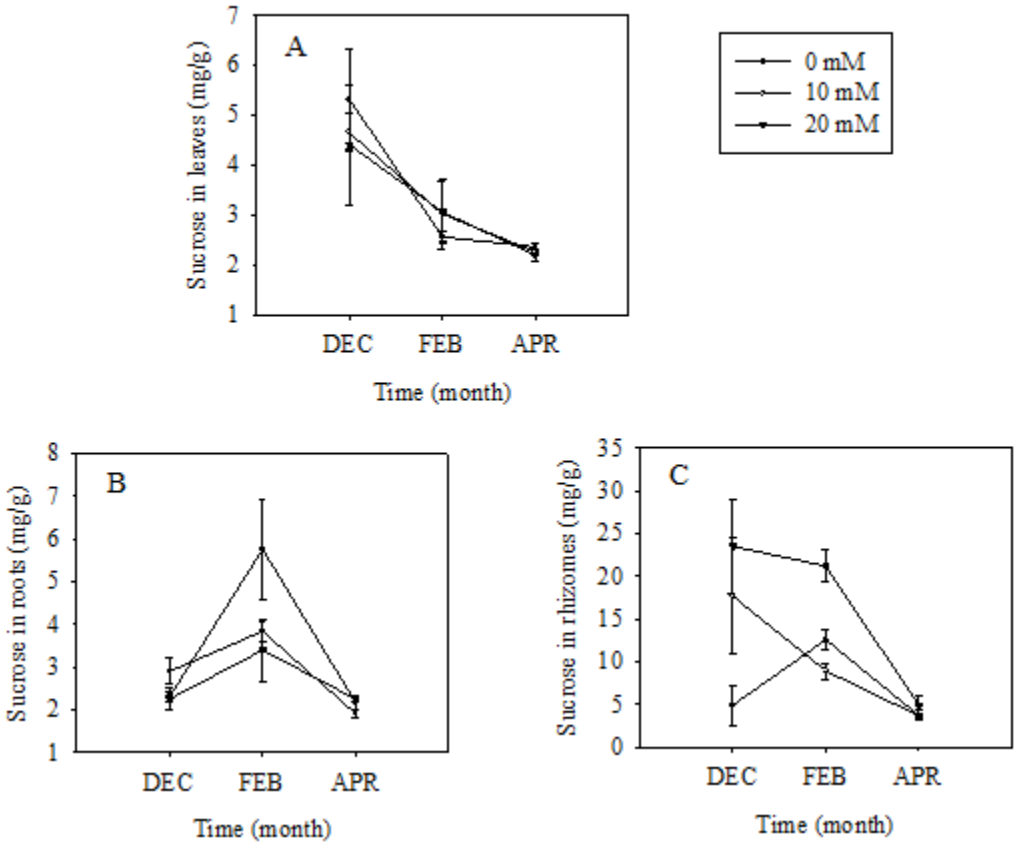

Figure 7.13 Sucrose concentration in Dec. 2014, Feb. 2015, and Apr. 2015 in leaves (A), roots (B), and rhizomes (C) of 'Immortality' TB iris plants (Expt. 2).

Rhizomes were planted in Aug. 2014 and plants were harvested in Dec. 2014, Feb. 2015, and Apr. 2015. Data points present the means, vertical bars represent ± SE, n=3. SE Bars are not shown when smaller than point markers.

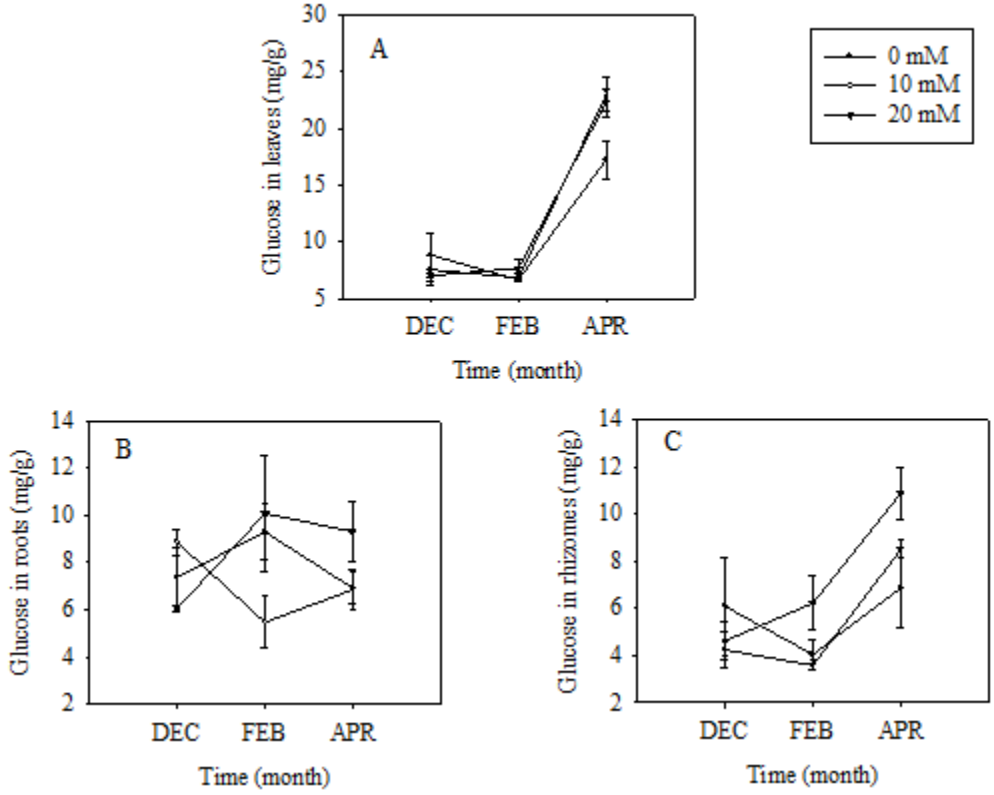

Figure 7.14 Glucose concentration in Dec. 2014, Feb. 2015, and Apr. 2015 in leaves (A), roots (B), and rhizomes (C) of 'Immortality' TB iris plants (Expt. 2).

Rhizomes were planted in Aug. 2014 and plants were harvested in Dec. 2014, Feb. 2015, and Apr. 2015. Data points present the means, vertical bars represent ± SE, n=3. SE Bars are not shown when smaller than point markers.

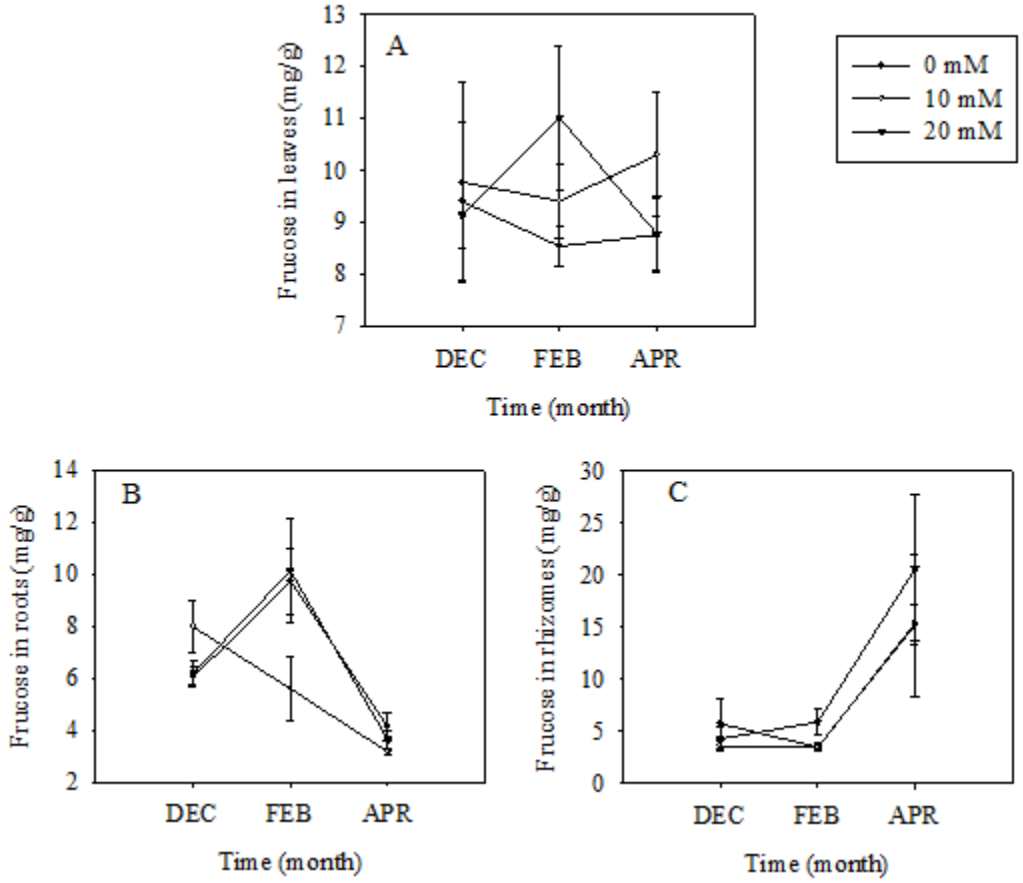

Figure 7.15 Fructose concentration in Dec. 2014, Feb. 2015, and Apr. 2015 in leaves (A), roots (B), and rhizomes (C) of 'Immortality' TB iris plants (Expt. 2).

Rhizomes were planted in Aug. 2014 and plants were harvested in Dec. 2014, Feb. 2015, and Apr. 2015. Data points present the means, vertical bars represent ± SE, n=3. SE Bars are not shown when smaller than point markers.

References

Addai, I.K. and P. Scott. 2011. Regulation of carbohydrates partitioning and metabolism of the common hyacinth. Agr. Biol. J. North Amer. 2:279-297.

Bi, G., C.F. Scagel, L. Cheng, S. Dong, and L.H. Fuchigami. 2004. Soil and foliar nitrogen supply affects the composition of nitrogen and carbohydrates in young almond trees. J. Hort. Sci. Biotechnol. 79:175-181.

Chapin, F.S., E.-D. Schulze, and H.A. Mooney. 1990. The ecology and economics of storage in plants. Annu. Rev. Ecol. Syst. 21:423-447.

Cheng, L., S. Dong, S. Guak, and L.H. Fuchigami. 2001. Effects of nitrogen fertigation on reserve nitrogen and carbohydrate status and regrowth performance of pear nursery plants. Acta Hort. 564:51-62.

Foyer C.H., S. Ferrario, and G.Noctor. 2001. Interactions between carbon and nitrogen metabolism, In: Lea P.J., Morot Gaudry J.F. (Eds.), Plant Nitrogen, Springer-Verlag, Berlin, 2001, p. 237-254.

Gloser, V. 2002. Seasonal changes of nitrogen storage compounds in rhizomatous grass *Calamagrostis epigejos*. Biol. Plant. 45:563-568.

Gloser, V., M. Košvancová, and J. Gloser. 2007. Regrowth dynamics of *Calamagrostis epigejos* after defoliation as affected by nitrogen availability. Biol. Plant. 51:501-506.

Halevy, A.H. 1990. Recent advances in control of flowering and growth habit of geophytes. Acta Hort. 266:35-42.

Invers, O., G.P. Kraemer, M. Pérez, and J. Romero. 2004. Effects of nitrogen addition on nitrogen metabolism and carbon reserves in the temperate seagrass *Posidonia oceanica*. J. Exp. Mar. Biol. Ecol. 303:97-114.

Khuankaew, T., S. Ruamrungsri, S. Ito, T. Sato, N. Ohtake, K. Sueyoshi, and T. Ohyama. 2010. Assimilation and translocation of nitrogen and carbon in *Curcuma alismatifolia* Gagnep. Plant Biol. 12:414-423.

Le Nard M and De Hertogh A.A. (1993) Bulb growth and development and flowering. In: De Hertogh A.A. and Le Nard M. (eds) The physiology of flower bulbs: a comprehensive treatise on the physiology and utilization of ornamental flowering bulbous and tuberous plants. Elsevier, Amsterdam, p. 29-43.

Millard, P. 1988. The accumulation and storage of nitrogen by herbaceous plants. Plant Cell Environ. 11:1-8.

Miller, W.B. and R.W. Langhans. 1990. Low temperature alters carbohydrate metabolism in Easter lily bulbs. HortScience 25:463-465.

Miller, W.B. 1992. A review of carbohydrate metabolism in geophytes. Acta Hort. 325:239-246.

Monson, R.K., T.N. Rosenstiel. T.A. Forbis, D.A. Lipson, and C.H. Jaeger. 2006. Nitrogen and carbon storage in alpine plants. Integr. Comp. Biol. 46:35-48.

Nordin, A. and T. Näsholm. 1997. Nitrogen storage forms in nine boreal understory plant species. Oecologia 110:487-492.

Ohtake, N., S. Ruamrungsri, S. Ito, K. Sueyoshi, T. Ohyama, and P. Apavatjrut. 2006. Effect of nitrogen supply on nitrogen and carbohydrate constituent accumulation in rhizomes and storage roots of *Curcuma alismatifolia* Gagnep. Soil Sci. Plant Nutr. 52: 711-716.

Ohyama,T., T. Ikarashi, A. Obata, and A. Baba. 1988. Role of nitrogen accumulated in tulip roots during winter season. Soil Sci. Plant Nutr. 34:341-350.

Ohyama, T. 1991. Assimilation and transport of nitrogen in tulip (*Tulipa gesneriana*) as pursued by ^{15}N. JARQ. 25:108-116.

Orthen, B. 2001. Sprouting of the fructan- and starch-storing geophyte *Lachenalia minima*: Effects on carbohydrate and water content within the bulbs. Physiol. Plant. 113:308-314.

Orthen, B. and A. Wehrmeyer. 2004 Seasonal dynamics of non-structural carbohydrates in bulbs and shoots of the geophyte *Galanthus nivalis*. Physiol. Plant. 120:529-536.

Quartieri, M., P., Millard, and M. Tagliavini. 2002. Storage and remobilisation of nitrogen by pear (*Pyrus communis* L.) trees as affected by timing of N supply. Eur. J. Agric. 17:105-110.

Rees, A.R. 1992. Ornamental bulbs, corms and tubers. CAB International, Wallingford, UK.

Rosnitschek-Schimmel, I. 1985. Seasonal dynamics of nitrogenous compounds in a nitrophilic weed II. The role of free amino acids and proteins as nitrogen store in *Urtica dioica*. Plant Cell Physiol. 26:177-183.

Ryan, F.J. 1994. Nitrogen and carbon concentrations, soluble proteins and free amino acids in subterranean turions of Hydrilla during overwintering. J. Aquat. Plant Manag. 32:67-70.

Steinbachová-Vojtišková, L., E. Tylova, A. Soukup, H. Novicka, O. Votrubová, H. Lipavská, and H. Čizková, 2006. Influence of nutrient supply on growth, carbohydrate, and nitrogen metabolic relations in *Typha angustifolia*. Environ. Exp. Bot. 57:246-257.

Theron, K.I. and G. Jacobs. 1996. Changes in carbohydrate composition of the different bulb components of *Nerine bowdenii* W. Watson (Amaryllidaceae). J. Amer. Soc. Hort. Sci. 12:343-346.

Toit, E.S., P.J. Robbertse, and J.G Niederwieser. 2004. Plant carbohydrate partitioning of *Lachenalia* cv. Ranina during bulb production. Sci. Hort. 102:433-440.

Tromp, J. and J.C. Ovaa. 1973. Spring mobilization of protein nitrogen in apple bark. Physiol. Plant. 29:1-5.

Vojtíšková, L., E. Munzarová, O. Votrubova, H. ýížková, and H. Lipavská. 2006. The influence of nitrogen nutrition on the carbohydrate and nitrogen status of emergent macrophyte *Acorus calamus* L. Hydrobiologia 563:73-85.

CHAPTER VIII

CONCLUSION

Tall bearded iris 'Immortality' is capable of repeated blooming in a growing season; however, the second bloom was largely influenced by N fertilization rate in the year of flowering. Thus, a relatively high N rate is needed to produce a second bloom. Flowering of plants in the spring was more dependent on N applied and stored from the previous year than N applied in the spring. Higher N rates in the previous year is recommended to improve production of flower stems the following spring.

Increasing N rates increased plant height, leaf SPAD reading, number of flower stems, plant dry weight, and plant N content. Greater N rates increased uptake of many essential elements, such as, potassium (K), calcium (Ca) and iron (Fe),which could be due to more vigorous growth. Nitrogen was discriminately allocated to rhizomes in December and to leaves in May. Spring N fertigation contributed more to leaf growth. The allocation of N and C to different tissues showed a trend similar to the allocation of dry weight. The C/N ratio in all tissues decreased with increasing N rate as a result of the influence of N rate on N concentration.

In spring, N uptake efficiency had a quadratic relation with increasing N rates and was highest with the 10 mM N treatment. Nitrogen use efficiency was not significantly affected by N rate, while N use efficiency of absorbed N decreased with increasing N

rate. The proportion of N derived from spring fertigation decreased due to a dilution effect by a greater amount of reserve N from the previous year.

NH_4:NO_3 ratios in fertilizer did not affect plant growth, flowering, dry weight, or N content. Plant height and leaf SPAD readings were affected by NH_4:NO_3 ratios in some months, but not across the whole growing season. Over the entire growing season, pH of leachate was increased by higher NH_4:NO_3 ratios. The net uptake of N was not affected by NH_4:NO_3 ratio, which indicates TB iris may not have a preference for either ammonium or nitrate N.

Phosphorus (P) rates did not affect plant height or dry weight and only increased leaf SPAD in October. Considering N:P ratio in this experiment was low, 5 mM P rate was sufficient for growth and development in TB iris.

Rhizomes likely function as main storage tissue for N in overwintering. Concentration of starch, sucrose, glucose, and fructose showed seasonal changes in all tissues, except for concentration of starch in leaf and concentration of glucose in roots. Starch was the major form of carbohydrates in December Glutamate, alanine, aspartate, serine, and tyrosine contributed more than 90% of the total free amino acids in all tissues. Total amino acids in all tissues did not fluctuate with seasonal changes. Late fall N application had significant influences on N concentration in all tissues. Nitrogen application influences carbohydrates concentration, but there was no clear increasing or decreasing trend.