Improving Nitrogen Use Efficiency of Potted Chrysanthemums Grown in a Subirrigation System

by

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ABSTRACT

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This thesis tested the hypothesis that nitrogen use efficiency of subirrigated potted chrysanthemum (*Chrysanthemum morifolium* Ramat.) can be improved by managing the NO_3^- status of the plant. Replacement of NO_3^- with water one week prior to flower development was the most effective method of reducing tissue NO_3^- content and improving the nitrogen usage index, as compared to various combinations of NO_3^- and Cl^-. Shoot or flower dry mass and N content were unaffected and the medium electrical conductivity was reduced. Supplying N in the NH_4^+ form in combination with NO_3^- did not affect nitrogen use indices. Reducing N supply improved various indices of nitrogen use efficiency, with a slight loss of quality. Elimination of NO_3^- prior to flower development and reduction in N supplied are effective strategies for improving nitrogen use efficiency in subirrigated potted chrysanthemums without sacrificing quality.

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LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
Cl^-	Chloride
CO_2	Carbon dioxide
DAT	Days after treatment
DM	Dry mass
EC	Electrical conductivity
FM	Fresh mass
GS1	Cytosolic glutamine synthetase
KCl	Potassium chloride
KNO_3	Potassium nitrate
LA	Leaf area
LSD	Least significant difference
m^3	Cubic metre
mM	millimolar
mmol	millimole
MOE	Ontario Ministry of the Environment
mS/cm	milliSiemens per centimetre
NADPH	Nicotinamide adenine dinucleotide phosphate
N	Nitrogen
NH_3	Ammonia
NH_4^+	Ammonium
NHI	Nitrogen harvest index

NO_2^-	Nitrite
NO_3^-	Nitrate
NR	Nitrate reductase
NRT1	Nitrate transporter 1
NUE	Nitrogen use efficiency
NUI	Nitrogen usage index
NUpE	Nitrogen uptake efficiency
NUtE	Nitrogen utilization efficiency
ppm	Parts per million
RO	Reverse osmosis
Rubisco	Ribulose bisphophate carboxylase-oxygenase
SE	Standard error
SO_4^{2-}	Sulphate

CHAPTER 1: Introduction

The Canadian floricultural industry is a large and diversified industry with annual gross sales of $1.42 billion in 2009 (Brown & Murphy, 2011). The Ontario industry at $742 million represents 52% of this industry. Recently, the Canadian, and more particularly, the Ontario floricultural industry has been undergoing some difficulty. Total sales decreased by 21% in 2008, compared to 2007, due primarily to the fallout from the financial crisis in North America, and the high value of the Canadian dollar.

Greenhouse floricultural operations can pose significant environmental risk due to the large input of fertilizers to the different crops (Cox, 2001). Leaching and runoff of nutrients such as nitrate (NO_3^-) pollute surface and ground waters. A number of factors contribute to this, including regular use of water-soluble fertilizers, frequent irrigation with large volumes of water, and earthen floors that facilitate runoff and leaching.

Currently, the Ontario Ministry of the Environment (MOE) is inspecting greenhouse operations to insure that they are compliant with water management legislation (Flowers Canada Growers, 2012). Section 30.(1) of the Ontario Water Resources Act states that "*Every person that discharges or causes or permits the discharge of any material of any kind into or in any waters or on any shore or bank thereof or into or in any place that may impair the quality of the water of any waters is guilty of an offence*" (Service Ontario, 2012). The Canadian water quality guidelines for the protection of aquatic life (freshwater) state that the limit for NO_3^- nitrogen (N) in the discharge water should be 0.2

mM (Canadian Council of Ministers of the Environment, 2012). Greenhouse operators are required to obtain and operate within an "Environmental Compliance Approval for Process Wastewater" from the MOE. Process Wastewater is defined as water with added nutrients, water that has been used for irrigation and washing of crops, recycled water, and water transported through floor drains located within the greenhouse operation, including water that mixes with storm water (Ontario Ministry of Environment, 2011).

In the face of these challenges, the floricultural industry is continually adapting to remain competitive and be environmentally responsible. For example, the industry has adopted recirculating systems for potted greenhouse plants, such as the subirrigated "ebb-and-flow" system on benches, troughs and concrete floors (Brown & Murphy, 2011). These systems effectively eliminate nutrient run-off and can reduce nutrient use (Molitor, 1990). However, subirrigation can result in the retention of nutrients and soluble salts in the growing medium. In contrast to traditional overhead irrigation, leaching of nutrients from the medium is not possible in subirrigation, as water flow is primarily from the bottom to the top of the pot by capillary action (Reed, 1996). The remainder of the nutrient solution is virtually unchanged in composition and is returned to the supply tank for subsequent irrigations. Subirrigation lends itself very well to recirculation as growers can continually reuse the nutrient solution and need only to replenish the supply tank with fresh solution as the volume decreases. Unfortunately, the accumulation of salts in the top of the medium can pose problems for the grower, especially for long-term crops.

One strategy for alleviating this problem is to reduce the fertilizer concentration and/or eliminate the fertilizer towards the end of the crop cycle, although growers may be reluctant to do this for fear of reducing crop quality (Hall et al., 2009). Research has

shown that elimination of fertilizer, especially N, towards the end of the crop cycle can enhance postharvest quality (Nell et al., 1989). The technology exists in the greenhouse floriculture industry to control nutrient application very precisely, as water-soluble nutrients are applied to the crop with each irrigation (i.e., fertigation), enabling the concentration or elimination of the nutrients to be adjusted according to stage of growth, weather and nutritional status of the crop.

Reduction of fertilizer-N application can theoretically improve N use efficiency (NUE), as plants tend to accumulate N in the form of NO_3^- when supply exceeds reduction and assimilation (Liu & Shelp, 1995,1996). Also, reducing N application after a period of adequate supply can increase the remobilization of N, particularly NO_3^-, from the metabolically inactive pools within the plant (e.g., vacuole), thereby improving NUE. For example, NO_3^- starvation rapidly deletes the NO_3^- pool in *Arabidopsis thaliana* [L.] Heynh. and this is associated with an increase in the protein pool, suggesting that NO_3^- is remobilized from storage and converted into N forms, resulting in the synthesis of protein (Richard-Molard et al., 2008).

Chrysanthemum (*Chrysanthemum morifolium* Ramat.) is the most commonly grown floricultural greenhouse crop species in the world and the potted type is well adapted to subirrigation (PickOntario, 2013). Reduction of fertilization is recommended towards the end of the crop cycle, especially in subirrigation, to improve the shelf life of cut and potted chrysanthemums (Lunt & Kofranek, 1958; Aris, 2010). Chrysanthemum is known to accumulate NO_3^- during exponential growth, which could theoretically limit N utilization; however, this NO_3^- pool could also be utilized later when exogenous NO_3^- is less available (Elliott & Nelson, 1983). During inflorescence development, large

quantities of reduced and NO_3^-- N are remobilized from vegetative tissues and directed to the developing flower. For example, at a constant NO_3^--N supply of 14.3 mM chrysanthemum plants grown in a peat:vermiculite medium remobilize significant amounts of NO_3^- from the stems plus petioles to be converted into reduced-N for the inflorescence, without any change in leaf NO_3^- content (Woodson & Boodley, 1983). Subsequent results with sand-cultured plants, where salts were not allowed to accumulate in the medium due to a large leaching fraction, indicate that regardless of the amount of NO_3^- supplied, the NO_3^- content of all vegetative tissues declines during inflorescence development, indicating dependence on previously accumulated NO_3^- (Woodson et al., 1984).

In this thesis, I investigated various strategies to increase the remobilization of stored NO_3^- in chrysanthemum during inflorescence development in order to improve the efficiency at which the supplied NO_3^- is used. Strategies tested included elimination of NO_3^-, replacement of NO_3^- with chloride (Cl^-), supplying combinations of NO_3^- and ammonium (NH_4^+), and reducing the amount of total N supplied. Elimination of NO_3^- at the mid point of the growth cycle was an effective method to improve the efficiency of utilizing the supplied NO_3^-, whereas the addition of Cl^- to the nutrient solution had no effect. Lowering the amount of total N supplied was also effective in improving nitrogen use efficiencies, whereas the addition of NH_4^+ to the nutrient solution did not affect the final outcome.

CHAPTER 2: Literature Review

2.1 Introduction

Nitrogen, an essential macronutrient in plants (Stern et al., 2008), is typically present at a concentration of approximately 5% on a dry mass (DM) basis in floricultural plants, but can reach as high as 7.6% (Dole & Wilkins, 2005). In the plant, N is an important component of proteins and chlorophyll. The major soluble protein in leaves is ribulose 1, 5-bisphosphate carboxylase-oxygenase (Rubisco), which constitutes 30 to 50% of the soluble protein and is the most abundant protein in nature (Goodwin & Mercer, 1983; Stern et al., 2008). In cereal leaves, up to 75% of the reduced N in mesophyll cells is present as Rubisco (Bertheloot et al., 2008). The mesophyll cells, which contain chloroplasts, are the location for harvesting light energy. Rubisco, a chloroplast stromal enzyme, is responsible for catalyzing the fixation of atmospheric CO_2 in the Calvin cycle (i.e., the dark reactions of photosynthesis), resulting in the accumulation of DM (Emes & Neuhaus, 1997; Hortensteiner & Feller, 2002; Stern et al., 2008). The energy used in the Calvin cycle, in the form of ATP and NADPH, is harvested as light energy via the light reactions of photosynthesis. The primary molecule involved in this process is chlorophyll, which is a chelate containing four N atoms, as well as magnesium as the central ion (Goodwin & Mercer, 1983). Thus, N plays central roles in both harvesting light energy for CO_2 fixation and producing the protein responsible for CO_2 fixation (Lawlor, 2002).

2.2 Uptake and Assimilation of Nitrogen

Plants obtain soil N primarily in two inorganic forms, NO_3^- and NH_4^+, with NO_3^- being the primary form (Crawford & Forde, 2002). The anionic NO_3^- form readily dissolves in water and has a high diffusion coefficient in soil, which makes it very mobile in soil or growing media, whereas the positively charged NH_4^+ is attracted to the negatively charged soil colloids making it less prone to leaching (Plaster, 2003; Miller & Cramer, 2004; Miller et al., 2007).

Soil water status affects NO_3^- availability and the form of N encountered by the plant in field soils. In moist soils, NO_3^- is readily available to the plant as it moves easily in the soil water, with the opposite being true in a dry soil. In a well-aerated soil the primary form is typically NO_3^- because of the rapid nitrification of NH_4^+ (Crawford & Forde, 2002; Miller & Cramer, 2004); however, in a waterlogged soil, such as a rice paddy field, NH_4^+ is the primary form (Tobin & Yamaya, 2001). pH also influences the form of N available, as the nitrifying bacteria *Nitrosomonas* and *Nitrobacter* grow optimally at pH 6.0-7.5. Therefore, NO_3^- is the primary form in neutral to basic soils, whereas NH_4^+ is the predominant form in acidic soils (Plaster, 2003).

In order for the plant to use the NO_3^- that is available in the soil solution, the plant root must first take it up. This uptake of NO_3^- requires energy, even when the external NO_3^- concentration is adequate and in the mM range (Crawford & Glass, 1998). Nitrate is actively transported across the plasma membranes of the epidermal and cortical root cells via an active H^+-mediated co-transport system (Crawford & Forde, 2002; Miller & Cramer, 2004; Miller et al., 2007). Once NO_3^- is in the cytoplasm, there are a number of pathways that it can take: (1) mobilization to the xylem for transport to the shoot; (2)

transport to the vacuole for storage; and (3) reduction and incorporation into amino acids (Crawford & Forde, 2002).

The first step in the reduction of NO_3^- to ammonia occurs via the cytosolic enzyme nitrate reductase (NR) (Crawford & Glass, 1998). The resultant nitrite is then transported to plastids in non-green tissue or chloroplasts in green tissue where it is further reduced to ammonia by nitrite reductase (Goodwin & Mercer, 1983; Crawford & Forde, 2002). The ammonia is then incorporated into organic-N by the collaborative activity of two enzymes, glutamine synthetase (GS) and glutamate synthase (GOGAT), resulting in the production of glutamine and glutamate, respectively. These amino acids are used in the synthesis of asparagine, aspartate and arginine, which are primary N transport compounds in xylem, along with glutamate and glutamine, the building blocks of proteins (Goodwin & Mercer, 1983).

2.3 Nitrate Uptake and Accumulation

Nitrate can be reduced and assimilated in the root for subsequent transport to the shoot or stored in the vacuoles of the root. However, in numerous plant species, NO_3^- is primarily transported to the shoot where it is reduced by the cytoplasmic NR or stored in the vacuole (Crawford & Glass, 1998; Masclaux-Daubresse et al., 2010). Plants supplied with NO_3^- in excess of their current demand, as when uptake exceeds reduction and subsequent assimilation, accumulate this excess NO_3^- (Liu & Shelp, 1995). It is well established that the vacuole is the site of NO_3^- storage in the plant cell and that this stored NO_3^- can serve as an osmoticum for turgor pressure or as a reserve for maintaining

cellular assimilation (Granstedt & Huffaker, 1982; van der Leij et al., 1998; Fan et al., 2007).

Nitrate uptake and accumulation, as measured by plant content, is usually very steady during the early phase of plant growth, but levels off at the onset of flowering and fruiting. Nitrate content in hydroponically grown oilseed rape (*Brassica napus* L.) supplied with 1 mM NO_3^--N, increases linearly for the first 24 d (coinciding with the start of flowering) and then levels off for the next 15 d (beginning of pod filling) because significant NO_3^- uptake does not occur (Rossato et al., 2001). The N content of the leaves also significantly decreases from day 24 to day 39, leading the authors to hypothesize that the N required for the flowers and pods is remobilized from leaves. The majority of N found in the grain at the conclusion of grain filling is derived from vegetative tissues, rather than by active uptake during podfill. Also, the NO_3^- uptake rate of *Arabidopsis* plants grown with a non-limiting N supply of 10 mM NO_3^--N is lower during the reproductive phase, than during the vegetative phase (Masclaux-Daubresse et al., 2010). Thus, due to the relatively lower NO_3^- uptake rate during the reproductive stage, and the larger demand for N during this phase, another N source, such as remobilized N, is necessary to fulfill the demands of developing sink tissues.

2.4 Remobilization of Stored Nitrogen

When sinks such as flowers, fruits and seeds develop, the uptake and assimilation of N by the plant is usually insufficient to supply their demands (Masclaux-Daubresse et al., 2010). During seed fill for example, previously accumulated N (endogenous) is required to supply the demand of the sink (Salon et al., 2001; Schiltz et al., 2005). This

endogenous N is primarily remobilized from vegetative parts such as leaves and stems. In legumes, remobilized N contributes 70% to the total seed N in field pea to 90% in soybean, with leaves and stems being the major sources (Schiltz et al., 2005).

Photosynthetic proteins, including light harvesting complex proteins, and to a large extent, Rubisco, represent the majority of total leaf N (Gastal & Lemaire, 2002). Rubisco has a low catalytic rate per mass of protein, so C_3 plants require large amounts of this protein in order to maintain high rates of CO_2 assimilation (Lawlor, 2002). As Rubisco can constitute up to 50% of the total soluble protein in the leaves of C_3 plants and 20% in C_4 plants, it has been considered a storage protein that can be subsequently remobilized (Sage et al., 1987; Lawlor, 2002). When a plant is in the latter stages of growth, the leaves transition from nutrient assimilation to nutrient remobilization and a large amount of N is remobilized to sink tissues (Viestra, 1993; Feller & Fisher, 1994). Proteins such as chloroplast stromal enzymes like Rubisco are degraded and the resultant amino acids are exported via the phloem to sink areas of the plant (Hortensteiner & Feller, 2002; Sondergaard et al., 2004).

The total N in a plant can be categorized into two pools, the structural and the remobilizable. The structural pool is found primarily in cell walls as glycine rich proteins (GRP), whereas the remobilizable N is composed primarily of amino acids and soluble proteins (Ringli et al., 2001; Rossato et al., 2001; Diaz et al., 2008). Nitrate can also represent a significant fraction in many plants, as it can represent as much as 30% of the total-N in vegetative chrysanthemums (Woodson & Boodley, 1983; Fan et al., 2009).

2.5 Fertilizer Nitrogen Usage

The efficient use of fertilizer is important for any crop, whether it is to produce a crop as economically as possible, or to prevent contamination of the environment. Growers are faced with the task of producing crops that achieve maximum yield, while limiting production costs, in order to maximize profit, minimize the environmental impact, and produce a crop of extremely high quality that is demanded by today's consumers (Jeuffroy et al., 2002).

There is much pressure to reduce fertilizer usage, in particular N, yet this can conflict with the need for greater crop yields (Lawlor, 2002; Good et al., 2004). The use of N fertilizers in the past 50 years has increased 20 fold, yet it is clear that the applied fertilizer N is commonly utilized very inefficiently, with only 30% to 40% of the N being taken up by the crop and the rest lost through leaching, denitrification, volatilization, soil erosion, and microbial consumption (Raun & Johnson, 1999; Glass, 2003; Kant et. al., 2008). Historically, N fertilizers have been relatively cheap and applied in relatively large quantities in order to insure maximal yield. However, fertilizer N costs have been very volatile in recent years due to the rising cost of fossil fuels (Glass, 2003; Garnett et al., 2009; Nelson, 2009; Ellis, 2010).

Inorganic fertilizer N is derived from the conversion of N_2 to NH_3 to make urea, NO_3^- etc., a process that requires large inputs of energy derived from fossil fuels (Lawlor, 2002). This increases the carbon footprint of N fertilizers, as 1.2% of the world's energy consumption is consumed in N fertilizer production (Ahlgren et al., 2008). Therefore, reducing fertilizer N usage can reduce input costs for the grower, thereby increasing

profits, and reduce the environment hazards associated with contamination of the water and atmosphere.

2.6 Nitrogen Use Efficiency

Many papers have been written on nitrogen use efficiency (NUE), and there are also many definitions of NUE. As Brauer & Shelp (2010) point out there is much confusion regarding the correct use of NUE and provide several examples from the scientific literature. These authors suggested that the "simple and ubiquitous definitions/formulae summarized by Good et al. (2004) be used as a starting point for NUE studies", and indicated that the choice of specific type of NUE to be studied "depends on the plant tissue of interest and whether changes in the efficiency of uptake or utilization of N are suspected".

In an experiment with Kentucky bluegrass cultivars (*Poa pratensis* L.), NUE is "defined as grams of dry matter per gram N present in the tissue" (Jiang et al., 2000). Good et al. (2004) point out that this does not account for an increase in biomass, and suggest the use of the Usage Index (UI) (Siddiqi & Glass, 1981), which does account for an increase in biomass. The UI, as defined by Siddiqi & Glass (1981), is Sw x (Sw/N), where Sw is the shoot weight and N is the N content of the shoot. When measuring the harvestable product such as grain, beans and melons (*Cucumis melo* L.) (Colla et al., 2010), NUE is defined as "the yield of harvestable product (dry mass)/N supply (g per plant)". This is often the definition of NUE that is used since it simply reflects how much "product" is obtained with the N supplied (Good et al., 2004; Brauer & Shelp, 2010).

NUE varies widely in crop plants as it can be influenced by many variables. A survey of field-grown crops in the UK indicated that sugar beet has the highest NUE (kg DM per N available) with a NUE of 69, followed by potato with an NUE of 40; oilseeds and peas have the lowest NUE, at 9 and 6, respectively (Sylvester-Bradley & Kindred, 2009). While this information may indicate that oilseeds and peas are very inefficient crops in regards to N usage, the low NUE could also result from an oversupply of N fertilizer and an underestimation of N fixation by the legume crops (Sylvester-Bradley & Kindred, 2009). Oversupplying N as "insurance" to obtain a higher yield is not an uncommon practice by growers (Glass, 2003). This wasteful use of fertilizer N, especially when crop prices are high, can influence the NUE of a crop, particularly where the optimum N requirement for each crop is not taken into consideration (Sylvester-Bradley & Kindred, 2009).

High value floricultural crops are no exception to over-fertilization since the perceived benefits have traditionally outweighed any extra costs involved. Typically, suppliers of materials for chrysanthemum production recommend supplying N as high as 400 ppm (28.6 mM) in the early stages of production (Aris, 2010). However, fertilizer costs are increasing and growers are now more closely evaluating the costs of fertilizer N and associated efficiencies (Ellis, 2010).

2.7 Improving Nitrogen Use Efficiency

Improved NUE could result from the capture of a greater proportion of the applied N, or more efficient use of the N captured so that crop yields are maintained even when the N supply is reduced. Both strategies would result in lower requirements for applied N and

consequently, lower fertilizer costs and less contamination of the environment (Zebarth et al., 2009; Brauer & Shelp, 2010).

The NUE of crops can be improved via bioengineering or management strategies. For example, enhanced expression of a pine *glutamine synthetase 1* (GS1) gene in poplar (*Populus tremula* X *P. alba*) increases total leaf area (LA), plant height and leaf DM under both low (0.3 mM) and high (10 mM) NO_3^--N as compared to an untransformed control, with the greater percentage increase occurring with low NO_3^- conditions (Man et al., 2005). This and other studies indicate that overexpression of GS1 may increase NUE in poplar (Kirby et al., 2006; Brauer & Shelp, 2010). To date, there appear to be no transgenic studies on ornamentals to increase NUE, and no further consideration will be given to this strategy in this thesis.

Matching the supply of N with plant-N demand is one of the most effective means to increase NUE (Zebarth & Rosen, 2007). Crops transition from the vegetative phase, wherein the absorption and assimilation of N by sink organs such as young developing roots and leaves is supported by direct N supply from the soil or substrate, to the remobilization or generative phase, wherein the formation of new developing sinks such as flowers, seeds and storage organs is supported by N supplied from senescing tissues (Hirel & Lemaire, 2005). Timing the delivery of N to coincide with the vegetative growth phase and increasing the remobilization of the accumulated N should lead to greater NUE. For example, timing fertilizer N application shortly before the rapid growth phase of wheat (*Triticum aestivum* L.) increases NUE (Howard et al., 2002).

Appropriate timing of fertilizer N application to match the N accumulation curve of plants can be used to increase N uptake efficiency (NUpE). For example, the use of a

variable supply rate of N that matches the biomass accumulation curve and a constant rate of supply at 14.3 mM N, gives NUpE of 58% and 38%, respectively, for poinsettias (*Euphorbia pulcherrima* Willd. Ex Klotz.) (Rose et al., 1994). All growth parameters, including DM, N content and quality are similar for the two treatments, yet the variable rate treatment results in 41% less N being applied. Greenhouse-grown broccoli (*Brassica oleracea* var. *italica* Plenck) has a 58% greater inflorescence fresh mass (FM) when the N rate is shifted from 17.9 mM to 10.7 mM at inflorescence initiation (Nkoa et al., 2001). Subsequent research with wheat demonstrated that N applied in small doses during the period of high N demand also maximizes crop yield and reduces losses of N (Barraclough et al., 2010).

Organic-N is degraded and remobilized in order to provide N sources for developing leaves, flowers and eventually seeds. A major source of this remobilized N is leaf protein in the form of Rubisco (Masclaux-Daubresse et al., 2010). Amino acids and proteins are easily mobilized from broccoli leaf tissue during inflorescence development, resulting in the majority of N in lower leaves in the form of NO_3^-, whereas the remaining canopy contains about 90% of the N in organic form (Liu & Shelp, 1993b; Liu & Shelp, 1995). Remobilization of the NO_3^- remaining in the lower canopy is important to sustain vigorous growth and contributes to improving the NUE of a plant; otherwise, this NO_3^- would be lost with senescing leaves (Fan et al., 2007).

Plants supplied with NO_3^- in excess of current demand have the ability to accumulate NO_3^-, primarily in the vacuole (Granstedt & Huffaker, 1982; van der Leij et al., 1998); thus, the leaf and stem tissue NO_3^- can be assumed to be storage NO_3^- (Fan et al., 2007; Miller et al., 2007). Removal of NO_3^- supply has been shown to lower NO_3^-

concentration of plants without lowering growth rate. Three- to five-day-old barley plants (*Hordeum vulgare* L.) grown with a nutrient solution containing 10 mM NO_3^--N for 24 h and then transferred to a NO_3^--free solution have lower NO_3^- concentrations in the root and shoot than control plants receiving 10 mM NO_3^--N continuously (van der Leij et. al, 1998). There was no difference in the relative growth rate or the root to shoot ratio over the experimental period. The authors interpret these findings as evidence for remobilization of vacuolar stored NO_3^- that is sufficient to maintain growth of the seedling, but indicate that the possible involvement of reduced N (amino acid pools) must also be assessed. Notably, cytosolic NO_3^- levels are maintained at a constant level when vacuolar NO_3^- levels are declining, suggesting that vacuolar release of NO_3^- is carefully regulated and that stored vacuolar NO_3^- can function as both an osmoticum and a reserve for future plant assimilation.

Comparison of two rice (*Oryza sativa* L.) cultivars that differ in N uptake efficiency (NUpE, designated as NUE in the paper) revealed that the cultivar with the higher NUpE depletes the root vacuolar NO_3^- pools faster during 24 h of NO_3^- starvation and maintains a steady concentration of cytosolic NO_3^- (Fan et al., 2007). The cultivar with the higher NUpE also remobilizes NO_3^- from the vacuoles of leaf epidermal cells faster and maintains a higher cytosolic NO_3^- level than the less efficient cultivar. The NO_3^- pool in *Arabidopsis* grown at 5 mM NO_3^--N for 28 d and then subjected to 7 d of NO_3^- starvation is quickly depleted, yet the soluble protein pool in the shoot and the insoluble pool in the roots increase, indicating that protein synthesis does not stop during the NO_3^- starvation, but relies on the previously accumulated and stored NO_3^- (Richard-Molard et al., 2008).

These results suggest that NO_3^- can be rapidly mobilized from reserves to maintain N metabolism under conditions of N shortage.

In studies of NO_3^- mobilization, the age and the organic-N content of the plant tissue should also be considered. For example, Liu & Shelp (1995) reported that the organic-N content decreases before NO_3^- from the lower canopy of broccoli during inflorescence development, indicating that vacuolar NO_3^- is mobilized only after organic-N. The amount of N supplied must also be considered as broccoli plants (fertilized with N in a single application), with rates from 0 to 625 kg N ha^{-1} in 125 kg N ha^{-1} steps, retranslocate N from the leaf to the developing inflorescence at the two lowest rates, 0 and 125 kg N ha^{-1}, but this is not evident at the higher N rates (Bowen et al., 1999).

Comparison of two lines of *Arabidopsis* that possess identical shoot biomass under optimum N supply revealed that the line with the higher N shoot concentration is better able to withstand NO_3^- starvation (Richard-Molard et al., 2008). Two recombinant inbred lines, 282 and 432, were grown hydroponically for 28 d in a complete nutrient solution and then subjected to 10 d of N starvation. Line 432 had a higher plant NO_3^- concentration at the beginning of the experiment and produced more biomass than line 282 thoughout NO_3^- starvation. The N remobilization dynamics and capacity to intercept light were similar for both lines, suggesting that these were not a factor in the greater ability of line 432 to withstand NO_3^- starvation. Line 432 had a significantly higher NO_3^- uptake capacity and displayed higher expresssion of two high affinity transport genes (*AtNRT2.1* and *2.4*) and one low affinity transport gene (*AtNRT1.1*). These results were interpreted as evidence for the importance of the size of the N storage pool in determining the capacity of the genotypes to withstand NO_3^- starvation.

Together, these studies provide evidence for the importance of remobilizing organic and NO_3^--N to improve NUE. Nitrate is stored in significant amounts in the vacuole and factors controlling the extent of this storage and remobilization are of particular interest in this thesis. Plants that efficiently remobilize accumulated NO_3^- from the vacuole should theoretically have a higher NUE. It is possible that NUE can be improved by selecting cultivars that are more efficient at remobilizing NO_3^- or by employing management strategies that improve remobilization of NO_3^- stored in the vacuole.

2.8 Strategies for Manipulating Nitrate Status

Management strategies such as the removal of N fertilization at selected points in the crop cycle, or substitution with other anions have been employed to reduce tissue NO_3^- levels for human health reasons. The concentration of NO_3^- decreases in lettuce (*Lactuca sativa* var. *capitata* L.) heads with little decline in yield by removing the N supply at the end of the growing cycle (Blom-Zandstra & Lampe,1983). This strategy is more effective than the addition of NH_4^+ or Cl^-, but the reduced or organic-N concentration is also decreased (van der Boon et al., 1990).

Removal of the NO_3^- supply at the initiation of inflorescence development, rather than its substitution with Cl^- or sulphate (SO_4^{2-}) anions, is an effective means to decrease the NO_3^- content of the inflorescence of broccoli plants, with an approximately 90% reduction in NO_3^- content (Liu & Shelp, 1995). Also, removal of NO_3^- fertilization at 35 or 54 d after sowing significantly reduces the NO_3^- concentration of the leaf tissue in butterhead lettuce (Broadley et al., 2003). Removal of NO_3^- at 35 d after sowing results in

an immediate and almost complete loss of tissue NO_3^-, whereas removal at 54 d results in a less dramatic decline in tissue NO_3^-.

Strategies to replace the vacuolar NO_3^- as an osmoticum or to compete with the uptake of NO_3^- have been employed in order to reduce the NO_3^- levels in plants. Tomato (*Solanum esculentum* L.) plants receiving a constant supply of NO_3^- display a decline in the tissue NO_3^- concentration with increasing Cl^- concentration in the nutrient solution (Kafkafi, 1982). Lettuce plants receiving NO_3^- for 53 d and then Cl^- or SO_4^{2-} instead of NO_3^- for 15 d, display a decrease in leaf NO_3^- concentration, together with an increase in Cl^-, but not SO_4^{2-} (Blom-Zandstra & Lampe, 1983). The NO_3^- concentration declines from 91 mmol kg^{-1} FM to 27 mmol kg^{-1} FM in the outer leaves, while the Cl^- concentration increases from 7 mmol kg^{-1} FM to 44 mmol kg^{-1} FM with Cl^- substitution, suggesting partial substitution of Cl^- for NO_3^- in the leaf.

Moderate amounts of Cl^- in the growing medium can reduce the NO_3^- content of broccoli plants grown under both field and greenhouse conditions (Liu & Shelp, 1996). The shoot NO_3^- content increases linearly with increasing ammonium nitrate (NH_4NO_3) application and decreases linearly with increasing Cl^- application. Notably, there is a significant negative correlation between shoot NO_3^- and Cl^- contents and the Cl^-/ NO_3^- replacement coefficient indicates that an increase of 1 mmol Cl^- g^{-1} DM results in a decrease of 0.3-0.5 mmol NO_3^- g^{-1} DM. The addition of Cl^- to the nutrient solution decreases the FM of the shoot and inflorescense but not the DM of these plant parts, leading the authors to suggest that the water relations of the plants are affected by the addition of Cl^-.

Replacement of potassium chloride (KCl) for potassium nitrate (KNO_3), either at the beginning or at a later point in the crop cycle (12 or 47 days after planting), in greenhouse tomato production has been shown to improve fruit quality and lower NO_3^- content (Chapagain et al., 2003). The fruit NO_3^- content is inversely proportional to the Cl^- content of the nutrient solution, whereas quality indices such as total soluble solids, glucose and DM trend to be higher with increasing Cl^-. As the authors note, economic benefits may result from the use of KCl as it is less expensive than KNO_3 and there would be less risk of environmental damage. Greenhouse tomatoes in a closed loop system can be grown with Cl^- levels as high as 12.7 mM without affecting yield or causing any physological disorders (Mizrahi et al., 1988).

Other research has focused on the replacement of the NO_3^- supply with NH_4^+; the NO_3^- concentration of winter and summer-grown lettuce for example, is reduced by 18% and 8%, respectively, when 20% of the N is supplied in the NH_4^+ form, as compared to supplying N solely in the NO_3^- form (van der Boon et al., 1990). Increasing the relative NH_4^+ content of the nutrient solution to 50% or 80% shortly before harvest markedly decreases the NO_3^- concentration and subsequently increases the reduced-N content of the lettuce. The NO_3^- concentration of head lettuce is significantly reduced when 40% of the N is supplied as NH_4^+, with no effect on FM or DM of the crop (McCall & Willumsen, 1998).

The optimal NH_4^+:NO_3^- ratio (mM/mM) for greenhouse-grown broccoli is 75:25 (Liu & Shelp, 1993a). At this ratio, the greatest inflorescence FM and shortest time to harvest are obtained, along with minimal accumulation of NO_3^-. The authors noted that optimal NH_4^+:NO_3^- ratios need to be determined for each crop species since a 50:50 ratio appears

to give maximal growth and minimal NO_3^- accumulation for spinach (Mills et al., 1976) and a ratio of 25:75 for chinese cabbage (*Brassica chinensis* L.) gives maximal growth, while increasing amounts of NH_4^+ in the nutrient solution decreases tissue NO_3^- content (Chen et al., 2005). In hydroponically-grown lettuce cultivars, the lowest tissue NO_3^- concentration is found at an NH_4^+:NO_3^- ratio of 50:50, while the highest biomass of roots and shoots is found at a ratio of 25:75 (Wang & Shen, 2011).

2.9 Chrysanthemum Nutrition

It has been well established that chrysanthemums have a high requirement for N early in their growth cycle. The first 7 weeks of a cut flower chrysanthemum crop is critical in regards to N nutrition, as application of N after this period has little effect on flower size or quality (Lunt & Kofranek, 1958). Chrysanthemum plants continually fed 14.3 mM N accumulate DM and N up to week 6, the start of flower development, and thereafter the accumulation rate decreases (Woodson & Boodley, 1983). Interestingly, the NO_3^--N content of the leaves remains steady at approximately 49 mg from week 6 onwards, while the reduced N of the leaves peaks and then remains steady at week 8. Nitrogen content (NO_3^- and reduced N) is lost from the stem plus petioles between weeks 6 and 9, leading the authors to speculate that during inflorescence development newly absorbed N is inadequate to supply the N needs of the inflorescence.

As previously noted, chrysanthemums accumulate large quantities of NO_3^- early in the growth cycle. Plants grown with 1 mM NO_3^- accumulate about 42% of the total-N as NO_3^- after 9 d, whereas over the next 21 d about 30% is accumulated as NO_3^- (Elliott & Nelson, 1983). When plants are grown subjected to increasing NO_3^--N concentrations

(0.25 – 5 mM), both NO_3^- and total-N accumulations are positively correlated with NO_3^- supply. Sand-cultured plants grown with 3.75 mM NO_3^--N remobilize a significant amount of reduced-N from vegetative tissues during inflorescence development compared to those grown with 15.0 mM NO_3^-. The NO_3^- content of the leaves and stem also declines during inflorescence development under both fertilization regimes, indicating an increasing dependence on previously accumulated NO_3^- for reduction, along with previously reduced-N, as newly absorbed N is inadequate to supply the N needs of the developing flower (Woodson et al., 1984). These authors suggest that N absorbed during infloresence development is inadequate to supply the developing infloresence, but it appears that uptake of N declines after visible bud (Yoon et al., 2000). Chrysanthemum plants must then rely on previously accumulated N for infloresence N supply, as the uptake of N declines at this point.

Commercial fertilizer recommendations for potted chrysanthemums suggest the feeding of 17.9 to 28.6 mM, primarily in the NO_3^- form, using soilless media (Dole & Wilkins, 2005). Aris Horticultural Services, a major supplier of chrysanthemum cuttings, recommends feeding 21.4 to 28.6 mM of N, and reducing NO_3^--N by 25% to 50% when using subirrigation rather than overhead irrigation (Aris, 2010).

2.10 Stay Green Character

Potted chrysanthemum cultivars have been bred and selected for intense green leaves and retention of lower leaves during flowering as this crop is sold solely for its aesthetic value (Elliott & Nelson, 1983). Dry mass continues to accumulate in leaves of chrysanthemum during inflorescence development, yet there is a significant loss of N

from the vegetative tissues, primarily the stems plus petioles; this pattern is similar to "stay green" cereal mutants (Woodson & Boodley, 1983).

In cereal stay green mutants, leaf chloroplasts do not degrade as early during grain development and grain DM increases (Borrell et al., 2001; Masclaux et al., 2001). The selection of cereal cultivars that store N in plant parts other than leaves, such as internodes, has been suggested as a means to increase N remobilization, without reducing plant photosynthetic capacity (Bertheloot et al., 2008). This selection process appears similar to the selection process for chrysanthemum breeding (Elliott & Nelson, 1983).

2.11 Greenhouse Plant Production and Subirrigation

Production of plants in greenhouses is an intensive form of agriculture that requires application of inputs such as fertilizer and pesticides. The intensive use of fertilizer can negatively influence the environment by leaching nutrients into the groundwater; furthermore, nutrients are often oversupplied even when technology can be used to regulate the supply of plant nutrients to meet plant needs (Molitor, 1990; Blom & Piott, 1992; Cox, 2001; Glass, 2003). As previously mentioned (see Thesis Introduction), the MOE is now enforcing existing legislation that restricts the discharge of nutrients from a greenhouse operation. Recently, the levels of NO_3^--N in Sturgeon Creek in Essex County were found to be almost 20 times the allowable limit and greenhouse discharge was found to be the source of that pollution (Ontario Ministry of the Environment, 2012). The average greenhouse NO_3^--N outfall, calculated from the mean of 94 samples at 13 sites in 2010 and eight sites in 2011 was 6.5 mM, well above the Canadian Council of the Ministers of the Environmental guideline of 0.2 mM.

The adaptation of closed systems (zero runoff) such as “ebb-and-flow” eliminates or at the very least, greatly reduces nutrient leaching from the greenhouse (Klock-Moore & Broschat, 1999; James & van Iersel, 2001). With this system plants are grown on benches, troughs or “flood floors” whereby the water and/or nutrient solution are pumped onto the bench and the nutrient solution enters the bottom of the pot non-selectively by capillary action (Reed, 1996; Cox, 2001). The unused water or nutrient solution remains virtually unchanged in composition, and unused nutrients within the pot can accumulate in the upper part of the substrate because drainage does not occur or is limited. This is in contrast to top irrigation systems (drip or hose watering) where the solution runs through the substrate and nutrients may accumulate in the leachate (Reed, 1996; Rouphael et al., 2008; Rouphael & Colla, 2009). With the closed “ebb-and-flow” system there is less fertilizer usage, particularly N, lower input costs, less salt build up in the nutrient solution, increased crop longevity, and the nutrient solution can be recirculated so that there is no runoff of nutrients from the greenhouse (Nell et al., 1989; Blom & Piott, 1992; Kent & Reed, 1996; Incrocci et al., 2006; Nelson, 2009).

2.12 Media Electrical Conductivity

The use of subirrigation systems with recirculation, while environmentally friendly, can pose serious problems in regards to soluble salt build-up in the top of the growing medium, due to the absence of leaching (Molitor, 1990; Cox, 2001). A comparison of chrysanthemum plants grown in a peat:rockwool medium using a high volume ‘Chapin’ and a subirrigation system revealed that the medium EC at flowering was significantly higher in the subirrigation system (Blom & Piott, 1992). Roots and root hairs were absent

at the surface of the pots in subirrigated plants, yet present in the 'Chapin' watered plants. Salt stratification of the medium has been observed in New Guinea impatiens (*Impatiens x hawkeri* Bull.) grown in a subirrigation system, as the top one third of the medium has EC readings two to five times those of the middle and bottom portions, independent of the N treatments (Kent & Reed, 1996). Similarly, subirrigation of poinsettias results in greater EC values in the top one third of the pot medium than the use of traditional, high volume, overhead irrigation (Cox, 2001).

Tomato plants grown under saline conditions using subirrigation have a strong gradient of salts in the substrate, as compared to high volume drip irrigation (Incrocci et al., 2006). Due to moisture evaporation from the medium (peat:perlite) surface the upper one-third of the substrate employing subirrigation accumulates salts, resulting in EC values up to 13 mS/cm, as compared to EC values of 4.0 mS/cm for drip irrigation, and corresponding losses in root growth as assessed by visual observations. The lower one third of the substrate employing subirrigation has a much lower EC value (approximately 4 mS/cm), whereas with drip irrigation the EC value is 6.0 mS/cm.

Salt build-up in the medium with subirrigation can be controlled to some extent by reducing fertilizer N rates. Baltic ivy (*Hedera helix* L.) plants grown with subirrigation produce satisfactory plants with fertilizer rates as low as 3.6 mM N, which is well below the recommended fertilizer rate for this crop (Holcomb et al., 1992). Reducing N from 17.9 to 12.5 mM in a subirrigation system results in poinsettia plants with approximately 10% greater height and DM. By contrast, the application of 12.5 mM N using overhead irrigation produces plants of less DM than with 17.9 mM N (Dole et al., 1994). Salt stratification of the medium for ornamental peppers (*Capsicum annuum* L.) does not

occur in a subirrigation system using low N concentrations (7.1 mM N) in the nutrient solution, although higher quality plants are produced with 14.3 mM N, where stratification does occur (Kang et al., 2004).

Medium EC recommendations of potted chrysanthemums, as determined by the saturated media extract (SME) method, are 0.8-1.5 mS/cm during root establishment, 1.7-3.0 mS/cm during the growing period, and 0.8-1.5 mS/cm during finishing (Aris, 2010). Reduction or elimination of fertilization is recommended 2 to 3 weeks prior to shipping.

2.13 Reduction of Fertilizer Prior to Shipping

The establishment of the need for N early in the crop cycle and lower demand later in the crop cycle of chrysanthemum led to research on the termination of fertilizer application as the plant enters the generative phase. Many early studies with chrysanthemum demonstrated that heavy N fertilization early in the production cycle, followed by lower N fertilization levels later in the cycle, produce the best quality chrysanthemum plants (Lunt & Kofranek, 1958; Waters, 1967; Roude et al., 1991a). Other work demonstrated that elimination of fertilizer N application 3 weeks before flowering in potted chrysanthemums increases the shelf life by up to 7 d, and heavy N fertilization decreases postharvest longevity, probably as a result of high medium conductance (Nell et al., 1989; Nell et al., 1997). Further experiments showed that reducing the fertilizer N concentration of water soluble fertilizer increases flower longevity of potted chrysanthemums from 21 to 34 d; this is also associated with a reduction in medium EC (Roude et al., 1991a). Damage to the root system and high

medium conductance (low osmotic potential) can limit water uptake and post harvest life of the plant (Roude et al., 1991b).

Reducing or eliminating fertilizer near the end of crop cycle also reduces the cost of production, as fertilizer costs are becoming a major production cost in floriculture. Fertilizer prices have been volatile lately, while margins have been declining due to downward pressure on prices (Nelson, 2009; Brown & Murphy, 2011).

2.14 Concluding Remarks

Reduction, correct timing, form or substitution of fertilizer N can possibly lead to the improvement of NUE in chrysanthemums. As previously mentioned, chrysanthemum plants accumulate N, and in particular NO_3^-, early in the growth cycle and later remobilize this N during floral development when uptake of NO_3^- declines. Employing management strategies such as the reduction of fertilizer N application during inflorescence development to increase the mobilization of NO_3^- could possibly improve NUE, as NO_3^- would move from the storage pool into the metabolically active pool. While this knowledge has been available for decades, commercial growers are reluctant to reduce or eliminate fertilizer N at the end of the crop cycle due to fear of compromising quality (Hall et al., 2009).

With the increased, and possibly mandated, use of subirrigation in commercial greenhouses, growers need to adapt growing methods to fit these systems. Problems can arise in subirrigation systems as these systems do not allow for leaching, as water and nutrient flow is unidirectional and salts accumulate in the upper layer of the pot.

Integration of methods to improve the mobilization of NO_3^-, such as reduction or elimination of NO_3^- application during floral development, could benefit growers by improving the use efficiencies of the applied NO_3^-, resulting in the same biomass with less N and reducing the salt build up common with subirrigation. Other strategies to improve the mobilization of NO_3^-, such as the replacement of NO_3^- with Cl^-, could also improve NUE. Addition of NH_4^+-N to the nutrient solution, with the subsequent reduction in applied NO_3^-, could also be a strategy to improve NUE in chrysanthemum.

2.15 Hypothesis and Objectives

The hypothesis of this thesis is that nitrogen use efficiency of subirrigated potted chrysanthemum (*Chrysanthemum morifolium* Ramat.) can be improved by managing the NO_3^- status of the plant.

To test this hypothesis, a subirrigation production system was employed to test the following management strategies: removal of fertilizer N at floral development; reduction in rate of fertilizer N applied over the growing period; application of Cl^- in the nutrient solution; and, addition of NH_4^+ to the nutrient solution.

CHAPTER 3: Materials and Methods

3.1 Experimental Set-up

Four ebb-and-flow benches measuring 1.7 m wide and 2.5 m long were used to grow potted chrysanthemums. Each ebb-and-flow bench was divided length-wise into three equal sections using 7.5 cm high plexiglass dividers (Fig. 3.1) so that each section was 0.55 m (wide) by 2.5 m (long). The result was 12 equal sized sections of ebb-and-flow benches.

Each of the 12 sections was irrigated using a submersible pump (Little Giant Pump Company, Oklahoma City, OK) that was placed in an 80-L Rubbermaid container (Rubbermaid Canada, Oakville, ON). Nutrient solution was pumped from the container into the bottom of the ebb-and-flow bench via a supply hose, to provide a solution depth of approximately 3 cm, and after 5 min was allowed to drain back into the container. The nutrient solution was in contact with the bottom of the pots for approximately 10 min.

The ebb-and-flow benches were in a glass-covered greenhouse at the University of Guelph in a side-by-side pattern, with 0.6 m between each bench. A metal frame was constructed over each ebb-and-flow bench to support a “black-out cloth”. This cloth was used for photoperiod control and was manually pulled daily at 1700 h over the benches and withdrawn at 0800 h in order to provide a flower inductive photoperiod (15 h of darkness). The first experiment (NO_3^- removal by substituting Cl^- for NO_3^-) was conducted during the Spring and Summer of 1990. A light white washing was applied to the greenhouse over the summer months. The second experiment was conducted in late

summer and fall of 1991, without white washing the greenhouse roof. No high intensity discharge lighting was added to the crop. Incandescent light bulbs (60W, GE Canada, Mississauga, ON), spaced 30 cm apart, were placed 120 cm above the crop and lit from 2200 to 0200 h to provide non flower-inductive long days. The temperature in the greenhouse was maintained at an average day/night temperature of 26 ^{o}C /15 ^{o}C.

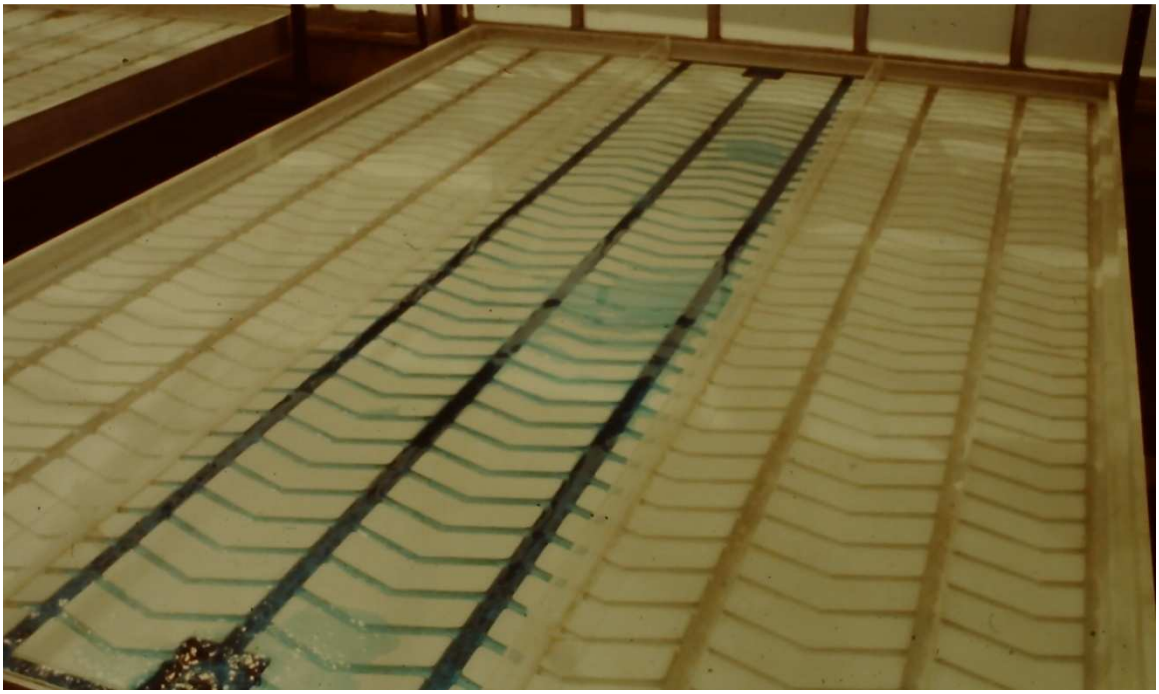


Figure 3.1 Flooding of an ebb-and-flow bench. Plexiglass dividers split the bench into three equal sections.

3.2 Plant Culture

Rooted cuttings of *Chrysanthemum morifolium* Ramat. 'Yellow Favor' were obtained from Aldershot Greenhouses Ltd., Burlington, ON. 'Yellow Favor' has a 9-week photoperiodic response time. The chrysanthemum cuttings were planted one per 10 cm diameter (0.42 L) azalea plastic pot (Kord Products Inc., Burlington, ON). The medium was composed of 50% Canadian sphagnum peat moss (Premier Horticulture, Rivière-du-Loup, PQ) and 50% perlite (Grace Canada, Ajax, ON) (v:v). This mixture was amended with 7.1 kg of ground limestone and 1.8 kg of 0-20-0 (super phosphate) per m^3 of peat moss. The initial medium pH and EC were 5.4 and 0.2 mS/cm, respectively. The plants were watered in with reverse osmosis (RO) water.

After transplanting (week=0), the chrysanthemum plants were subjected to 2 weeks of non flower-inductive long days, after which time the plants were subjected to flower-inductive short days as described above until flowering (Section 3.1). As plants developed, all axillary buds were removed, allowing only the terminal bud to continue, resulting in single stemmed flowering plants.

3.3 Plant Sampling

Destructive plant sampling was performed every 2 weeks. The shoots were removed at soil level and separated into leaves, stem plus petioles, and flower, when present.

Measurements of the following parameters were taken: height, measured as the distance from the soil surface to the highest point on the plant; number of leaves; total leaf FM and DM; total stem plus petiole FM and DM; and flower FM and DM when appropriate. Leaves were removed during destructive harvesting and leaf area (LA) was

measured using a LI-COR area meter (model 3100, LI-COR Environmental, Lincoln, Nebraska, USA).

In the *Ammonium and Nitrate Nitrogen Nutrition* experiment (section 3.6.2) plants were separated into upper and lower leaves and stem plus petioles. This was done by measuring the bottom twelve leaves separately from the leaves and stem plus petioles that developed above these leaves.

All plant parts were triple rinsed with RO water to remove any adhering surface particles, and then rapidly dried between paper towels before FM was obtained. The plant parts were oven dried at 70 ^{o}C for 72 h. Prior to chemical analysis, the plant parts were ground to a fine powder using an electric coffee grinder. The individual plant parts (stem plus petioles, leaves and flower) were analyzed for NO_3^--N and total-N as described below (Sections 3.5.1 and 3.5.2)

3.4 Medium Sampling

The soil medium was removed from the pot and cut in half across the vertical axis. The top and bottom halves were analysed for pH and EC by the SME method (Warncke & Krauskopf, 1983). Samples, which were not analyzed immediately, were frozen for later analysis.

3.5 Chemical Analysis

3.5.1 *Total Nitrogen*

Total-N was determined by micro-Kjeldahl with salicylic pre-digestion for recovery of NO_3^- (Eastin, 1978). Approximately 250 mg of oven dried plant material was placed in a

75 mL digestion tube. Twenty-five grams of salicylic acid was dissolved in 1 L of concentrated H_2SO_4 and 10 mL of the mixture was added to each tube and allowed to sit overnight. Then 0.5 g of sodium thiosulfate, $Na_2S_2O_3$, was added to each tube and one "Kjel-tab" was added to each tube as a catalyst. Each Kjel-tab contains 1.5 g K_2SO_4 + 0.15 g $CuSO_4$. This mixture was heated in an electrically heated aluminum block at 375 ^oC and digested for 1.5 h. The clear digest was allowed to cool and diluted to the dilution line of 75 ml with distilled water. The NH_4^+ formed was determined colorimetrically after reaction with salicylic acid in the presence of hypochlorite and nitroprusside to form an emerald green complex. Color intensity was measured on a Technicon Auto Analyzer II at 660 nm, with a standard curve prepared at the beginning and mid point of each day from a series of prepared standards (Kalra & Maynard, 1991).

3.5.2 *Nitrate Nitrogen*

NO_3^--N was determined as the nitrification of salicylic acid according to the method described by Cataldo et al. (1975). Approximately 100 mg of oven dried plant material was placed in each 50 mL Erlenmeyer flask and 30 mL of distilled water was added. This mixture was shaken at a speed of 250 rpm for 30 min and then filtered through Whatman #42 filter paper. Nitrate concentrations in the filtrate were determined colorimetrically by the cadmium reduction method on a Technicon Auto Analyzer II at 520 nm, with a standard curve prepared at the beginning and mid point of each day from a series of prepared standards (Kalra & Maynard, 1991).

3.5.3 *Nitrogen Reporting*

With our methods, NO_3^--N was recovered in total-N; therefore, organic-N was estimated as total-N minus NO_3^--N. All organic-N and NO_3^--N values are reported as mg N.

3.6 Treatments

3.6.1 *Nitrate Removal and Substitution of Chloride for Nitrate*

3.6.1.1 Planting, short day and final harvest dates

Rooted cuttings were obtained on June 11, 1990, and planted the following day. The plants were then subjected for 2 weeks to non flower-inductive long days, followed by flower-inductive short days beginning on June 26. The experiment ended on August 21, 1990, when the flowers first displayed pollen, 10 weeks after transplanting, and 8 weeks after the beginning of short days. 'Yellow Favor' is classified as a 9-week response time cultivar, but some cultivars are known to flower earlier under summer conditions as occurred with this experiment (Ball, 1991).

3.6.1.2 Nutrient solution

All experimental units received a modified Sonneveld solution (Sonneveld & Kreij, 1987) for the first 5 weeks after transplanting. The macronutrient solution was prepared using RO water with (in mM): 2.6 KH_2PO_4, 6.75 $Ca(NO_3)_2$, 5 KNO_3, 0.75 K_2SO_4, and 1.5 $MgSO_4$, resulting in 18.5 NO_3^-, 2.6 $H_2PO_4^-$, 2.25 SO_4^{2-}, 9.1 K^+, 6.75 Ca^{2+} and 1.5 Mg^{2+}. Micronutrients were added to the macronutrient solution and prepared (in µM): 25 FeEDTA, 5.0 $MnSO_4$, 3.5 $ZnSO_4$, 5.0 $Na_2B_4O_7$, 0.75 $CuSO_4$, 0.5 Na_2MoO_4, resulting in (µM) 25 Fe^{3+}, 5 Mn^{2+}, 3.5 Zn^{2+}, 5 $B_4O_7^{2-}$, 0.75 Cu^{2+}, and 0.5 MoO_4^{2-}.

3.6.1.3 Substitution nutrient solutions

Six treatments were applied to the experimental units commencing 5 weeks after transplanting (July 17): the modified Sonneveld nutrient solution was continued for the duration of the experiment (18.5 mM NO_3^--N); the nutrient solution was replaced with RO water at every other irrigation (18.5:0 mM NO_3^--N); the nutrient solution was replaced with RO water (0 mM NO_3^--N); the nutrient solution was modified to contain 15.5 mM NO_3^- by reducing the $Ca(NO_3)_2$ to 5.25 mM from 6.75 mM and adding 1.5 mM $CaCl_2$ (15.5 mM NO_3^--N); the nutrient solution was modified to contain 12.5 mM NO_3^- by reducing the $Ca(NO_3)_2$ to 3.75 mM from 6.75 mM and adding 3.0 mM $CaCl_2$ (12.5 mM NO_3^--N); and, the nutrient solution was modified to contain 9.5 mM NO_3^- by reducing the $Ca(NO_3)_2$ to 2.25 mM from 6.75 mM and adding 4.5 mM $CaCl_2$ (9.5 mM NO_3^--N). Plants were irrigated every second day up to week 6 of the experiment (July 24, 1990), at which point the plants were irrigated every day due to their larger size.

3.6.1.4 Experimental design

Fifty-six individually potted plants were placed in each of the 12 ebb-and-flow sections. The six treatments were randomly assigned to the 12 sections, so that each treatment was replicated twice. Thus, the experimental design was a Completely Randomized Design with two replicates. Five plants were randomly subsampled from each section every two weeks. The use of extra plants in the design ensured that border plants were not collected and that more than five plants were available at the end of the experiment.

3.6.2 *Ammonium and Nitrate Nitrogen Nutrition*

3.6.2.1 Planting, short day and final harvest dates

Rooted cuttings were obtained on August 26, 1991 and planted the next day. The cuttings were subjected to two weeks of non flower-inductive long days, followed by eight weeks of flower-inductive short days. The experiment ended on November 5, 1991, 10 weeks after transplanting when the flowers first displayed pollen.

3.6.2.2 Nutrient solutions

Two nutrient solutions were employed in this experiment, with the first nutrient solution containing 18 mM NO_3^--N and 0 mM of NH_4^+-N (18:0) and the second solution containing 9 mM NO_3^--N and 9 mM of NH_4^+-N (9:9). Each solution was supplied at either 200 or 400 mg N per pot, resulting in four treatments. The macronutrient solution for the 18:0 treatment was prepared using RO water with (in mM): 3.0 KH_2PO_4, 4.5 $Ca(NO_3)_2$, 5 KNO_3, 1.0 $MgSO_4$, 2.0 $Mg(NO_3)_2$, resulting in 18.0 NO_3^-, 3.0 PO_4^-, 1.0 SO_4^{2-}, 8.0 K^+, 4.5 Ca^{2+}, and 3.0 Mg^{2+}. The macronutrient solution for the 9:9 treatment was prepared using RO water with (in mM): 3.0 KH_2PO_4, 4.5 $Ca(NO_3)_2$, 2.5 K_2SO4, 3.0 $MgSO_4$, 4.5 $(NH_4)_2SO_4$, resulting in 9.0 NO_3^-, 3.0 PO_4^- , 10.0 SO_4^{2-}, 8.0 K^+, 4.5 Ca^{2+}, 3.0 Mg^{2+}, and 9.0 NH_4^+. Micronutrients were added to the macronutrient solution (µM): 25 FeEDTA, 5.0 $MnSO_4$, 3.5 $ZnSO_4$, 5.0 $Na_2B_4O_7$, 0.75 $CuSO_4$, 0.5 Na_2MoO_4, resulting in 25 Fe^{3+}, 5 Mn^{2+}, 3.5 Zn^{2+}, 5 $B_4O_7^{2-}$, 0.75 Cu^{2+}, and 0.5 MoO_4^{2-}. Fresh solutions were made up on the following dates: August 29, September 4, September 13 and September 19.

3.6.2.3 Determination of nitrogen supplied

The 80 L Rubbermaid containers were graduated in 500 mL increments in order that the amount of nutrient solution applied to the crop could be determined. Preliminary irrigations were done without a crop on the benches and it was determined that 500 mL of nutrient solution did not return to the Rubbermaid container as this solution remained in crevasses on the ebb and flow benches and did not enter a pot. This 500 mL of remaining nutrient solution was not taken into account for nutrient solution calculations as the water evaporated within 24 h and the remaining fertilizer adhered tightly to the bench.

Determination of the amount of nutrient solution delivered to each pot was calculated by the volume of nutrient solution delivered to the pots divided by the number of pots in each section of the ebb and flow bench.

Two hundred and 400 mg of N were delivered to the pots by 3 and 5 weeks after transplanting, respectively. After this point the plants were irrigated with RO water. Plants were irrigated every second day during the experiment.

3.6.2.4 Experimental design

Fifty-six individually potted plants of 'Yellow Favor' were placed in each of the 12 sections of the ebb and flow benches. The four treatments were randomly assigned to the 12 sections so that each treatment had three replicates. Thus, the experimental design was a Completely Randomized Design with three replicates for each treatment. Four plants were randomly subsampled from each section every two weeks. The use of extra plants in the design ensured that border plants were not collected and that more than five plants were available at the end of the experiment. It was necessary to pool two plant subsamples to create a sample large enough for analysis of total N and NO_3^-;

consequently, the number of subsamples for this chemical analysis was two, rather than four as described as above.

3.7 Nitrogen use indices formulae

Nitrogen use indices were calculated according to formulae summarized by Good et al. (2004). Nitrogen harvest index (NHI) was calculated by the formula given by Lecoeur & Sinclair (2001). Where grain N content and DM was defined in the original formula, flower N content and DM was used in our calculations. A summary of these formulae is given in Table 3.1.

Table 3.1. Formulae of nitrogen use efficiencies used in this thesis from Good et al. (2004) and nitrogen harvest index (NHI) from Lecoeur & Sinclair (2001).

Term	Formula
Nitrogen Usage Index (NUI)	NUI = shoot DM * (shoot DM / shoot N content)
Nitrogen Uptake Efficiency (NUpE)	NUpE = shoot N content / N supply
Nitrogen Utilization Efficiency (NUtE)	NUtE = flower DM / shoot N content
Nitrogen Use Efficiency$_{flower}$ (NUE)	NUE$_{flower}$ = flower DM / N supply
Nitrogen Harvest Index (NHI)	NHI = flower N content / shoot N content

3.8 Statistical Analysis

All data were analysed using CropStat for Windows version 7.2 2007. 3 (International Rice Research Institute, DAP Box 7777 Metro Manila, Philippines). The data are presented as the mean of the subsamples ± SE, but significance was determined with the planned comparison LSD test at the 95% confidence level. Analysis of variance of a CRD with equal subsample numbers was conducted according to Steele and Torrie (1980). Means and residual values were analyzed to ensure the assumptions of variance were met. Homogeneity of variance, normal and independent distribution, and transformable non-additivity were tested with Bartlett's test, the Anderson-Darling statistic and Tukey's test, respectively. No transformation of the data was required.

CHAPTER 4: Results

4.1 Nitrate Removal and Chloride Substitution for Nitrate

4.1.1 *Electrical Conductivity of Pot Media as a Function of Plant Development*

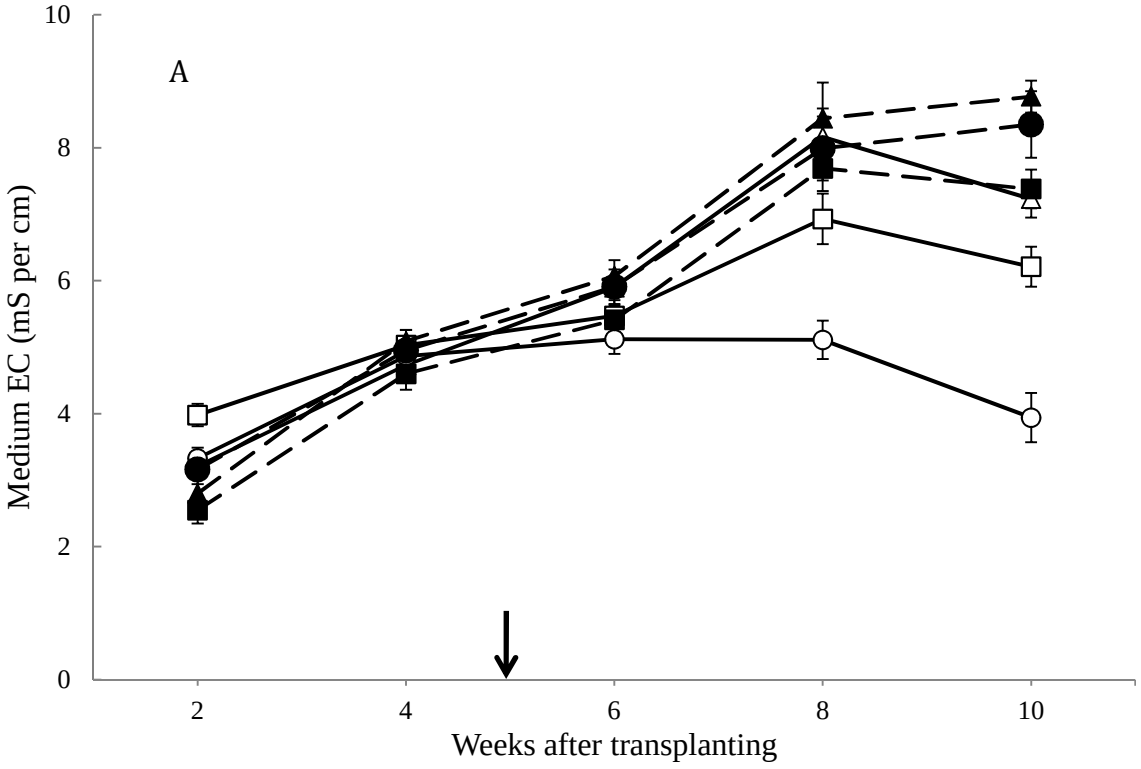
During the 10-week experiment, the EC of the medium in the upper and lower halves of each pot was determined to assess the effectiveness of the various NO_3^- and Cl^- treatments. Replacement of the full NO_3^- supply in the final five weeks with water (0 mM) or an alternating supply of full NO_3^- and water (18.5:0 mM) increasingly reduced the EC of the upper and lower halves over the remainder of the time course, with the lower half of the pot in the 0 mM treatment decreasing to nearly 0 mS/cm (Fig. 4.1). By contrast, partial replacement of the NO_3^- with Cl^- generally had no significant effect on the EC, either in the top or bottom of the pot. Thus, the EC of the pot medium responded to the treatments as predicted.

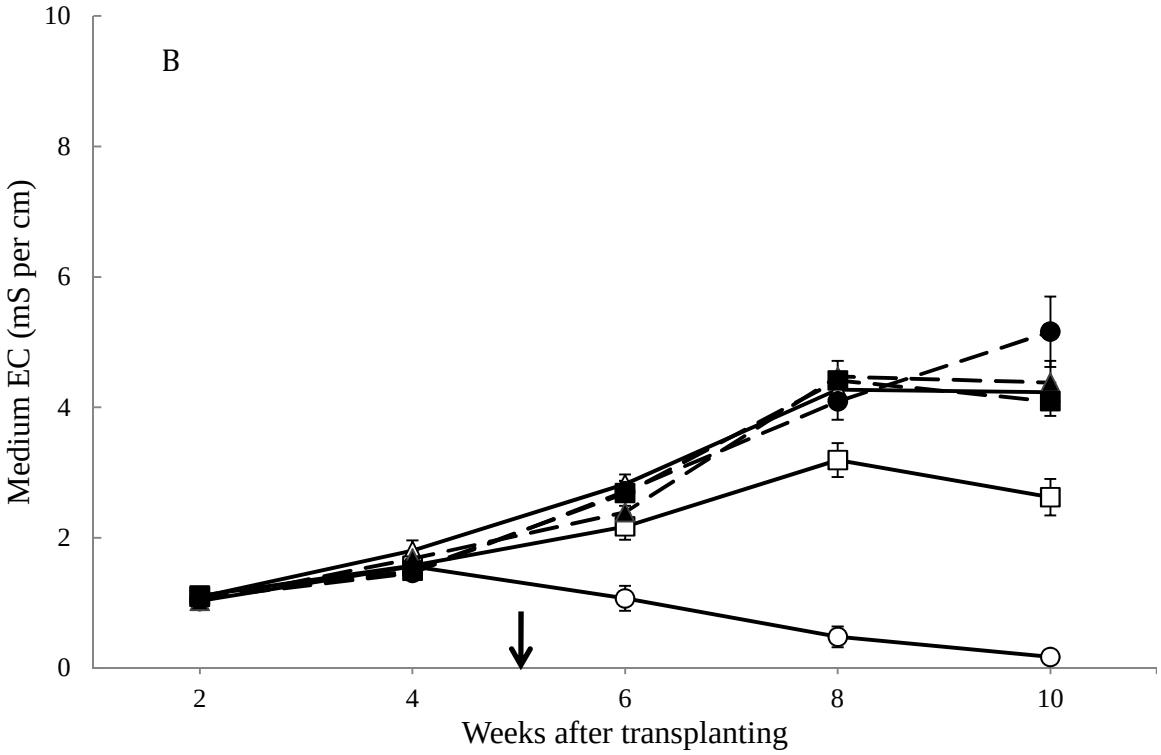
4.1.2 *Yield and N Accumulation as Function of Plant Development*

The impact of the various NO_3^- and Cl^- treatments on the accumulation of FM and DM of various plant parts, as well as their N contents (total-N, NO_3^--N and organic-N), over the growth cycle is shown in Figs 4.2-4.7. In all treatments, leaf FM accumulated over the entire experiment, but the FM of stems did not continue to accumulate after approximately 6 weeks (Figs. 4.2-4.7, panel A). Overall, there was only limited DM accumulation after week 8, at which time the flower started to develop. The flowers accumulated 4.8-6.0 g and 0.8-0.9 g of FM and DM, respectively, by the end of the experiment. The flowers accounted for approximately 10% of the shoot DM.

The leaves, stem plus petioles, and flower accumulated considerably more organic-N than NO_3^--N throughout development of the plant. The leaves in particular continued to accumulate organic-N to the end of the experiment, with no apparent difference in trends among the treatments (Figs. 4.2-4.7, panel B). The leaves also accumulated NO_3^- over time, but the rate of accumulation toward the end seemed to be positively related to the NO_3^- supply. On the other hand, the accumulation of organic-N in the stem plus petioles increased in a linear fashion only up to 8 weeks for all treatments, and this was followed by a decline (Figs. 4.2-4.7, panel C). However, NO_3^- accumulation late in development seemed to be positively related to the NO_3^- supply. The flower (including calyx) accumulated primarily organic-N and only minor amounts of NO_3^-, while total-N accumulation appeared to be positively related to NO_3^- supply (Figs. 4.2-4.7, panel D).

Figure 4.1. Medium electrical conductivity (EC) of the upper (A) and lower (B) halves of the pots containing *Chrysanthemum morifolium* 'Yellow Favor' supplied with various combinations of NO_3^- and Cl^-. In all cases, 18.5 mM NO_3^--N was supplied in each irrigation for the first 5 weeks, whereas NO_3^- was supplied continuously (18.5 mM, open triangles, Δ), in alternate irrigations with water (18.5:0 mM, open squares, □), removed completely (0 mM, open circles, ○), or replaced partially with Cl^- (15.5 mM, closed triangles, ▲; 12.5 mM, closed squares, ■; or 9.5 mM, closed circles, ●) in the second five weeks. The treatment description indicates the NO_3^--N concentration (mM) supplied in the second five weeks of the experiment. Data represent the mean ± SE (N = 10); where the bar is not shown, it is within the symbol. The x-axis shows the number of weeks after transplanting and the arrow on x-axis represents commencement of treatments.


A
Medium EC (mS per cm)
10
8
6
4
2
0
2
4
6
8
10
Weeks after transplanting


B
Medium EC (mS per cm)
10
8
6
4
2
0
2
4
6
8
10
Weeks after transplanting

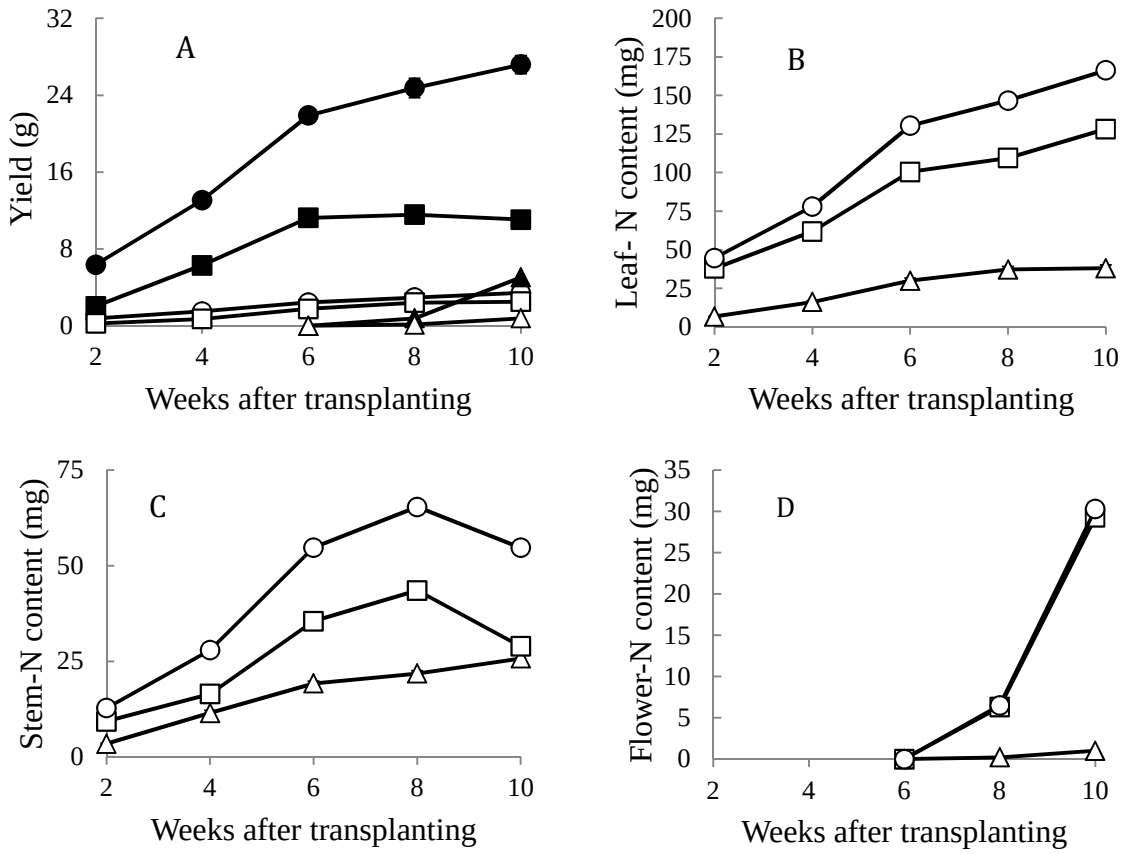


Figure 4.2. Cumulative characteristics of *Chrysanthemum morifolium* 'Yellow Favor' supplied with 18.5 mM NO_3^--N for the second 5 weeks of the growth cycle. See Figure 4.1 for full description of treatments. A. Yield: leaf fresh mass (FM), closed circles (●); leaf dry mass (DM), open circles (○); stem plus petioles FM, closed squares (■); stem plus petioles DM, open squares (□); flower FM, closed triangles (▲); flower DM, open triangles (Δ). B. Leaf-N content: NO_3^--N, open triangles (Δ); organic-N, open squares (□); total-N, open circles (○). C. Stem plus petioles-N content: NO_3^--N, open triangles (Δ); organic-N, open squares (□); total-N, open circles (○). D. Flower-N content: NO_3^--N, open triangles (Δ); organic-N, open squares (□); total-N, open circles (○). The data represent the mean ± SE (N = 10); where bar is not shown, it is within the symbol.

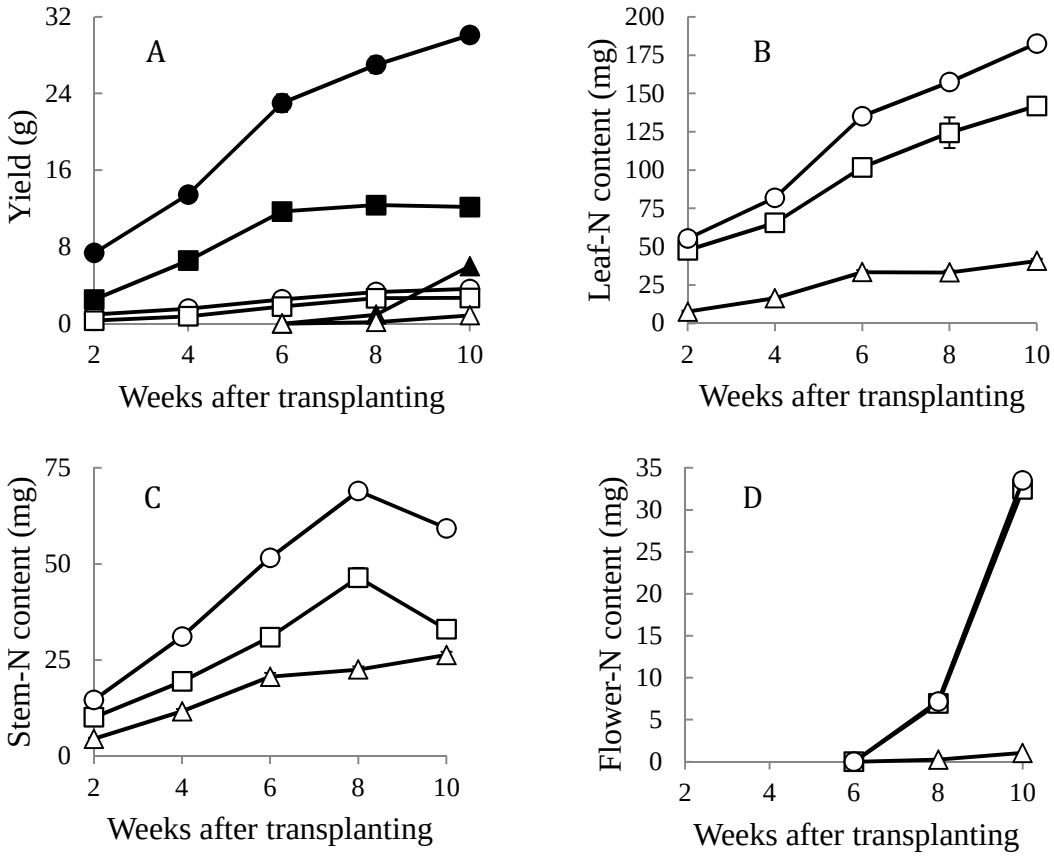


Figure 4.3. Cumulative characteristics of *Chrysanthemum morifolium* 'Yellow Favor' supplied with alternating 18.5 mM NO_3^--N and water for the second 5 weeks of the growth cycle. See Figure 4.1 for full description of treatments. A. Yield: leaf fresh mass (FM), closed circles (●); leaf dry mass (DM), open circles (○); stem plus petioles FM, closed squares (■); stem plus petioles DM, open squares (□); flower FM, closed triangles (▲); flower DM, open triangles (Δ). B. Leaf-N content: NO_3^--N, open triangles (Δ); organic-N, open squares (□); total-N, open circles (○). C. Stem plus petioles-N content: NO_3^--N, open triangles (Δ); organic-N, open squares (□); total-N, open circles (○). D. Flower-N content: NO_3^--N, open triangles (Δ); organic-N, open squares (□); total-N, open circles (○). The data represent the mean ± SE (N = 10); where bar is not shown, it is within the symbol.

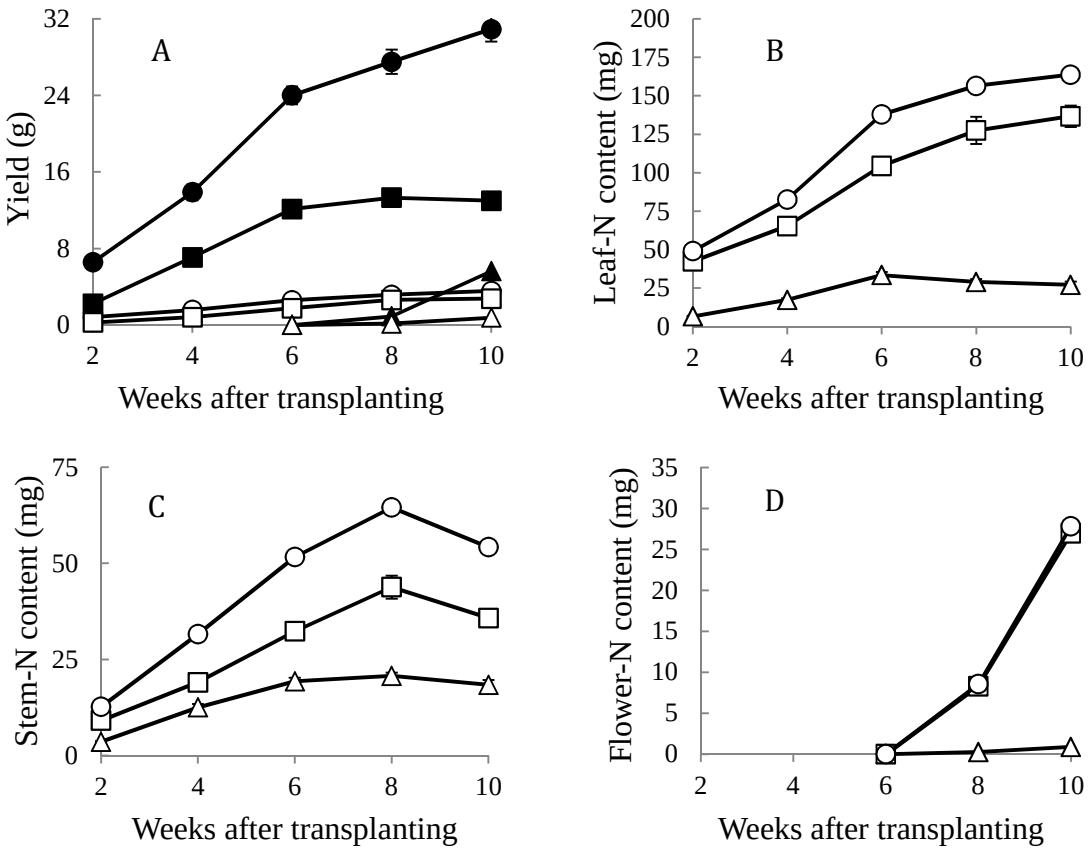


Figure 4.4. Cumulative characteristics of *Chrysanthemum morifolium* 'Yellow Favor' supplied with water (0 mM NO_3^--N) for the second 5 weeks of the growth cycle. See Figure 4.1 for full description of treatments. A. Yield: leaf fresh mass (FM), closed circles (●); leaf dry mass (DM), open circles (○); stem plus petioles FM, closed squares (■); stem plus petioles DM, open squares (□); flower FM, closed triangles (▲); flower DM, open triangles (Δ). B. Leaf-N content: NO_3^--N, open triangles (Δ); organic-N, open squares (□); total-N, open circles (○). C. Stem plus petioles-N content: NO_3^--N, open triangles (Δ); organic-N, open squares (□); total-N, open circles (○). D. Flower-N content: NO_3^--N, open triangles (Δ); organic-N, open squares (□); total-N, open circles (○). The data represent the mean ± SE (N = 10); where bar is not shown, it is within the symbol.

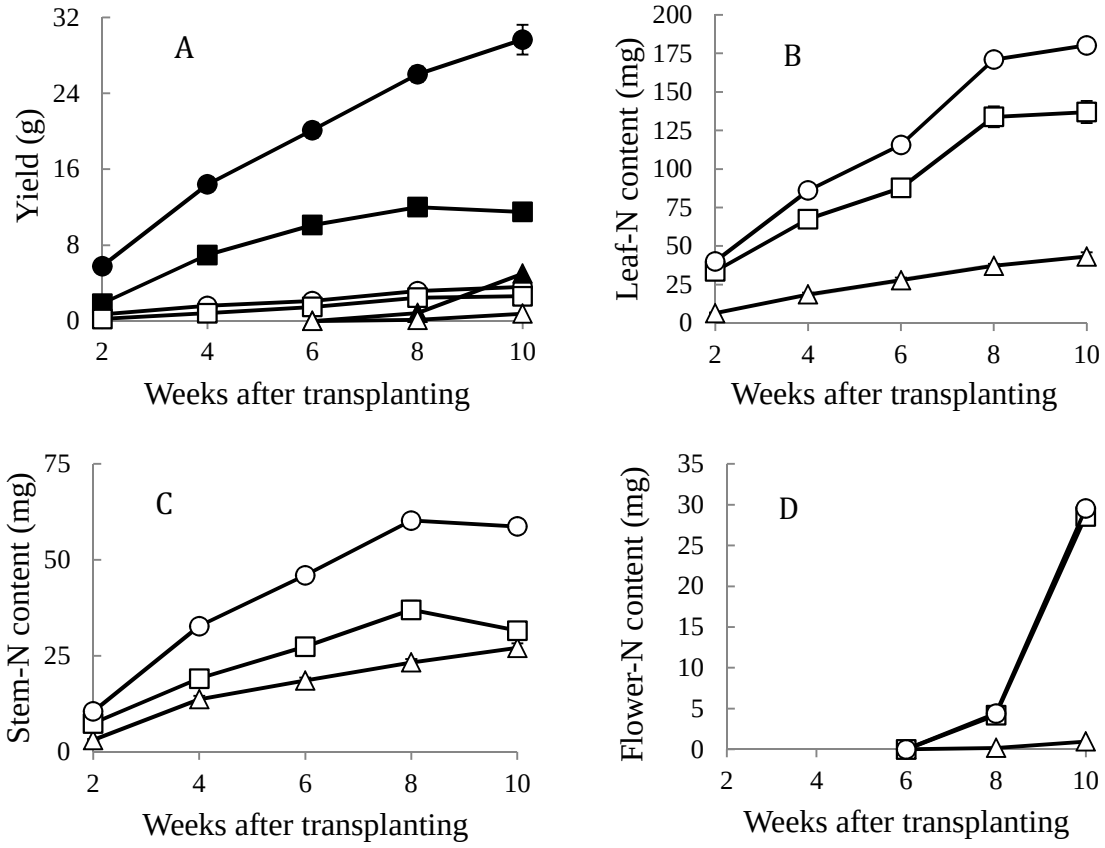


Figure 4.5. Cumulative characteristics of *Chrysanthemum morifolium* 'Yellow Favor' supplied with 15.5 mM NO_3^--N and 3 mM Cl^- for the second 5 weeks of the growth cycle. See Figure 4.1 for full description of treatments. A. Yield: leaf fresh mass (FM), closed circles (●); leaf dry mass (DM), open circles (○); stem plus petioles FM, closed squares (■); stem plus petioles DM, open squares (□); flower FM, closed triangles (▲); flower DM, open triangles (Δ). B. Leaf-N content: NO_3^--N, open triangles (Δ); organic-N, open squares (□); total-N, open circles (○). C. Stem plus petioles-N content: NO_3^--N, open triangles (Δ); organic-N, open squares (□); total-N, open circles (○). D. Flower-N content: NO_3^--N, open triangles (Δ); organic-N, open squares (□); total-N, open circles (○). The data represent the mean ± SE (N = 10); where bar is not shown, it is within the symbol.

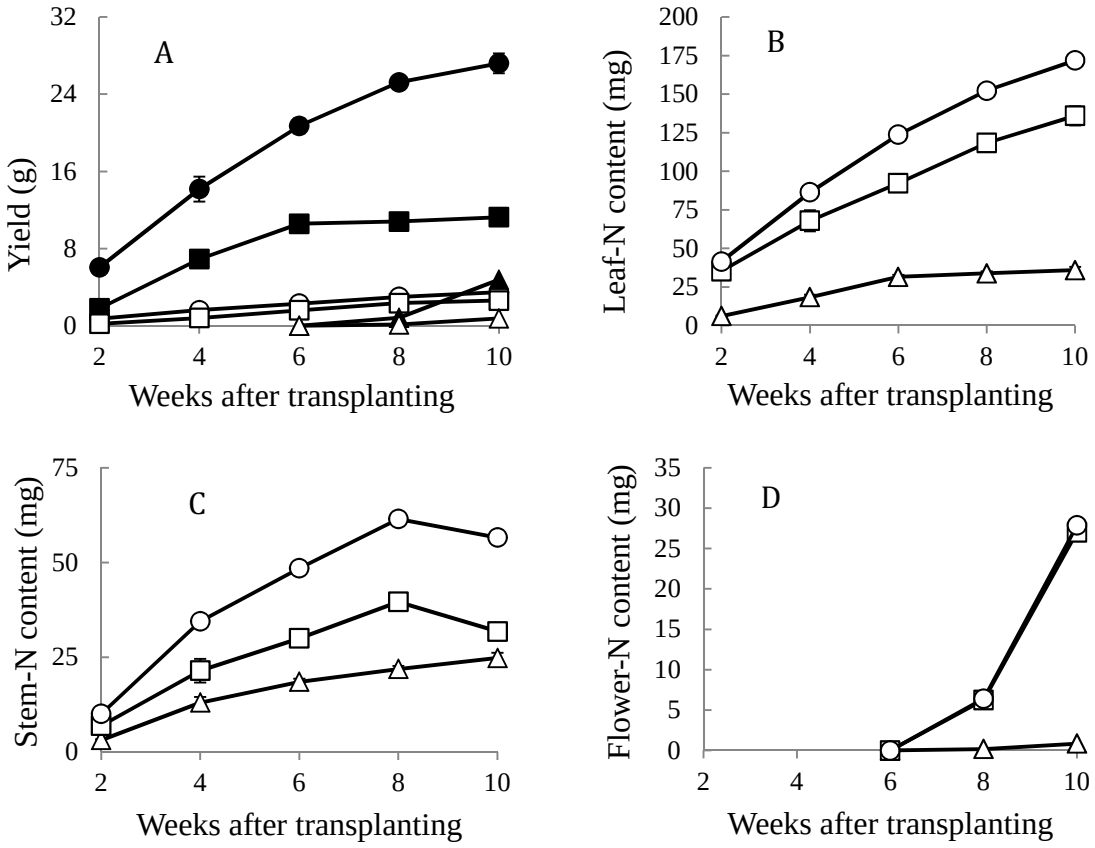


Figure 4.6. Cumulative characteristics of *Chrysanthemum morifolium* 'Yellow Favor' supplied with 12.5 mM NO_3^--N and 6 mM Cl^- for the second 5 weeks of the growth cycle. See Figure 4.1 for full description of treatments. A. Yield: leaf fresh mass (FM), closed circles (●); leaf dry mass (DM), open circles (○); stem plus petioles FM, closed squares (■); stem plus petioles DM, open squares (□); flower FM, closed triangles (▲); flower DM, open triangles (Δ). B. Leaf-N content: NO_3^--N, open triangles (Δ); organic-N, open squares (□); total-N, open circles (○). C. Stem plus petioles-N content: NO_3^--N, open triangles (Δ); organic-N, open squares (□); total-N, open circles (○). D. Flower-N content: NO_3^--N, open triangles (Δ); organic-N, open squares (□); total-N, open circles (○). The data represent the mean ± SE (N = 10); where bar is not shown, it is within the symbol.

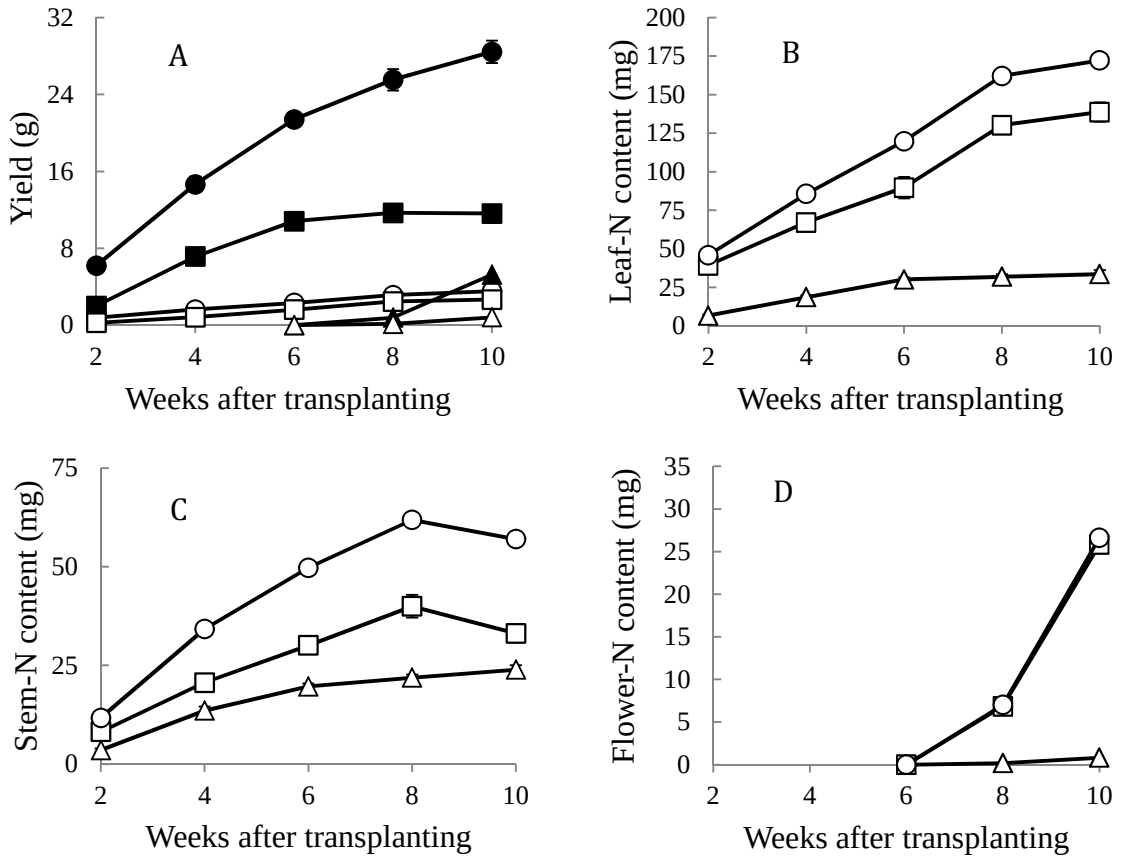


Figure 4.7. Cumulative characteristics of *Chrysanthemum morifolium* 'Yellow Favor' supplied with 9.5 mM NO_3^--N and 9 mM Cl^- for the second 5 weeks of the growth cycle. See Figure 4.1 for full description of treatments. A. Yield: leaf fresh mass (FM), closed circles (●); leaf dry mass (DM), open circles (○); stem plus petioles FM, closed squares (■); stem plus petioles DM, open squares (□); flower FM, closed triangles (▲); flower DM, open triangles (Δ). B. Leaf-N content: NO_3^--N, open triangles (Δ); organic-N, open squares (□); total-N, open circles (○). C. Stem plus petioles-N content: NO_3^--N, open triangles (Δ); organic-N, open squares (□); total-N, open circles (○). D. Flower-N content: NO_3^--N, open triangles (Δ); organic-N, open squares (□); total-N, open circles (○). The data represent the mean ± SE (N = 10); where bar is not shown, it is within the symbol.

4.1.3 *Nitrogen Use Efficiency During Flower Development*

4.1.3.1 Plant characteristics

Figures 4.8 and 4.9 illustrate the detailed characteristics of plants after 6 and 10 weeks of growth. Supply of water (0 mM) over the second 5 weeks of the growth cycle resulted in plants with a greater FM at 6 weeks than plants supplied with a decreasing NO_3^- supply and a corresponding increase in Cl^- (15.5, 12.5 and 9.5 mM NO_3^--N). At 10 weeks this treatment (0 mM) had a greater FM than the continuous supply of NO_3^- (18.5 mM) and the 12.5 mM treatment, although not different from that obtained with the alternating supply of full NO_3^- and water (18.5:0 mM) (Fig. 4.8). This result could be attributed to an increase in leaf FM. The FM of plants supplied with a decreasing NO_3^- supply and a corresponding increase in Cl^- was not different from that for plants supplied with the full NO_3^- supply. At 10 weeks, there were no differences in LA, plant height, and FM and DM of flowers between plants supplied solely with NO_3^- or water (Figs. 4.8 and 4.9). Interestingly, total DM did not differ among the treatments (Fig. 4.8), suggesting that water relations of the plants varied somewhat among treatments.

4.1.3.2 Nitrogen accumulation and mobilization

During the period from week six to 10, the 0 mM NO_3^--N treatment was the only one to exhibit a reduction in NO_3^- content compared to the full NO_3^- supply (Table 4.1). In this case, both leaf and stem plus petioles NO_3^- contents declined, whereas all the other treatments, particularly the 15.5 mM treatment, resulted in an increase in NO_3^- content. The only treatment to exhibit a reduction in organic-N was the full NO_3^- treatment.

As noted earlier, during the period from eight to 10 weeks when the flower gained most of its final DM and N, the only treatment wherein NO_3^- declined in leaves and stem

plus petioles was 0 mM NO_3^- (Table 4.2). However, there was a decline in the organic-N content of stem plus petioles in all treatments. Overall, the loss of NO_3^- from the stem plus petioles in the 0 mM NO_3^- treatment represents 12% of the total N in the flower at the end of the experiment, whereas the loss in organic-N represents 42%, for a total of 54% of the flower N coming from the stem plus petioles. This compares to a loss of organic-N from stem plus petioles that represents 51-61 % of the flower total-N in the 18.5 and 18.5:0 treatments and 22-26% of the flower total-N in the 15.5, 12.5 and 9.5 mM NO_3^--N treatments. Interestingly, the overall loss in total-N from the stem plus petioles was similar in the 18.5 and 0 mM NO_3^- treatments, as was total-N accumulation of the flower.

Notably, the shoot continued to accumulate N to the end of the experiment in all treatments, including the 0 mM treatment, indicating that N may be coming from the root medium or the roots themselves (Fig. 4.10).

Figure 4.8. Yield of *Chrysanthemum morifolium* 'Yellow Favor' supplied with various combinations of NO_3^- and Cl^- on a fresh mass (FM) and dry mass (DM) basis at 6 weeks (A, C) and 10 weeks (B, D). See Figure 4.1 for full description of treatments. The treatment description indicates the NO_3^--N concentration (mM) supplied in the second 5 weeks of the experiment. The data represent the mean (N = 10). Bars not sharing the same letter are significantly different at $P \leq 0.05$ ($LSD_{0.05}$). Letters within the bars represent comparisons among plant parts from the various treatments and letters above the bars represent comparisons among totals of shoot parts. White, black and grey bars represent stems plus petioles, leaves and flowers, respectively.

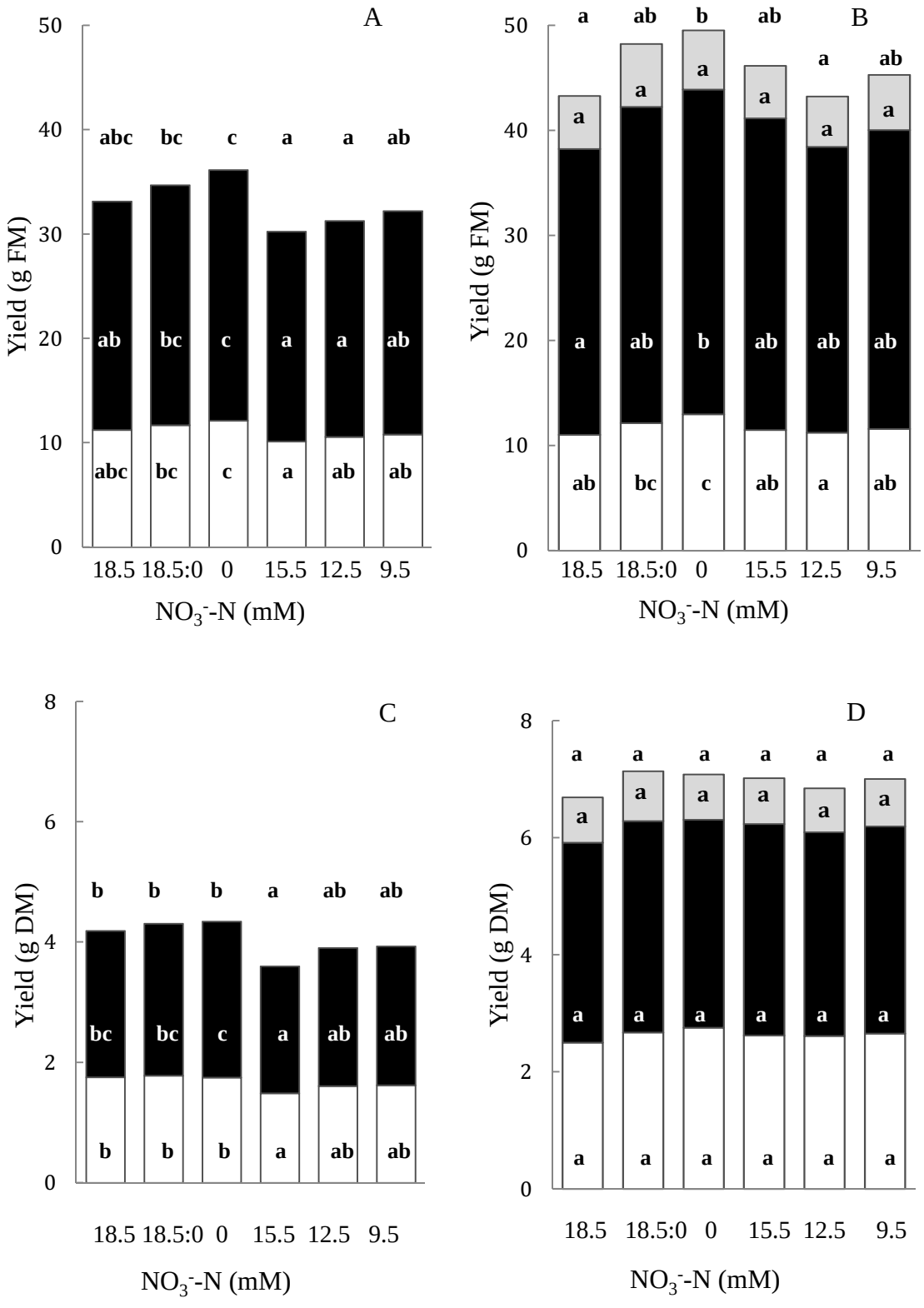
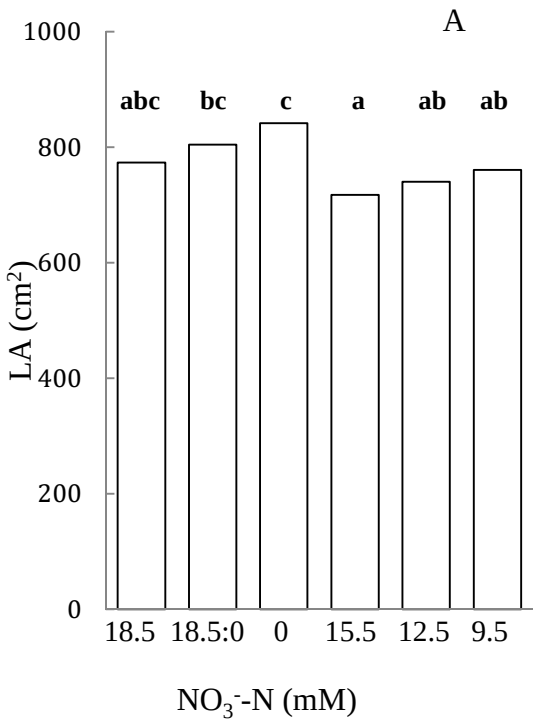
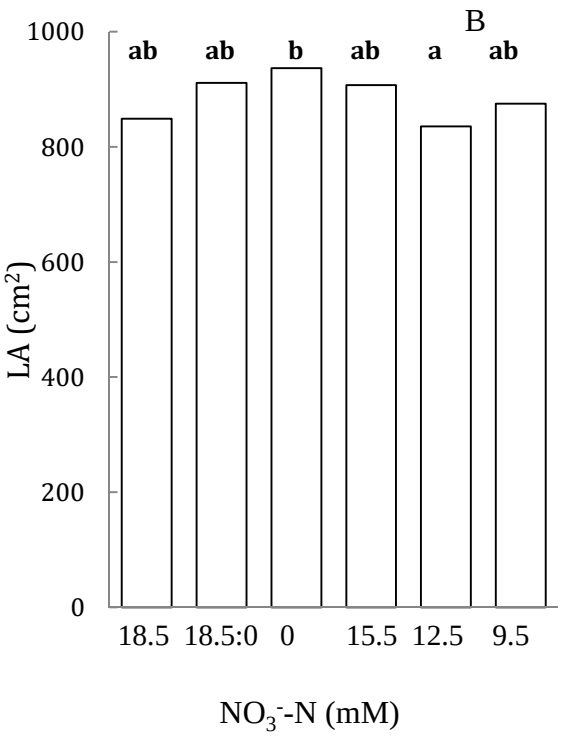
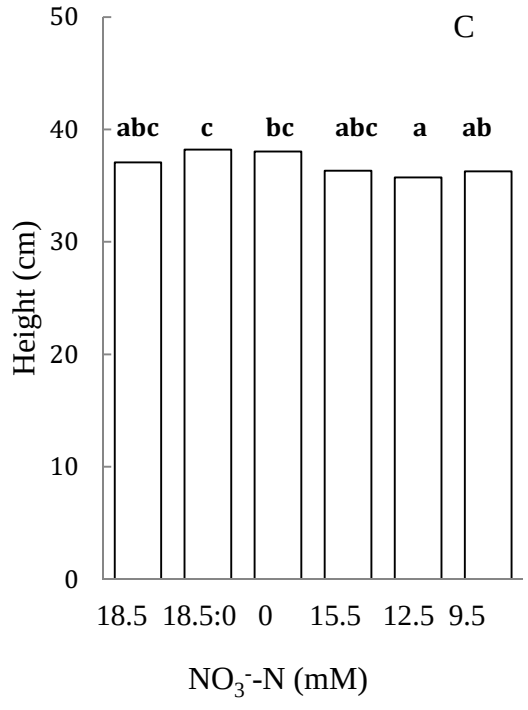

A
B
C
D
Yield (g FM)
Yield (g DM)
NO_3^--N (mM)
18.5
18.5:0
0
15.5
12.5
9.5

Figure 4.9. Leaf area (LA) and plant height at 6 weeks (A, C) and 10 weeks (B, D) of *Chrysanthemum morifolium* 'Yellow Favor' supplied with various combinations of NO_3^- and Cl^-. See Figure 4.1 for full description of treatments. The treatment description indicates the NO_3^--N concentration (mM) supplied in the second 5 weeks of the experiment. The data represent the mean (N = 10). Bars not sharing the same letter are significantly different at $P \leq 0.05$ ($LSD_{0.05}$).


A
1000
800
600
400
200
0
LA (cm2)
abc bc c a ab ab
18.5 18.5:0 0 15.5 12.5 9.5
NO3-N (mM)


B
1000
800
600
400
200
0
LA (cm2)
ab ab b ab a ab
18.5 18.5:0 0 15.5 12.5 9.5
NO3-N (mM)


C
50
40
30
20
10
0
Height (cm)
abc c bc abc a ab
18.5 18.5:0 0 15.5 12.5 9.5
NO3-N (mM)

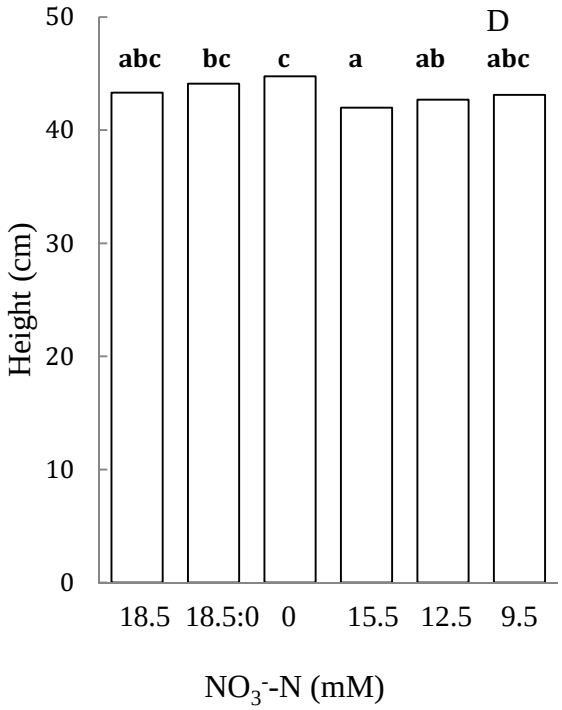

D
50
40
30
20
10
0
Height (cm)
abc bc c a ab abc
18.5 18.5:0 0 15.5 12.5 9.5
NO3-N (mM)

Table 4.1. Change in N content over the 6-10 week period of parts of *Chrysanthemum morifolium* 'Yellow Favor' supplied with various combinations of NO_3^- and Cl^- for the second 5 weeks of the growth cycle. The treatment description indicates the NO_3^--N concentration (mM) supplied; see Figure 4.1 for full description. The data represent the mean difference (± SE) in N content between 10 and 6 weeks (N = 10). Data not sharing the same letter within columns of the same N fraction are significantly different ($P \leq 0.05$).

Treatment NO_3^- (mM)	N fraction	Change in N contents (mg N)		
		Leaves	Stem + petioles	Flower
18.5	Nitrate	+ 8.1 ± 1.9 b	+ 6.5 ± 1.1 bc	+ 1.0 ± 0.1 a
	Organic	+ 27.8 ± 5.6 x	- 6.6 ± 2.5 x	+ 29.3 ± 1.9 xy
	Total	+ 35.9 ± 7.1 A	- 0.1 ± 3.1 A	+ 30.3 ± 2.0 AB
18.5:0	Nitrate	+ 7.3 ± 0.9 b	+ 5.7 ± 0.9 bc	+ 1.1 ± 0.1 a
	Organic	+ 40.0 ± 5.1 x	+ 2.1 ± 2.3 y	+ 32.4 ± 1.3 y
	Total	+ 47.3 ± 5.7 AB	+ 7.8 ± 3.1 AB	+ 33.5 ± 1.4 B
0	Nitrate	- 6.3 ± 2.8 a	- 0.9 ± 1.2 a	+ 0.9 ± 0.1 a
	Organic	+ 32.2 ± 9.1 x	+ 3.5 ± 2.9 y	+ 27.0 ± 1.6 xy
	Total	+ 25.9 ± 11.3 A	+ 2.6 ± 3.5 AB	+ 27.9 ± 1.7AB
15.5	Nitrate	+ 15.4 ± 3.3 c	+ 8.5 ± 1.6 c	+ 1.0 ± 0.1 a
	Organic	+ 49.2 ± 9.5 x	+ 4.2 ± 2.1 y	+ 28.6 ± 2.8 xy
	Total	+ 64.6 ± 12.5 B	+ 12.7 ± 3.4 B	+ 29.6 ± 2.9 AB
12.5	Nitrate	+ 4.4 ± 2.1 b	+ 6.3 ± 1.6 bc	+ 0.9 ± 0.1 a
	Organic	+ 43.8 ± 7.6 x	+ 1.9 ± 1.8 y	+ 27.0 ± 1.8 xy
	Total	+ 48.2 ± 9.5 AB	+ 8.2 ± 3.1 AB	+ 27.9 ± 1.8 AB
9.5	Nitrate	+ 3.4 ± 2.7 b	+ 4.2 ± 1.0 b	+ 0.8 ± 0.1 a
	Organic	+ 49.0 ± 8.5 x	+ 3.0 ± 2.4 y	+ 25.8 ± 3.6 x
	Total	+ 52.4 ± 9.7 AB	+ 7.2 ± 3.0 AB	+ 26.6 ± 3.7 A

Table 4.2. Change in N content over the 8-10 week period of parts of *Chrysanthemum morifolium* 'Yellow Favor' supplied with various combinations of NO_3^- and Cl^- for the second 5 weeks of the growth cycle. The treatment description indicates the NO_3^--N concentration (mM) supplied; see figure 4.1 for full description. The data represent the mean difference (± SE) in N content between 10 and 8 weeks (N = 10). Data not sharing the same letter within columns of the same N fraction are significantly different ($P \leq 0.05$).

Treatment	N fraction	Change in N content (mg N)		
NO_3^- mM		Leaves	Stem + petioles	Flower
18.5	Nitrate	+ 0.8 ± 3.2 ab	+ 3.9 ± 1.5 b	+ 0.8 ± 0.1 a
	Organic	+ 17.8 ± 7.7 x	- 14.6 ± 2.6 x	+ 23.0 ± 2.0 xy
	Total	+ 34.4 ± 18.6 A	- 10.7 ± 3.9 A	+ 23.8 ± 2.1 AB
18.5:0	Nitrate	+ 7.6 ± 2.3 b	+ 3.8 ± 1.0 b	+ 0.8 ± 0.0 a
	Organic	+ 6.9 ± 5.2 x	- 13.5 ± 1.9 xy	+ 25.5 ± 1.2 y
	Total	+ 25.1 ± 13.7 A	- 9.7 ± 2.2 A	+ 26.3 ± 1.2 B
0	Nitrate	- 1.9 ± 2.8 a	- 2.3 ± 1.5 a	+ 0.6 ± 0.1 a
	Organic	+ 9.2 ± 10.7 x	- 8.0 ± 4.3 xyz	+ 18.7 ± 2.0 x
	Total	+ 7.4 ± 12.8 A	- 10.3 ± 4.4 A	+ 19.3 ± 2.1 A
15.5	Nitrate	+ 6.1 ± 2.7 b	+ 3.8 ± 1.6 b	+ 0.8 ± 0.1 a
	Organic	+ 3.1 ± 8.1 x	- 5.4 ± 2.4 z	+ 24.4 ± 2.8 xy
	Total	+ 9.2 ± 10.5 A	- 1.6 ± 3.4 A	+ 25.1 ± 2.8 AB
12.5	Nitrate	+ 2.1 ± 2.8 ab	+ 2.9 ± 1.7 b	+ 0.7 ± 0.0 a
	Organic	+ 17.6 ± 7.8 x	- 7.8 ± 2.0 xyz	+ 20.8 ± 1.5 xy
	Total	+ 19.7 ± 9.8 A	- 4.9 ± 3.3 A	+ 21.4 ± 1.5 AB
9.5	Nitrate	+ 1.6 ± 2.1 ab	+ 2.0 ± 1.4 b	+ 0.6 ± 0.1 a
	Organic	+ 8.4 ± 8.7 x	- 6.9 ± 3.8 yz	+ 22.4 ± 3.3 xy
	Total	+ 10.0 ± 9.6 A	- 4.9 ± 5.0 A	+ 23.0 ± 3.3 AB

4.1.3.3 Nitrogen use indices

For the NUI at 6 weeks, the only treatment that was significantly different from the 18.5 mM control was the 15.5 mM treatment (Fig. 4.11). At 10 weeks, the 0 mM treatment resulted in a higher NUI than the 18.5 mM treatment, but was not significantly different from any of the other treatments.

While the 0 and 9.5 mM treatments appeared to produce the highest N utilization efficiency (NUtE) at 10 weeks, these were found not to be significantly different from any of the treatments (data not shown). Similarly, the N harvest index (NHI) at 10 weeks was not found to be significantly different among treatments (data not shown).

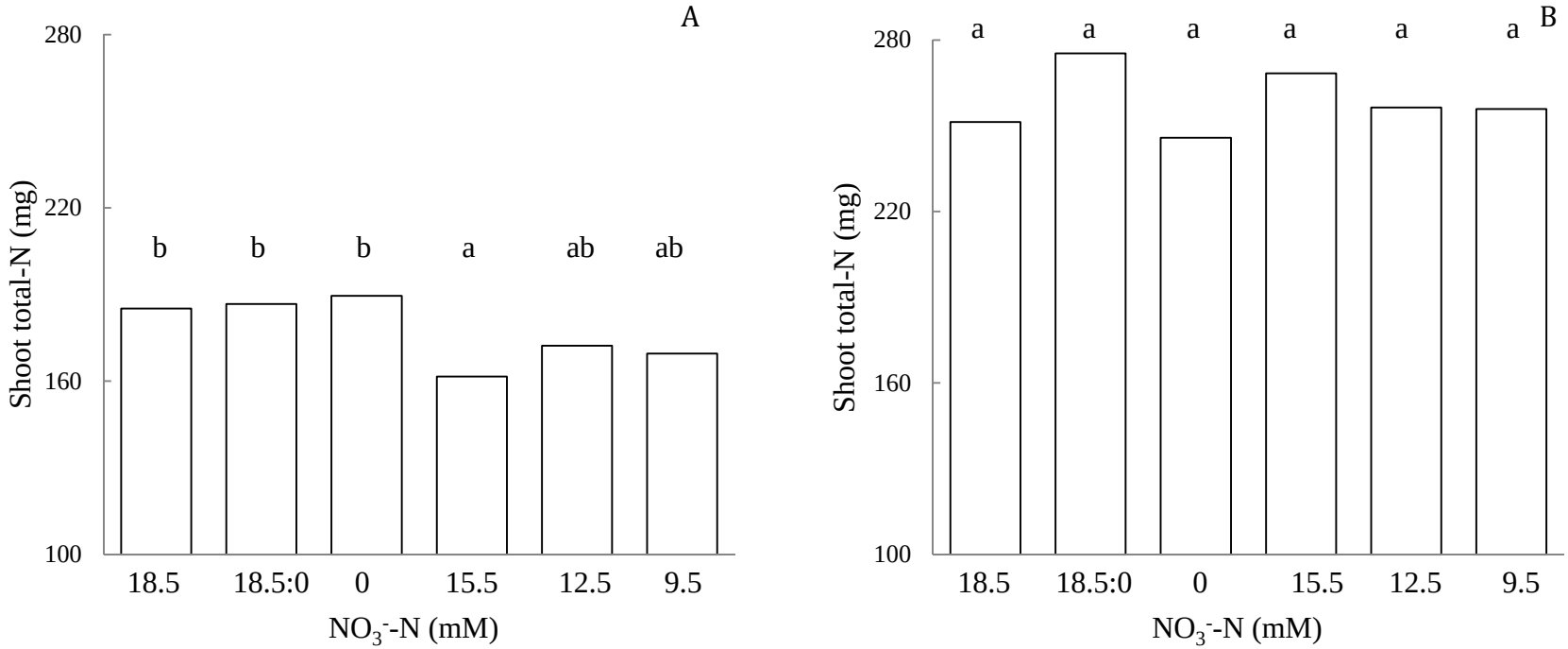


Figure 4.10. Shoot total-N of *Chrysanthemum morifolium* 'Yellow Favor' at 6 weeks (A) and 10 weeks (B) supplied with various combinations of NO_3^- and Cl^- in the second 5 weeks of the growth cycle. The treatment description indicates the NO_3^--N concentration (mM) supplied; see Figure 4.1 for full description. The data represent the mean (N = 10). Bars not sharing the same letter are significantly different at $P \leq 0.05$ ($LSD_{0.05}$).

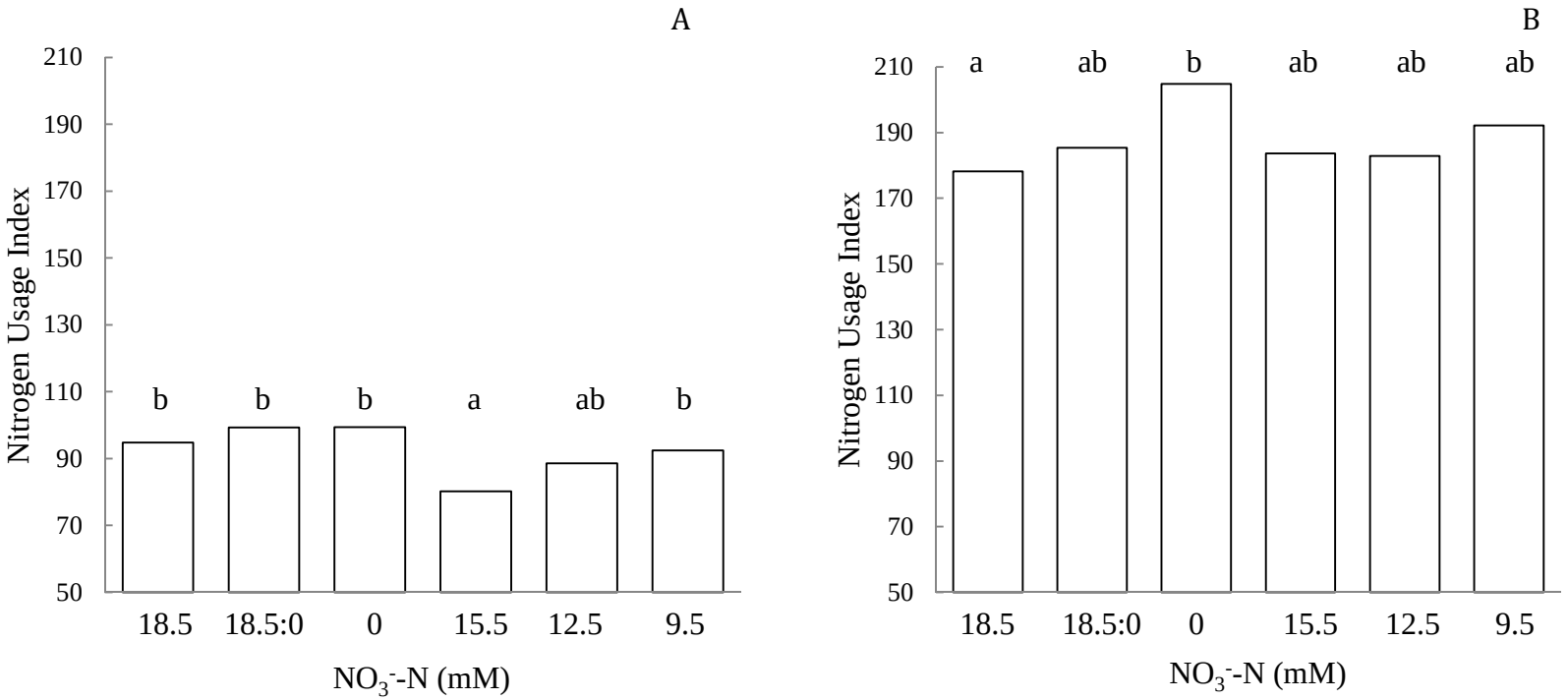


Figure 4.11. The Nitrogen Usage Index (NUI) of *Chrysanthemum morifolium* 'Yellow Favor' at 6 weeks (A) and 10 weeks (B) supplied with various combinations of NO_3^- and Cl^- in the second 5 weeks of the growth cycle. The treatment description indicates the NO_3^--N concentration (mM) supplied; see Figure 4.1 for full description. The data represent the mean (N = 10). Bars not sharing the same letter are significantly different at $P \leq 0.05$ ($LSD_{0.05}$). NUI = shoot DM * (shoot DM / shoot N content).

4.2 Ammonium and Nitrate Nutrition

4.2.1 *Electrical Conductivity of Pot Media as a Function of Plant Development*

The mean medium EC of the treatments containing NH_4^+ in the nutrient solution was greater than the treatments containing no NH_4^+, with the 9:9(400) having the highest medium EC reading throughout the experimental period (Fig. 4.12). Higher SO_4^{2-} levels in the medium of the treatments containing NH_4^+ could have contributed to the increased medium EC as sulphate salts were substituted for NO_3^- stoichiometrically in the preparation of these nutrient solutions. By the end of the experiment, the 18:0(200) and 18:0(400) treatments had mean medium EC readings that were below 1.0 mS/cm.

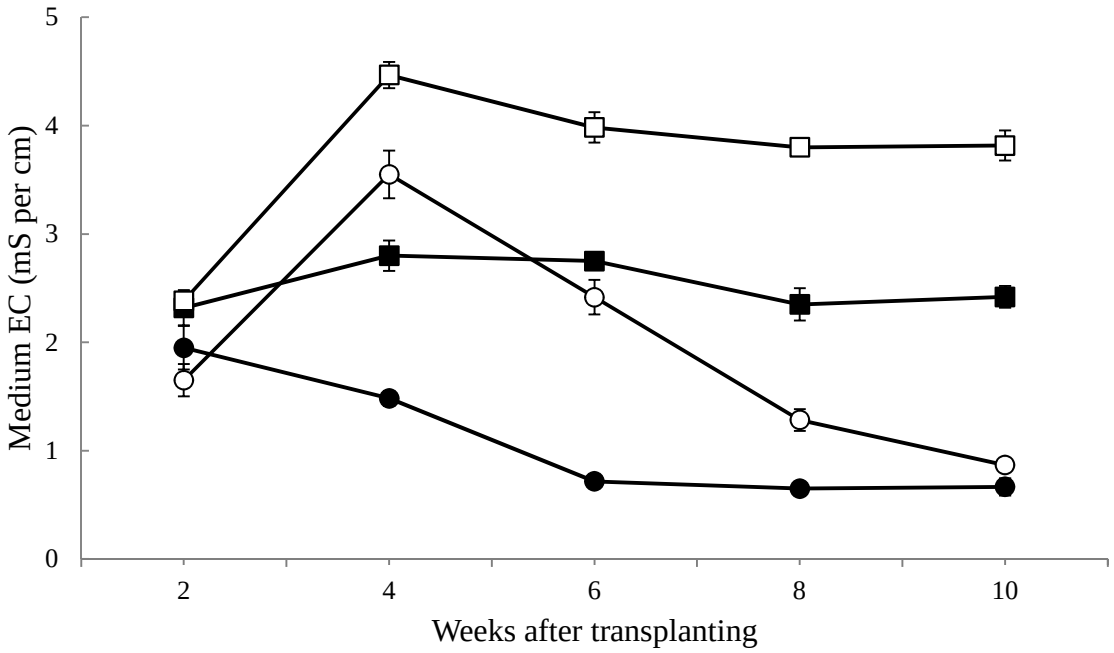


Figure 4.12. Medium electrical conductivity (EC) of the pots containing *Chrysanthemum morifolium* 'Yellow Favor' supplied with 18 mM of NO_3^--N (18:0), or a combination of 9 mM NO_3^--N and 9 mM NH_4^+-N (9:9). Pots in the 18 mM NO_3^--N and the 9 mM NO_3^- / 9 mM NH_4^+-N combination were supplied with either 200 mg total N, (18:0(200) closed circles, ●; 9:9 (200) closed squares, ■) or 400 mg total N (18:0(400) open circles, ○; 9:9(400) open squares, □). 200 mg total N was supplied by 3 weeks after planting and 400 mg total N was supplied by 5 weeks after planting. After the required amount of total N (either 200 or 400 mg) was reached, the plants were irrigated with RO water until flowering. The data represent the mean ± SE (N = 12); where bar is not shown, it is within the symbol.

4.2.2 *Nitrogen Accumulation as a Function of Plant Development*

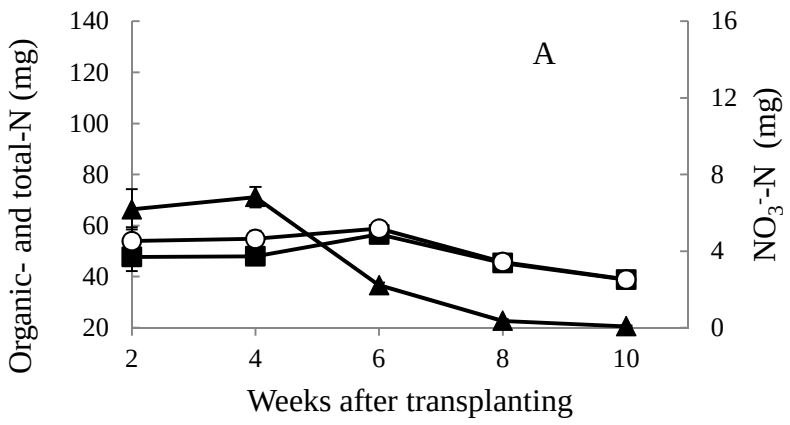
The addition of NH_4^+ to the nutrient solution increased the organic-N content of the lower leaves, as compared to the treatments without NH_4^+. This was evident during the early stage of the chrysanthemum plant growth cycle, although this was not evident at the end of the experiment (Figs. 4.13–4.16, panel A). In the treatments supplied with 400 mg of N, the organic-N content of the upper leaves increased steadily with development, whereas in plants receiving 200 mg N, the organic-N content peaked at six weeks, and declined thereafter (Figs. 4.13-4.16, panel B).

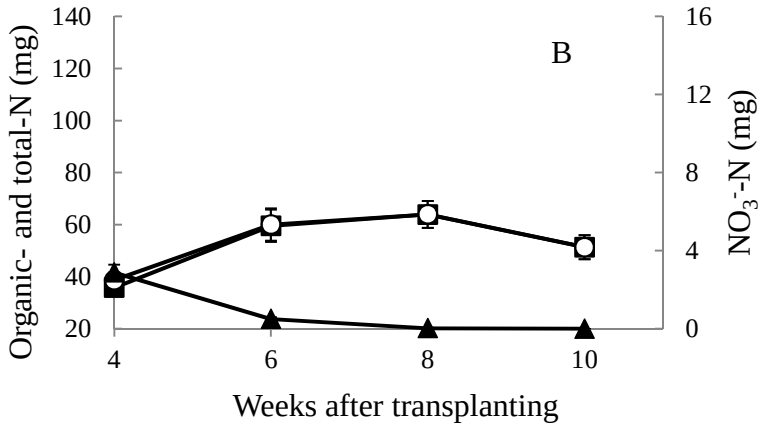
The 18:0(400) and the 9:9(400) treatments displayed similar NO_3^- accumulation patterns in the upper and lower leaves, with NO_3^- being accumulated early in the growth cycle, up until week six (Figs. 4.14 and 4.16, panels A and B). Thereafter, both treatments showed a steep decline in NO_3^- content. The treatments supplied with 200 mg of N also accumulated NO_3^- early in the growth cycle and commencing at week four, the NO_3^- content of the upper and lower leaf tissue declined and was virtually zero in both treatments by the end of the experiment, irrespective of the form of N fed (Figs. 4.13 and 4.15, panels A and B).

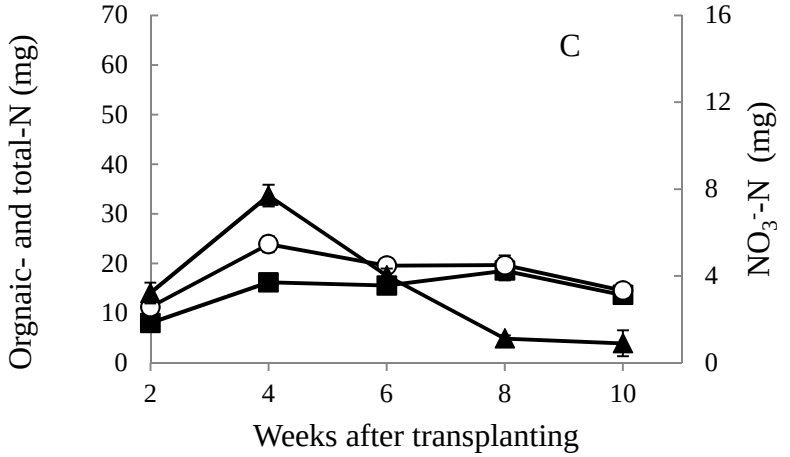
The addition of NH_4^+ to the nutrient solution increased the organic-N content of the stem plus petioles tissue in both the 200 mg and 400 mg feeding regimes (Figs. 4.13-4.16, panels C and D). The 9:9(400) treatment had the greatest organic-N content in both the upper and lower stem plus petioles tissue at all but the final sampling period. The organic and total-N contents of the upper and lower stems plus petioles tissue in plants receiving 200 mg N increased up until week four and then declined thereafter.

There was no difference in the flower organic-N between the two N forms, although it was proportional to the amount of N supplied (Fig. 4.17, panels A-D). The accumulation of NO_3^- in the flowers was also proportional to the N supply regardless of N form, and was higher with the 18:0(400) treatment than with the 9:9(400) treatment. It should be noted that the floral tissue also included the calyx.

Figure 4.13. Weekly cumulative characteristics of vegetative parts of *Chrysanthemum morifolium* 'Yellow Favor' supplied with 18 mM NO_3^--N, with fertilization terminated when 200 mg of total N was supplied to the pot. See Figure 4.12 for full description of treatments. A. lower leaves, B. upper leaves C. bottom stem, D. upper stem; total-N, open circles (○); organic-N, closed squares (■); NO_3^--N, closed triangles (▲). The data represent the mean ± SE (N = 6); where bar is not shown, it is within the symbol.


A
Organic- and total-N (mg)
NO_3^--N (mg)
Weeks after transplanting


B
Organic- and total-N (mg)
NO_3^--N (mg)
Weeks after transplanting


C
Orgnaic- and total-N (mg)
NO_3^--N (mg)
Weeks after transplanting

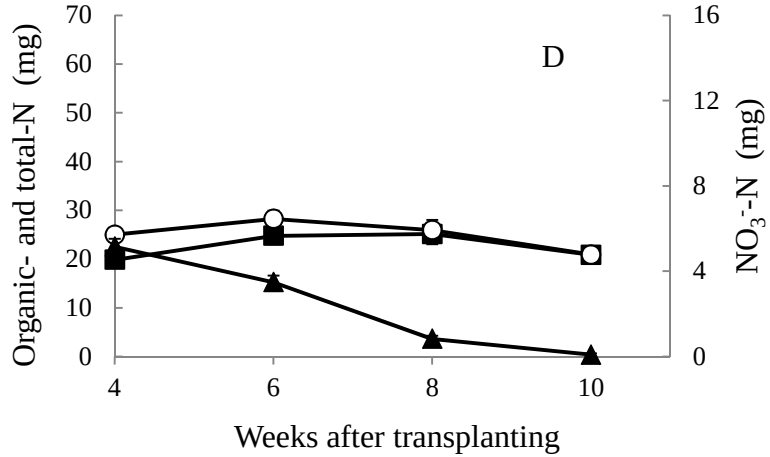
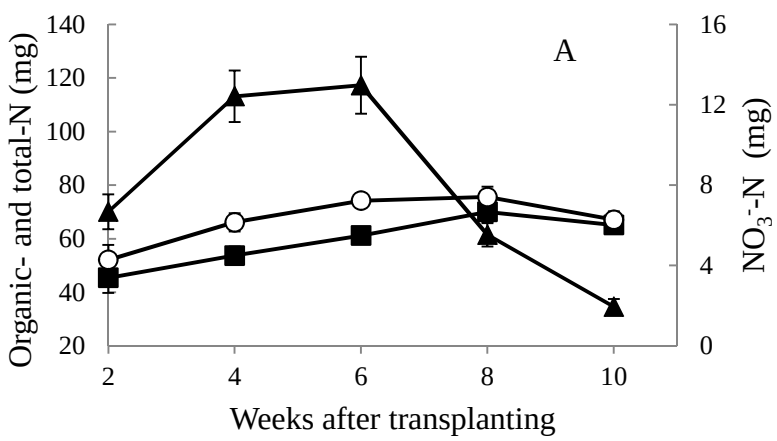
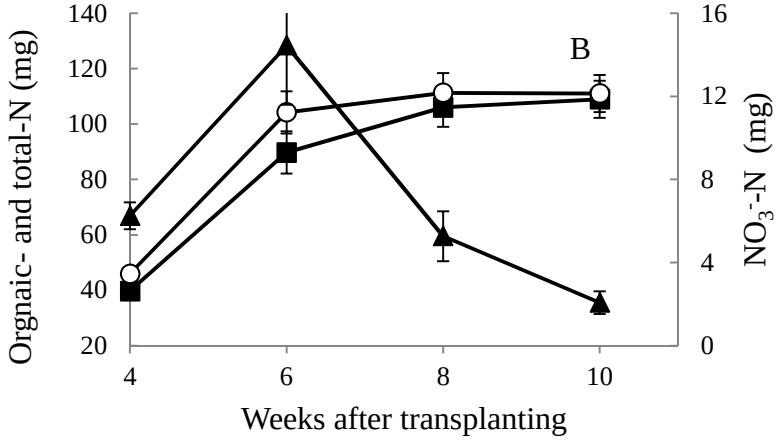
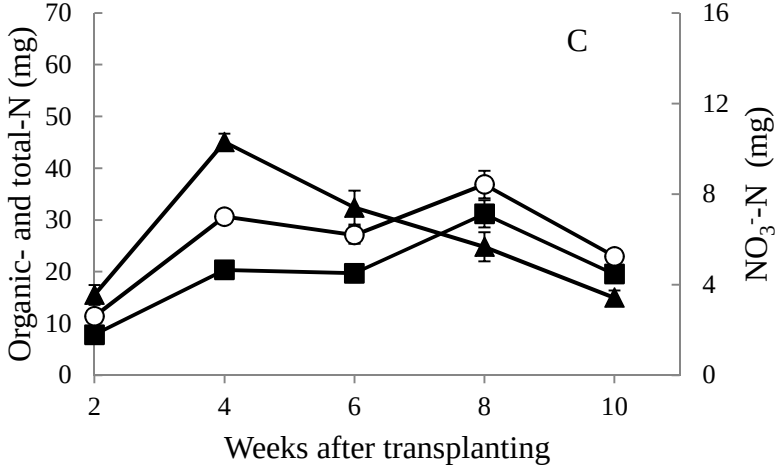

D
Organic- and total-N (mg)
NO_3^--N (mg)
Weeks after transplanting

Figure 4.14. Weekly cumulative characteristics of vegetative parts of *Chrysanthemum morifolium* 'Yellow Favor' supplied with 18 mM NO_3^--N, with fertilization terminated when 400 mg of total N was supplied to the pot. See Figure 4.12 for full description of treatments. A. lower leaves, B. upper leaves C. bottom stem, D. upper stem; total-N, open circles (○); organic-N, closed squares (■); NO_3^--N, closed triangles (▲). The data represent the mean ± SE (N = 6); where bar is not shown, it is within the symbol.


A
Organic- and total-N (mg)
NO_3-N (mg)
Weeks after transplanting


B
Orgnaic- and total-N (mg)
NO_3-N (mg)
Weeks after transplanting


C
Organic- and total-N (mg)
NO_3-N (mg)
Weeks after transplanting

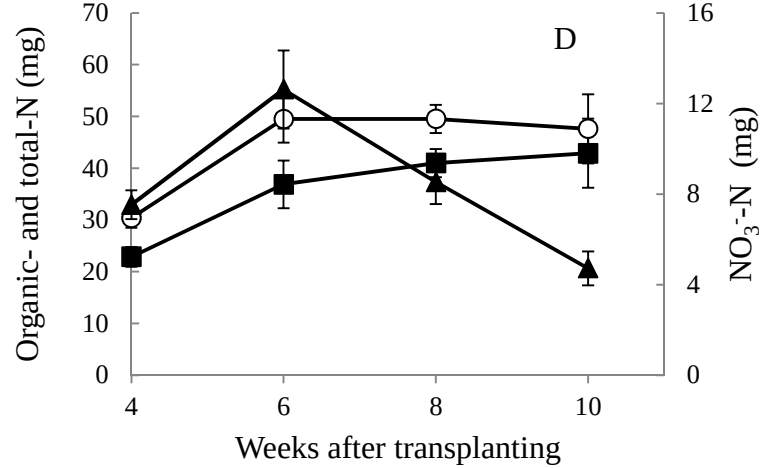
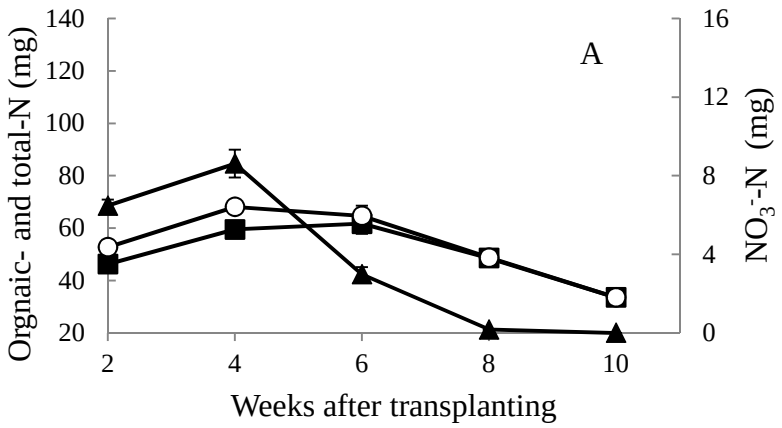
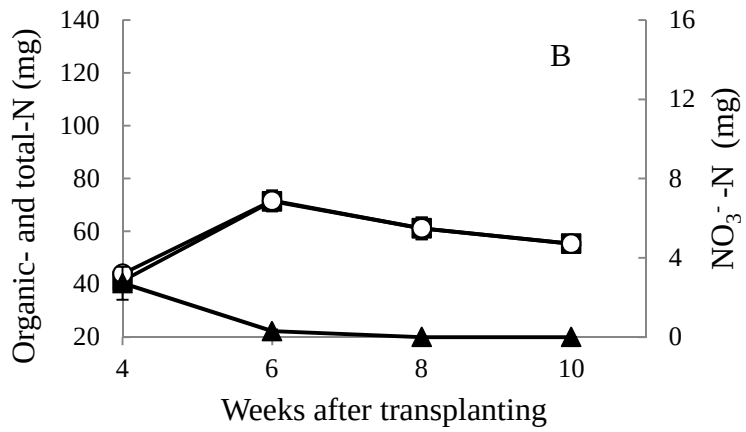
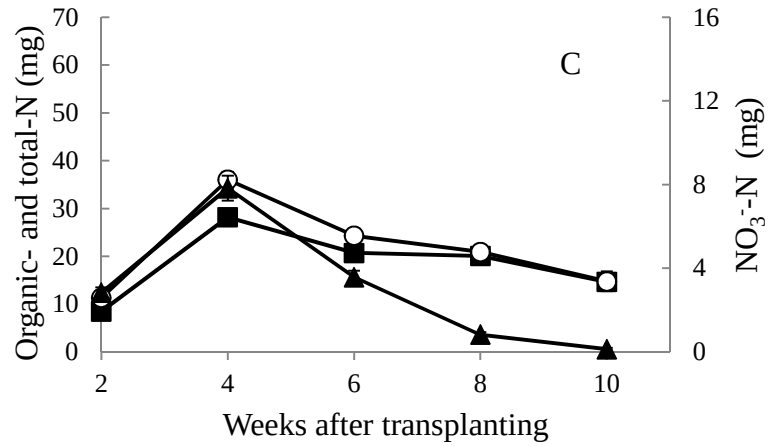

D
Organic- and total-N (mg)
NO_3-N (mg)
Weeks after transplanting

Figure 4.15. Weekly cumulative characteristics of vegetative parts of *Chrysanthemum morifolium* 'Yellow Favor' supplied with a combination of 9 mM NO_3^--N and 9 mM of NH_4^+-N, with fertilization terminated when 200 mg of total N was supplied to the pot. See Figure 4.12 for full description of treatments. A. Lower leaves, B. upper leaves C. bottom stem, D. upper stem; total-N, open circles (○); organic-N, closed squares (■); NO_3^--N, closed triangles (▲). The data represent the mean ± SE (N = 6); where bar is not shown, it is within the symbol.


A
Orgnaic- and total-N (mg)
NO3-N (mg)
Weeks after transplanting


B
Organic- and total-N (mg)
NO3- -N (mg)
Weeks after transplanting


C
Organic- and total-N (mg)
NO3-N (mg)
Weeks after transplanting

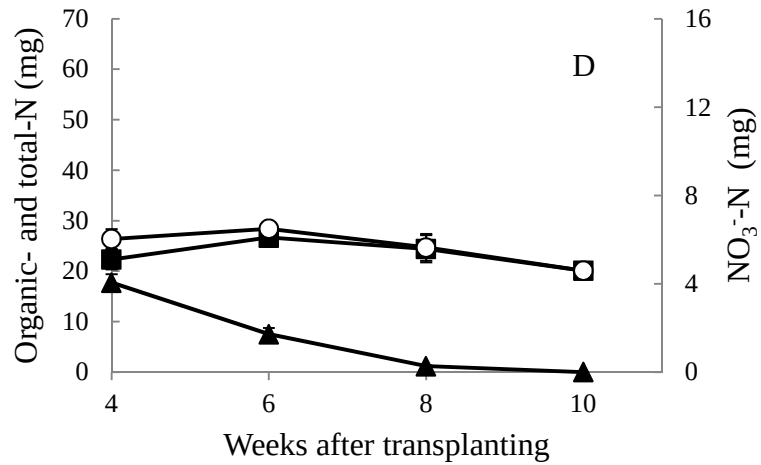
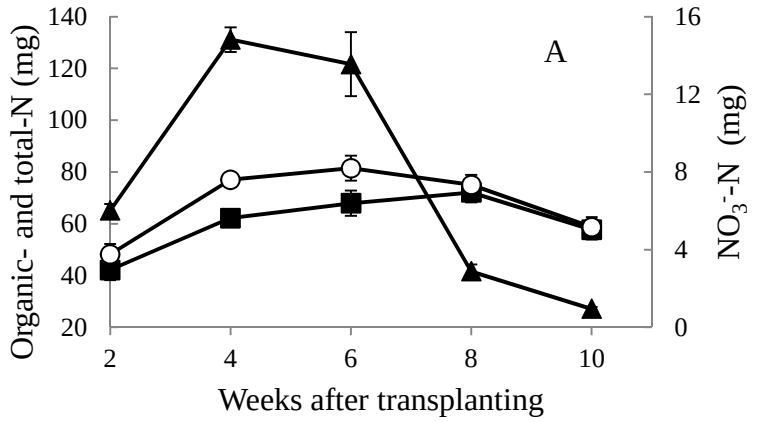
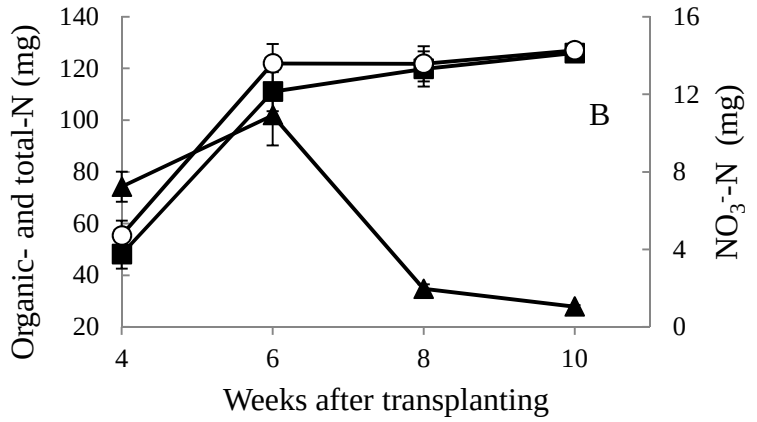
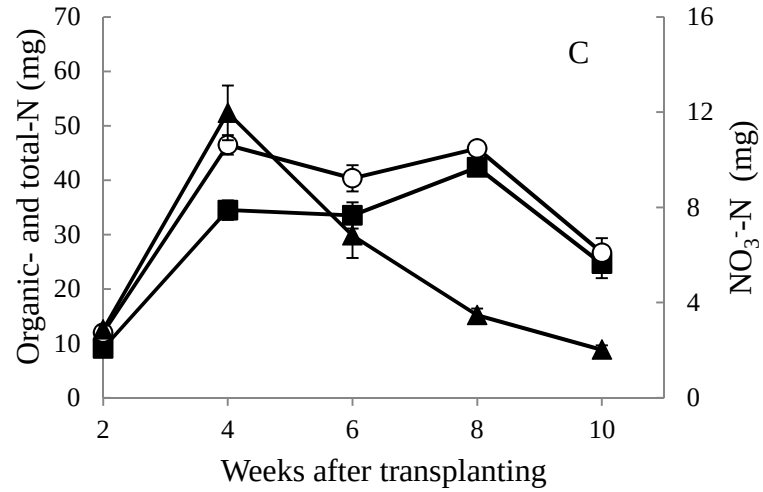

D
Organic- and total-N (mg)
NO3-N (mg)
Weeks after transplanting

Figure 4.16. Weekly cumulative characteristics of vegetative parts of *Chrysanthemum morifolium* 'Yellow Favor' supplied with a combination of 9 mM NO_3^--N and 9 mM of NH_4^+-N, with fertilization terminated when 400 mg of total N was supplied to the pot. See Figure 4.12 for full description of treatments. A. lower leaves, B. upper leaves C. bottom stem, D. upper stem; total-N, open circles (○); organic-N, closed squares (■); NO_3^--N, closed triangles (▲). The data represent the mean ± SE (N = 6); where bar is not shown, it is within the symbol.


A
Organic- and total-N (mg)
NO_3-N (mg)
Weeks after transplanting


B
Organic- and total-N (mg)
NO_3-N (mg)
Weeks after transplanting


C
Organic- and total-N (mg)
NO_3-N (mg)
Weeks after transplanting

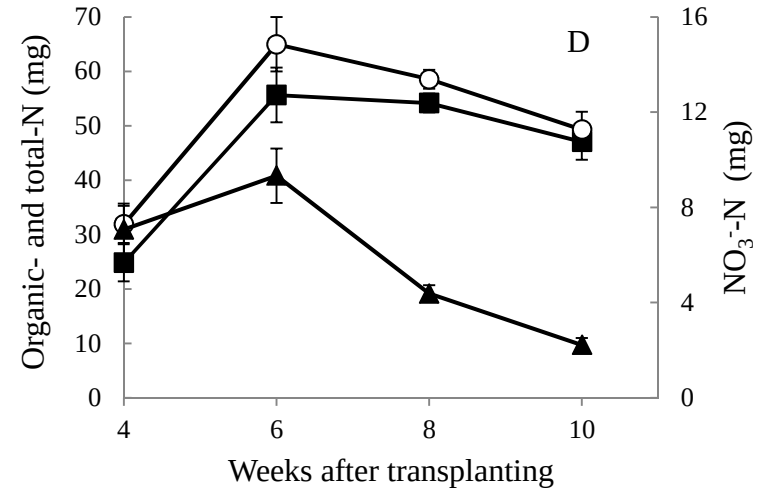
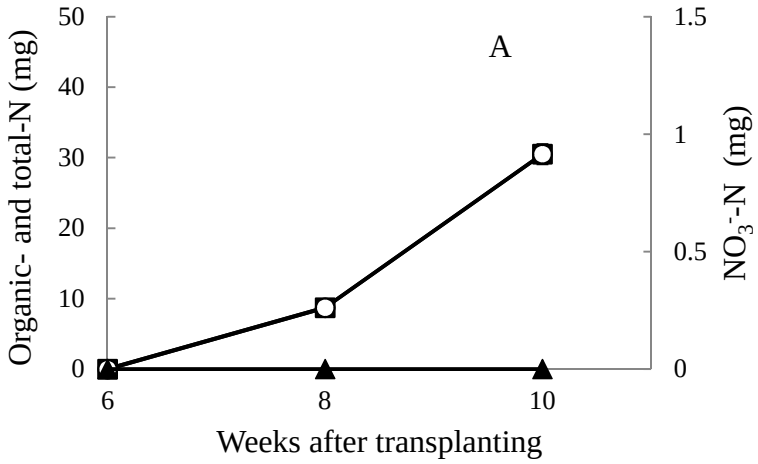
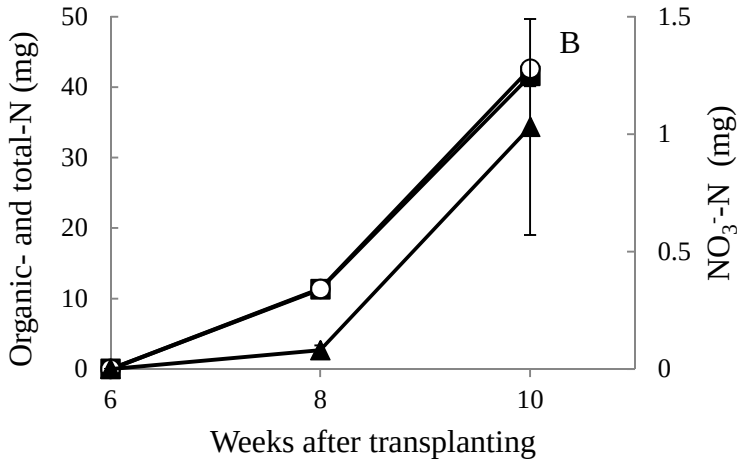
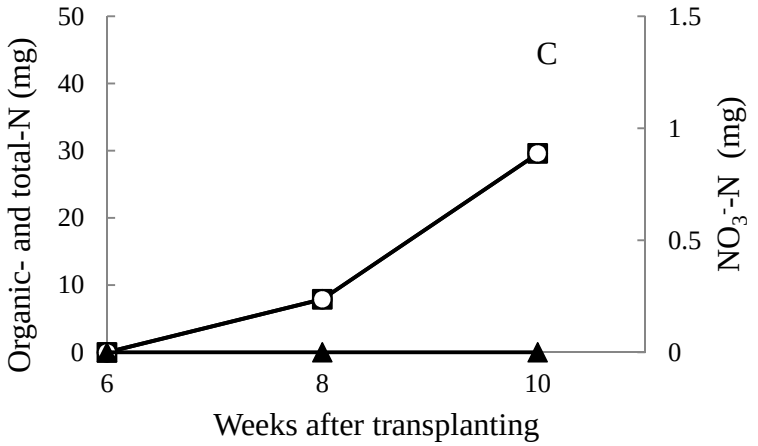
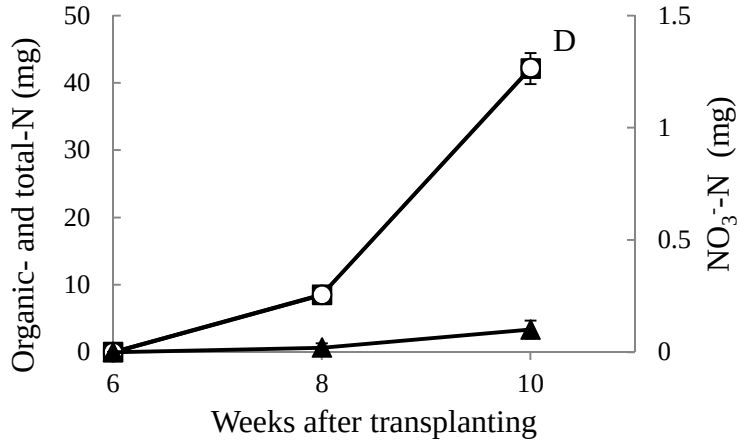

D
Organic- and total-N (mg)
NO_3-N (mg)
Weeks after transplanting

Figure 4.17. Weekly cumulative characteristics of flowers of *Chrysanthemum morifolium* 'Yellow Favor' supplied with A. 18 mM of NO_3^--N at 200 mg N B. 18 mM of NO_3^--N at 400 mg N C. 9 mM NO_3^--N / 9 mM NH_4^+-N at 200 mg N D. 9 mM NO_3^--N / 9 mM NH_4^+-N at 400 mg N. See Figure 4.12 for full description of treatments. Flower total-N, open circles (○); flower organic-N, closed squares (■); flower NO_3^--N, closed triangles (▲). The data represent the mean ± SE (N = 6); where bar is not shown, it is within the symbol.


A
Organic- and total-N (mg)
NO_3^--N (mg)
Weeks after transplanting
0
10
20
30
40
50
0
0.5
1
1.5
6
8
10


B
Organic- and total-N (mg)
NO_3^--N (mg)
Weeks after transplanting
0
10
20
30
40
50
0
0.5
1
1.5
6
8
10

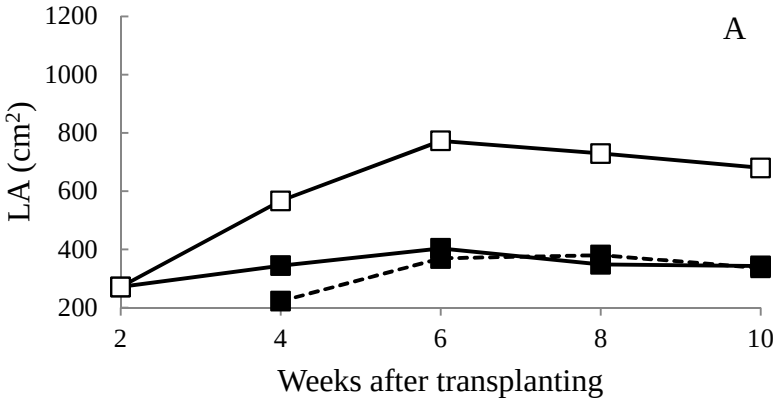

C
Organic- and total-N (mg)
NO_3^--N (mg)
Weeks after transplanting
0
10
20
30
40
50
0
0.5
1
1.5
6
8
10

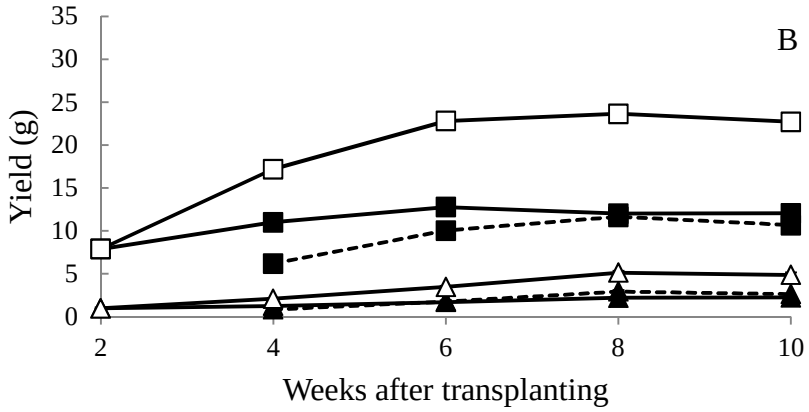

D
Organic- and total-N (mg)
NO_3^--N (mg)
Weeks after transplanting
0
10
20
30
40
50
0
0.5
1
1.5
6
8
10

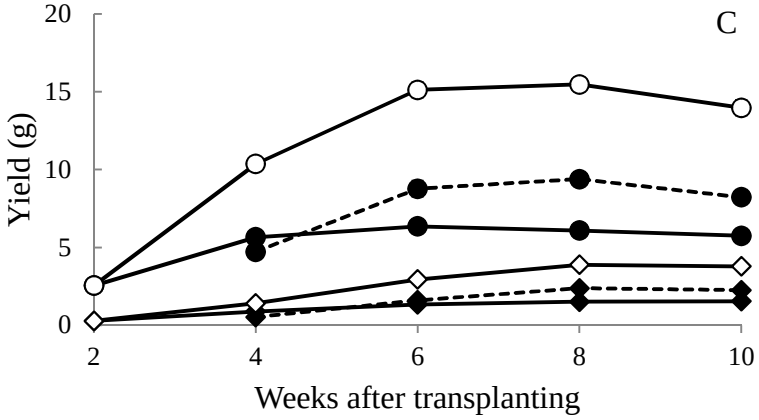
4.2.3 *Leaf Area and Yield as a Function of Plant Development*

The addition of NH_4^+ and NO_3^-, rather than NO_3^- alone, produced higher LA and FM for the upper and lower leaves and greater FM for upper and lower stem plus petioles for up to the first 6 weeks in the growth cycle (Figs. 4.18-4.21, panels A-C). This trend did not continue thereafter, as the only treatment effect was the amount of supplied N; the 400 mg N treatments produced greater LA and FM of both upper and lower leaves and FM of upper and lower stem plus petioles. At the conclusion of the experiment, treatment had no effect on the DM of the lower leaves, and upper and lower stems plus petioles. The upper leaves of plants receiving 400 mg of N had greater DM than those from plants supplied with 200 mg N, but effect of N form was not significant. The treatments receiving 200 mg of N did not continue to accumulate leaf and stem plus petioles FM or DM after week 6. Similarly, very little leaf and stem plus petiole FM or DM accumulated in the 400 mg N treatments after week 6, during which time the flower was developing. The yield characteristics of the flower followed similar trends (Fig. 4.18-4.21, panel D). The 18:0(400) treatment produced plants with greater mean flower FM and DM than the other treatments. The 9:9(400) treatment resulted in greater flower FM and DM than found with either the 18:0(200) or the 9:9(200) treatment; these latter two treatments were not different from each other.

Figure 4.18. Weekly cumulative yield characteristics of *Chrysanthemum morifolium* 'Yellow Favor' supplied with 18 mM NO_3^--N, with fertilization terminated when 200 mg of total N was supplied to the pot. See Figure 4.12 for full description of treatments. A. Leaf area (LA): lower leaves, closed squares (■) solid line; upper leaves, closed squares (■) dashed line; total LA, open squares (□) solid line. B. Leaf fresh mass (FM) and dry mass (DM): lower leaf FM, closed squares (■) solid line; upper leaf FM, closed squares (■) dashed line; total leaf FM, open squares (□) solid line; lower leaf DM, closed triangles (▲) solid line; upper leaf DM, closed triangles (▲) dashed line; total leaf DM, open triangles (Δ) solid line. C. Stem plus petioles FM and DM: lower stem plus petioles FM, closed circles (●) solid line; upper stem plus petioles FM, closed circles (●) dashed line; total stem plus petioles FM, open circles (○) solid line; lower stem plus petioles DM, closed diamonds (**♦**) solid line; upper stem plus petioles DM, closed diamonds (**♦**) dashed line; total stem plus petioles DM, open diamonds ( ) solid line. D. Flower: FM, saltire (⨰) solid line; DM saltire (⨰) dashed line. The data represent the mean ± SE (N = 12); where bar is not shown, it is within the symbol.


A
LA (cm2)
1200
1000
800
600
400
200
2
4
6
8
10
Weeks after transplanting


B
Yield (g)
35
30
25
20
15
10
5
0
2
4
6
8
10
Weeks after transplanting


C
Yield (g)
20
15
10
5
0
2
4
6
8
10
Weeks after transplanting

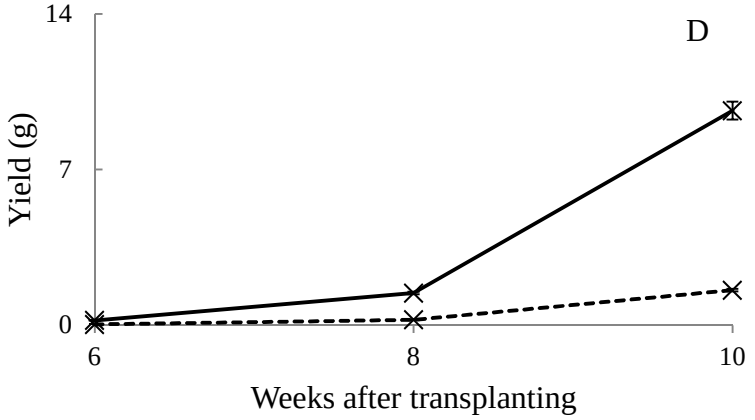
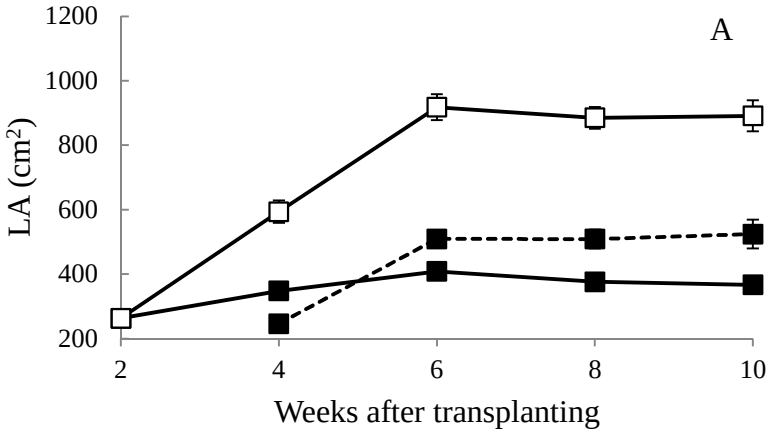
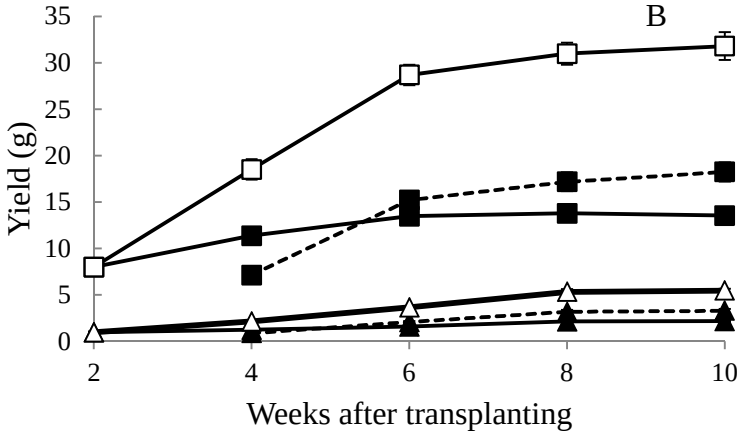
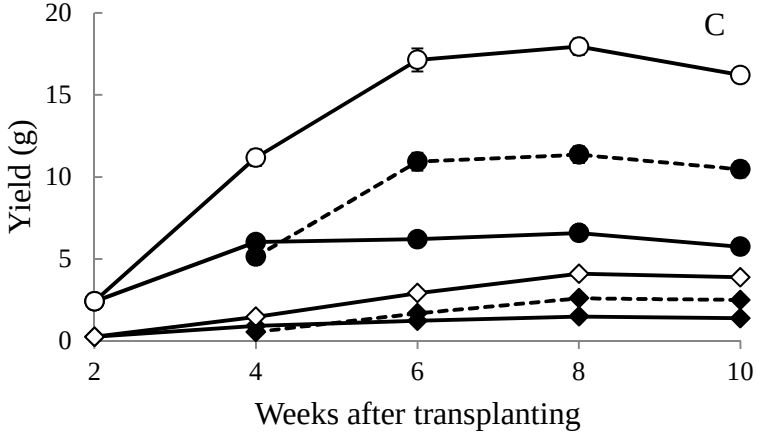

D
Yield (g)
14
7
0
6
8
10
Weeks after transplanting

Figure 4.19. Weekly cumulative yield characteristics of *Chrysanthemum morifolium* 'Yellow Favor' supplied with 18 mM NO_3^--N, with fertilization terminated when 400 mg of total N was supplied to the pot. See Figure 4.12 for full description of treatments. A. Leaf area (LA): lower leaves, closed squares (■) solid line; upper leaves, closed squares (■) dashed line; total LA, open squares (□) solid line. B. Leaf fresh mass (FM) and dry mass (DM): lower leaf FM, closed squares (■) solid line; upper leaf FM, closed squares (■) dashed line; total leaf FM, open squares (□) solid line; lower leaf DM, closed triangles (▲) solid line; upper leaf DM, closed triangles (▲) dashed line; total leaf DM, open triangles (Δ) solid line. C. Stem plus petioles FM, and DM: lower stem plus petioles FM, closed circles (●) solid line; upper stem plus petioles FM, closed circles (●) dashed line; total stem plus petioles FM, open circles (○) solid line; lower stem plus petioles DM, closed diamonds (♦) solid line; upper stem plus petioles DM, closed diamonds (♦) dashed line; total stem plus petioles DM, open diamonds ( ) solid line. D. Flower: fresh mass (FM), saltire (⨯) solid line; dry mass (DM), saltire (⨯) dashed line. The data represent the mean ± SE (N = 12); where bar is not shown, it is within the symbol.


A
LA (cm²)
1200
1000
800
600
400
200
2
4
6
8
10
Weeks after transplanting


B
Yield (g)
35
30
25
20
15
10
5
0
2
4
6
8
10
Weeks after transplanting


C
Yield (g)
20
15
10
5
0
2
4
6
8
10
Weeks after transplanting

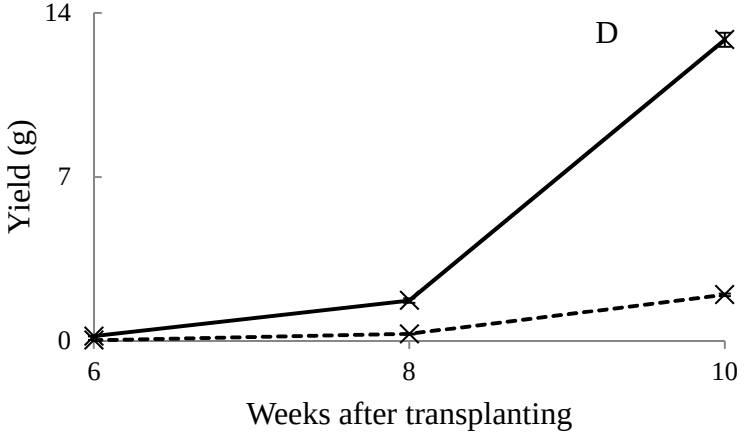
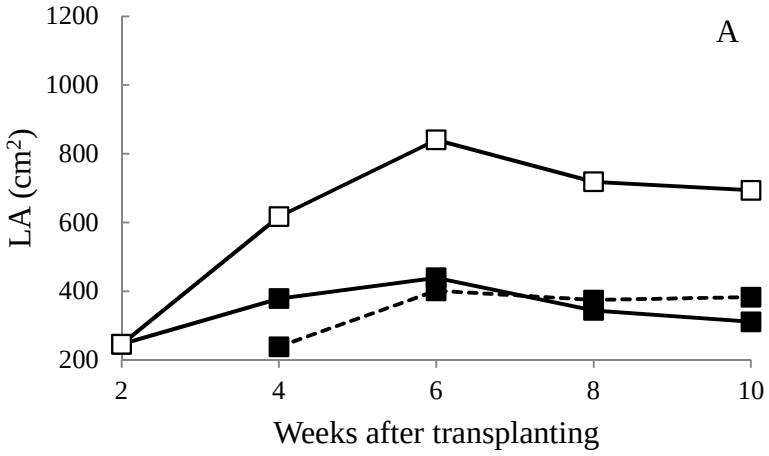
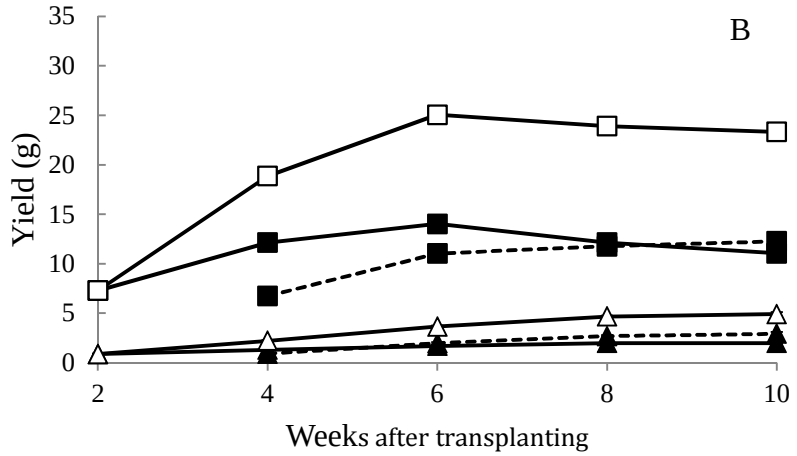
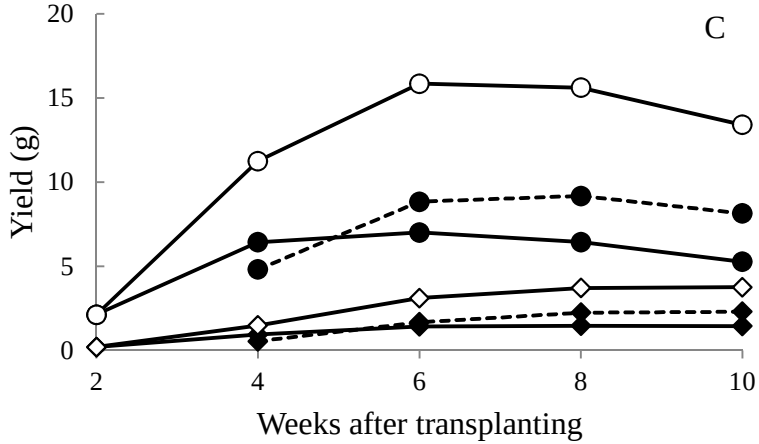

D
Yield (g)
14
7
0
6
8
10
Weeks after transplanting

Figure 4.20. Weekly cumulative yield characteristics of *Chrysanthemum morifolium* 'Yellow Favor' supplied with a combination of 9 mM NO_3^--N and 9 mM of NH_4^+-N, with fertilization terminated when 200 mg of total N was supplied to the pot. See Figure 4.12 for full description of treatments. A. Leaf area (LA): lower leaves closed squares (■) solid line; upper leaves, closed squares (■) dashed line; total LA, open squares (□) solid line. B. Leaf fresh mass (FM) and dry mass (DM): lower leaf FM, closed squares (■) solid line; upper leaf FM, closed squares (■) dashed line; total leaf FM, open squares (□) solid line; lower leaf DM, closed triangles (▲) solid line; upper leaf DM, closed triangles (▲) dashed line; total leaf DM, open triangles (Δ) solid line. C. Stem plus petioles FM and DM: lower stem plus petioles FM, closed circles (●) solid line; upper stem FM, closed circles (●) dashed line; total stem plus petioles FM, open circles (○) solid line; lower stem plus petioles DM, closed diamonds (♦) solid line; upper stem plus petioles DM, closed diamonds (♦) dashed line; total stem plus petioles DM, open diamonds ( ) solid line. D. Flower FM, saltire (⨯) solid line; DM, saltire (⨯) dashed line. The data represent the mean ± SE (N = 12); where bar is not shown, it is within the symbol.


A
LA (cm2)
1200
1000
800
600
400
200
2
4
6
8
10
Weeks after transplanting


B
Yield (g)
35
30
25
20
15
10
5
0
2
4
6
8
10
Weeks after transplanting


C
Yield (g)
20
15
10
5
0
2
4
6
8
10
Weeks after transplanting

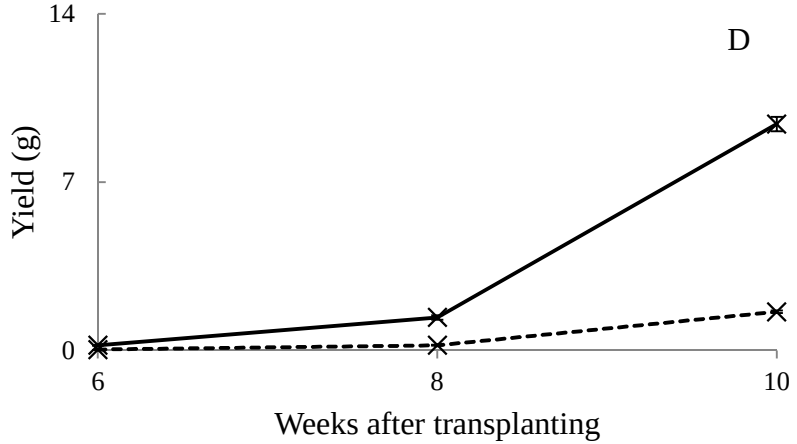
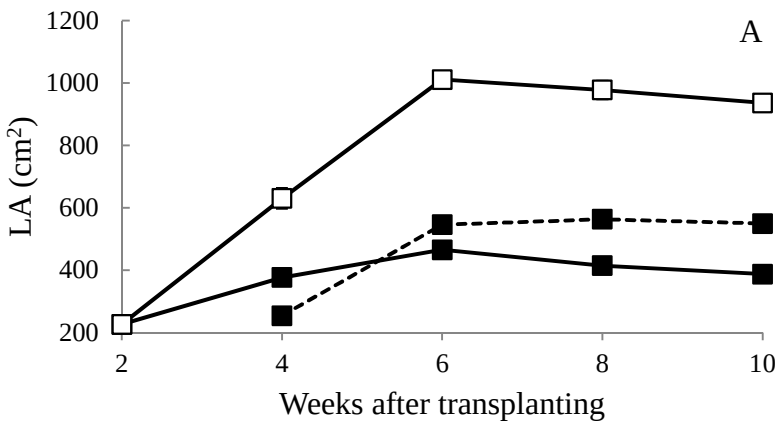
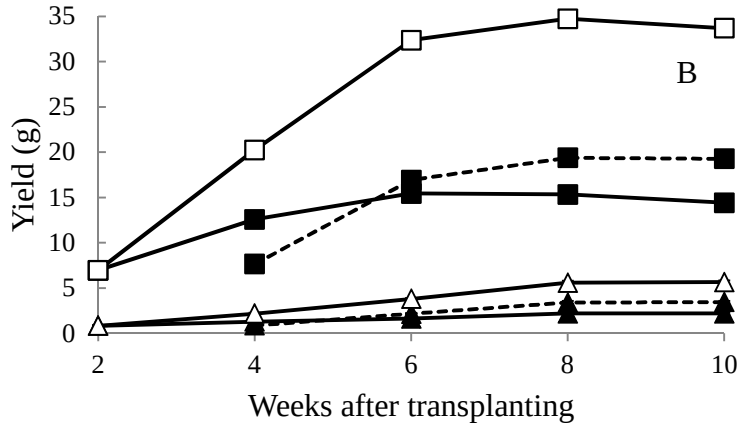
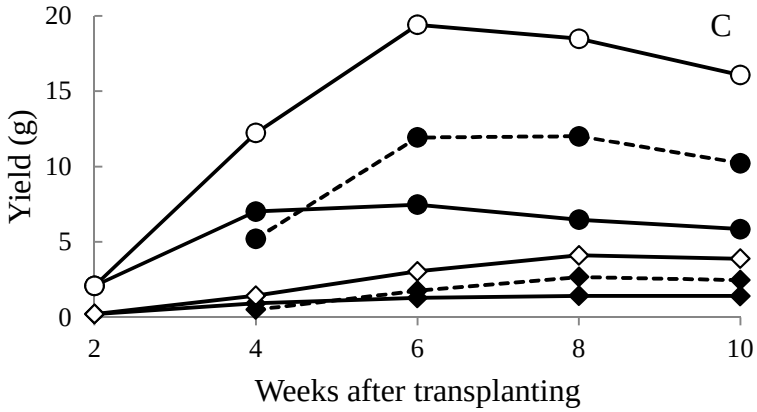
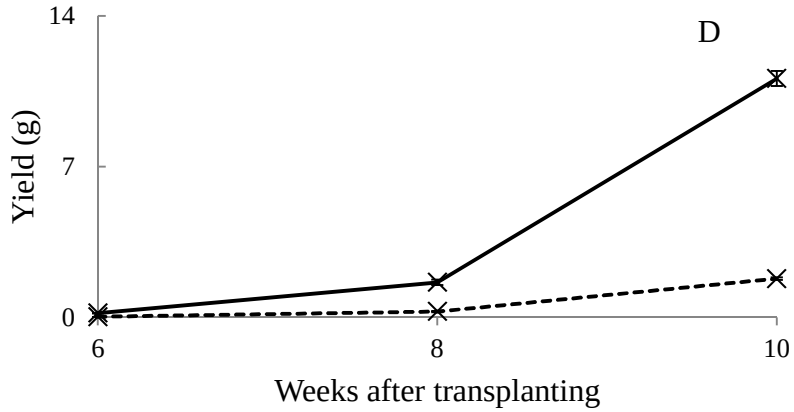

D
Yield (g)
14
7
0
6
8
10
Weeks after transplanting

Figure 4.21. Weekly cumulative yield characteristics of *Chrysanthemum morifolium* 'Yellow Favor' supplied with a combination of 9 mM NO_3^--N and 9 mM of NH_4^+-N, with fertilization terminated when 400 mg of total N was supplied to the pot. See Figure 4.12 for full description of treatments. A. Leaf area (LA): lower leaves, closed squares (■) solid line; upper leaves, closed squares (■) dashed line; total LA, open squares (□) solid line. B. Leaf fresh mass (FM) and dry mass (DM): lower leaf FM, closed squares (■) solid line; upper leaf FM, closed squares (■) dashed line; total leaf FM, open squares (□) solid line; lower leaf DM, closed triangles (▲) solid line; upper leaf DM, closed triangles (▲) dashed line; total leaf DM, open triangles (Δ) solid line. C. Stem plus petioles FM and DM: lower stem plus petioles FM, closed circles (●) solid line; upper stem plus petioles FM, closed circles (●) dashed line; total stem plus petioles FM, open circles (○) solid line; lower stem plus petioles DM, closed diamonds (♦) solid line; upper stem plus petioles DM, closed diamonds (♦) dashed line; total stem plus petioles DM, open diamonds ( ) solid line. D. Flower FM, saltire (⨯) solid line; DM, saltire (⨯) dashed line. The data represent the mean ± SE (N = 12); where bar is not shown, it is within the symbol.


A
LA (cm2)
1200
1000
800
600
400
200
2
4
6
8
10
Weeks after transplanting


B
Yield (g)
35
30
25
20
15
10
5
0
2
4
6
8
10
Weeks after transplanting


C
Yield (g)
20
15
10
5
0
2
4
6
8
10
Weeks after transplanting


D
Yield (g)
14
7
0
6
8
10
Weeks after transplanting

4.2.4 *Nitrogen Use Efficiency During Flower Development*

4.2.4.1 Nitrogen accumulation and mobilization

Over the 6 to 10 week period, during which the flower was developing, the NO_3^- content in the lower leaves of plants receiving 400 mg N declined more than those from plants receiving 200 mg N (Table 4.3). The organic-N content of the lower leaves declined in all but the 18:0(400) treatment, which happened to gain organic-N. Interestingly, the loss in organic-N content of the lower leaves of plants receiving the 9:9(200) treatment was greater than those of the 9:9(400) treatment. The treatments with NH_4^+ added to the nutrient solution showed a greater loss in total-N from the lower leaves than the 18:0(400) treatment. The NO_3^- content of the upper leaves declined during the flowering period, with the plants receiving 400 mg N losing more NO_3^- than those receiving 200 mg N. The upper leaves of plants receiving 400 mg N gained both organic-N and total-N, while those receiving 200 mg lost both N forms. The NO_3^- and organic-N contents of the lower stem plus petioles declined in all cases, with no difference between the treatments in regards to NO_3^- content, but 9:9(400) treatment showed a greater loss in organic-N than the plants receiving strictly NO_3^-, the 18:0(400) treatment. The NO_3^- content in the upper stem plus petioles of plants receiving 400 mg N decreased more than that in plants receiving 200 mg N. All treatments showed a decrease in organic-N content with exception of 18:0(400), which gained organic-N. The flowers of plants supplied with 400 mg N gained more total and organic-N than those of plants supplied with 200 mg, but there was no effect of N form.

Table 4.3. Change in N content over the 6-10 week period of parts of *Chrysanthemum morifolium* 'Yellow Favor' supplied with 18 mM NO_3^--N or a combination of 9 mM NO_3^--N and 9 mM NH_4^+-N. Pots in the 18 mM NO_3^--N or the 9 mM NO_3^-/ NH_4^+-N combination were supplied with either 200 or 400 mg total N. The data represent the mean difference (± SE) in N content between week 10 and 6 (N = 6). Data not sharing the same letter within columns of the same N fraction are significantly different ($P \leq 0.05$).

N treatment	N fraction	Change in N content (mg N)				
		Lower Leaves	Upper Leaves	Lower Stem	Upper Stem	Flower
18:0(200)	Nitrate	- 2.2 ± 0.1 b	- 0.5 ± 0.1 b	- 3.1 ± 0.4 a	- 3.4 ± 0.3 b	0.0 ± 0.0 a
	Organic	- 17.8 ± 2.9 xy	- 8.2 ± 9.1 xy	- 1.9 ± 1.2 xy	- 3.9 ± 2.4 x	+ 30.5 ± 1.4 x
	Total	- 19.9 ± 2.9 AB	- 8.7 ± 9.1 A	- 5.0 ± 1.1 AB	- 7.3 ± 2.7 A	+ 30.5 ± 1.4 A
18:0(400)	Nitrate	- 11.0 ± 1.1 a	- 12.4 ± 2.5 a	- 4.0 ± 0.7 a	- 7.9 ± 1.7 a	+ 1.0 ± 0.5 b
	Organic	+ 3.9 ± 3.4 z	+ 19.2 ± 10.8 z	- 0.2 ± 2.0 y	+ 6.0 ± 9.1 x	+ 41.6 ± 1.5 y
	Total	- 7.1 ± 4.3 B	+ 6.8 ± 11.2 A	- 4.1 ± 2.6 B	- 1.9 ± 10.2 A	+ 42.7 ± 1.4 B
9:9(200)	Nitrate	- 3.0 ± 0.4 b	- 0.3 ± 0.1 b	- 3.4 ± 0.3 a	- 1.7 ± 0.3 b	0.0 ± 0.0 a
	Organic	- 28.2 ± 3.6 x	- 16.0 ± 6.2 x	- 6.1 ± 1.7 xy	- 6.6 ± 1.3 x	+ 29.6 ± 0.9 x
	Total	- 31.1 ± 3.9 A	- 16.3 ± 6.1 A	- 9.5 ± 1.7 AB	- 8.3 ± 1.5 A	+ 29.6 ± 0.9 A
9:9(400)	Nitrate	- 12.6 ± 1.6 a	- 9.9 ± 1.6 a	- 4.8 ± 0.9 a	- 7.1 ± 1.0 a	+ 0.1 ± 0.0 a
	Organic	- 10.2 ± 5.4 y	+ 15.0 ± 8.6 yz	- 8.9 ± 4.6 x	- 8.6 ± 3.8 x	+ 42.1 ± 2.3 y
	Total	- 22.3 ± 6.9 A	+ 5.1 ± 9.3 A	- 13.7 ± 5.4 A	- 15.7 ± 4.4 A	+ 42.2 ± 2.3 B

4.2.4.2 Shoot total N and nitrogen use indices

At six weeks the 9:9(400) treatment had the greatest content of total-N in the shoot, even 21% more total-N than the 18:0(400) treatment (Fig 4.22). The 9:9(200) treatment had 13% more total-N in the shoot than the 18:0(200) treatment, although it was not significantly different from this treatment. By week 10 the total-N content was directly proportional to the amount of N supplied. The NUI of plants receiving 200 mg N had a greater NUI than the plants receiving 400 mg N (Fig. 4.23). There was no difference in NUpE among the treatments. Regardless of the N form or the amount of N supplied, approximately 80% of the supplied N was absorbed by the plant (Fig. 4.24, panel A). However, the treatments receiving the lower amount of N utilized the N better, with a greater DM being produced per unit of N in the shoot. The form of N supplied did not affect the NUI. NUtE, NUE (flower) and NHI (flower) all displayed the same trend. Plants supplied with lower amounts of N were better able to utilize the supplied N, with all of these indices being higher with the 200 mg N treatments (Fig. 4.24, panels B-D). Plants supplied with 200 mg N generally had slight yellowing of the lower leaves (Fig. 4.25).

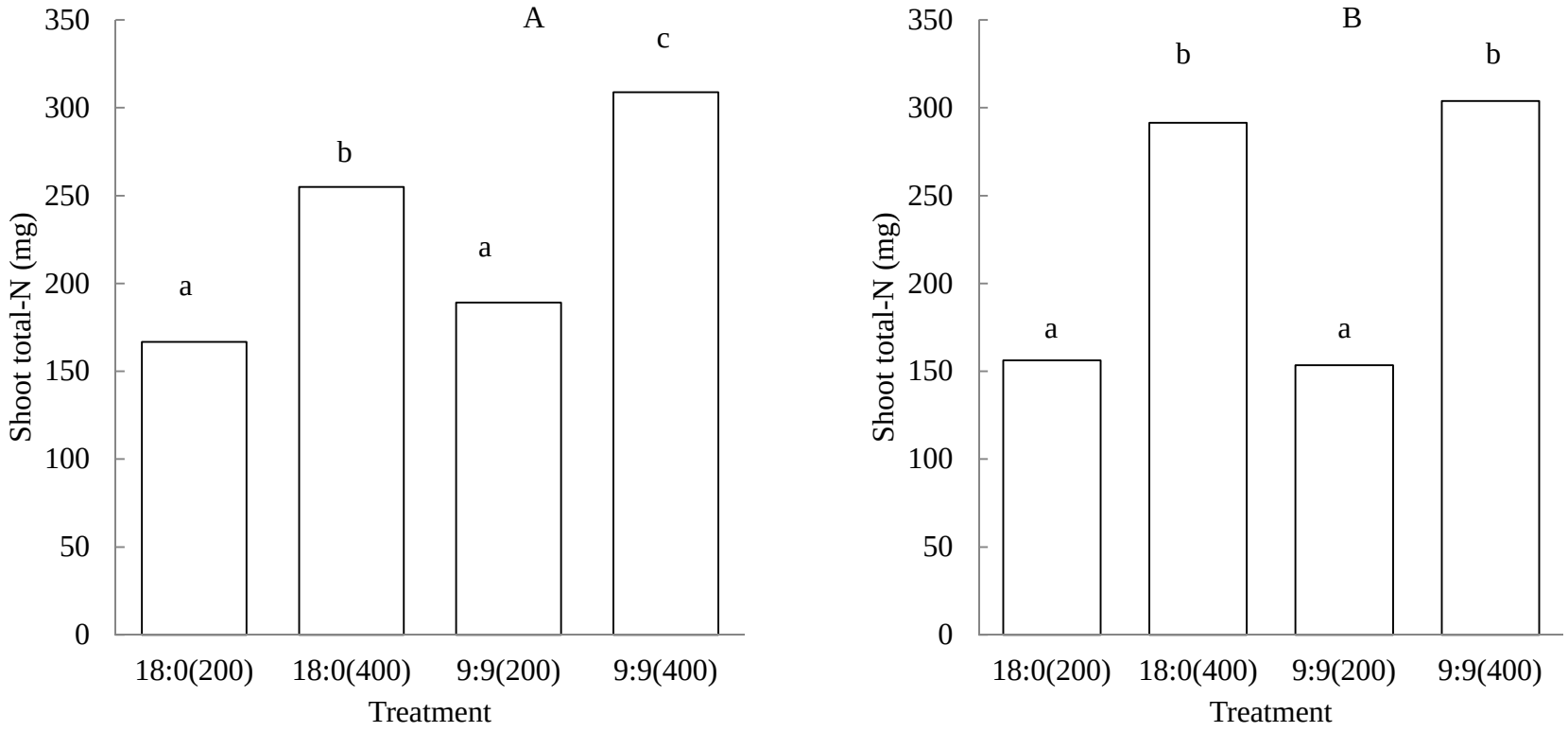


Figure 4.22. Shoot total-N of *Chrysanthemum morifolium* 'Yellow Favor' at 6 weeks (A) and 10 weeks (B) supplied with 18 mM NO_3^--N and 9 mM NO_3^-/NH_4^+-N combination at 200 and 400 mg total N per pot. See Figure 4.12 for full description of treatments. The data represent the mean (N = 6). Bars not sharing the same letter are significantly different at $P \leq 0.05$ ($LSD_{0.05}$).

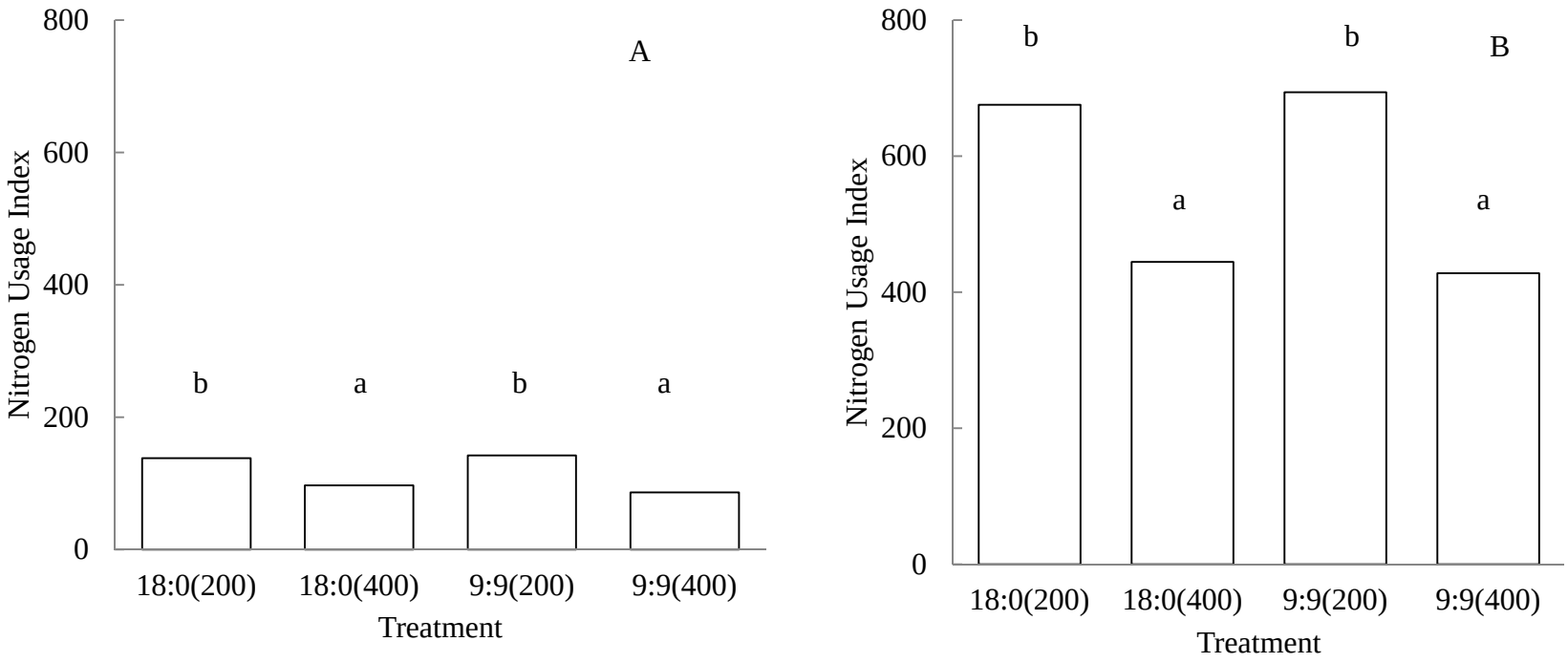
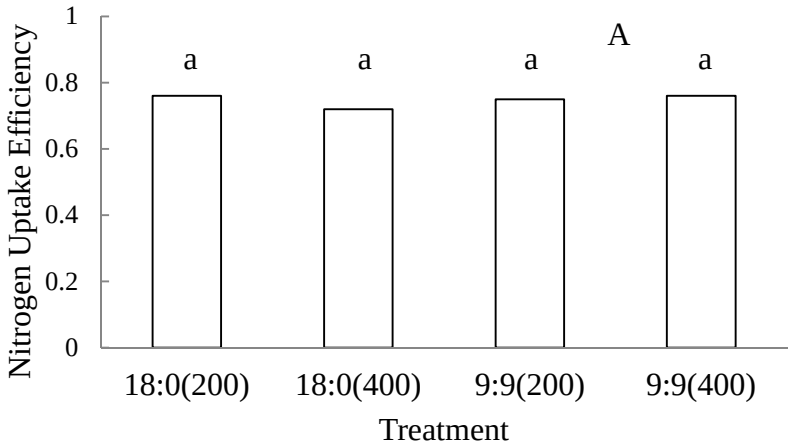
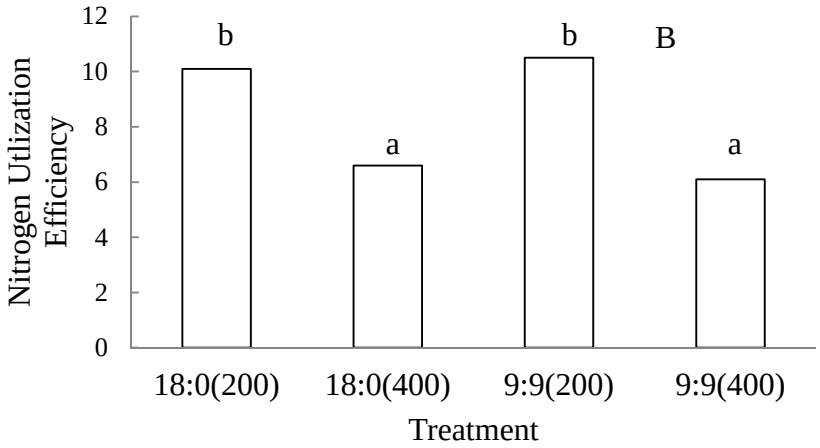
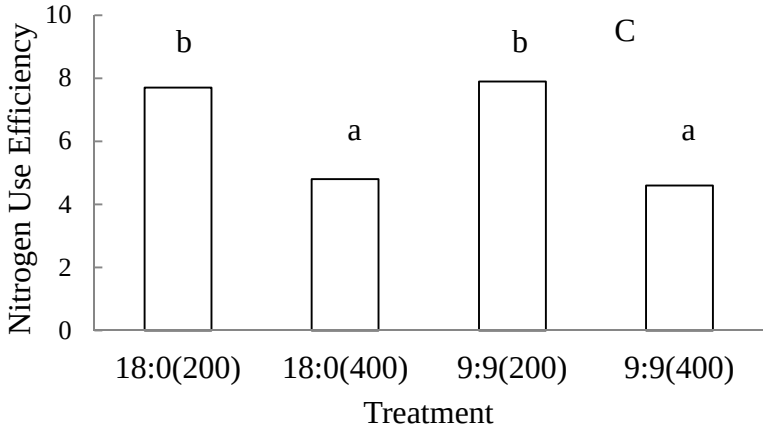


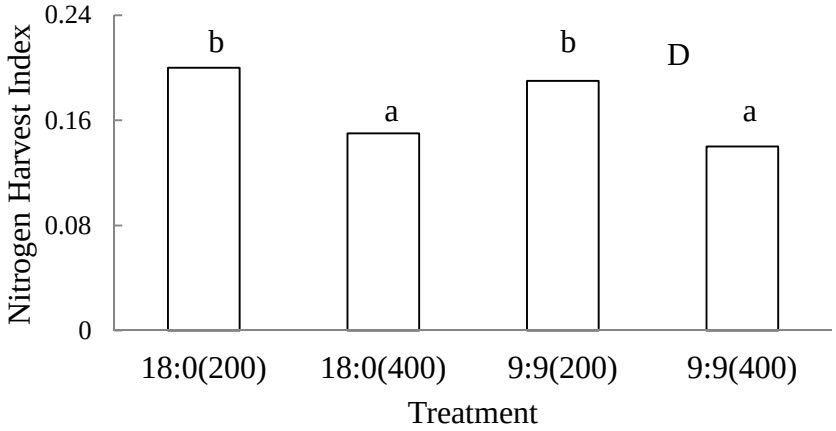
Figure 4.23. The Nitrogen Usage Index (NUI) of *Chrysanthemum morifolium* 'Yellow Favor' at 6 weeks (A) and 10 weeks (B) supplied with 18 mM NO_3^--N and 9 mM NO_3^--N/NH_4^+-N combination at 200 and 400 mg total N per pot. See Figure 4.12 for full description of treatments. The data represent the mean (N = 6). Bars not sharing the same letter are significantly different at $P \leq 0.05$ ($LSD_{0.05}$). NUI = shoot DM * (shoot DM / shoot N content).

Figure 4.24. The Nitrogen use indices of *Chrysanthemum morifolium* 'Yellow Favor' supplied with 18 mM NO_3^--N and 9 mM NO_3^-/NH_4^+-N combination at 200 and 400 mg total N per pot. See Figure 4.12 for full description of treatments. A. Nitrogen Uptake Efficiency (NUpE). B. Nitrogen Utilization Efficiency (NUtE). C. Nitrogen Use Efficiency (flower) (NUE). D. Nitrogen Harvest Index (NHI). The data represent the mean (N = 6). Bars not sharing the same letter are significantly different at $P \leq 0.05$ ($LSD_{0.05}$). NUpE = shoot N content / N supply; NUtE = flower DM / shoot N content; NUE = flower DM / N supply; NHI = flower N content / shoot N content.


Nitrogen Uptake Efficiency
1
0.8
0.6
0.4
0.2
0
A
a
a
a
a
18:0(200)
18:0(400)
9:9(200)
9:9(400)
Treatment


Nitrogen Utlization Efficiency
12
10
8
6
4
2
0
B
b
a
b
a
18:0(200)
18:0(400)
9:9(200)
9:9(400)
Treatment


Nitrogen Use Efficiency
10
8
6
4
2
0
C
b
a
b
a
18:0(200)
18:0(400)
9:9(200)
9:9(400)
Treatment


Nitrogen Harvest Index
0.24
0.16
0.08
0
D
b
a
b
a
18:0(200)
18:0(400)
9:9(200)
9:9(400)
Treatment

9:9(200) 9:9(400) 18:0(200) 18:0(400)

Figure 4.25. Representative chrysanthemum plants at the conclusion of the experiment. See Figure 4.12 for full description of treatments.

CHAPTER 5: Discussion

5.1 Nitrate Removal and Chloride Substitution for Nitrate

The goal of greenhouse floricultural production is to produce a quality product in a cost effective manner while minimizing the impact on the environment. Here, research was conducted to establish an effective approach for reducing the overall N requirements for producing high quality potted chrysanthemums in a commercial growing system. Several management strategies were employed to limit the accumulation of NO_3^- (storage) during flowering, without sacrificing plant quality, thereby reducing the exogenous requirement for N during this period.

The first strategy involved the elimination of NO_3^-. Control plants receiving the full NO_3^- supply (18.5 mM) accumulated NO_3^- during the entire course of the experiment in the leaves and stem plus petioles (Fig. 4.2, panels B & C). The NO_3^- content of the stem plus petioles was 47 % of the total-N at flowering, while the NO_3^- in the leaves was 23% of the total-N. These plants lost organic-N from the stem plus petioles over the 6 to 10 week period, while continuing to accumulate NO_3^- (Table 4.1). During the last two weeks, the stem plus petioles gained 3.9 mg of NO_3^- N, as compared to a 14.6 mg decline in organic-N content, resulting in a net loss of 10.7 mg of total-N. This remobilized N accounted for 45% of that accumulated by the flower. Thus, when N was in adequate supply, organic-N in the stem plus petioles was the predominant form of N remobilized for the growth of reproductive tissue. Previous work demonstrated that organic-N is mobilized from the lower canopy of broccoli before NO_3^- when the N supply is adequate (Liu & Shelp, 1995). In the present study, the leaves gained organic and NO_3^--N during

flower development, and there was no evidence for remobilization from this tissue. The flower accumulated 30.3 mg N and 0.8 g DM by the conclusion of the experiment. The EC of the upper and lower medium was 7.2 and 4.2 mS/cm, respectively, at the conclusion of the experiment, which is greater than the recommended value of 0.8-1.5 mS/cm (Aris, 2010).

Complete removal of the NO_3^- supply (0 mM) reduced the NO_3^- content of the leaves and stem plus petioles during flower development, but increased the organic-N fraction in stem plus petioles (Table 4.1). The NO_3^- contents of the stem plus petioles and leaves, respectively, represented 34% and 16% of the total-N at flowering (Fig. 4.4, panels B & C). During the last two weeks, the loss of organic and NO_3^--N from the stem plus petioles was 8.0 mg and 2.3 mg, respectively, with the remobilized N accounting for 53% of the N accumulated by the flower (Table 4.2). Overall, there was no evidence for remobilization of N from the leaves. The EC of the upper and lower medium of these plants declined significantly during flowering, indicating that a large proportion of the N recovered in the flower originated from the medium (Fig. 4.1).

Plants receiving no NO_3^- lost similar amounts of total-N from the stem plus petioles as control plants over the last two weeks. In the control plants, the remobilized N was strictly in the organic form, whereas in the absence of NO_3^- supply 22% was in the NO_3^- form. It could be assumed that NO_3^- was being remobilized from the vacuole when the NO_3^- supply was eliminated because tissue NO_3^- concentration is primarily a measure of NO_3^- stored in the vacuole (van der Liej et al., 1998; Fan et al., 2007; Richard-Molard et al., 2008). These plants were able to produce the same amount of flower DM and total-N

as plants receiving the full NO_3^- supply, suggesting that the remobilized NO_3^- was used to support growth during flower development.

Previous research using barley supports this interpretation. Removal of NO_3^- supply reduces tissue NO_3^- concentration, without affecting the relative growth rate, and this is accompanied by a decline in vacuolar NO_3^- level but no change in cytosolic NO_3^-, leading the authors to suggest that the growth of the seedlings is maintained via the remobilization of vacuolar NO_3^- (van der Leij et al., 1998). It has been demonstrated that the ability of *Arabidopsis* to withstand NO_3^- starvation depends on the extent of NO_3^- storage prior to NO_3^- starvation (Richard-Molard et al. 2008). In the present study, the stem plus petioles of chrysanthemums with or without NO_3^- supply contained a large proportion of their total-N as NO_3^- at the conclusion of flowering (50 and 34%, respectively) (Figs. 4.2 & 4.4, panel C). It has been established that this plant species has the ability to store large amounts of NO_3^-, particularly in the stems plus petioles (Woodson & Boodley, 1983).

Other research has identified the petiole as a major storage site for NO_3^- (Rabb & Terry, 1995; Chiu et al., 2004; Dechorgnat et al., 2011; Wang et al., 2012). Two-week-old sugar beet (*Beta vulgaris* L.) plants receiving NO_3^--N only have significantly greater NO_3^- concentration in the petioles than in the leaf lamina, and 3 and 21 d after the treatment begins 93% and 63%, respectively, of the petiolar N exists as NO_3^-, whereas approximately 0.5% and 1.5% of the laminar N exists as NO_3^- (Rabb & Terry, 1995). Plants receiving NH_4^+ only have markedly less NO_3^- in the leaf lamina and petioles (to be further discussed below). Broccoli, grown strictly with NO_3^--N until inflorescence emergence, followed by substitution of NO_3^- with Cl^- or SO_4^{2-}, experience varying

reductions in shoot NO_3^- contents, yet regardless of treatment, 70-90% of the decrease in shoot NO_3^- is from the stems, petioles and midribs even though this stratum accounts for only 43% of the shoot DM. These findings indicate that petioles are a major site for the storage of NO_3^- (Liu & Shelp, 1995).

The petioles of wild type *Arabidopsis* have higher NO_3^- concentration and lower NR activity than those of *atnrt 1:4-1* knockout mutants (Chiu et al., 2004). Wild type plants also have lower NO_3^- concentration and higher NR activity higher in the lamina than in the petioles. The laminar NO_3^- concentration is higher in the *atnrt 1:4-1* mutants than in the wild type, but the NR activity is similar. These results suggest that petiolar *AtNRT 1:4* gene regulates NO_3^- homeostasis in the leaves of *Arabidopsis*. Another *NRT1* gene in *Arabidopsis*, *NRT1.7*, is expressed in the phloem of minor veins of older leaves and could be responsible for phloem loading of NO_3^- from older source leaves to younger sink leaves (Fan et al., 2009). Together, these studies support the feasibility of using management strategies for enhancing the remobilization of NO_3^- from older tissues, thereby increasing the efficiency of utilization for the NO_3^- supplied. This was evident in the stem plus petioles of chrysanthemums receiving no NO_3^- during flowering, as the percentage of total-N remaining as NO_3^- at the conclusion of flowering was 34 %, compared to 47% in control plants. During weeks 8-10, the stem plus petioles of plants receiving no NO_3^- remobilized 22% of the total-N as NO_3^-, as compared to 0% in the control (Table 4.2). The EC of the upper and lower halves of the growing medium for plants receiving no NO_3^- was significantly lower than that for the control, and the shoot FM was greater (Fig. 4.1 & Fig. 4.8, panel B). Notably, plants with the lower medium EC would have a greater shelf life (Nell et al., 1989; Roude et al., 1991a; Roude et al. 1991b;

Nell et al., 1997) and the greater FM is desirable when plants are produced in a commercial setting using plant growth regulators (Ball, 1991).

The second strategy involved the substitution of Cl^- for NO_3^- in the nutrient solution (i.e., the 15.5, 12.5 and 9.5 mM treatments) to provide the plant with an anion that could possibly replace NO_3^- in the vacuole. The plants for all the Cl^- treatments gained NO_3^- in the leaves and stem plus petioles during flower development; only the leaves of plants receiving 15.5 mM NO_3^- accumulated more NO_3^- than the control plants (Table 4.1). In the 15.5 mM NO_3^- treatment, 46% of total-N was present as NO_3^- in the stems plus petioles at flowering, whereas in the 12.5 and 9.5 mM NO_3^- treatments 42-44% of the total-N was present as NO_3^- (Figs. 4.5-4.7, panel C). The leaves of plants from these treatments also gained NO_3^- during flower development, as 19-24% of the total-N was present as NO_3^- at the conclusion of flowering (Figs. 4.5-4.7, panel B). The stem plus petioles and leaves of these plants were similar to the control plants that contained 47% and 23% of total-N as NO_3^-, respectively. During the last 2 weeks, the loss of total-N from the stem plus petioles was 1.6 to 4.9 mg, with the remobilized N accounting for only 6-23% of the N accumulated by the flower, and there was no evidence of N being remobilized from the leaves (Table 4.2). The additional N recovered in the flower must have originated from the medium, although there was not a decline in the medium EC, which could be explained by the accumulation of Cl^- in the medium (Fig 4.1). Thus, the complete elimination of NO_3^- was the only management strategy that reduced the NO_3^- contents of both the leaf and stem plus petiole tissue. Similar results have been found for soil and hydroponically-grown lettuce (van der Boon et al., 1990; McCall & Willumsen, 1998). Also, the substitution of NO_3^- with Cl^- or SO_4^{-2} is effective in lowering the NO_3^-

content of the broccoli inflorescence, but results are similar to those obtained with the removal of NO_3^- only (Liu & Shelp 1995). Thus, it would appear that elimination of NO_3^-, as opposed to replacement of NO_3^- with another anion, is the most effective method of remobilizing NO_3^- in chrysanthemum.

The total-N content of the chrysanthemum shoots at the conclusion of the experiment did not differ among the NO_3^-/Cl^- treatments, indicating that N uptake was similar (Fig. 4.10). However, the partitioning of N between NO_3^- (storage) and organic-N (metabolic) did differ, and this influenced the NUI, which reveals the efficiency with which N is used to produce biomass, while also considering the yield component (Siddiqi & Glass, 1981). The NUI was significantly greater with the elimination of NO_3^- than with the full NO_3^- treatment (Fig. 4.11). Plants receiving no NO_3^- during the last 5 weeks of the growth cycle mobilized stored NO_3^- for conversion into organic-N, resulting in a greater biomass per unit of N than plants receiving full NO_3^-, which continued to accumulate and store NO_3^-. Thus, elimination of NO_3^- at the onset of flower development appears to be an effective strategy to lower N use and improve NUI in a subirrigation system growing chrysanthemums.

5.2 Ammonium and Nitrate Nutrition

Control plants receiving 400 mg N at 18 mM NO_3^--N (18:0(400)), accumulated a large proportion of the total-N in the stem plus petioles as NO_3^-, with a smaller proportion as NO_3^- in the leaves. At 6 weeks, 25-27% of the total N contents in the lower and upper stem plus petioles was in the NO_3^- form. However, at flowering NO_3^- comprised 10-15% of the total-N contents in these tissues (Fig. 4.14, panel C & D), as well as 2-3% of the

total-N content in the lower and upper leaves (Fig. 4.14, panels A & B). During development of, and NO_3^- gain by, the flower (week 6 to 10), all vegetative tissues lost NO_3^- . This was accompanied by gains in organic-N in both lower and upper leaves, yet there was a net loss of total-N, all as NO_3^-, from the lower leaves (Table 4.3). Both upper and lower stems plus petioles lost N during this time, and combined with the loss of N from the lower leaves, accounted for 31% of the flower N at the conclusion of the experiment. There was a steep decline in the medium EC during this time, suggesting that a significant amount of the N in the flower originated from the root medium (Fig. 4.12).

The addition of NH_4^+ to the nutrient solution, at the same N supply level (9:9(400)), reduced NO_3^- accumulation in the upper and lower stems plus petioles (Fig. 4.16, panels C & D). At 6 weeks, 14-17% of the total-N content in the upper stems plus petioles and the lower stems plus petioles was present as NO_3^-. At the conclusion of flowering, this was reduced to 4-8% of the total-N as NO_3^-. The upper and lower leaves responded similarly to added NH_4^+, with less of the total-N content present as NO_3^- (Fig. 4.16, panels A & B). These changes were accompanied by increased organic-N content early in the growth cycle. At week 6 the organic-N content was greater in all tissues, with the exception of the lower leaves, and by week 8 the organic-N content of both the upper and lower stems plus petioles was greater (Fig. 4.16, panels A-D). The only effect of the NH_4^+ treatment at the conclusion of the experiment was the increased organic-N content of the upper leaves (Fig. 4.16, panel B). The amount of total-N remobilized from the leaves and stems plus petioles of 9:9(400) plants was 46.6 mg, while the amount of N recovered in the flower was 42.2 mg. In contrast to the medium EC of the 18:0(400) plants, the medium EC of the 9:9(400) plants did not decline during flowering (Fig. 4.12).

Together, these data indicate that the organic-N accumulated early in the growth cycle of 9.9(400) plants and subsequently remobilized, could account for the N accumulated by the flower.

Plants supplied with 200 mg N, either in the NO_3^- or NH_4^+ form, contained virtually no NO_3^- in any plant tissue at flowering (Figs 4.13 & 4.15, panels A-D), and the greatest amount of N was lost during the last 4 weeks from the lower leaves: 19.9 and 31.1 mg N from the 18:0(200) and 9:9(200) treatments, respectively (Table 4.3). This was accompanied by slight yellowing of the lower leaves, an indication of N loss, but overall quality, while less than that of plants receiving 400 mg N, was acceptable (Fig. 4.25). The N use indices of plants receiving 200 mg N were generally greater than those of plants receiving 400 mg N (Fig. 4.23, panels A & B; Fig. 4.24, panels A-D); the only exception was NUpE, indicating similar uptake of available N, regardless of N form. Thus, the plants receiving the reduced N supply were better able to utilize the available N, resulting in more total DM and an improved NUI, more flower DM and an improved NUtE and NUE, and more mobilization of N to the flower and an improved NHI.

These findings are in agreement with earlier results with chrysanthemums (Elliot & Nelson, 1983) and leafy vegetables, which have decreased NO_3^- concentration and increased levels of reduced-N upon the addition of NH_4^+ to the nutrient solution (van der Boon et al., 1988; 1990; McCall & Willumsen, 1998; Chen et al., 2005; Wang & Shen, 2011). Similar reductions in NO_3^- concentration are found in broccoli with the addition of NH_4^+, and in sugar beets grown with NH_4^+ as the sole N source, petiolar NO_3^- concentration declines to virtually zero within 6 d after the treatment began (Liu & Shelp, 1993a; Rabb & Terry, 1995).

The amount of N remobilized from all vegetative strata of the plants receiving 200 mg N was substantially more than that recovered in the flower: 134% and 220% in the 18:0(200) and 9:9(200) treatments, respectively (Table 4.3). This N was presumably supplying another sink, as chrysanthemums are a perennial plant and in contrast to annuals, woody and herbaceous perennials store N in tissues such as roots at the end of the seasonal growth cycle (Millard, 1996). In woody perennials, remobilization of nutrients such as N increases with low or deficient nutrient supply (Salifu & Timmer, 2001; Salaün et al., 2005). The 200 mg N supplied to these plants would be considered suboptimal for growth, as these plants accumulated approximately 50% of the total N of the plants supplied with 400 mg N and as noted earlier, there was yellowing of the lower leaves (Fig. 4.22, panel B; Fig. 4.25). With deficient amounts of N supplied, N was remobilized to the developing sink of the flower, but presumably to a storage sink in the roots as well.

5.3 Concluding Remarks

Decreasing the amount of fertilizer N, especially NO_3^--N, used in the production of potted chrysanthemums reduces the risk to the environment, but more important to a grower, the NUE is improved. This research provided evidence that while chrysanthemums accumulate large quantities of NO_3^-, elimination of NO_3^- prior to flowering remobilizes this NO_3^- from storage in the vacuole to the metabolic pool in the cytoplasm. Most important to a grower, neither the flower DM or N content was changed by this management method, as indicated by the greater NUI. Elimination of NO_3^- prior to flowering significantly lowered the medium EC, which would result in increased shelf

life (Nell et al., 1989; Nell et al., 1997). Substitution with an anion such as Cl^- did not appear to be an effective method of increasing remobilization of NO_3^-, and the addition of Cl^- substantially increased the medium EC. The addition of NH_4^+ to the nutrient solution decreased the NO_3^- content, especially in the stem plus petioles, and increased the reduced N content early in the growth cycle, but did not improve NUE at flowering. The use of a lower N supply, either as NH_4^+ or NO_3^-, eliminates NO_3^- accumulation prior to flowering, thereby improving the NUE of potted chrysanthemums.

Literature Cited

Ahlgren, S., Baky, A., Bernesson, S., Nordberg, Å., Norén, O. & Hansson, P. A., 2008. Ammonium nitrate fertiliser production based on biomass - envrionmental effects from a life cycle perspective. *Bioresource Technology*, 99(17): 8034-41.

Aris Horticulture, 2010. *Pot Mum Cultural Information*. [Online] Available at: http://www.glplants.com/index.php?option=com_docman&task=cat_view&gid=98&Itemid=60 [Accessed 2 Janury 2013].

Ball, V., 1991. Chrysanthemum. *In: The Ball Red Book*. 15th ed. West Chicago, IL. George. J. Ball Publishing, p. 435-62.

Barraclough, P. B., Howarth, J. R., Jones, J., Lopez-Bellido, R., Saroj P., Shepherd, C. E. & Hawkesford, M. J., 2010. Nitrogen efficiency of wheat: Genotypic and environmental variation and prospects for improvement. *European Journal of Agronomy*, 33(1): 1-11.

Bertheloot, J., Marte, P. & Andrieu, B., 2008. Dynamics of light and nitrogen distribution during grain filling within wheat canopy. *Plant Physiology*, 148(3): 1717-20.

Blom, T.J. & Piott, B.D., 1992. Preplant moisture content and compaction of peatwool using two irrigation techniques on potted chrysanthemums. *Journal of the American Society for Horticultural Science*, 117(2): 220-23.

Blom-Zandstra, G. & Lampe, J.E.M., 1983. The effect of chloride and sulphate salts on the nitrate content in lettuce plants (*Lactuca sativa* L.). *Journal of Plant Nutrition*, 6(7): 611-28.

Borrell, A., Hammer, G. & Van Oosterom, E., 2001. Stay-green: A consequence of the balance between supply and demand for nitrogen during grain filling? *Annals of Applied Biology*, 138(1): 91-95.

Bowen, P.A, Zebarth, B.J. & Toivonen, P.M.A., 1999. Dynamics of nitrogen and dry-matter partitioning and accumulation in broccoli (*Brassica oleracea* var. *italica*) in relation to extractable soil organic nitrogen. *Canadian Journal of Plant Science*, 79(2): 277-86.

Brauer, E.K. & Shelp, B.J., 2010. Nitrogen use efficiency: reconsideration of the bioengineering approach. *Botany*, 88:103-09.

Broadley, M.R., Seginer, I., Burns, A., Escobar-Gutiérrez, A.J., Burns, I.G. & White, P.J., 2003. The nitrogen and nitrate economy of butterhead lettuce (*Lactuca sativa* var. *capitata* L.). *Journal of Experimental Botany*, 54(390): 2081-90.

Brown, W. & Murphy, G., 2011. *The Ontario Greenhouse Floriculture Industry*. [Online] Available at: http://www.omafra.gov.on.ca/english/crops/facts/greenflor.htm [Accessed 2 January 2013].

Canadian Council of Ministers of the Environment, 2012. Canadian water quality guidelines for the protection of aquatic life: Nitrate Ion. In: Canadian environmental quality guidelines, Canadian Council of Ministers of the Environment, Winnipeg. Excerpt from Publication No. 1299; ISBN 1-896997-34-1 [Online] Available at: http://www.ec.gc.ca/lcpe-cepa/default.asp?lang=En&n=E9DBBC31-1 [Accessed 23 December 2012].

Cataldo, D.A., Haroon, M., Schrader, L.E. & Youngs, V.L., 1975. Rapid colorimetric determination of nitrate in plant tissues by nitration of salicylic acid. *Communications in Soil Science and Plant Analysis*, 6(1): 71-80.

Chapagain, B.P., Wiesman, Z., Zaccai, M., Imas, P. & Magen, H, 2003. Potassium chloride enhances fruit appearance and improves quality of fertigated greenhouse tomato as compared to potassium nitrate. *Journal of Plant Nutrition*, 26(3): 643-58.

Chen, W., Luo, J.-K. & Shen, Q.-R., 2005. Effect of NH_4^+-N/NO_3^--N ratios on growth and some physiological parameters of Chinese cabbage cultivars. *Pedosphere*, 15(3): 310-18.

Chiu, C.-C., Lin, C.-S., Hsia, A.-P., Su, R.-C., Lin, H.L. & Tsay, Y.-F, 2004. Mutation of a nitrate transporter, AtNRT1:4, results in reduced petiole nitrate content and altered leaf development. *Plant and Cell Physiology*, 45(9): 1139-48.

Colla, G., Suárez, C. M. C, Cardarelli, M. & Rouphael, Y., 2010. Improving nitrogen use efficiency in melon by grafting. *HortScience*, 45(4): 559-65.

Cox, D.A., 2001. Growth, nutrient content, and growth medium electrical conductivity of poinsettia irrigated by subirrigation or from overhead. *Journal of Plant Nutrition*, 24(3): 523-33.

Crawford, N.M. & Forde, B.G., 2002. Molecular and developmental biology of inorganic nitrogen nutrition. *The Arabidopsis Book 1*: e0011. doi:10.1199/tab.0011.

Crawford, N.M. & Glass, A.D.M., 1998. Molecular and physiological aspects of nitrate uptake in plants. *Trends in Plant Science*, 3(10): 389-95.

Diaz, C., Lemaître, T., Christ, A., Azzopardi, M., Kato, Y., Sato, F., Morot-Gaudry, J.F., Le Dily, F. & Masclaux-Daubresse, C, 2008. Nitrogen recycling and remobilization are differentially controlled by leaf senescence and development stage in *Arabidopsis* under low nitrogen nutrition. *Plant Physiology*, 147(3): 437-49.

Dechorgnat, J., Chi Tam Nguyen, Armengaud, P., Jossier, M., Diatloff, E., Filleur, S. & Daniel-Vedele, F., 2011. From the soil to the seeds: the long journey of nitrate in plants. *Journal of Experimental Botany*, 62(4): 1349-59.

Dole, J.M., Cole, J.C. & von Broembsen, S.L., 1994. Growth of poinsettias, nutrient leaching, and water-use efficiency respond to irrigation methods. *HortScience*, 29(8): 858-64.

Dole, J.M. & Wilkins, H.F., 2005. *Floriculture, Principles and Species*. 2nd ed. Upper Saddle River, New Jersy, USA: Pearson Education Inc. p. 1-995.

Eastin, E.F., 1978. Total nitrogen determination for plant material containing nitrate. *Analytical Biochemistry*, 85(2): 591-94.

Elliott, G.C. & Nelson, P.V., 1983. Relationships among nitrogen accumulation, nitrogen assimilation and plant growth in chrysanthemum. *Physiologia Plantarum*, 57(2): 250-59.

Ellis, S., 2010. *Farms.com*. 2011 Crop production costs should still yield profits. [Online] Available at: http://www.farms.com/FarmsPages/Commentary/DetailedCommentary/tabid/192/Default.aspx?NewsID=35044 [Accessed 2 January 2013].

Emes, M.J. & Neuhaus, H.E., 1997. Metabolism and transport in non-photosynthetic plastids. *Journal of Experimental Botany,* 48(12): 1995-2005.

Fan, S.-C, Lin, C.-S., Hsu, P.-K., Lin, S.-H. & Tsay, Y.-F., 2009. The *Arabidopsis* nitrate transporter NRT1.7, expressed in phloem, is responsible for source-to-sink remobilization of nitrate. The Plant Cell 21(9): 2750-61.

Fan, X. Jia, L., Li, Y., Smith, S.J, Smith, A.J. & Shen, Q., 2007. Comparing nitrate storage and remobilization in two rice cultivars that differ in their nitrogen use efficiency. Journal of Experimental Botany 58(7): 1729-40.

Feller, U. & Fisher, A., 1994. Nitrogen metabolism in senescing leaves. *Critical Reviews in Plant Science,* 13(2): 241-73.

Flowers Canada Growers, 2012. *Flowerscanadagrowers.com A blueprint for environmentally sustainable greenhouse farming.* [Online] Available at: http://www.flowerscanadagrowers.com/flower-growers/fcg-current-projects/a-blueprint-for-environmentally-sustainable-greenhouse-farming [Accessed 23 December 2012].

Garnett, T., Conn, V. & Kaiser, B.N., 2009. Root based approaches to improving nitrogen use efficiency in plants. *Plant, Cell and Environment,* 32(9): 1272-83.

Gastal, F. & Lemaire, G., 2002. N uptake and distribution in crops: an agronomical and ecophysical perspective. *Journal of Experimental Botany*, 53(370): 789-99.

Glass, A.D.M., 2003. Nitrogen use efficiency of crop plants: physiological constraints upon nitrate absorption. *Critical Reviews in Plant Sciences*, 22(5): 453-70.

Good, A.G., Shrawat, A.K. & Muench, D.G., 2004. Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? *Trends in Plant Science*, 9(12): 597-605.

Goodwin, T.W. & Mercer, E.I., 1983. *Introduction to Plant Biochemistry*. 2nd ed. Toronto: Permagon Press, p. 1-626.

Granstedt, R.C. & Huffaker, R.C., 1982. Identification of the leaf vacuole as a major nitrate storage pool. *Plant Physiology*, 70(2): 410-13.

Hall, T.J., Dennis, J.H., Lopez, R.G. & Marshall, M.I., 2009. Factors affecting growers' willingness to adopt sustainable floriculture practices. *HortScience*, 44(5): 1346-51.

Hirel, B. & Lemaire, G., 2005. From agronomy and ecophysiology to molecular genetics for improving nitrogen use efficiency in crops. *Journal of Crop Improvement*, 15(2): 213-58.

Holcomb, E.J., Gamez, S., Beattie, D. & Elliott, G.C., 1992. Efficiency of fertigation programs for baltic ivy and asiatic lily. *HortTechnology*, 2(1): 43-46.

Hortensteiner, S. & Feller, U., 2002. Nitrogen metabolism and remobilization during senescence. *Journal of Exerimental Botany*, 53(370): 927-37.

Howard, D.D., Newman, M.A., Essington, M.E. & Percell, W.M., 2002. Nitrogen fertilization of conservation-tilled wheat. II. Timing of nitrogen application of two nitrogen sources. *Journal of Plant Nutrition*, 25(6): 1329-39.

Incrocci, L., Malorgio, F., Bartola, .A.D. & Pardossi, A., 2006. The influence of drip irrigation or subirrigation on tomato grown in closed-loop substrate culture with saline water. *Scientia Horticulturae,* 107(4): 365-72.

James, E. & van Iersel, M.W., 2001. Ebb and flow production of petunias and begonias as affected by fertilizers with different phosphorus content. *HortScience*, 36(2): 282-85.

Jeuffroy, M.H., Ney, B. & Ourry, A., 2002. Integrated physiological and agronomic modelling of N capture and use within the plant. *Journal of Experimental Botany*, 53(370): 809-23.

Jiang, Z.-C., Sullivan, W.M. & Hull, R.J., 2000. Nitrate uptake and nitrogen use efficiency by Kentucky bluegrass cultivars. *HortScience*, 35(7): 1350-54.

Kafkafi, U., Valoras, N. & Letey, J. 1982. Chloride interaction with nitrate and phosphate nutrition in tomato (*Lycopersicon esculentum* L.). *Journal of Plant Nutrition*, 5(12): 1369-85.

Kalra, Y.P. & Maynard, D.G., 1991. *Methods Manual for Forest Soils and Plant Analysis*. Natural Resources Canada, Ministry of Supply and Services Canada. Information report NOR-X-319: p. 1-108.

Kang, J.-G., van Iersel, M.W. & Nemali, K.S., 2004. Fertilizer concentration and irrigation method affect growth and fruiting of ornamental pepper. *Journal of Plant Nutrition*, 27(5): 867-84.

Kant, S., Bi, Y.-M., Weretilnyk, E., Barak, S. & Rothstein, S.J., 2008. The arabidopsis halophyltic relative *Thellungiella halophila* tolerates nitrogen-limiting conditions by maintaining growth, nitrogen uptake, and assimilation. *Plant Physiology*, 147(3): 1168-80.

Kent, M.W. & Reed, D.W., 1996. Nitrogen nutrition of New Guinea Impatiens 'Barbados' and Spathiphyllum 'Petite' in a subirrigation system. *Journal of the American Society for Horticultural Science*, 121(5): 816-19.

Kirby, E. G., Gallardo, F., Man, H. & El-Khatib, R., 2006. The overexpression of glutamine synthetase in transgenic poplar: a review. *Silvae Genetica*, 55(6): 278-84.

Klock-Moore, K.A. & Broschat, T.K., 1999. Differences in bedding plant growth and nitrate loss with a controlled-release fertilizer and two irrigation systems. *HortTechnology*, 9(2): 206-09.

Lawlor, D.W., 2002. Carbon and nitrogen assimilation in relation to yield: mechanisms are the key to understanding production systems. *Journal of Experimental Botany*, 53(370): 773-87.

Lecoeur, J. & Sinclair, T.R. 2001. Analysis of nitrogen partitioning in field pea resulting in linear increase in nitrogen harvest index. *Field Crops Research*, 71(3): 151-58.

Liu, L. & Shelp, B.J., 1993a. Nitrogen partitioning in greenhouse-grown broccoli in response to varying NH4:NO3 ratios. *Communications in Soil Science and Plant Analysis*, 24(1-2): 45-60.

Liu, L. & Shelp, B.J., 1993b. Broccoli yield and nitrogen composition in response to different management regimes. *Communications in Soil Science and Plant Analysis*, 24(1-2): 61-84.

Liu, L. & Shelp, B.J., 1995. Mobilization of stored nitrate in broccoli (*Brassica oleracea* var. *italica*). *Canadian Journal of Plant Science*, 75(3): 709-15.

Liu, L. & Shelp, B.J., 1996. Impact of chloride on nitrate absorption and accumulation by broccoli (*Brassica oleracea* var. *italica*). *Canadian Journal of Plant Science*, 76(2): 367-77.

Lunt, O.R. & Kofranek, A.M., 1958. Nitrogen and potassium nutrition of chrysanthemums. *Proceedings of the American Society of Horticultural Science*, 72: 487-97.

Man, H. M., Boriel, R., El-Khatib, R. & Kirby, E. G., 2005. Characterization of transgenic poplar with ectopic expression of pine cytosolic glutamine synthetase under conditions of varying nitrogen availability. *New Phytologist*, 167(1): 31–39.

Maslaux, C., Quillere, I., Gallais, A. & Hirel, B., 2001. The challenge of remobilisation in plant nitrogen economy. A survey of physio-agronomic and molecular approaches. *Annals of Applied Biology*, 138(1): 69-81.

Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L. & Suzuki A., 2010. Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. *Annals of Botany*, 105(7): 1141-57.

McCall, D. & Willumsen, J.R., 1998. Effects of nitrate, ammonium and chloride application on the yield and nitrate contents of soil-grown lettuce. *Journal of Horticultural Science and Biotechnology,* 73(5): 698-703.

Millard, P., 1996. The ecophysiology of the internal cycling of nitrogen for tree growth. *Zeitschrift für Pflanzenernährung und Bodenkunde,* 159(1): 1-10.

Miller, A.J. & Cramer, M.D., 2004. Root nitrogen acquisition and assimilation. *Plant and Soil*, 274(1-2): 1-36.

Miller, A.J., Fan, X., Orsel, M., Smith, S.J. & Wells, D.M., 2007. Nitrate transport and signalling. *Journal of Experimental Botany*, 58(9): 2297-306.

Mills, H.A., Barker, A.V. & Maynard, N.D., 1976. Effects of nitrapyrin on nitrate accumulation in spinach. *Journal of American Society for Horticultural Science,* 101(3): 202-04.

Mizrahi, Y., Taleisnik, E., Kagan-Zur, V., Zohar, Y., Offenback, R., Matan, E. & Golan, R. 1988**.** A saline irrigation regime for improving tomato fruit quality without reducing yield. *Journal of American Society for Horticultural Science*, 113(2): 202-05.

Molitor, H.-D., 1990. Bedding and pot plants: The European prospective with emphasis on subirrigation and recirculation of water and nutrients. *Acta Horticulturae*, 272: 165-71.

Nell, T.A., Barrett, J.E. & Leonard, R.T., 1989. Fertilization termination influences postharvest performances of pot chrysanthemum. *HortScience*, 24(6): 996-98.

Nell, T.A., Barrett, J.E. & Leonard, R.T., 1997. Production factors affecting postproduction of quality of flowering potted plants. *HortScience*, 32(5): 817-19.

Nelson, A., 2009. *Greenhouse technology saves on input costs*. [Online] Available at: http://www.thepacker.com/fruit-vegetable-news/shipping-profiles/western-greenhouse-vegetables/greenhouse_technology_saves_on_input_costs_122156019.html [Accessed 2 Janury 2013].

Nkoa, R., Coulombe, J., Desjardins, Y. & Tremblay, N., 2001. Towards optimization of growth via nutrient phasing: nitrogen supply increases broccoli (*Brassica oleracea* var. *italica*) growth and yield. *Journal of Experimental Botany*, 52(357): 821-27.

Ontario Ministry of the Environment, 2011. Greenhouses and Sewage. *The Queen's Printer for Ontario*, PIBS, 8946e. January 2011. [Online] Available at: http://www.ene.gov.on.ca/stdprodconsume/groups/lr/@ene/@resources/documents/resource/stdprod_095566.pdf [Accessed June 15 2012].

Ontario Ministry of the Environment, 2012. Greenhouse Wastewater Monitoring Project (2010 and 2011). *The Queen's Printer for Ontario*, PIBS 8688. January 2012. [Online] Available at: http://www.ene.gov.on.ca/stdprodconsume/groups/lr/@ene/@resources/documents/resource/stdprod_095363.pdf [Accessed 21 September 2012].

PickOntario, 2013. *Pick Ontario.ca*. [Online] Available at: http://www.po.flowerscanadagrowers.com/our-products/chrysanthemum-commerical [Accessed 2 January 2013].

Plaster, E.J., 2003. *Soil Science and Management*. 4th ed. New York: Delmar Learning. p. 1-336.

Rabb, T.K., & Terry, N., 1995. Carbon, nitrogen, and nutrient interactions in *Beta vulgaris* L. as influenced by nitrogen source, NO_3^- versus NH_4^+. *Plant Physiology*, 107(2): 575-84.

Raun, W.R. & Johnson, G.V., 1999. Improving nitrogen use efficiency for cereal production. *Agronomy Journal*, 91(3): 357-63.

Reed, D.W., 1996. Closed Production Systems for Containerized Crops: Reciruculating Subirrigation and Zero-Leach Systems. In D.W. Reed, ed. *Water, Media, and Nutrition for Greenhouse Crops*. Batavia, Illinois, USA: Ball Publishing. p.221-45.

Richard-Molard, C., Krapp, A., Brun, F., Ney, B., Daniel-Vedele, F. & Chaillou, S., 2008. Plant response to nitrate starvation is determined by N storage capacity matched by nitrate uptake capacity in two Arabidopsis genotypes. *Journal of Experimental Botany*, 59(4): 779-91.

Ringli, C., Keller, B. & Ryser, U. 2001. Glycine-rich proteins as structural components of plant cell walls. *Cellular and Molecular Life Sciences*, 58 (10): 1430-41.

Rose, M.A., White, J.W. & Rose, M.A, 1994. Maximizing nitrogen-use efficiency in relation to the growth and development of Poinsettia. *HortScience*, 29(4): 272-76.

Rossato, L., Laine, P. & Ourry, A., 2001. Nitrogen storage and remobilization in *Brassica napus* L. during the growth cycle: nitrogen fluxes within the plant and changes in soluble protein patterns. *Journal of Experimental Botany*, 52(361): 1655-63.

Roude, N., Nell, T. A. & Barret, J. E., 1991a. Nitrogen source and concentration, growing medium and cultivar affect longevity of potted chrysanthemum. *HortScience*, 26(1): 49-52.

Roude, N., Nell, T. A. & Barrett, J. E., 1991b. Longevity of potted chrysanthemums at various nitrogen and potassium concentrations and NH_4:NO_3 ratios. *HortScience*, 26(2): 163-65.

Rouphael, Y., Cardarelli, E., Rea, E. & Colla, G., 2008. The influence of irrigation system and nutrient solution concentration on potted geranium production under various conditions of radiation and temperature. *Scientia Horticulturae*, 118(4): 328-37.

Rouphael, Y. & Colla, G., 2009. The influence of drip irrigation or subirrigation on zucchini squash grown in closed-loop substrate culture with high and low nutrient solution concentrations. *HortScience*, 44(2): 306-11.

Sage, R.F., Pearcy, R.W. & Seeman, J.R., 1987. The nitrogen use efficiency in C3 and C4 plants. III. Leaf nitrogen effects on the activity of carboxylating enzymes in *Chenopodium album* (L.) and *Amaranthus retroflexus* (L.). *Plant Physiology*, 85(2): 355-59.

Salifu, K.F. & Timmer, V.R., 2001. Nutrient retranslocation response of *Picea mariana* seedlings to nitrogen supply. *Soil Science Society of America Journal*, 65(3): 905-13.

Salon, C., Munier-Jolain, N.G., Duc, G., Voisin, A.-S., Grandgirard, D., Larmure, D., Emery, R.J.N. & Ney, B., 2001. Grain legume seed filling in relation to nitrogen acquisition: A review and prospects with particular reference to pea. *Agronomie*, 21(6-7): 539–52.

Salaün, M., Guérin, V., Huché-Thélier, L., Charpentier, S. & Dily, F. Le., 2005. Nitrogen storage and mobilization for spring growth in *Ligustrum* cultivated in container. *Scientia Horticulturae*, 103(4): 461-71.

Schiltz, S., Munier-Jolain, N., Jeudy, C., Burstin, J. & Salon, C., 2005. Dynamics of exogenous nitrogen partitioning and nitrogen remobilization from vegetative organs in pea revealed by ^{15}N in vivo labelling throughout seed filling. *Plant Physiology*, 137(4): 1463-73.

Service Ontario, 2012. *e-laws.gov.on.ca Ontario water resources act.* [Online] Available at: http://www.e-laws.gov.on.ca/html/statutes/english/elaws_statutes_90o40_e.htm#Top [Accessed 23 December 2012].

Siddiqi, M.Y. & Glass, A.D.M., 1981. Utilization Index: A modified approach to the estimation and comparison of nutrient utilization efficiency in plants. *Journal of Plant Nutrition*, 4(3): 289-302.

Steele, R.G.D. & Torrie, J.H., 1980. *Principles and Procedures of Statistics: A Biometrical Approach*. 2nd ed. New York: McGraw-Hill. p. 1-569.

Sondergaard, T. E., Schulz, A. & Palmgren, M. G., 2004. Energization of transport processes in plants. Roles of plasma membrane H+ -ATPase. *Plant Physiology*, 136(1): 2475-82.

Sonneveld, C. & Kreij, C. de 1987. Nutrient solutions for vegetables and flowers grown in water or substrates, sixth edition. *Glasshouse Crops Research Station, Naaldwijk, The Netherlands.* Series: Voedingsoplossingen Glastuinbouw no 8. p. 1-45.

Stern, K.R., Bidlack, J.E & Jansky, S.H., 2008. *Introductory Plant Biology*. 11th ed. New York: McGraw-Hill. p. 1-509.

Sylvester-Bradley, R. & Kindred, D.R., 2009. Analysing nitrogen responses of cereals to prioritize routes to the improvement of nitrogen use efficiency. *Journal of Experimental Botany*, 60(7): 1939-51.

Tobin, A.K. & Yamaya, T., 2001. Cellular compartmentation of ammonium assimilation in rice and barley. *Journal of Experimental Botany*, 52(356): 591-604.

van der Boon, J., Steenhuizen, J.W. & Steingröver, E.G., 1988. Effect of EC, and Cl and NH_4 concentration of nutrient solutions on nitrate accumlulation on lettuce. *Acta Horticulturae*, 222: 35-42.

van der Boon, J., Steenhuizen, J. & Steingröver, E.G., 1990. Growth and nitrate concentration of lettuce as affected by total nitrogen and chloride concentration, NH_4/NO_3 ratio and temperature of the recirculating nutrient system. *Journal of Horticultural Science*, 65(3): 309-21.

van der Leij, M., Smith, S.J. & Miller, A.J., 1998. Remobilisation of vacuolar stored nitrate in barley root cells. *Planta*, 205(1): 64-72.

Viestra, R.D., 1993. Protein degradation in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, 44: 385-410.

Wang, B. & Shen, Q. 2011. NH_4^+-N/NO_3^-N ratios on growth and NO_3^-N remobilization in root vacuoles and cytoplasm of lettuce genotypes. *Canadian Journal of Plant Science,* 91(2): 411-17.

Wang, Y.-Y., Hsu, P.-K. & Tasy, Y.-F., 2012. Uptake, allocation and signalling of nitrate. *Trends in Plant Science*, 17(8): 458-67.

Warncke, D. & Krauskopf D., 1983. Greenhouse growth media: testing and nutrition guides. Michigan State University Agriculture; Ext. bull. E-1736

Waters, W.E., 1967. Effects of fertilization schedules on flower production, keeping quality, disease susceptibility, and chemical composition at different growth stages of *Chrysanthemum morifolium*. *Proceedings of the American Society of Horticultural Science*, 91: 627-32.

Woodson, W.R. & Boodley, J.W., 1983. Accumulation and partitioning of nitrogen and dry matter during the growth of chrysanthemum. *HortScience*, 18(2): 196-97.

Woodson, W.R., Negrm, F.B. & Boodley, J.W., 1984. Relationship between nitrate reductase activity, nitrogen accumulation, and nitrogen partitioning in chrysanthemum. *Journal of the American Society for Horticultural Science*, 109(4): 491-94.

Yoon, H.S., Goto, T. & Kageyama, Y., 2000. Developing a nitrogen application curve for spray chrysanthemums in hydroponic system and its practical use in NFT system. *Journal of Japanese Society for Horticultural Science*, 69(4): 416-22.

Zebarth, B.J. & Rosen, C.J., 2007. Research prospectives on nitrogen BMP development for potato. *American Journal of Potato Research*, 84(1): 3-18.

Zebarth, B.J., Drury, C.F., Tremblay, N. & Cambouris, A.N., 2009. Opportunities for improved fertilizer nitrogen management in production of arable crops in eastern Canada: A review. *Canadian Journal of Soil Science*, 89(2): 113-32.