ANALYZING THE EFFECTS OF NITROGEN ON FLOWER BUD INDUCTION AND WINTER DORMANCY IN DAY-NEUTRAL STRAWBERRY

By

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i. Abstract

In recent years, strawberry cultivation has been increasing in interest and profit within Quebec. The introduction of day-neutral strawberry (DN) as an alternative to short-day (SD) has advanced this interest. These varieties significantly increase strawberry yield potential through their ability to grow irrespective of photoperiod, offering a solution to increasing strawberry demands within Canada and reducing the need for international imports. However, research into the ideal environmental conditions needed for these varieties to produce effectively in Quebec is limited, particularly in optimal nitrogen fertilizer usage. This study aimed to close this knowledge gap by further understanding N fertilizer's role in DN strawberry growth during production and pre-dormancy, applying these findings to determine a targeted fertilizer guide for Quebec climates. Two separate experiments were conducted; the first experiment considered N fertilizer concentration and source during production and the effect on flower bud development, growth rate, and photosynthetic rate. The second experiment considered these same N concentrations and sources, observing the effect on nutrient storage and flower bud induction before, during, and after winter dormancy. Both experiments implemented nitrate, ammonium, and urea as the primary sources of N delivered at three concentration intervals of either 50 ppm, 100 ppm, or 150 ppm per plant. A significantly greater number of flower buds were produced in the 150 ppm urea treatment than the 150 ppm ammonium treatment, and a greater photosynthetic rate among all urea treatments compared to the nitrate and ammonium treatments was observed from experiment one. The second experiment found no significant data regarding N source and concentration on DN nutrient storage or flower bud induction. However, some significance was noted between N treatments and simple sugar levels within the crown. Furthermore, a relationship between N and K fertilizer was determined, finding that a 1:2 N:K ratio produced the most flower buds before dormancy compared to a 1:1 or 1:4 N:K ratio. From these results, Quebec strawberry farmers can implement greater use of urea fertilizer at high concentrations for DN transplants during production, while placing more emphasis on the N:K ratio of fertilizers in pre-dormancy transplants to increase flower bud induction and annual yield.

i. Résumé

Depuis quelques années, la culture de la fraise suscite de plus en plus d'intérêt et de profit au Québec. L'introduction de la fraise à jour neutre (DN) comme alternative à la fraise à jour court (SD) a fait progresser cet intérêt. Ces variétés augmentent considérablement le potentiel de rendement des fraises grâce à leur capacité de croître quelle que soit la photopériode, offrant une solution à la demande croissante de fraises au Canada et réduisant le besoin d'importations internationales. Cependant, la recherche sur les conditions environnementales idéales nécessaires à la production efficace de ces variétés au Québec est limitée, notamment dans l'utilisation optimale d'engrais azotés. Cette étude visait à combler ce manque de connaissances en comprenant davantage le rôle des engrais azotés dans la croissance des fraises DN pendant la production de transplants et la pré-dormance, en appliquant ces résultats pour déterminer un guide d'engrais ciblé pour les climats québécois. Deux expériences distinctes ont été menées; la première expérience a examiné la concentration et la source d'engrais azotés pendant la production et l'effet sur la production de bourgeons floraux, le taux de croissance et le taux photosynthétique. La deuxième expérience a examiné ces mêmes concentrations et sources d'azote, en observant l'effet sur le stockage des nutriments et l'induction des boutons floraux avant, pendant et après la dormance. Les deux expériences ont considéré le nitrate, l'ammonium et l'urée comme les principales sources de N délivrées à trois intervalles de concentration, soit 50 ppm, 100 ppm ou 150 ppm par plante. Un nombre significativement plus grand de bourgeons floraux a été produit dans le traitement d’urée à 150 ppm comparativement au traitement avec l'ammonium à 150 ppm. Un taux de photosynthèse plus élevé avec les traitements à l'urée comparativement aux traitements avec du nitrate et de l'ammonium a été observé durant la première expérience. La deuxième expérience n'a trouvé aucune différence significative concernant la source et la concentration de N sur le stockage des nutriments ou l'induction des bourgeons floraux. Cependant, une certaine signification a été notée entre les traitements à l'azote et les niveaux de sucre simples dans la couronne. En outre, une relation entre l'engrais N et K a été déterminée, constatant qu'un rapport N:K de 1:2 produisait le plus de bourgeons floraux avant la dormance par rapport à un rapport N:K de 1:1 ou 1:4. À partir de ces résultats, les producteurs de fraises du Québec peuvent mettre en œuvre une plus grande utilisation d'engrais à base d'urée à des concentrations élevées pour les greffes de DN pendant la production, tout en mettant davantage l'accent sur le rapport N:K des engrais dans les greffes de pré-dormance pour augmenter l'induction des boutons floraux et le rendement annuel.

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iii. Contribution of Authors

All experimental data collected and presented in both manuscript 1 and manuscript 2 was obtained and analyzed by author AP. VG oversaw the data collection and analysis process from all experiments and contributed to the revisions made to the final manuscripts.

1. Introduction

Cultivated strawberry, *Fragaria* x *ananassa*, is a perennial flowering plant, belonging to the Rosaceae family. *F.* x *ananassa* species of strawberry is a hybrid produced from the cross of two wild octoploid Fragaria species, *F. chiloensis* and *F. virginiana* (Tenessen *et al.*, 2014). *F.* x *ananassa* was introduced in France during the 17th century and has become a novel species in strawberry cultivation, replacing woodland strawberry species, *F. vesca*, in commercial production (Liston *et al.*, 2014). As a species, *F.* x *ananassa* is of the greatest commercial interest, with numerous cultivars bred for use in strawberry production. These cultivars include the more recently developed day-neutral varieties, which are of rising interest to growers (Strawberry Plants.org, 2018; Liston *et al.*, 2014).

Strawberry is a highly valuable crop worldwide, generating approximately 22 billion CAD globally each year with global strawberry production exceeding twice the amount of all other berry production combined (360 Research Reports 2020; Stewart *et al.*, 2011). Demand for strawberry has also been expanding globally, causing an approximate 4% increase in production with each consecutive year (Index Box Marketing and Consulting, 2018). Within Canada, Quebec is leading as the primary producer of strawberry, contributing to nearly half of the total strawberry grown throughout the country (Agri-Food Canada, 2016). However, recent trends have shown that Canadian strawberry production has been falling significantly short of annual consumer demands, relying heavily on imports from elsewhere in North America (Statistics Canada, 2018). This unmet demand is partly caused by both a shorter strawberry harvest season from colder seasonal temperatures in the spring and the use of primarily short-day varieties, which only produce fruit within a brief window of time (Vilsack, 2009; Weebadde *et al* 2008). Furthermore, there is a lack of targeted research into the ideal cultivation practices for higher-yielding, day-neutral varieties in Canadian environments (Wang *et al.*, 2010). The production of day-neutral strawberries in 1990 introduced several varieties bred with an insensitivity to photoperiod length. These varieties would continually bear fruit throughout the season if other conditions, such as temperature and fertilizer, remained stable, allowing for a significant increase in strawberry yield (Durner *et al.*, 1984; Ahmadi and Bringhurst, 1991). However, these day-neutral varieties were developed to perform optimally in temperate climates and controlled environments, where they originated. While breeding programs in Canada have succeeded in developing day-neutral varieties that are more

resistant to colder climates, there have yet to have been abundant studies on optimizing these varieties within Quebec (Weebadde *et al.,* 2008).

Current research on the behavior and growth of day-neutral variety strawberries in Canada is limited when considering optimal environmental conditions for increasing yield. Thus, to gain the most from the advantages of day-neutral cultivars, significant research determining the environmental influence, particularly the effects of fertilizer on day-neutral strawberry yield, will become crucial for advancing production and profitability for Quebec farmers. When considering improving fertilizer practices for increasing yield, nitrogen is the most critical nutrient to observe in day-neutral strawberry, as nitrogen has the most significant role in yield quantity and quality (Haifa Group, 2019). Presently, scarce research has delved into nitrogen as a factor of increasing strawberry yield for day-neutral strawberries, particularly how nitrogen source and concentration can significantly affect yield observations. With greater knowledge of the role nitrogen plays in day-neutral strawberry, farmers will have more control over the yield of their crops and can expect to add up to 14 weeks to their production schedule (Petran *et al.,* 2016). Furthermore, nitrogen optimization can also improve the survival rate of day-neutral transplants through winter dormancy. Currently, Quebec farmers are dissuaded from favoring day-neutral varieties to short day as day-neutral varieties tend to suffer heavier plant losses following winter than their hardier alternatives. An ideal nitrogen application framework, however, will allow for sufficient nutrient storage within the crown to survive through dormancy and break dormancy earlier in the Spring.

This study focused on bridging the current knowledge gap concerning nitrogen fertilizer in day-neutral strawberries by considering several nitrogen fertilization sources and concentrations and observing how they influence flower bud induction during production and prior to winter dormancy in day-neutral strawberry transplants. Nitrogen fertilizer practices were analyzed in terms of nitrogen source and nitrogen concentration on transplants of the day-neutral variety of *F.* x *ananassa*, cv. “Albion”. Several experiments were conducted, examining how nitrogen fertilizer impacts the development of flower buds of strawberry transplants. The impacts of nitrogen fertilizer were then tested on nutrient storage during dormancy and survivability of strawberry transplants over-winter, determining how nitrogen influences the duration of dormancy and transplant survivability into the spring season. From these experiments, a more comprehensive

fertilizer guide can be provided for day-neutral transplants in Quebec. This guide will allow Quebec farmers to optimize annual yield through increased flower bud induction and improve over-winter transplant survival rates through sufficient nutrient storage. Furthermore, a more precise and climate-tailored fertilizer guide will benefit Quebec strawberry farms economically by providing exact volumes of fertilizer required annually, reducing costs from unnecessary fertilizer application and irrigation. A more precise fertilizer guide can also mitigate environmental harm caused by fertigating more frequently and at higher concentrations than is needed for optimal growth.

2. Literature Review

2.1 Strawberry Production in Quebec

Cultivated strawberry is a substantial crop within Quebec, Quebec being the primary producer of strawberry within the country (Agri-Food Canada, 2016). In 2018, 1,921 cultivated hectares of the total 4,110 hectares dedicated to strawberry production within Canada were located within the province, making up 47% of the cultivated strawberry produced in Canada (Agri-Food Canada, 2018).

Several strawberry cultivars are grown within Quebec, including short-day and day-neutral varieties (Fig.1). While several strawberry varieties are cultivated around the province, most of the varieties grown are short-day compared to day-neutral. Currently, there are 29 recommended short-day varieties of strawberry optimal for Quebec with only five day-neutral varieties being recommended in comparison (Strawberry Plants.org, 2021). This can be attributed to challenges in growing day-neutral strawberry varieties in northeastern climates, since these varieties tend to suffer yield loss caused by fluctuating summer temperatures in these regions (Wang *et al.,* 2010; Weebadde *et al.,* 2008). Since day-neutral strawberries were originally bred in California, these varieties have adapted to tolerate milder temperatures that stay within a stable daily range throughout the season. This is more variable during northeastern summers, resulting in limited flower bud development in temperatures exceeding 28°C and falling below 4ºC (Hancock *et al.,*2008). However, further research in the genotype, cultural practices, and environmental influence within day-neutral varieties has shown promise in the development of cultivars that are more tolerant in Quebec climates (Claire *et al.,* 2018).

Table 1: Common strawberry cultivars grown in Quebec and their respective growth patterns.

Cultivar	Developer	Growth Pattern
Albion	University of California	Day-neutral
Annapolis	AAFC*	Short-day
Cavendish	AAFC	Short-day
Chambly	AAFC/ McGill University	Short-day
Charlotte	French Breeding Program	Day-neutral
Clé de Champs	AAFC	Short-day
Harmonie	AAFC	Short-day
Honeoye	Cornell University/ NYSAES*	Short-day
Kent	AAFC	Short-day
Mara des Bois	French Breeding Program	Day-neutral
Mira	AAFC	Short-day
Monterey	University of California	Day-neutral
Saint Laurent	AAFC	Short-day
Saint Pierre	AAFC	Short-day
Seascape	University of California	Day-neutral
Yamaska	AAFC	Short-day

Source: Strawberryplants.org

*Abreviated for Agriculture and Agri Food Canada (AAFC) and New York Agriculture Experiment Station (NYSAES)

While strawberry production is prominent in Quebec, marketed strawberry within the province heavily relies on imports from regions outside the country, primarily from California and Florida, to meet burgeoning consumer demands. This is reflected throughout Canada, which imported roughly 135,229 metric tons of strawberry in 2017 compared to the exported strawberry only equating to 1,220 metric tons (Statistics Canada, 2018). The need for significant strawberry imports is partially due to the use of short-day strawberry varieties whose physiology provides a shorter growing season and fewer harvests. Supply of strawberry can likely be improved upon, however, with the growing implementation of day-neutral varieties.

2.2. Strawberry Varieties

To fully understand the yield benefits with increased development of day-neutral strawberry, it is important to know what sets these varieties apart. In cultivated strawberry, three behaviors of strawberry production are currently commercialized: Short-day or June-bearing, day-neutral and everbearing varieties. Short-day strawberries are the principal strawberry varieties used in production within Quebec, which produce an ample harvest only within a three-week window in June (Hancock, 2000). The production cycle of short-day varieties begins when either transplants or dormant bare roots are planted in April. From the end of the previous production season and during winter dormancy, short-day plants undergo flower bud induction within the crown, developing all the flowers that will bloom the following season. Once the temperature and photoperiod begin to increase, flower bud induction will stop within the crown and the plant will break dormancy and begin to develop and mature. By early June, these varieties will reach full maturation and will begin to produce ample fruit for up to three weeks. After this three-week period, these varieties will then undergo a period of vegetative growth only, with only leaves and runners produced, before slowing down growth in the fall and re-entering dormancy once temperatures begin to drop below 0°C. Environmentally, short-day varieties perform best in short-day photoperiods of 8 hours of natural light and in lower temperatures, with significant reduction in yield in temperatures exceeding 28ºC (Strik, 1985; Durner *et al.*, 1984).

On the other hand, everbearing varieties follow a similar production cycle to short-day varieties, however, unlike short-day, these varieties produce two significant harvest periods in early summer and again in early fall with a period of vegetative growth or small sporadic growth of fruit in between harvests (Smeets, 1980). Comparing environmental conditions, everbearing varieties also have been observed to prefer a long day photoperiod, exceeding 8 hours of natural light to produce optimal yields (Smeets, 1980). While the two-harvest period for everbearing varieties seemingly has the potential for increasing annual yield, everbearing fruits are relatively smaller and less numerous compared to short-day, with average annual yields at 7,316kg of berries per 1 ha of mature plants (Strawberryplants.org, 2020). This is about half of the yield quantity of average short-day yields (~14,645kg of berries per 1 ha of mature plants), which dissuades farmers from choosing these varieties when wanting to maximize yields (Strawberryplants.org, 2020). Everbearing varieties also produce fewer runners since more energy is spent on producing fruit

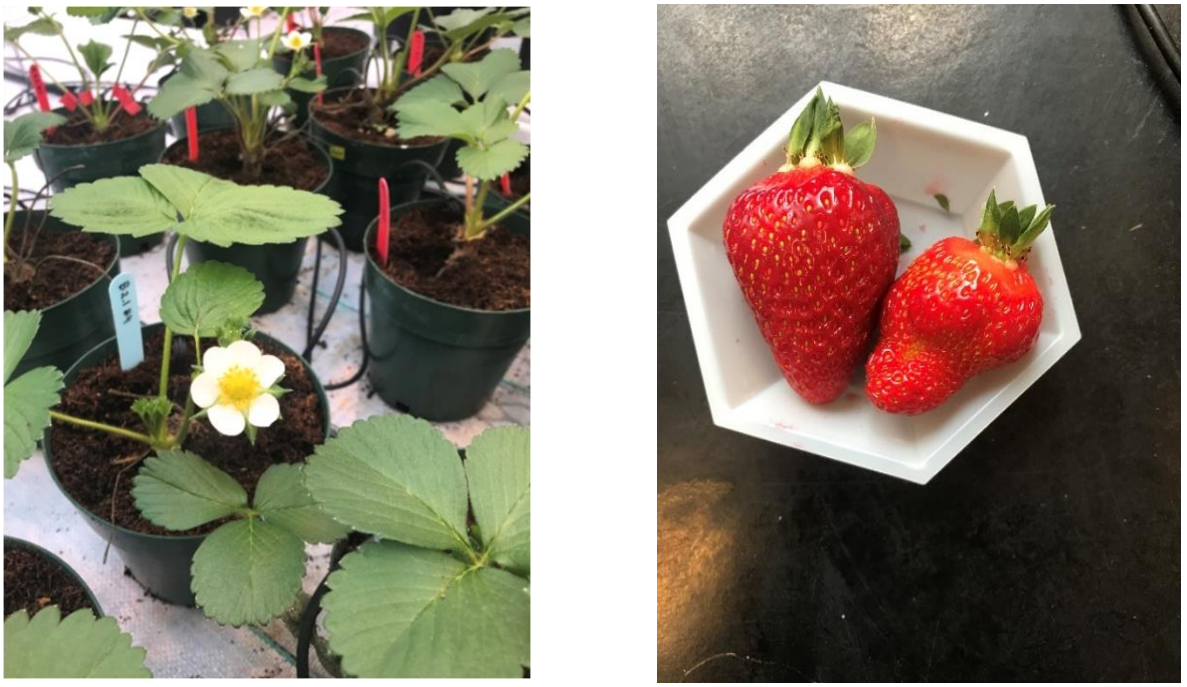
(Smeets, 1980). While this trait makes everbearing plants more manageable in the field, it is not ideal for annual cropping systems used by Quebec strawberry farmers, as they can expect a significantly reduced number of new transplants at the end of the production season needed for the following spring. Thus, lower yields and fewer runners make everbearing varieties less economically viable for Quebec growers hoping to improve yield and winter loss.

Lastly, the final variety, day-neutral, are the most recent commercialized cultivars of strawberry and likely the most profitable in terms of yield. These varieties were originally developed and marketed from a breeding program by the University of California in 1990 which bred *F.* x *ananassa* everbearing varieties with influence from *F. chiloensis* species of strawberry, giving rise to the day-neutrality trait (Bringhurst and Voth, 1984). The resulting bred cultivars were thus observed to produce fruit independent of photoperiod. With this trait, it was observed that, unlike short-day and everbearing varieties, day-neutral varieties did not undergo a vegetative state following the first harvest, instead producing fruit at a continuous rate from early-summer to mid-autumn if environmental conditions are adequate (Durner *et al.,* 1984; Liston *et al.,* 2014). While day-neutral strawberries provide unique disadvantages, such as an intolerance for northeastern climates as well as shortage in runner production similar to everbearing varieties, these cultivars often are regarded as the best alternative for increasing yield (Rowley *et al.,* 2010). Studies comparing yields from all three varieties have found that day-neutral have outperformed short-day and everbearing varieties as they add an additional 14 weeks of harvest time, allowing nearly double the yield produced over the harvest season (Rowley *et al.,* 2010). Fruit produced from day-neutral strawberry is also similar to short-day strawberry in terms of size and taste. Therefore, regarding Quebec farmers, it is of the greatest interest to further research into optimal growing conditions and practices for day-neutral strawberry varieties which will indubitably allow for more significant annual yields of strawberry within the province.

2.3. *Fragaria* x *ananassa* cv. Albion

Within this study, a single variety of day-neutral strawberry from the *Fragaria* x *ananassa* species will be examined. *Fragaria* x *ananassa* cv. Albion (Fig 1.) was chosen for this project, as it is observed to be a hardy variety with ample disease resistance compared to other day-neutral varieties (Shaw *et al.,* 2006). Albion strawberry grows within zones 4a to 7b of the North American

hardiness zones determined by the USDA, (which encompasses several regions of Quebec). Albion is also superior in tolerating temperature extremes and day to night fluctuations which is commonly experienced during Quebec production seasons (Strawberry Plants, 2018; Baessler, 2020). The variety itself was developed by the University of California in 1998 as a cross between the "Diamante" day-neutral cultivar and advanced selection Cal 94.16 strawberry (Shaw *et al.*, 2006). The variety was initially bred to develop traits of disease resistance but also saw benefits in production quality, making Albion a top choice amongst day-neutral strawberries for farmers (Shaw *et al.*, 2006).



a) **b)**

Figure 1. **a)** *Fragaria x ananassa* strawberry variety Albion transplant at 4 weeks of development. **b)** Mature fruit produced by Albion plants at 5 weeks of development

In production, Albion has been observed to produce continually throughout the season with high quality fruits. Fruits produced are large and conical in shape with darker, firmer, and often sweeter fruit compared to other day-neutral varieties (Baessler, 2020). Albion also exhibits more significant disease resistance compared to its predecessors (Shaw *et al.*,2006). Through trial studies, compared to Diamante and Seascape, Albion provides greater disease resistance to Phytophthora crown rot (*Phytophthora cactorum*) and Verticillium wilt (*Verticillium albo-atrum*) compared to other day-neutral cultivars, as well moderate resistance to powdery mildew

(*Podosphaera macularis*), common leaf spot (*Mycosphaerella fragariae*) and Anthracnose crown rot (*Colletotrichum gloeosporioides*) (Shaw *et al.,* 2006). In terms of cultivation, Albion strawberries provide the most optimal yield under arid climates and moderate temperatures. However, research studying how cultural practices effect its yield has been limited. Since this day-neutral cultivar is suitable for growth in Quebec's environmental conditions, further research into flower bud induction and dormancy through this study, allows for potential advances in growing Albion within Quebec.

2.4 Strawberry Physiology

Physiology and Development

Fragaria x *ananassa* species of strawberry are perennial, herbaceous rosettes belonging to the family *Rosaceae*. This species of strawberry was derived from a cross of *F. chiloensis* and *F. virginiana* in the 18th century, which led to higher yielding strawberry cultivars (Edger *et al.,* 2019). *F.* x *ananassa*, like its ancestors, is octoploid, triecious and largely self-pollinating (Edger *et al.,* 2019). Starting from the beginning of the growth cycle for strawberry, juvenile plants can be obtained from two routes of reproduction: through seeds or daughter runner plants. Growth from seeds begins in early spring when temperatures begin to increase. Once temperatures become ideal, seedlings will sprout and establish roots, form there growing exponentially until reaching maturity. Strawberry plants grown from seedlings will be genetically unique from parent plants, allowing for introduction of new traits and genetic diversity (Hancock, 2000). Alternatively, strawberry also reproduces through daughter shrubs produced from stolons. These plants, unlike seedlings, will be genetically identical to the mother plant (Hancock, 2000). Plants grown from stolons will also be further matured than seedlings with primary shoots already developed. These plants can be removed from the mother plant via cuttings in the fall as transplants which are then planted in late spring once temperatures have begun to increase. All growth within plants develop as shoots from a central crown, making up the core of the strawberry plant (Fig 2.). Strawberry crowns then produce axillary meristems which differentiate into leaf stalks, or petioles, terminal inflorescences or into stolons which later develop additional crowns (Polling, 2012)

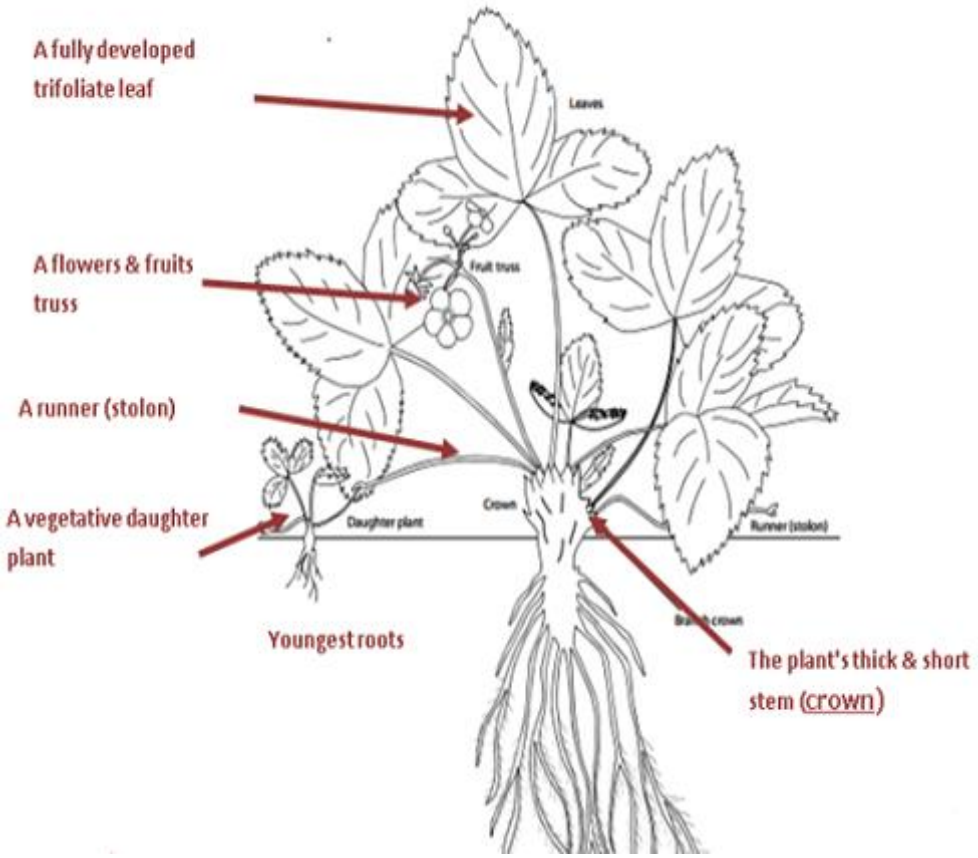


Figure 2. Architectural model of *Fragaria* x *ananassa* strawberry species. Source: Haifa Group, 2018

Development of cultivated strawberry follows a periodic growth cycle which continues throughout the growing season. In most cultivated strawberry varieties, new plants reach full maturity by early to mid-summer (Savini *et al*., 2005). Once strawberry plants have fully matured, flower induction will occur within the crown at varying stages over a period beginning in mid-summer and lasting until mid-autumn in day-neutral strawberry varieties. Strawberry flowers then undergo self-pollination at the receptacle before flower sepals close around the receptacle allowing the fruit to form. Strawberry sepals will reopen, becoming the calyx of the fruit, allowing the fruit to ripen fully (Petran *et al*., 2017). Strawberry ripening occurs as fruits mature, reaching full maturity when strawberries are a vibrant red. In day-neutral cultivars, plants will produce fruit throughout the season, as new inflorescences will continually develop from the crown during the harvest season. This occurs alongside growth of new leaves and stolons throughout the harvest season. At the end of the season, plants will enter a period of dormancy through winter that is triggered once daytime temperatures are consistently below a threshold of 10ºC to 5°C (Kronenburg and Wassenaar, 1972). Strawberry dormancy requires the plant to reduce cellular processes and cease all growth at the meristem to preserve the plant until the next growing season,

in which the growth cycle repeats (Gillespie *et al.*, 2017). Cultivated strawberry can undergo this cycle 3 to 5 times within each plants' lifespan (Gillespie *et al*., 2017).

Flower Bud Induction

Within the last century, the process of flower bud induction in strawberry has been a subject of increased interest, along with the factors affecting this process. Depending on the strawberry variety, flower bud induction occurs within the crowns of mature plants beginning in mid-summer and slows down once temperatures begin to cool and the onset of dormancy occurs (Savini *et al.,* 2008). At the point of winter dormancy, nearly all flower buds that will grow into fruit during the season have been produced, except in the case of day-neutral strawberry where new flower buds are continually produced in the crown until dormancy. The flower buds induced within the crown continue to develop until dormancy is broken once environmental condition are adequate for plant growth. These buds will then mature along with the plant, developing as alternating nodes off inflorescences (Savini *et al.,* 2008). In day-neutral strawberry, flower bud induction begins once the plant reaches maturity at 4-6 weeks after planting and continues through the growing season with some induced flower buds maturing before the end of the season allowing for a continuous yield (Durner *et al.,* 1984).

With an understanding of the process of flower bud induction, the next step is to determine the outside factors affecting flower bud induction and the best ways to manipulate these factors to allow for significant increases in flower bud growth. The main factors thought to control flower bud induction are photoperiod, temperature, and availability of fertilizer, with other factors such as genotype and cultivar playing a significant role in induction (Strik, 1985*)*. Photoperiod is the leading factor affecting induction in short-day cultivars as a shorter day period must occur for flower bud production (Strik, 1985*)* This is not the case so much in day-neutral strawberry, since daylength does not affect growth as significantly, however, light quality does have some effect on flower bud induction (Stuemsky and Uchanski, 2019). Temperature also greatly affects flower bud induction during production, as well as during dormancy. In production, temperatures need to fall below 20 ºC at night for short-day strawberries to produce flowers (Takeda *et al,* 2009). In day-neutral, temperature variance will still allow for the production of flowers, although lower daytime/nighttime temperatures of 20ºC/15°C produced greater quantities of flower buds while

higher temperatures of 30°C/25°C D/N produced better quality flower buds and greater quality fruits (Sønsteby and Heide, 2006; Nishimura and Kanahama, 2002).

Lastly, research has also examined the presence of nitrogen (N-) as a contributing factor to flower bud induction. It was found that the timing and concentration of N in short-day could optimize flower bud induction to allow for greater flowering from one year to the next (Human and Kotze, 1990). While there has yet to be much research covering the role of nitrogen in flower bud induction for day-neutral strawberry, it can be assumed that availability of nitrogen would play a significant role in flower bud growth and perhaps more so than short-day strawberry since more energy is allocated to flower bud induction throughout the season for day-neutral. By providing the ideal nitrogen needed by the plant, the quality and quantity of flower buds can likely be affected to allow for optimal production of flower buds.

Dormancy

Dormancy is a naturally occurring state that evolved in woody plants and other perennial plants, including strawberry, to allow these plants to conserve energy and survive through extreme environmental conditions, such as winter temperatures and a reduced photoperiod (Gillespie *et al.,* 2017). Processes of dormancy and reduction of growth are often initiated by changes in the environment, with temperature being the primary catalyst of dormancy in strawberry. With the change in temperature and photoperiod, strawberry plants will begin to reduce productivity in all parts of the plant until a full dormant state is reached when the temperature reaches between 0°C and -5°C and plants significantly reduce any new growth. Once transplants have entered dormancy, they will remain in a state of reduced growth until temperature and photoperiod begin to increase again in the spring. However, within greenhouse conditions, dormancy can be broken earlier by affecting temperature and photoperiod (Kronenburg and Wassenaar, 1972). The first factor of breaking dormancy early is by chilling transplants from long periods within a specialized freezer. It has been observed from experiments working with short-day strawberry transplants that dormancy was significantly reduced in transplants kept within freezers for an extended period compared to transplants that were not chilled (Lee *et al.,* 1970). Aside from chilling, the availability of nitrogen in the soil has also been observed to affect how early transplants can

metabolize nutrients and begin new growth in the spring (Khayyat *et al.,* 2010; Gagnon *et al.,*1990).

As strawberry plants enter dormancy, changing environmental conditions begin to regulate the effects of dormancy by signally genes linked to dormancy, as well as shifting phytohormone production. In most plants that undergo dormancy, it is observed that there is steady decrease in auxin production as well as a concurrent rise in abscisic acid (ABA) production signaled to the plant from changing environmental conditions (Zhang *et al.,* 2011). Changes in auxin and ABA hormone levels significantly decreases the rate of cell division occurring within the plant, allowing for reduced growth and conservation of energy (Horvath *et al.,* 2008). Environmental shifts and phytohormone changes also influence gene regulation in strawberry plants from the presence of epigenetic mechanisms: DNA methylation and histone modification (Zhang *et al.,* 2011). DNA methylation in this case is affected in dormant strawberry plants as greater methylation allows for silencing of certain genes not necessary to the plant in a dormant state and allowing for greater conservation of energy. Histone modification similarly increase gene silencing through making it more difficult for DNA transcription factors to bind to DNA. From these changes occurring within the strawberry plant, observable growth significantly decreases during winter dormancy. Instead, the majority of plant growth is focused internally to develop flower buds for the following growth season.

Winter dormancy has been observed to in all varieties of strawberry; however, studies have found that the survival rate of plants in spring is related to the variety of strawberry (Kronenburg and Wassenaar 1972; Fujime and Yamazaki, 1988). In short-day cultivars, dormancy survival is more likely to occur in varieties bred for northern climates, whereas southern bred cultivars usually suffer more plant loss (Kronenburg and Wassenaar 1972). Scarce research has been conducted measuring the extent of crop loss through winter dormancy in day-neutral strawberry compared to short-day cultivars although previous research has found day-neutral transplants to be susceptible to colder temperatures (Weebadde *et al.,* 2008). Therefore, it is crucial to consider the limitations of these cultivars when developing cultural practices to maintain plants over-winter.

2.5 Transplant Production

Strawberry cultivation within Quebec relies on transplant culture to store young strawberry plants over winter that will be planted for the following production season. These transplants are collected from stolons, or runners, of mother plants and are propagated in soil or substrate to allow for root formation. Often farms collect their own transplants from productions plants during the harvest season, however, transplants are also supplied to farms by plant nurseries in some instances. In terms of transplanting, there are three primary methods of transplant culture within Quebec: bare-root propagation and plug propagation in the form of dormant and fresh plugs (Fig.3)

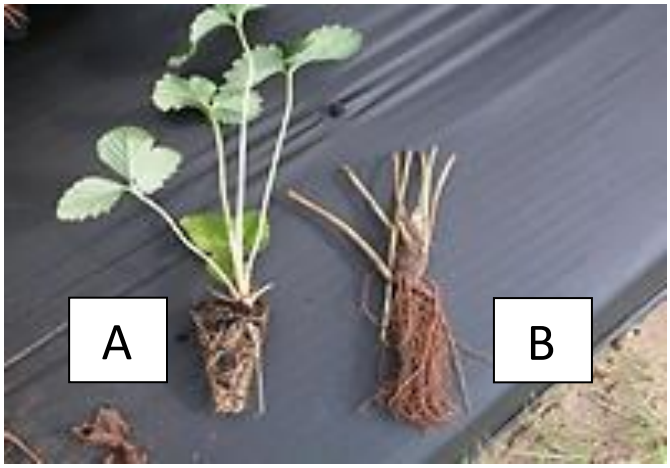


Figure 3. Strawberry Transplant Cultures. Transplant A is an example of the transplant plug method whereas transplant B is an example of the bare-root method. Source: Strawberries.ces.ncsu.edu.

Bare Root Transplanting

Bare-root transplant propagation involves removing mature plants from soil or collecting rooted stolons from mother plants to be preserved for the following season. For this method, stolons are allowed to root from the mother plant in July (Table 2), where these daughter plants will develop base roots, in around 4-6 weeks. The daughter plants are grown up until the end of the season, where they are harvested, often by machine, once they have become dormant (Hochmuth *et al.*, 2006). The roots of the transplants are cleaned thoroughly to avoid mold development and all leaves and flower stalks are pruned before being stored and refrigerated between 0-1^{o}C over-winter (Lieten *et al.*, 2005). While bare-root transplants offer a fairly cheap method of producing transplants for the following season, some setbacks with this method is that production of these transplants requires significantly more water than plug methods (Hochmuth *et al.*, 2006). Furthermore, these transplants also tend to get damaged through the harvesting

process, resulting in many plants with stunted growth when planted in the spring (Cocco *et al.,* 2011).

Table 2. Harvest and propagation timeline comparison for strawberry transplanting methods. Source: Lareault.com

*Transplant development (Devt.)

Month	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April
Bare-Root	Stolons Harvested	Propagation	Devt.*	Root Cleaning /Pruning	Cold Storage 0ºC	Cold Storage 0ºC	Cold Storage 0ºC	Cold Storage 0ºC	Cold Storage 0ºC	Planting
Dormant Plugs	Stolons Harvested	Propagation in plugs	Devt.*	Devt*.	Cold Storage 0ºC	Cold Storage 0ºC	Cold Storage 0ºC	Cold Storage 0ºC	Cold Storage 0ºC	Planting
Fresh Plugs								Stolons Harvested	Propagation in plugs and Devt.*	Planting

Plug Transplanting

The second method of transplant culture used in Quebec is plug transplanting, which similarly harvests stolons of a parent plant and cultivates them in substrate plugs. Within plug transplanting, two practices are employed in Quebec. The first is the use of dormant plugs, or fridge plants. In this practice, stolons are harvested like bare roots, harvesting from parent plants in July and propagating in individual soil plugs within plastic trays. Transplants are grown for 4-6 weeks, typically in greenhouse conditions, before being prepared for winter storage in early Autumn (Durner *et al.,* 2002). Leaves are typically left on the plants, unlike bare roots transplants, before refrigeration at 0ºC. Transplants are then removed from chilling in the Spring and grown within the greenhouse until new growth is observed 1-3 weeks after removing from cold storage. These transplants are then moved to the field or greenhouse where the whole plug can be planted. This method of transplant culture has been phasing out the bare-root method, as this method has been observed to respond more favorably to cold storage (Lieten *et al.,* 1994) and results in earlier flower bud induction and fruit production, resulting in a higher yield compared to the bare-root method (Hochmuth *et al.,* 2006).

Alternatively, a more recent approach to plug transplants has been the use of fresh plugs, or tray plants. Unlike the dormant plug method, this method does not require a period of chilling for the transplants, which has been shown to significantly decrease transplant damage, growth delay, and transplant loss that is common after refrigeration (Lieten *et al,* 1994). In this method, transplants are similarly harvested from the stolons of mother plants. In this instance, however, transplants are harvested from mature mother plants that have broken winter dormancy and have begun to produce new growth. These mature plants are often forced out of dormancy by shifting environmental conditions, such as temperature and photoperiod, to initiate new growth (Durner and Poling, 1998). Once stolons are produced from the mother plant after 3-4 weeks, daughter tips are harvested and planted in substrate plugs for 2-3 weeks before propagation in April-May. This method has been a prioritized method of transplant culture since it allows for easy transferring from individual tray plugs and suffers the least amount of transplant loss compared to the fridge or bare-root methods, where winter chilling can affect survivability as well as promoting delayed development which significantly shortens the harvest season (Durner *et al.,* 2002).

Furthermore, plug transplants have seen a significant reduction in soil-borne pathogens as runner transplants have not been exposed to these pathogens (Poling and Maas, 1997; Durner *et al.,*2002). Pathogen resistance in plug transplants has also reduced the need to treat the soil before strawberry planting, which is often necessary for bare-root transplants (Poling and Maas, 1997). Currently, most transplant culture in Quebec continues to utilize the bare-root method since it is cheaper and more feasible; however, with the incentive of higher yield and increased disease resistance, the use of plug transplants has grown in favor among strawberry producers in Quebec.

2.6 Soilless Systems

First commercialized in the 1980's, the use of soilless growing media has become a more commonly accepted practice when growing strawberry to reduce the need for crop rotation or fumigation, as soilless substrates are not contaminated with plant pathogens, and advancing organic cultivation models. Soilless substrate use also offers benefits towards sustainable use of water and fertilizer, as these substrates often have superior water retention qualities and are less likely to leach fertilizer compared to most soils (Jensen, 1997). As opinions shift towards precision

agriculture and efficient water use along the threats of climate change, employing soilless alternatives will likely become essential to strawberry production (Depardieu *et al.,* 2015). Therefore, soilless substrate was used as an alternative to soil within the methods of this project. Soilless media is composed mainly of organic substrates, such as bark mulch, peat, biochar, rockwool, vermicompost, and coconut coir (Raviv *et al.,* 2007). Each of these alternatives to soil has specific qualities that can be suited to match most closely to the desired soil conditions of the target plant, such as the pH, porosity, and readily available nutrients.

Soilless media as an alternative also offers disease prevention for strawberry plants, particularly more susceptible day-neutral varieties. This is especially true in the instance of soil-borne pathogens affecting strawberries, such as Anthracnose (*Colletotrichum acutatum*) and grey mold (*Botrytis cinerea*), which can be significantly reduced using non-soil alternatives (Raviv *et al.,* 2007). Reduced risk of these pathogens allows for a lessened need for chemical pesticides in strawberry, which improves the overall sustainability of production. The most popular soilless media sued for growing strawberries are peat moss, rockwool, and coconut coir.

Peat moss

Peat moss substrate is one of the most popular soilless substrates used for horticultural crops, including strawberry. Derived from bogs and other moisture saturated environments, peat is composed of 98% organic material, formed from the decomposition of plant material in water. As a growth medium, peat offers superior water and oxygen retaining abilities, making this medium suitable for plants that require a high moisture content at the roots, as well as providing adequate oxygen levels for the phytosphere. The water-retaining properties of peat also allow for excellent nutrient retention with more significant absorption of nutrients by plant roots and concurrently reduced nutrient leaching.

However, some disadvantages of peat are that plants grown in the media are at greater risk of developing plant diseases as high moisture environments allow for easier movement of pathogens into plant roots. Peat substrate employs some pathogen suppressing abilities with the presence of saprophytes, as well as *Bacillus* and *Streptomyces* strains that produce natural antibiotics against bacterium but it is not impenetrable (Madiyah *et al.,* 2020). Peat moss is also observed to be

susceptible to fluctuations in both pH and electrical conductivity (EC), requiring consistent monitoring to mitigate severe fluctuations that could damage plant roots (Eurofins Agro, 2019). Furthermore, the harvesting of peat has some negative environmental connotations as many sites of peat extractions are fragile ecosystems at risk of endangerment from excessive mining (Kitir *et al.*,2018).

Rockwool

Another option for soilless culture is the use of basaltic rockwool. This substrate, used formerly as an insulant for homes and shelters, is composed of thin, porous rock fibers, created by aerating, and spinning strands of molten basalt into threads. These threads are then stacked upon one another into slabs used as growing media. As a substrate, rockwool performs exceptionally well with horticultural plants, as the media creates a balanced water table with the bottom of the substrate column retaining plentiful nutrients and moisture. At the same time, the top layer remains relatively dry, allowing for proper aeration of soil (Morgan, 2020). Rockwool, unlike peat and coco coir, is also initially sterile as it is not derived from organic material, allowing for a reduced risk of pathogen contamination.

However, some disadvantages of rockwool are that while it supplies a balanced water table for plant roots, since the top of the substrate remains dry; it is susceptible to over-watering as it is hard to tell if the plant is dry or not. This, in turn, can result in nutrient deficiencies, as they are being flushed from the soil before uptake can occur in the roots. Another disadvantage with rockwool, is that as an inorganic substrate, there are no naturally occurring microbial communities present that can facilitate plant growth or offer disease resistance (Morgan, 2020).

Coconut Coir

Compared to peat moss and rockwool substrates for strawberry, coconut coir, made from ground and compressed coconut husks, is a favorable alternative and is the media of choice for all experimentation within this project. Coconut coir is composed of a fiber byproduct from coconut shells that is a cheap and sustainable substrate to produce as the shells are often accumulated as

waste products from coconut production. This medium is advantageous to strawberry growth as it provides adequate water and oxygen retention while also displaying greater aeration compared to peat moss while not appearing as dry as rockwool. Coir also has an average pH of 5.5 without the need for pH balancing compounds such as lime or calcium, which are often needed in peat moss (Xiong *et al.,* 2017). This is also beneficial to strawberry production as coir pH corresponds to the optimal pH level needed to grow strawberries between 5.5 and 6.5. Previous studies have also found that coconut coir has shown significant resistance to plant disease, including grey mold (*B. cinerea*) and Verticillium wilt (*V. dahliae*) in strawberry grown within this media (Chong, 2008).

The disadvantage of coco coir is that it is an inert substance that does not contain any nutrients needed for plant growth; therefore, all nutrients will need to be supplied through fertilization (Survase *et al.*,2010). This characteristic proves ideal for this experiment; however, as all observed data can be attributed to the applied fertilizer detailed in the experiment and not to any nutrients already present within the soilless media. Thus, coconut coir is the most optimal soilless growth medium for determining optimal N fertilization in day-neutral strawberries.

2.7 Nitrogen Fertilizer and Day Neutral Strawberry

Effects of Nitrogen on Flower Bud Induction

From numerous studies, it has been observed that nitrogen fertilizer has a direct effect on increased vegetative growth in strawberry through the promotion of leaf, stolon, and inflorescence growth in *F. x ananassa* species as well as influencing the development of specific plant parts, such as the growth of leaves over flower buds (Human and Kotze, 1990; Haynes *et al.,* 1987; Massetani *et al.,* 2010). From these observations, it is suggested that by providing cultivated strawberry with an optimal rate and recipe of nitrogen fertilizer that strawberry plants can be influenced to produce more significant numbers of inflorescences and flower buds during the growing season. The effect of nitrogen fertilizer on cultivated strawberry, however, can vary depending on the concentration and source of nitrogen as well as the timing of fertilizer application and the strawberry cultivar. Thus, thorough research is required to understand how nitrogen fertilizer interacts with different strawberry varieties, especially since knowledge of flower induction of cultivated strawberry is limited (Ariza *et al.,* 2015).

Several studies have considered how the concentration of nitrogen effects flower induction, which has provided mixed results. In Haynes *et al*. (1987), a study was conducted on short-day variety strawberries in New Zealand comparing how different concentrations of nitrogen fertilizer affected the architecture of the strawberry plants. It was concluded from the study that nitrate (NO_3^-) fertilizer administered at levels between 150kg/ha and 200kg/ha, approximately 17g to 23g per strawberry plant, produces an increase in strawberry inflorescences and provided a higher yield (Haynes *et al.,* 1987). Although, it was also observed that nitrogen concentrations over 200kg/ha, 23g per plant, resulted in increased growth of stolons and leaves of the plant rather than inflorescences, inhibiting yield (Haynes *et al.,* 1987). This is reaffirmed in Massetani *et al.,* (2010) which applied an increased use of nitrogen fertilizer in day-neutral strawberry using fertigation techniques. They found that increased use of nitrogen solution resulted in more significant flower bud induction. However, a solution that was more concentrated with nitrogen resulted in the growth of only stolons and leaves (Massetani *et al.,* 2010). Other studies have found no correlation between nitrogen concentration and increased flower bud induction (Iatrou *et al.,* 2013; Netsby *et al*¸1998). This suggests that strawberry variety and cultural practices are potential factors that can interfere with flower bud induction and must be considered when administering concentrations of nitrogen fertilizer.

The source of nitrogen has also found varying results in improving flower bud induction of strawberry. In Cárdenas-Navarro *et al*., (2006) nitrogen was supplied to strawberry at constant concentrations through fertigation techniques in the form of nitrate, NO_3^- , or ammonium, and NH_4^+ order to compare strawberry architecture and yield between to two study groups (Cárdenas-Navarro *et al.,* 2006). From the results of the study, there was no observed difference in flower bud induction in either treatment. However, plants treated with NH_4^+ were observed to produce more stolons than plants treated with NO_3^- (Cárdenas-Navarro *et al.,* 2006). An earlier study compared the use of NO_3^-, NH_4^+ as well as organic urea on strawberry and found similar results with no significant difference in flower induction between the use of NO_3^- or NH_4^+, however, the use of urea found a higher yield of strawberry compared to the other treatments (Papadopoulos, 1987). From each of these studies, there are observable differences in strawberry growth depending on the source of nitrogen, which requires this factor to be considered when optimizing fertilizer use.

Effects of Nitrogen on Winter Dormancy

While a period of winter dormancy is obligatory for the proper maintenance of strawberry, the length of the dormancy period, notably a timing needed for dormant transplants to begin growing new vegetation, results in a shortened growing season and a smaller yield. However, the presence of excess nitrogen in growing media during dormancy of strawberry transplants has been observed to accelerate the ability of metabolic processes to resume in early Spring, allowing for strawberry plants to begin bearing fruit earlier in the season (Human and Kotze, 1990). Thus, it is imperative to further research how nitrogen fertilizer affects the transition into dormancy and the length of dormancy in strawberry.

Prior research into the effects of nitrogen fertilizer on the length of day-neutral strawberry dormancy is limited, however, it can be hypothesized from what is known about strawberry dormancy and the effects of nitrogen in increasing plant growth, that the presence of abundant nitrogen in the soil during dormancy will be taken up by the plant more readily and will result in earlier growth in the Spring. A study by Khayyat *et al.,* (2010) tested this hypothesis by growing short-day strawberry cultivars where certain transplant groups were supplemented with potassium nitrate fertilizer prior to entering dormancy. During dormancy, chilling was also applied to compare whether nitrogen was as effective as chilling through winter in breaking dormancy earlier in transplants (Khayyat *et al*., 2010). It was observed that in groups where both chilling and nitrogen were applied, resulting in the earliest break in dormancy, however, treatment groups that only received nitrogen fertilizer tended to break dormancy earlier than transplants that were only chilled (Khayyat *et al.,* 2010). In another study, nitrogen fertilizer was applied to strawberry transplants either in the fall at the beginning of strawberry dormancy or in the Spring at the end of strawberry dormancy (Human and Kotzé, 1990). From this study, it was concluded that nitrogen applied at the beginning of strawberry dormancy in fall resulted in an earlier production of fruit than plants supplied with no nitrogen or only Spring nitrogen, suggesting the importance of nitrogen in optimizing the length of strawberry dormancy (Human and Kotzé, 1990). Further research can also consider the concentration and source of nitrogen on the effects of strawberry dormancy as these factors have shown significant differences in strawberry growth in other studies and have potential implications for the length of dormancy (Papadopoulos, 1987; Massetani *et al.,* 2010).

3. Analyzing the Effects of Nitrogen Fertilizer Source on Flower Bud Induction in Day-Neutral Strawberry

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Abstract: Day-neutral strawberry (DN) have been developed aside from short-day and everbearing varieties to produce fruit irrespective of photoperiod, allowing for an extended harvest season and higher yields. The use of day-neutral strawberry in Quebec, as an alternative to short-day, is a promising pursuit to address increasing strawberry demands and reducing imports from outside of Canada. However, previous research has observed that DN cultivars tend to yield significantly below their potential in northeastern climates. Optimizing nitrogen fertilization in transplants has been shown to improve strawberry yields and is inferred to be a solution to the decrease in productivity seen in DN strawberry cultivars grown in sub-optimal climates. Thus, this study aimed to further understand the role of N fertilizer in day-neutral transplant production and apply these findings to determine an optimal fertilizer guide for Quebec climates. DN cultivar Albion transplants were grown under greenhouse conditions and were given either nitrate, ammonium, and urea as the primary sources of N with each source being supplied at one of three concentrations, 50 ppm, 100 ppm, and 150 ppm per transplant. Phenology data, rate of photosynthesis, and relative growth rate were observed. Results suggested that the use of higher concentrations of urea produced more observable flower buds compared to the highest concentrations of ammonium whereas no significance was found among the other treatments. A relationship was also established between the photosynthetic performance of the transplants and the source of nitrogen used, with a greater rate of gas exchange noted in all urea treatments compared to nitrate and ammonium. This suggests that use of urea nitrogen sources allows for a more efficient photosynthetic rate that can affect flower bud induction in Albion strawberry transplants. Thus, Quebec strawberry farmers should consider increasing the use of urea as the primary source of nitrogen in DN transplants for improvements in photosynthetic efficiency and yield.

3.1 Introduction:

Strawberry is an economically important horticultural crop in Quebec, with approximately 47% of Canada's strawberry production occurring within the province (Agri-Food Canada, 2016). Strawberry farm gate value has also been increasing consecutively in Quebec since 2012, rising 18.1% in 2017 alone (Agri-Food Canada, 2017). Despite increasing production trends, however, the import value for strawberry is vastly outweighing the export value within Quebec and Canada as a whole. Nearly 95% of all marketed strawberry within the Canada is sourced from outside the country with increasing demand only widening the gap between import and export values annually (Agri-Food Canada, 2017). With high demand for strawberry and a need for significant yield increases, several methods have been considered in Canada, particularly Quebec, to address the problem. A promising method for increasing yield has been shifting from the predominant use of short-day, single harvest strawberries in Quebec to multi-harvest day-neutral strawberries. Through this shift in cultivars, Quebec farmers would see an increase in fruit production with harvestable fruit produced from June to mid-October (Rana and Gu, 2020). As such, the total strawberry yield will inarguably increase the more these day-neutral cultivars become prioritized as the principle strawberry cultivars grown in Quebec.

Compared to short-day (SD), day-neutral strawberry (DN) are mainly self-pollinating, octoploid cultivars bred by back-crossing wild *Fragaria virginiana* species with everbearing *F.* x *ananassa* varieties to produce day-neutrality traits (Bringhurst and Voth, 1984; Durner *et al.,* 1984). These cultivars were initially made available commercially in 1990 due to a breeding program headed by the University of California to increase yield. DN strawberry differ from SD and everbearing varieties as they are capable of flowering and bearing fruit irrespective of photoperiod, allowing the production season to extend until mid-October as long as temperatures remain moderate (Hancock, 2000; Weebadde *et al.*,2008). With these advantages, day-neutral traits provide a promising solution for increasing Quebec yield by providing nearly 14 more weeks of harvest time (Rana and Gu, 2020). However, despite these advantages, there are still some setbacks that can affect the adoption of DN strawberry in place of SD on Quebec farms. It was observed in previous trials that DN cultivars yield significantly below their potential in northeastern climates of the United States, and Canada compared to the west coast United States where these cultivars were initially bred (Hancock *et al.*, 2002; Weebadde *et al.*, 2008). From

these trials it was found that environmental factors, including fluctuating temperatures in the summer months of the northeast, would cause DN yield to significantly drop far below the potential seen in the same cultivars grown in the western and southern United States. These findings have highlighted the need for a more in-depth analysis towards developing region-specific practices for growing DN strawberry. By developing specific environmental conditions for DN strawberry in Quebec, it is hoped that the loss of yield can be mitigated, allowing for farmers to have greater confidence in adopting these cultivars to boost annual yields.

While Quebec climates are incomparable to west coast climates, other methods can be utilized to allow DN strawberry to overtake short-day production. A long-term solution can occur through the breeding of climate resistant cultivars, which has seen progress through Quebec-developed DN cultivars (Khanizadeh *et al.,* 2002; Dale *et al.,* 2013). However, until these cultivars can be marketed, DN yield will likely fall behind its competitive potential. Thus, an immediate solution is optimizing other environmental factors, particularly nitrogen fertilization. Flower bud induction in DN cultivars, directly linked to fruit yield, occurs continuously throughout all plant growth stages, unlike SD plants that only induce flower buds right before entering dormancy in the winter. From previous studies, flower bud induction is observed to be significantly influenced by nitrogen levels at early development stages, and thus, it is inferred that by adjusting N concentrations to ideal levels, flower bud production can be maximized throughout the growing season to allow for significant yield increases (Haynes *et al.,* 1987). Concurrently, the nitrogen source also has been observed to play a crucial role in determining differentiation in strawberry meristems and earlier flowering, improving yield (Papadopoulos, 1987). Depending on whether the predominate nitrogen source is either nitrate, ammonium or urea, it can affect the parts of the plant that grow including the ratio of flower stalks and flower buds compared to leaves and runners (Papadopoulos, 1987). Through testing various nitrogen concentrations and sources, a custom fertilizer model for DN strawberry can be developed for Quebec DN strawberry production to improve yields.

The purpose of this study was therefore to determine the most suitable nitrogen fertilizer practices for DN strawberry in Quebec by testing the relationship of nitrogen source and concentration on the induction of flower buds in DN strawberry under greenhouse conditions. For

this study nitrogen practices were primarily focused on improving flower bud induction at the transplant stage for these DN strawberry. By focusing on transplants, a better understanding of how N fertilization affects early flower bud production can be observed, as well as how quickly the plant matures to allow for fruit harvesting.

3.2 Materials and Methods:

Environmental Conditions

This experiment was conducted under greenhouse conditions at the Macdonald Campus of McGill University, Sainte Anne de Bellevue, QC (Lat. 45.408454, Long. -73.939977). The experiment lasted for 8 weeks beginning April 15th, 2019 and concluding June 7th, 2019. The 8-week period allowed for the monitoring of plants starting at the transplant stage and up until plant maturity at 6 weeks with two additional weeks during production to observe the effects of N throughout the strawberry growth stages. Plants were kept at a stable median daytime temperature of 25°C and a nighttime temperature of 18°C. Humidity was kept at a range of 70-80% with a daily photoperiod balanced at 12 hours of light input and 12 hours of darkness using high-pressure sodium lamps.

Bare-root transplants of day-neutral variety Albion were obtained from G.W. Allen Nursery Ltd. (Centreville, NS) for use in the experiment. Transplants were kept in refrigeration at 4°C for one week before transferring each transplant to a 15cm diameter growing pot filled with pre-moistened coconut coir substrate (Jiffy Products of America Inc., Lorain, USA). Transplants were arranged in the greenhouse, keeping 15cm of space between each container and outfitting each pot with a drip irrigation plug. For 10 days prior to the start of data collection, transplants were irrigated with only water daily to allow for initial root growth.

Experimental Groups

Transplants were allocated into nine separate treatment groups analyzing both a source and concentration of nitrogen. The three N sources of interest were urea, ammonium, and nitrate which made up for three available treatments. Within each of the source treatments, three concentrations were also tested at 50 ppm, 100 ppm, or 150 ppm of N delivered per plant, respectively. These

values were based on the average nitrogen ppm supplied to DN transplants at a value between 100-120 ppm per plant (Cantliffe *et al.* 2007). The 100 ppm value was defined as the average or 'moderate' concentration of nitrogen applied while 50 ppm was chosen as the 'low' concentration and 150 ppm as the 'high' concentration. These values were selected to determine if either of the nitrogen sources are more effective in increasing flower bud induction at lower or higher concentrations to create a more precise fertilizer model for Quebec farmers. In total, six transplants were randomly assigned to each of the nine treatments using a randomized complete block design. Each of the nine treatment blocks were replicated three times so that 18 transplants were assigned to each treatment, for a total of 18 treatment blocks and 162 transplants grown within the study.

Fertilizer Model

Solid, concentrated fertilizers were used for this experiment and are listed in Table 2. For the N treatment groups, the primary fertilizer selected was potassium nitrate as the nitrate source, ammonium sulphate as the ammonium source, and urea. Since N source was supplied to treatment groups prioritizing nitrate, ammonium and urea sources, no overlap of N sources in treatment groups was crucial. Thus, fertilizers containing nitrates, such as magnesium nitrate, were substituted with their sulfate alternatives to order to eliminate overlapping N influences. A fertilizer model was then calculated beforehand to ensure proper nutrition balance for transplants while adjusting for increased N concentrations.

Table 3. List of fertilizers used in the experimental model and the percent of active nutrients present per 100% volume of fertilizer

Fertilizers		Nutrient Content (%)
Macronutrient Fertilizers	***Potassium Nitrate (KNO_3)**	K 38.7%, N 13.5%
	***Ammonium Sulfate (NH_4SO_4)**	N 21%,
	***Urea**	N 46%
	Calcium Carbonate ($CaCO_3$)	Ca 40%
	Magnesium Sulfate ($MgSO_4$)	Mg 36%,
	MKP	K 52%, P 34%
	Potassium Sulfate ($K2SO_4$)	K 48%,
Micronutrient Fertilizers	Iron chelate (Fe EDTA)	Fe 10%
	Manganese sulfate ($Mn(SO_4)_2$)	Mn 36%
	Zinc sulfate ($ZnSO_4$)	Zn 36%
	Copper sulfate ($CuSO_4$)	Cu 25%
	Boric acid (H_3BO_3)	B 17.5%
	Molybdic acid (MoO_3)	Mo 60%

*Nitrogen fertilizers used in the experiment.

The macronutrient and micronutrient guidelines mentioned in Cantliffe *et al.,* (2007) and Paranjpe *et al.,* (2003) were consulted for targeted nutrient volumes in parts per million (ppm) for strawberry transplants (Cantliffe *et al.,* 2007 ; Paranjpe *et al.,* 2003). These values can be found in Appendix 1.

Using the recommended macronutrient ppm, the applied fertilizer was balanced to reach the desired ppm for each nutrient by adjusting the amount of fertilizer used. In the case of fertilizers that had overlapping nutrients, e.g. the use of MKP and K_2SO_4 fertilizer which both contained potassium, MKP concentrations were determined first to account for the ppm of phosphorus used, since MKP was the only fertilizer used containing phosphorus. The potassium gained from the amount of MKP used was then adjusted up to the desired ppm by adding additional K_2SO_4 (Appendix 2).

Once the fertilizer values were balanced, the amount supplied in solution was calculated using the formula:

Fertilizer per 1L= desired ppm x 1mg/L / % of nutrient

Where the desired ppm is the ppm needed for optimal strawberry growth and the percent of nutrient is the amount of the desired macronutrient in the whole fertilizer. From this calculation, the milligrams of fertilizer added per one liter of water to reach the desired ppm of macro and micronutrients was determined (Appendix 2).

Since the use of KNO_3 as a source of nitrate for the experiment also serves as a source of potassium, to avoid disparity in treatment groups, excess potassium also needed to be applied to the ammonium and urea treatment groups. To determine how much potassium was needed, the ppm of potassium supplied to the nitrate treatments was found by multiplying the percent of potassium in KNO_3 by the amount of KNO3 for the three nitrate concentrations. Once the ppm of potassium needed for the ammonium and urea treatments based off the nitrate treatments was found, K_2SO_4 was added to the ammonium and urea treatments to keep consistency.

Calcium fertilization was also calculated and applied separately as a fertilizer solution. This was carried out due to the need for a calcium source that did not contain nitrogen to prevent bias between overlapping nitrogen sources. Therefore, as a suitable alternative, agricultural lime ($CaCO_3$) was used as the primary source of calcium for this experiment which was delivered to plants as a separate stock solution.

After calculating the nitrogen, potassium and calcium fertilizer values, the amount applied to each strawberry transplant is shown in Table 4.

Table 4. Fertilizer values applied to individual transplants weekly during the 8-week experimental setup

Fertilizer	Nitrogen ppm	Fertilizer in Solution (g/L)	Fertilizer Applied Weekly per Transplant (g/plant)
Nitrate (KNO_3)	50 ppm	0.386g/L	**0.058g**
	100 ppm	0.769 g/L	**0.115g**
	150 ppm	1.153 g/L	**0.173g**
Ammonium (NH_4SO_4)	50 ppm	0.238 g/L	**0.036g**
	100 ppm	0.476 g/L	**0.071g**
	150 ppm	0.714 g/L	**0.107g**
Urea	50 ppm	0.109 g/L	**0.016g**
	100 ppm	0.217 g/L	**0.033g**
	150 ppm	0.326 g/L	**0.049g**
***Potassium (K_2SO_4)**	50 ppm	0.310 g/L	**0.047g**
	100 ppm	0.608 g/L	**0.091g**
	150 ppm	0.929 g/L	**0.139g**
Calcium ($CaCO_3$)	50 ppm	0.350 g/L	**0.053g**
	100 ppm	0.350 g/L	**0.053g**
	150 ppm	0.350 g/L	**0.053g**

*Potassium, in the form of K_2SO_4, was only applied to ammonium and urea treatments to balance the amount of excess potassium supplied from nitrate treatments.

For macronutrients and micronutrients other than the N treatments, fertilizer was made up in concentrated stock solutions that was delivered to transplants via fertilizer injector. To calculate for the concentrated solution that following calculation was used to determine the final mg/L values of fertilizer needed.

Nutrient per 1L= nutrient ppm x dilution factor / % nutrient in fertilizer x C

Where, the dilution factor represents the dilution employed in the fertilizer injectors which was set at 1L of stock solution per 80L of water, and C represents the constant which is 10. These values were calculated and separated into three stock solutions between macro and micronutrients to avoid precipitate buildup between reactive fertilizers (Appendix 2).

Fertilizer Production and Application

Nitrogen fertilizer treatments were applied to transplants using hand fertigation for each treatment group. Fertilizer solution was mixed at the start of each week, with each plant within the treatment group receiving 50mL of fertilizer solution 3 times a week. Before fertilizer solutions were applied to the treatment groups, each solution was measured for pH and EC. For each solution pH was adjusted to fall within the range ideal for strawberries between 5.5 and 6.5. To achieve this, agricultural lime ($CaCO_3$) powder was added in increments of 0.10mg to fertilizer solutions that were acidic. Similarly, citric acid powder was added at increments of 0.01mg to basic solutions until a pH of 6.0 was reached. In this experiment, lime was added to both the ammonium and urea sourced treatment groups and citric acid was added to the nitrate treatment groups. Electrical conductivity (EC) was also monitored between treatments to account for variance with an acceptable EC value ≥1.8mS.

The calcium solution was also delivered by hand to each of the treatment groups as 50mL of stock solution administered per plant 3x a week. These treatments were given concurrently with the nitrogen treatments each week. Calcium was supplied so that a concentration of 140 ppm was delivered per transplant each week (Appendix 1). Using the aforementioned calculation to determine the amount of fertilizer needed for a 140 ppm concentration of calcium, the final result was 0.05g of $CaCO_3$ delivered per transplant each week. For the stock solution, 0.95g of $CaCO_3$ was added per 1L of DI water. However, unique solutions were made for each of the nitrogen treatments, adjusting the 0.95g/L concentration by subtracting the amount of calcium added to each of the ammonium and urea solutions as a pH balance in order to keep consistency. The stock solutions were well agitated before watering to avoid precipitation of $CaCO_3$ fertilizer in water.

For all other macronutrients, drip irrigation was delivered to each of the treatment groups using fertilizer injectors supplying each of the three concentrate solutions connected to a main water line, suppling all plants. All injectors were set to deliver fertilizer stock at a ratio of 1:80mL water:stock solution. Fertilizer and water were supplied to all plants twice a day in the morning and evening for a duration of 5 minutes of continuous irrigation.

Relative Growth Rate

Relative growth rate in the transplants was measured by taking the dry weight of an initial transplant at the start of the experimental period at 0 days and comparing it to the dry weight of transplants at the end of the growth period at 42 days. These measurements were taken to understand which treatment resulted in the greatest amount of growth in different parts of the plant during the 8 weeks of maturation. The growth rate was measured using the following calculation sourced from Hunt (2002):

$$\mathbf{RGR = (W1 – W2) / (t1 – t2)}$$

Where W1 represents the initial total dry weight, W2 represents the final dry weight, t1 represents the starting time in days and t2 represents the final time in days (Hunt, 2002). At the start of the experiment, one of the six transplants in each treatment group was randomly selected to represent the initial weight of the transplant groups. This was replicated three times per treatment group for a total of 27 plants. These transplants were dissected into leaves, petioles, inflorescences, stolons, crowns and roots which were weighed separately to account for the fresh weight as well as the total combined fresh weight of plant parts. These groups were then dried in an oven overnight at 60°C and weighed again to account for the dry weight. At the end of the experimental period, a second sampling occurred, taking 27 more transplants randomly selected from each treatment groups to calculate the growth rate over the 8-week growth period. The initial and final dry weights for all plant materials were determined between the treatment groups before using the calculation to find the relative growth rate.

Phenology

For the 8-week period, growth in three randomly selected transplants per treatment group was monitored for new observable petioles, inflorescences and stolons. All initial growth was tagged I0 to represent initial growth at 0 weeks. All new growth of stolons, petioles and inflorescences were tagged. Transplant growth was observed at the start of each new week with all new growth labelled on this day. The number of petioles, stolons, inflorescences, as well as flower buds were counted for each plant. Fruit count produced by transplants starting at week 5

was also recorded with fruits being collected at the start of each week. Collected fruits were then weighed for each treatment group to account for fruit size.

Photosynthetic Rate

Rate of photosynthesis was also accounted for in this experiment to determine the treatment that would allow for a heightened plant metabolism, contributing to greater growth and higher yield. Photosynthetic rate was measured using a LI-6800 portable photosynthesis system (Li-Cor Biosciences®, Lincoln, USA) which measured the rate of CO_2 gas exchange in the transplants. The photosynthetic rate was measured every 2.5 weeks during the experiment to account for changes over stages of development. The first measurement was taken on the 2^{nd} week of the experiment with concurrent readings on the 5^{th} and 8^{th} weeks. One transplant was randomly selected from each of the treatment groups, replicated three times, to calculate the photosynthetic rate for a total of 27 plants. Furthermore, the newest petiole growth from the upper canopy of the plant with leaves measuring at least 4cm in diameter were selected. From each of the selected leaves, the middle leaflet of the three-leaf petiole was selected for analysis. The Li-6800 was calibrated to the environmental conditions of the greenhouse at 25ºC and 80% humidity. Light intensity was individually adjusted for each leaf using the built-in light receptor. CO_2 gas exchange was measured four times for each leaf and averaged to improve data accuracy.

pH and EC Monitoring

Coir pH and EC was also monitored throughout the experiment by collecting coir samples from each treatment and saturating with water at a 1:1 v/v ratio (Thunjai *et al.*, 2007). The soil-water mixture was agitated with a stir rod and left to rest for 15 minutes before decanting slurry into a clean flask. The pH and EC of the slurry was then measured using portable pH and EC meters. This experiment was repeated three times during the 8-week trial period to monitor pH and EC levels over time and detect any changes in coir pH from acidic fertilizers and EC change from nutrient buildup.

Statistical Analysis

All data is expressed by their mean ± standard deviation between each replication. For each data parameter, three replications for each treatment group were collected for phenology, RGR and photosynthesis rate. A mixed-model ANOVA analysis using the SAS statistical package, version 9.4 (SAS Institute Inc., Cary, USA), was used to determine the interaction between N source, concentration, and week. The week was included into the analysis to account for repeated measurements taken over time for the RGR, phenology and photosynthetic rate data. For all observed values, an alpha value of 0.05 was predetermined to be considered significant. In the instance of significant values, the mean of the data was separated and compared among the treatment groups.

3.3 Results:

Relative Growth Rate

From the data observed in Figure 4, it was determined that the interaction between N source and concentration was not significant for RGR (p-value = 0.510). There was also no significance noted between N sources or N concentrations, p-value of 0.577 and 0.951 N concentrations. All p-values exceeded the predetermined alpha value of 0.05, suggesting no significance in the relative growth rates of the N treatment groups.

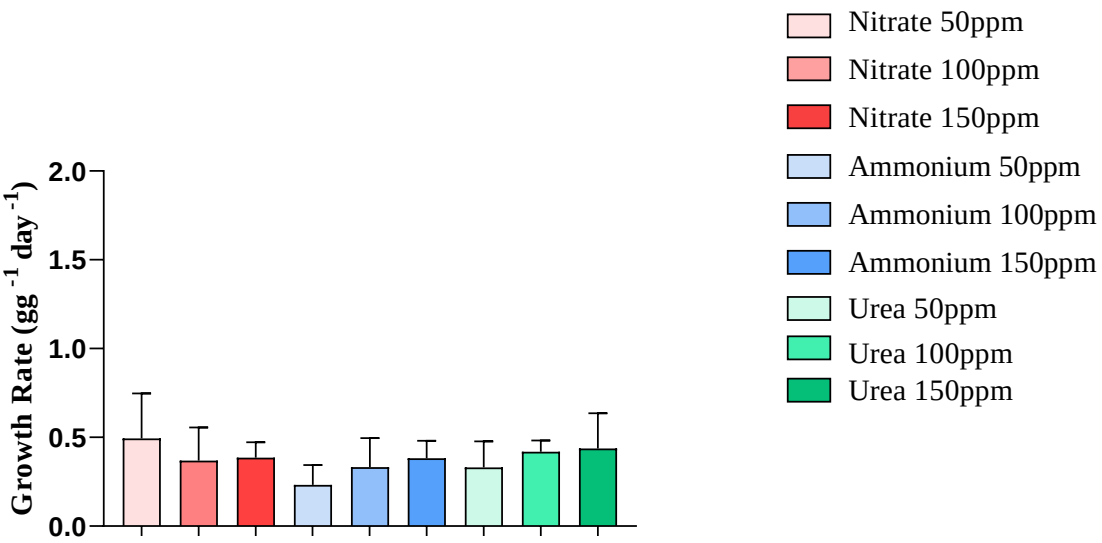


Figure 4. Relative growth rate (RGR) observed between the N treatments in DN strawberry transplants during an 8-week (56 day) growth period. RGR was calculated using RGR = (W1 – W2) / (t1 – t2), where W1 represents the initial weight at week 0, W2 represents the final weight at the end of 8-weeks, t1 represents the starting time (0 days) and t2 represents the final time (56 days). Growth rate was quantified in initial grams multipled by final grams minus one multiplied by the number of days minus one (gg^{-1} day^{-1}). Three transplants were selected from each of the nine treatments at the beginning and end of the experimental period and were dried in a 40°C oven before weighing to determine growth rate.

Phenology Data

From the data it was observed that there was significance between N concentration and source when comparing the number of petioles and flower buds produced over time (Fig.5). The number of leaves observed between N source and concentration was significant (p-value = 0.001). Comparing the treatment groups, it was observed that the greatest number of leaves were produced in the 150 ppm urea treatment group which was higher than all other treatment groups with a mean value of 8.7 leaves produced among the transplants. There were also significantly more leaves produced in the 50 ppm ammonium treatment, with a mean value of 6.84 leaves per transplant, when compared to the 150 ppm nitrate treatment. There was no other significance in the number of leaves produced from the other treatment groups.

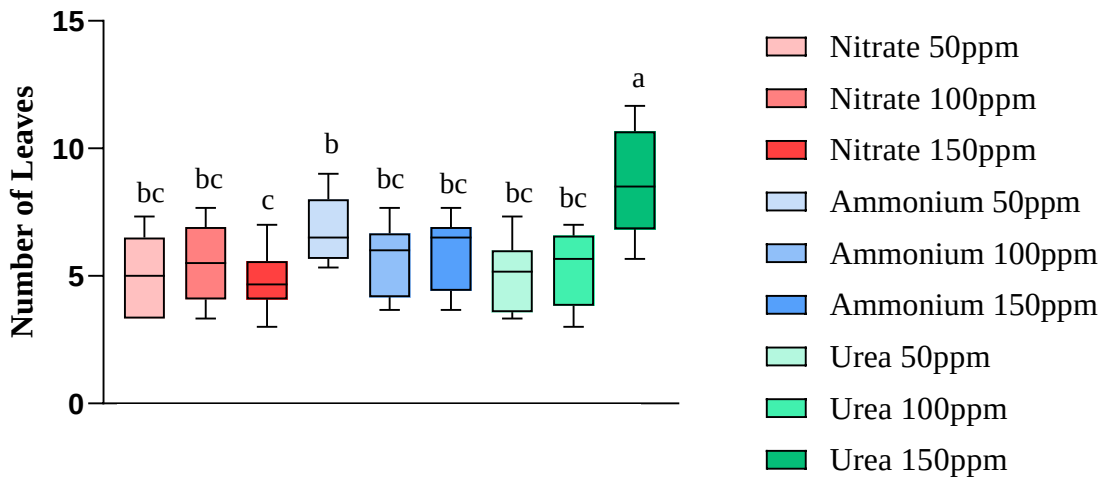


Figure 5. Average number of leaves observed from the source and concentration of the different N treatments in DN strawberry transplants. Leaves were quantified and tagged once per week for eight weeks from three transplants from each of the nine nitrogen treatments. Significance in leaf quantity between treatments was determined using two-way ANOVA.

From the phenology data, (Fig. 6), some significance was noted in the number of flower buds produced by comparing the N source and concentrations (p-value = 0.003). Although, this significance was found only between the 150 ppm urea treatment which was observed to produce significantly more flower buds than the 150 ppm ammonium treatment group. The 150 ppm urea treatment did not show the same significance when compared to the other N treatments. Furthermore, all other N treatments showed no significance compared to the other treatments in the production of flower buds. Thus, the results suggests that a N treatment of 150 ppm of urea per transplant will allow for a greater production of flower buds when compared to the same concentration of ammonium given to transplants.

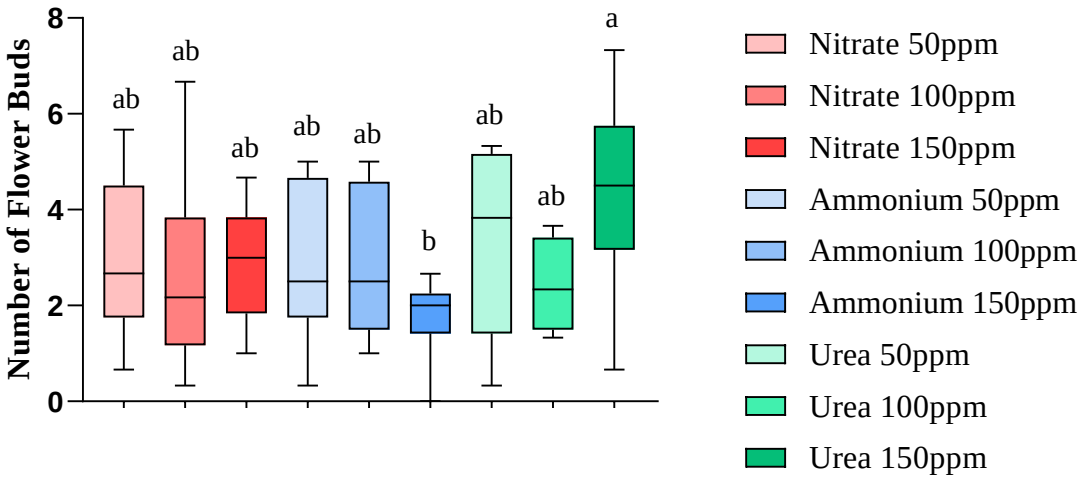


Figure 6. Average number of flower buds observed from the source and concentration of the different N treatments in DN strawberry transplants. Flower buds were quantified and tagged once per week for eight weeks from three transplants from each of the nine nitrogen treatments. Significance in flower bud quantity between treatments was determined using two-way ANOVA.

Rate of Photosynthesis

The photosynthetic rate was not significantly different when comparing N sources and concentrations together (Fig. 7). However, significance was noted depending on the N sources. From the data, it was observed that all urea treatments had a higher photosynthetic rate compared to both the nitrate and ammonium treatments with a significant p-value of 0.024. This suggests that urea applied as a source of N will likely bring about the most efficient exchange of CO_2 to accelerate the process of photosynthesis.

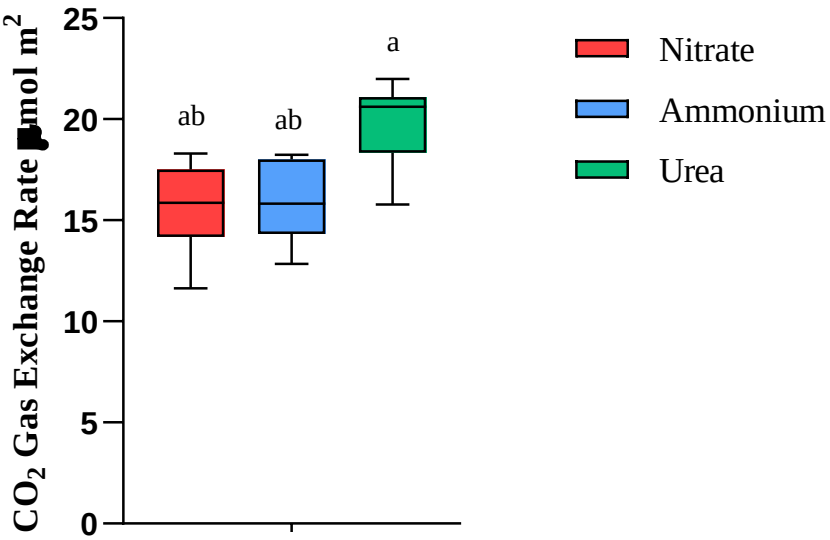
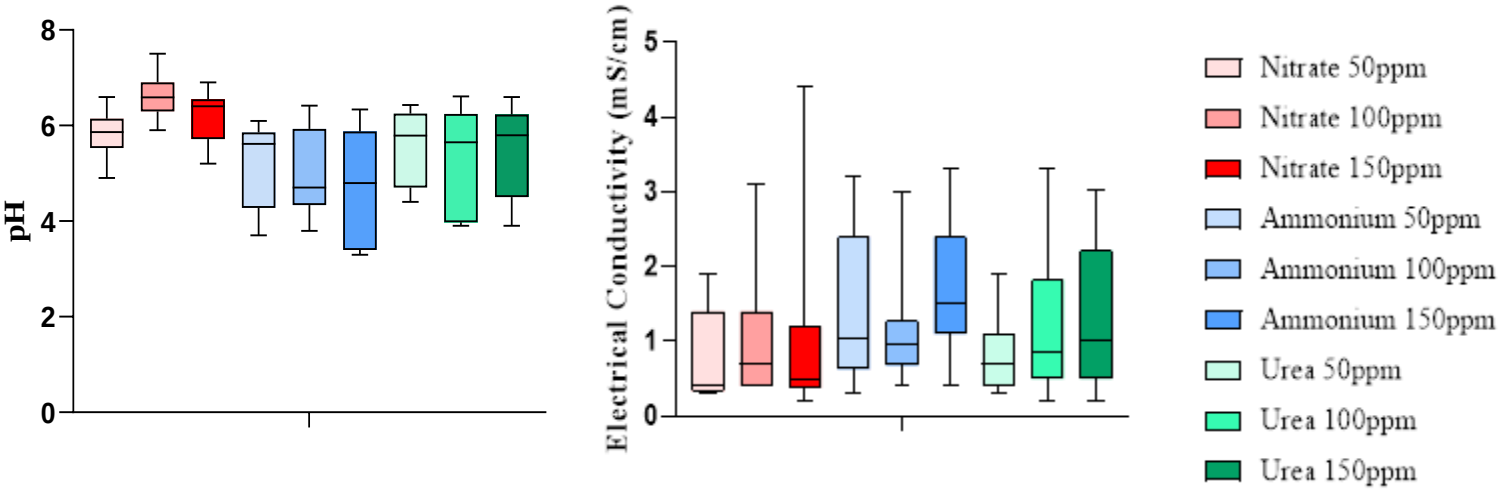


Figure 7. CO_2 gas exchange rate was quantified in µmol of CO_2 gas fixed per m^2 of the leaf's surface using a LI-6800 portable photosynthesis system (Li-Cor Biosciences®, Lincoln, USA. Three transplants were selected from each of the nine treatments and were sampled from every 1.5 weeks for 8-weeks total. Data shows the significance of the gas exchange rate between transplants of the different N sources in all three concentrations of 50 ppm, 100ppm and 150ppm respectively. Significance between the gas exchange rate was determined using two-way ANOVA, with significance found only when observing the nitrogen source (p-value= 0.024) .

pH and EC Monitoring

The pH and EC monitored during the 8-week trial found notable differences in the values obtained between the treatment groups (Fig. 8&9). While the differences noted in the measured pH and EC values between N treatments was not statistically significant, certain trends were noted among treatment groups. In Figure 5, the average recorded pH was compared, with ammonium and urea treatment groups observed to have a lower pH average compared to the nitrate treatments, with 6.1 as the average pH among nitrate treatments and 5.4 and 4.8 as the average pH among urea and ammonium treatments respectively. EC values saw no significant differences in recorded values between treatments, however, nearly all treatments observed one or more EC readings that exceeded the ideal threshold of 1.8mS/cm.



Figures 8&9. Average pH and EC measured from N treatments obtained from soil leachate during the 8-week experimental period. pH and EC was sampled from three transplants from each of the nine nitrogen treatments. pH was quantified on a 1-14 scale using a pH meter and EC was quantified in microsiemens of electrical conductance observed between two electrodes at 1 cm distance apart.

3.4 Discussion:

From the conclusion of the phenology experiment, it was found that 150 ppm of urea will allow for a greater production of leaves compared to the other N treatments as well as a greater number of flower buds compared to a treatment of 150 ppm ammonium. The phenology experiment results coincide with what was observed in the literature for short-day strawberry, with urea being the N source that most significantly affects yield of strawberry transplants. In Papadoupoulos (1987), 150 ppm of urea fertilizer was observed to produce the most significant number of flower buds in short-day variety strawberries when compared to the use of nitrate and ammonium (Papadopoulos, 1987). Since the number of flower buds produced from the 150 ppm urea treatment was not significantly greater amongst all the other treatments, it cannot be confidently determined as the best-suited treatment for increasing flower bud production among DN strawberry. However, these results do suggest that high concentrations of urea tend to outperform high concentrations of ammonium in flower bud production. From these results,

farmers can perhaps lean towards using urea in their DN strawberry fertilizer model rather than ammonium to improve flower bud induction.

The highest concentration of urea producing the most flower buds conflicts with other studies on the effects of N concentration and flower bud induction. In Massetani *et al.* (2010), a higher concentration of N tended to reduce the induction of flower buds, instead promoting the growth of more stolons and petioles at the transplant stage (Massetani *et al.,* 2010). Since this data was attained from growing SD strawberry varieties, it can be argued that perhaps DN strawberries have a greater tolerance of higher concentrations of N when producing flower buds. Another explanation could be that urea as a source of N in DN strawberry transplants allows for a greater N concentration tolerance compared to nitrate and ammonium used in Massetani *et al.* (2010). This would suggest that greater concentrations of urea, compared to ammonium and nitrate, can be applied to transplants to further increase flower bud induction before growth of flower buds no longer significantly increases. In an experiment comparing urea concentrations in SD transplants, it was found that higher concentrations of urea double that of the recommended dosage of nitrogen, allowed for the most significant number of flower buds produced as well as earlier flowering (Wan *et al.,* 2017). Unlike nitrate and ammonium, it was observed from the study that flower bud induction began to occur sub-optimally only when the urea concentration was x4 the recommended dosage, at 400-500 ppm (Wan *et al.,* 2017). Thus, it can be argued from this study and from the results of the phenology, that a greater concentration of urea can be used among DN transplants to significantly boost yield with fewer risks of triggering accelerated vegetative growth. To expand on this, a follow-up trial applying a broader range of urea concentrations can better illustrate the maximum optimal concentration with the greatest induction of flower buds before the production of flower buds begins to plateau or decrease.

Urea was also the most statistically significant treatment group when observing the rate of photosynthesis between treatments. The rate of CO2 gas exchange between all three urea treatment groups was greater compared to both nitrate and ammonium treatments, suggesting that urea is the most efficient N source for increasing plant metabolism in DN strawberry. These results are important to consider, as a more efficient rate of photosynthesis will improve growth, including the growth of flower buds, and accelerate the time it takes for initial fruits to be ready for harvest. However, while a significant rate of photosynthesis was observed in each of the urea treatments, only one urea treatment, 150 ppm, had significant effects on growth of leaves and flower buds,

observed in the phenology data. Furthermore, RGR data also found no significance in an accelerated growth rate linked to a more efficient photosynthetic rate in any of the treatments. This conflicting data can be further examined by repeating this experiment and comparing the effect of photosynthesis on the short-term and long-term growth and development of DN strawberries.

The relative growth rate of DN strawberry plants between treatments was observed not to yield significant growth rates. Growth rates were compared solely between N source, solely between N concentration, and through the interaction of source and concentration, which produced no significant or skewed data towards any of the treatment groups. From this, the source and concentration of N is assumed to likely not be significantly linked to improving the overall rate of growth in DN strawberries. A further analysis of growth factors could give better insight into how the N source and concentration affects transplant growth at the molecular level, including the production of gibberellic acid within plant tissue. Previous research has found that gibberellic acid is crucial for increasing the growth rate of plant tissue and earlier flowering in strawberry, with these effects even more prominent in DN cv. "Seascape" plants compared to SD cvs. "Camarosa" and "Laguna" plants (Paroussi *et al.,* 2002). Since nitrogen is involved simple sugar production, needed for signaling gibberellic acid production within plants (Perata *et al.,* 1997), a future analysis of gibberellic acid concentration from the transplants in this experiment could indicate if any of the N treatments are significantly affecting this production, despite no observable differences in growth rate. Thus, further analysis of phytohormone production within DN transplants given N treatments could support the findings of this experiment in finding the most optimal N treatment.

Since $CaCO_3$ could not be added to the drip irrigation system as it would precipitate, causing potential damage to the system, it had to be applied manually to transplants by means of $CaCO_3$ solution. The percent calcium was adjusted based on the calcium added to the fertilizer solutions to balance pH and was delivered to plant roots as a 50mL dissolved solution. While calcium was supplemented to meet strawberry requirements beginning on the fifth week of data collection, observable symptoms of calcium deficiency were noticed in the leaves (Fig. 7).

Figure 10. Observed effects of calcium deficiency in strawberry plants at Week 8 of the experiment. Transplants showed telltale signs of calcium deficiency in underdeveloped leaves, displaying “tip burn” and a crimped appearance from the deterioration of cell walls (Bolda, 2009). Photo was obtained on May 24th, 2019.

This deficiency was diagnosed based on a comparison to strawberry crop reports, which found similar leaf curling in the presence of deficient calcium within cultivated strawberry (Haifa, 2010). This curling is in response to deterioration of the cell walls of the plant tissue from lack of structure provided by calcium, resulting in malformed leaves with necrosis at the tip from loss of polyphenols (Bolda, 2009). As a response, calcium application was increased from 140 ppm per plant to 200 ppm at week 5 of the experiment. Despite these corrections, calcium deficiency symptoms persisted even in new leaf growth up until the eighth week. While the contribution of the calcium deficiency is unknown in the data retrieved, in repeat measures, a supplemental calcium foliar spray is suggested to mitigate any results that could have been stunted due to a lack of proper calcium delivery.

EC and pH were also variable factors within the experiment. Even with pH-adjusting before fertigating plants, pH leachate from measuring plant leachate was observed to produce pH and EC values outside of the optimal parameter for strawberries (Fig. 5,6). pH from soil samples was measured at values as low as 3.3 in ammonium treatment groups, with EC exceeding a 1.8mS threshold in all treatments. Strawberry crop profiles often recommend that the soil pH remain in a range of 6.5 and 5.5 and the soil leachate EC to remain below a value of 1.8mS/cm (Haifa Group,

2019) The effects on growth in this experiment are undetermined, although the recorded values could have contributed to reduction in growth and proper nutrient allocation. However, the 150 ppm urea treatment also suffered from low pH and high EC yet still produced significantly more flower buds compared to all other treatments. To best determine whether the effects of pH and EC affected the induction of flower buds in strawberry, future experiments could compare the observed range of different pH and EC levels from transplant leachate to observe if the different values significantly impact flower bud development within transplants.

3.5 Conclusion:

Day-neutral (DN) strawberry cultivars offer Quebec a solution to unsatisfactory annual strawberry years through providing farmers with a longer harvest season with multiple harvests throughout the year. Optimization of nitrogen fertilizer by determining an ideal source and concentration can increase these yields by inducing more flower buds and promoting increased growth. The data collected found significance in the observed phenology, which determined a greater number of flower buds produced and observed from strawberry plants that had received 150 ppm of urea sourced N compared to 150 ppm of ammonium. However, 150 ppm of urea did not significantly affect flower bud induction compared to the other nitrogen treatments, suggesting that the nitrogen source does not significantly affect the number of observed flower buds produced in phenology data. The photosynthesis rate, however, was found to be significantly greater in all urea treatments compared to nitrate and ammonium treatments, suggesting that urea treatments allow for greater efficiency of plant growth which could subsequently affect yield. Overall, the inadequacies with proper calcium delivery to plants and fluctuations of soil pH and EC suggest that this data would benefit from a repeat study to obtain similar results to reaffirm the data that was deemed significant.

4. Connecting Statements

At the conclusion of the first experiment, the use of urea was observed to be the optimal N treatment group observed in DN strawberry Albion, in terms of increasing photosynthetic efficiency compared to nitrate and ammonium. The number of flower buds in a concentration of 150 ppm urea was also significantly greater compared to 150 ppm of ammonium. With this knowledge, it was then inferred that these results could also be similarly observed when determining the optimal N fertilizer model to produce the most flower buds, increase transplant survivability, and energy storage in the crown in pre-dormancy DN Albion transplants. Thus, a second experiment was proposed following the same N treatment groups with the same sources and concentrations. From this experimental setup, it was hypothesized that a similar outcome would be reached between treatment groups, suggesting that urea would be the most optimal N treatment for pre-dormancy DN transplants. In conjunction with the second experiment, an additional trial was conducted to observe the relationship between N and K fertilizer ratios on the optimizing effects in DN strawberry. Since the first fertilizer experiment saw the need for increased K concentrations in relation to increased N concentrations, some of the significant effects observed in the experiment could also have been correlated to elevated concentrations of K. Thus, to verify this possibility, three ratios of N:K fertilizer were also tested in pre-dormancy transplants during the second experiment.

5. Analyzing the Role of N and K Fertilizer in After-Dormancy Yield of Day-Neutral Strawberry

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Abstract: Day-neutral strawberry (DN) has been developed aside from short-day and everbearing varieties to produce fruit irrespective of photoperiod, allowing for an extended harvest season and higher yields. DN varieties, however, tend to suffer in yield potential following winter dormancy through transplant loss and delayed flower bud formation in spring. A viable solution is the optimization of N and K fertilizer. An ideal source and concentration of N and balancing N and K ratios was hypothesized to increase nutrient storage in DN transplants and facilitate optimal growth after breaking dormancy. This study aimed to understand the role of N and K fertilizer role in DN transplants before, during, and after dormancy to establish a fertilizer guide for pre-dormancy transplant production. Two trials were conducted: Trial 1 considered N source and concentration, testing nitrate, ammonium, and urea supplied at 50 ppm, 100 ppm, and 150 ppm. Trial 2 compared N:K ratios at 1:1, 1:2, and 1:4 intervals. Both trials monitored weekly phenology data and took weekly plant samples for dissection, biomass assays, and sugar content analysis in the crown. Transplants for both trials were kept in cold storage in October to initiate dormancy, and samples were taken monthly for the same data parameters. Transplants were again sampled after dormancy. Results found no significance in data obtained in Trial 1 before or during dormancy. Trial 2 observed that a 1:2 ratio of N:K resulted in greater observed flower buds before dormancy and more significant flower buds counted within the crown from dissections taken during dormancy. Thus, results suggest that a 1:2 N:K ratio is optimal for increasing flower bud production and yield in DN transplants after dormancy, which can be incorporated into DN transplant fertilizer models to optimize annual yields.

5.1 Introduction:

Strawberry is an increasingly economically important horticultural crop in Quebec, with nearly half of Canada's strawberry production occurring within the province (Agri-Food Canada, 2016). The introduction of day-neutral (DN) strawberry cultivars offers further economic potential for strawberry, by extending the annual production season up to 14 weeks compared to the 4-5 weeks of short-day (SD) strawberry varieties (Rowley *et al.,* 2010). This is primarily due to these varieties' ability to continually produce flower buds independent of photoperiod, which results in fruit being produced well into October, significantly increasing annual yield (Durner *et al.,* 1984). However, current use of DN strawberry in Quebec has encountered setbacks in yield throughout the season.

While an increased use of DN strawberries over SD offer greater yield and economic potential to Quebec farmers, growing DN strawberry in northeastern climates has encountered challenges which can affect the adoption of these varieties if not mitigated. Previous studies have observed a significant drop in yield for DN varieties due to fluctuating hot temperatures in the summer and a greater temperature range between daytime and nighttime temperatures (Weebadde *et al.*,2008). Greater sensitivity to temperature is also seen at the transplant stage for DN varieties, with transplants observed to be more susceptible to colder temperatures and produce lower yields after breaking dormancy in spring compared to their SD counterparts (Gagnon *et al.,* 1990). This temperature sensitivity is related to DN varieties originally being developed for more temperate climates and temperature-controlled environments (Durner *et al.*,1984). In the case of transplants, a solution to increase strawberry survivability and yield potential following dormancy is of high priority for these DN cultivars to provide less risk for Quebec farmers losing significant potential yield annually. Therefore, a simple and effective method to increase DN survivability over winter was suggested by optimizing N and K levels during pre-dormancy treatments for DN transplants.

Day-neutral strawberry cultivars were bred by back-crossing wild *F. virginiana* species with *F. x ananassa* to produce day-neutrality traits (Bringhurst and Voth, 1989). These cultivars differ from short-day and everbearing varieties as they are capable of flowering and bearing fruit independent of the photoperiod, allowing for fruit yield if environmental temperatures remain moderate (Hancock, 2000; Weebadde *et al.,* 2008). Day-neutral traits provide a promising advantage for increasing yield in Quebec, as the harvest window for DN strawberries has the

potential to stretch into October in most regions of Quebec. However, as transplants, these cultivars need to be to be kept in cold storage over winter, at temperatures between 1°C and -1°C, to effectively preserve transplants for the following harvest and trigger winter dormancy. While periods of dormancy are innate to almost all strawberry varieties, DN strawberries tend to be more susceptible to cold storage and can have a harder time breaking dormancy in the spring (Gagnon *et al.*, 1990). One explanation for this was observed in a study comparing carbohydrate storage in DN and SD cultivars following winter dormancy. The study observed DN strawberry having a significantly reduced storage potential for carbohydrates and overall lower cold resistance when compared to most of the SD cultivars (Gagnon *et al.*, 1990).

One cause of this yield discrepancy between DN and SD cultivars could be in part due to sub-optimal fertilizer practices for DN cultivars in Quebec, particularly in the application of N and K. From the observations of Gagnon *et al.*, (1990), the optimal storage of carbohydrates within the roots and crowns of strawberry plants played a role in yield potential and survival after dormancy in the spring which is linked to the uptake and utilization of N fertilizer (Gagnon *et al.*, 1990). The source and concentration of N has been shown to correlate to the amount of carbohydrate storage in the crown (Lopez *et al.*, 2002; Gagnon *et al.*, 1990) as well as having a significant role in earlier flower bud induction after breaking dormancy in spring (Papadopoulos, 1987). Likewise, K fertilizer has also been observed to play a role in the uptake and efficient usage of N within the crown of strawberry plants when present in ideal concentrations in relation to N concentration (Ahmad *et al.*, 2014). Therefore, since DN cultivars have been observed to have significantly less carbohydrate storage within the crown during winter dormancy compared to short-day, increasing the ration of potassium to nitrogen could facilitate more efficient nutrient storage over-winter (Gagnon *et al.*, 1990). Aside from these studies, however, there is scarce information covering ideal fertilizer practices for pre-dormancy DN transplants within Quebec. With this knowledge gap, fertilizer practices on pre-dormancy transplants could continue to result in below optimal yields. Thus, for DN strawberry production to grow within Quebec, a custom fertilizer model for DN strawberry, prioritizing ideal N and K application, is crucial.

This study aimed to observe the response of DN strawberry cv Albion transplants during pre-dormancy treatments with both the N source and concentration as well as specific N:K ratios in two trial groups prior to over-winter dormancy. From these observations, it is hoped that a

particular treatment will provide more optimal results for DN transplant survivability and yield after winter dormancy, which can be used to construct a distinct fertilizer model for Quebec farmers.

5.2 Materials and Methods:

Environmental Conditions

This experiment was conducted as two distinct trials in separate locations in Quebec. The first trial observed the effects of nitrogen source and concentration on pre-dormancy DN strawberry transplants and was conducted under greenhouse conditions at the Horticultural Research Center located at the Macdonald Campus of McGill University, Sainte-Anne-de-Bellevue, QC (Lat. 45.408454, Long. -73.939977). This trial lasted six weeks beginning September 9th, 2019 and concluding October 18th, 2019. Plants were kept under polyethylene covered tunnels with ventilated sides to regulate humidity. Temperature under greenhouse conditions fluctuated based on daily recorded temperatures in Sainte-Anne-de-Bellevue. Average daytime temperatures were measured at a range of 12-22°C and average night temperatures ranged from 5-15°C for the 6-week period. Average relative humidity was also measured between 75%-85% daily. Photoperiod was determined by natural daylight with a daylight cycle at approximately 12 hours of light input and 12 hours of darkness during the 6-week period.

For Trial 1, 360 DN transplants cv Albion were collected from Ferme Onésime Pouliot Inc. for use in the experiment by harvesting runner tips from mother plants. The runner tips were chilled and were transported to the Macdonald Campus of McGill University and were immediately transferred to 5x5 centimeter diameter, 10cm deep plastic growth trays. Growth trays were filled with pre-moistened coconut coir substrate (Jiffy Products of America Inc., Lorain, USA) as the chosen growth medium. Each tray had the capacity to hold 12 transplants for a total of 43 trays used. Once propagated in the substrate trays, transplants were arranged in the greenhouse and for 10 days prior to the start of data collection, transplants were irrigated with only water daily to allow for root initiation. Any pre-existing growth in transplants prior to propagating was also pruned at the crown before collecting data. At the start of the trial, all irrigation of the transplants was delivered by hand watering.

The second trial of this experiment compared the ratios of N:K on pre-dormancy DN strawberry transplants and was conducted under greenhouse conditions at Ferme Onésime Pouliot Inc. in Île d'Orleans, QC (Lat. 46.981189, Long. -70.85058). This trial ran in conjunction with Trial 1 for the 6-week period beginning on September 9th and concluding on October 18th, 2019. Transplants were kept at regulated temperature and humidity levels throughout the trial, with temperatures between 15-20°C and a relative humidity of approximately 80%. Photoperiod was supplied by natural daylight with a balanced photoperiod of around 12 hours of light and 12 hours of darkness.

For Trial 2, 1,000 transplants were allocated to each of the three tested treatment groups from runner tips harvested from mature plants. These transplants were propagated by Ferme Onésime Pouliot Inc. in identical plastic growth trays as described in Trial 1 and were kept within an on-site greenhouse. Each of the transplant trays were irrigated using an overhead sprinkler system that would deliver the appropriate fertilizer ratio to each of the treatment groups for the 6-week duration. Like in Trial 1, transplants also received only water for the first 10 days following propagation to allow for proper initial root establishment before taking data.

Experimental Groups

For Trial 1, transplants were separated into individual treatment groups testing, ammonium, and nitrate as the sources of nitrogen at concentrations of either 50 ppm, 100 ppm, or 150 ppm nitrogen. Alongside the nitrogen treatments, a control group was tested which supplied plants with a fertilizer model employed by Ferme Onésime Pouliot Inc. annually for their DN transplants (Appendix 4). For each of the ten treatment groups, the 360 transplants were randomly assigned across all trays. This was replicated three times per treatment group for a total of 18 plants assigned to a treatment using a random complete block design.

In Trial 2, treatment groups were separated into three ratios of N:K fertilizer, focusing primarily on how the level of K effects transplant growth and survivability when the level of N is held constant. The treatment groups were separated into three distinct ratios, with the first treatment employing a 1:1 N:K ratio and the two subsequent treatments having K at 2x and 4x the amount of supplied N at a 1:2 and 1:4 N:K respectively. One thousand transplants were separated

into each of the three treatment groups and were treated as per instruction. Concentrations of these fertilizers evolved over time following the fertilizer model used by Ferme Onésime Pouliot Inc. which was adapted on a week-by-week basis (Appendix 4).

Fertilizer Model

Solid, concentrated fertilizers were used for this experiment in both Trials 1 and 2. For Trial 1, potassium nitrate was selected as the nitrate source, ammonium sulphate as the ammonium source, and urea. Since the N source was supplied to treatment groups prioritizing nitrate, ammonium, and urea sources, it was crucial that no overlap of N sources in treatment groups occurred. Therefore, nitrate fertilizers, such as magnesium nitrate, were substituted with sulfate alternatives to mitigate N source overlap between treatments. A fertilizer model was then calculated beforehand to ensure proper nutrition balance for transplants while adjusting for increased N concentrations.

Table 5. List of fertilizers used in the fertilizer model for Trial 1 and the percent of active nutrients per 100% volume of fertilizer

Fertilizer		**Nutrient Percent**
Macronutrient Fertilizers	***Potassium Nitrate (KNO_3)**	K 38.7%, N 13.5%
	***Ammonium Sulfate (NH_4SO_4)**	N 21%,
	***Urea**	N 46%
	Calcium Carbonate ($CaCO_3$)	Ca 40%
	Magnesium Sulfate ($MgSO_4$)	Mg 36%,
	MKP	K 52%, P 34%
	Potassium Sulfate ($K2SO_4$)	K 48%,
Micronutrient Fertilizers	Iron chelate (Fe EDTA)	Fe 10%
	Manganese sulfate ($Mn(SO_4)_2$)	Mn 36%
	Zinc sulfate ($ZnSO_4$)	Zn 36%
	Copper sulfate ($CuSO_4$)	Cu 25%
	Boric acid (H_3BO_3)	B 17.5%
	Molybdic acid (MoO_3)	Mo 60%

*Indicates nitrogen fertilizers used for N treatments

The macronutrient and micronutrient guideline used for this experiment was based off the fertilizer model used by Ferme Onésime Pouliot Inc. using targeted ppm of nutrients provided to transplants which adapted weekly alongside transplant establishment (Appendix 4). Where fertilizers used in the Ferme Onésime Pouliot Inc. model overlapped the use of N sources, the sulphate alternative of these fertilizers was used: e.g. magnesium nitrate (MgNo3) was substituted with magnesium sulfate (MgSo4). All fertilizers were balanced in to meet the desired ppm.

Once the fertilizers were balanced, the amount needed to be supplied in solution was calculated using the formula:

Nutrient per 1L= desired ppm x 1mg/L / % of nutrient in fertilizer

Where the desired ppm is the ppm of nutrient needed from each fertilizer and the percent of nutrient in fertilizer is the percent of the desired macronutrient in 100% of fertilizer. The mg/L needed in solution for each of the nine N treatment groups was calculated using this formula (Table 2).

Since the use of KNO_3 as a source of nitrate for the experiment also serves as a source of K, to avoid disparity in treatment groups, excess potassium also needed to be applied to the ammonium and urea treatment groups. To determine how much K was needed, the ppm of K supplied within the 50 ppm, 100 ppm, and 150 ppm ammonium and urea treatments needed to be found by using the amount of N fertilizer needed in for one liter and multiplying this value by the percent of K in KNO_3. Once the additional ppm of K was determined, the amount of K_2SO_4 needed to supplement K levels was calculated using the same formula used to find the N levels.

Table 6. Fertilizer values applied to individual transplants in Trial 1 each week from September 9th to October 18th, 2019

Fertilizer	Nitrogen ppm	Fertilizer in Solution (g/L)	Fertilizer Applied Weekly per Transplant (g/plant)
Nitrate (KNO_3)	50 ppm	0.386g/L	**0.058g**
	100 ppm	0.769 g/L	**0.115g**
	150 ppm	1.153 g/L	**0.173g**
Ammonium (NH_4SO_4)	50 ppm	0.238 g/L	**0.036g**
	100 ppm	0.476 g/L	**0.071g**
	150 ppm	0.714 g/L	**0.107g**
Urea	50 ppm	0.109 g/L	**0.016g**
	100 ppm	0.217 g/L	**0.033g**
	150 ppm	0.326 g/L	**0.049g**
***Potassium (K_2SO_4)**	50 ppm	0.310 g/L	**0.047g**
	100 ppm	0.608 g/L	**0.091g**
	150 ppm	0.929 g/L	**0.139g**

*Potassium values were only given to ammonium and urea treatments to balance the amount of potassium supplied from nitrate treatments.

For macronutrients and micronutrients other than the N treatments, fertilizer was made up in three concentrated 1L stock solutions that were added together along with 7.8L of DI water before watering transplants. These fertilizers changed based on the ppm specified weekly by the Ferme Onésime Pouliot Inc.model (Appendix 4) and were calculated using the same formula used to find the N and K values.

In Trial 2, transplants were separated into the 1:1, 1:2 and 1:4 treatment groups and were supplied fertilizer based on a weekly model (Appendix 4). In the fertilizer model, N:K fertilizer was balanced each week so that the N:K ratio would remain as either 1:1, 1:2 or 1:4. N in the form of nitrate was the primary source of N which was supplied through potassium nitrate (KNO_3) and magnesium nitrate ($MgNO_3$). However, to adjust the fertilizer ratios, ammonium also was used as

a source of N to keep Mg and K concentrations balanced. All other nutrients were adjusted as well following the Ferme Onésime Pouliot Inc. fertilizer model (Appendix 4).

Fertilizer Production and Application

For Trial 1, nitrogen fertilizer treatments were applied to transplants using hand fertigation for each treatment group. Fertilizer stock solution were mixed at the start of each week, with each transplant within the treatment group receiving 30mL of fertilizer solution 3 times a week. The control treatment likewise received 30ml of solution based on the farm model. Each treatment group received its intended N ppm, at 50 ppm, 100 ppm and 150 ppm per plant for each N source and ammonium and urea treatments also received K in the form of K_2SO_4, corresponding to the level of K in the KNO_3 treatments. Before fertilizer solutions were applied to the treatment groups, each solution was measured for pH and EC. For each solution, pH was adjusted to fall within the range ideal for strawberries, which is between 5.5 and 6.5. To achieve this, agricultural lime ($CaCO_3$) at increments of 0.10mg were added to fertilizer solutions that were acidic and citric acid was added at increments of 0.01mg to basic solutions until a pH of 6.0 was reached. In this experiment, lime was added to both the ammonium and urea sourced treatment groups and citric acid was added to the nitrate treatment groups. Electrical conductivity (EC) was also monitored between treatments to account for variance with an acceptable EC range of 1.8mS.

For all other macronutrients and micronutrients, three stock solutions were mixed ahead of time in 1L of DI water. These stock solutions were kept separate until watering where each of the solutions were mixed along with 7.8L of DI water and delivered to plants immediately to avoid precipitation of incompatible fertilizers. The final solution was applied to all transplant treatments except the control group which instead received only water since all micro and macronutrients were delivered at the same time in accordance with the farm model. To prevent overwatering, the three stock solutions were applied twice a week on the days between N fertilizer application with 30mL of the final diluted stock solution given to each of the N treatment groups and 30mL water to the control group.

For Trial 2, fertilizers were delivered to transplants through overhead sprinklers provided by Ferme Onésime Pouliot Inc. farm, with fertilizer being injected into solution via automatic

injector. Before injection, the fertilizers used were grouped into three concentrated stock solutions, A, B and C, to avoid precipitation within the irrigation lines from incompatible compounds (Appendix 4). Each of these stock solutions included were measured and dissolved in separate tanks containing 270L of water weekly following the farm model and balanced N:K model. These tanks of fertilizer stock were connected to automatic injectors set at a dilution of 1:100 to a main water line. A separate irrigation line was used for each treatment group so that there would be no overlap of treatments occurred. The stock solutions were delivered to transplants once daily with irrigation lasting for five minutes at a flow rate of 8.7L/min. Each tank was supplied with the nutrients that needed to be delivered each given week and were emptied at the start of each week to measure the following week's treatment.

Phenology

For the six-week period, growth in randomly selected transplants per treatment groups was monitored once a week for new observable petioles, inflorescences and stolons in both trials 1 and 2. Six transplants were randomly selected to be observed from each of the treatment groups in each of the trials. All initial growth was tagged I0 to represent initial growth at 0 weeks. All new growth of stolons, petioles and inflorescences were tagged N1 to 6 to represent new growth in week 1 to 6. Transplant growth was observed at the start of each new week with all new growth labelled on this day. The number of petioles, stolons, inflorescences, and flower buds were counted for each plant. After counting weekly flower buds, these buds were removed from the transplant to facilitate new flower bud growth.

Crown Dissection

For both Trial 1 and Trial 2 of the experiment, transplants were collected to dissect the crowns and observe the formation of flower buds in relation to stolons and leaves within the crown of the transplants. During the fertilizer trial these plants were taken every two weeks starting at Week 2, Week 4 and Week 6 for Trial 1. Three transplants were selected each time for each of the ten N treatment groups for a total of 30 dissected transplants. In Trial 2, three transplants were collected from each of the three N:K ratios for 9 dissected transplants. When dissecting transplants,

all dead or wilted plant material was removed as well as the roots. After removing dead material, each petiole, stolon, or flower bud was then counted starting from the outmost layer of the crown and counting inward. All data counts were recorded and sketched to locate where the concentration of stolons, petioles and leaves are in relation to the outmost and inmost layers of the plant. The remaining experimental transplants at the end of the experiment were kept in cold storage with three transplants per treatment being removed every five weeks for Trial 1 and 2 to observe crown dissections during dormancy. The first dormancy sample was taken on the 11th of December 2019 with additional samples taken on January 15th, 2020 and on the 19th of February 2020.

Biomass

Biomass was also measured in both Trials 1 and 2. Biomass was collected as a method of measuring the growth of transplants leading up to dormancy and the effects of the different nitrogen treatments on growth of different plant parts such as the growth of leaves versus the growth of flower stalks. Like the collection of transplants for crown dissections, three transplant samples were taken on Weeks 2, 4 and 6 for Trial 1 and weekly for Trial 2. These transplants were cleaned of all substrate prior to being separated into individual components of the plant. Each transplant was separated into their roots, crown, stems, inflorescences, leaves and stolons. These plant parts were weighed for their initial fresh weight before they were dried in a drying oven (Isotemp 650D Incubator, Fisher Scientific, Waltham, USA) set at 60°C for 48 hours. Dried samples were then weighed again to measure the dry weight. Biomass data was again collected during the period of winter dormancy every five weeks with samples taken on December 11th, 2019, January 15th, 2020, and February 19th, 2020.

Crown Sugar Analysis

Lastly, the sugar content of transplant crowns was analyzed during winter dormancy. This analysis hoped to identify a nitrogen treatment group that produced the greatest concentration of simple carbohydrate sugars: glucose, fructose, and sucrose, within the crown during dormancy. Only Trial 1 plants were analyzed for sugar content. For the experiment, three transplants from each of the ten treatment groups were randomly selected for a total of 30 transplants. Transplants

were sampled twice during the period leading up to dormancy as well as twice during dormancy for a total of 120 transplants analyzed.

Sugars within the crown of the transplants were quantified using enzyme reaction assays based on a method adapted from Landhausser *et al.* (2018). Within this method, free glucose, sucrose, and fructose were measured from transplant crown material by NAD^- related enzymatic assays. For the experiment, crown material was broken down by boiling it in ethanol, releasing free sucrose. This free sucrose was then broken down into free glucose and fructose using invertase derived from baker's yeast (*S. cerevisiae*) which was then phosphorylated to glucose-6-P and fructose-6-P using hexokinase dehydrogenase (Landhausser *et al.,* 2018). Fructose-6-P is then further converted into solely glucose-6-P using isomerase also derived from baker's yeast (*S. cerevisiae*). The remaining glucose-6-P is then quantified through the oxidative reaction of glucose-6-P to gluconate-6-P caused by the presence of NAD^+ catalyzed from the dehydrogenase in the hexokinase solution. Alongside the oxidative reaction of glucose-6-P and NAD^+, a reductive reaction also occurs when NAD^+ is converted to NADH, which results in an increased absorbance observed at 340nm. From this process, the relative free sugar content of sucrose, glucose and fructose can be quantified by the measured absorbance of the samples taken at 340nm (Landhausser *et al.,* 2018).

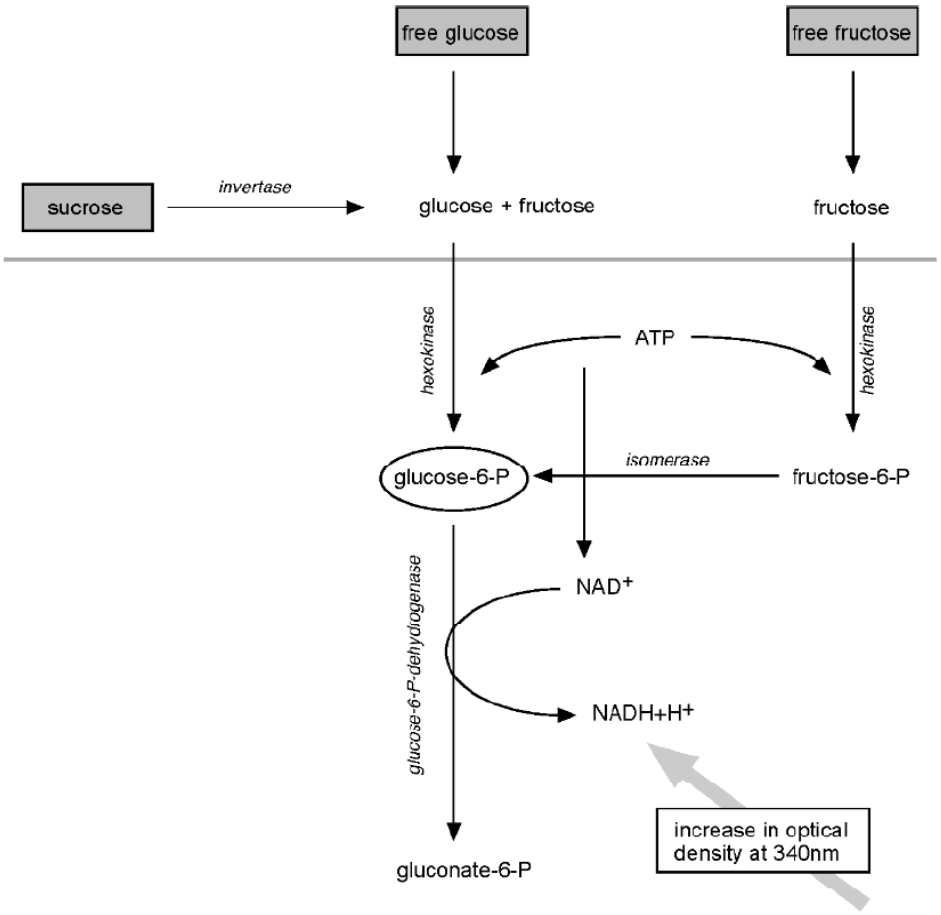

Figure 11. Reaction pathway of free sugars, fructose, sucrose and glucose, into quantifiable gluconate-6-P. Source: Landhausser *et al.*,2018

For the experimental setup, strawberry crown samples were collected from Trial 1 on September 18th and October 16th 2019 before dormancy, as well as on December 16th and January 27th 2019 during dormancy. These crowns were presevered at -80°C until the sugar assay setup could be completed. Frozen crowns were removed in September 2020 and were dried for 48 hours in a drying oven at 60ºC. Once crowns were completely dry, crowns were mechanically ground into coarse powder. The dried crown material was then weighed out to 30mg per transplant crown for all of the treatments. Weighed material was transferred to 2mL centrifuge tubes which were pre-weighed to account for the actual weights of the samples. From there, 1.5mL of 80% ethanol was added to each of the 2mL centrifuge tubes of sample before agitating using a vortex mixer and placing the tubes in a 90ºC water bath for 10 minutes. Samples were boiled within the hot water

bath before removing from heat and letting cool after 10 minutes. Samples were then centrifuged for 1 minute at 13,000rpm to separate the solid plant material from the supernatant. Supernatant was pipetted into two new 2mL centrifuge tubes with 200µL added to each tube for analysis. These new tubes were then placed in a drying oven set at 60ºC for 4 hours to evaporate all ethanol from the samples, leaving only sugar residue behind in the bottom of the tubes. Once ethanol was evaporated, 1mL of DI water was added to each of the tubes to resuspend the sugar residue. For each of the sampling dates, pre-dormancy and during dormancy, a black control tube as well as three tubes containing 30mg of pure glucose, fructose and sucrose standard were also prepared using the described method to validate sample results. In order to measure the absorbance of the sugars within the samples, three reagents were also prepared to catalyze the reaction pathway shown in Figure 11. For analysis of glucose, glucose hexokinase assay reagent (GHK), (Sigma G3293-50mL) was used by combining 50mg of GHK powder with an equal volume of DI water and gently agitating the solution. Fructose analysis followed a similar protocol to the glucose assay, however, 31µg of phosphoglucose isomerase (Sigma P5381), was added to the 50mL solution of GHK and water in order to quantify this sugar. For the sucrose analysis, a sodium acetate buffer solution was first prepared by combining 1.025g of sodium acetate to 450mL of DI water. This buffer was then adjusted to a pH of 4.6 by adding acetic acid and then bringing the final volume of the solution up to 500mL. 600 active units (U), one active unit representing the amount of enzyme that will hydrolyze 1.0µmole of sucrose to glucose and fructose at a pH of 4.6, of invertase solution (Sigma I9274) was then added to 10mL of the sodium acetate buffer. This solution was then agitated gently before use in sucrose assays.

After preparing each of the crown samples, absorbance was measured using a microplate reader to quantify the amount of free sugar present within each of the crown samples. For this part of the experiment, 96-well clear, flat-bottom microplates were used to measure the absorbance at 340nm for glucose, frustose and sucrose. For the glucose and fructose assay, two microplates were prepared, one microplate with wells filled with 200µL of DI water and another filled with 200µL of GHK, or 200µL of GHK with phosphoglucose isomerase for the fructose assays. Absorbance was measured for both microplates before adding 20µL of the collected sample to the wells, with three wells filled per sample per microplate. Also added to the microplate wells were the sugar standards and blank made within the sample preparation as well as six glucose standard serial dilutions, including 1mL, 0.5mL, 0.25mL, 0.125mL, 0.062mL and 0mL volumes of glucose

standard, as well as fructose and sucrose standards at 1000µg/mL in solution. For the sucrose analysis, 100µL of the sample supernatants as well as the sugar standards and blank were added to an empty microplate. From there, 50µL of the invertase dissolved in the 10mL of acetate buffer was added to each of the microplate wells to quantify sucrose.

Once the wells were filled for each of the three assays, microplates were agitated on a microplate shaker for 20 minutes before reading the absorbance using a Tecan Spark 96-well microplate reader (Tecan Life Sciences®, Mannedorf, Switzerland). Absorbance readings were taken 3 times, at 20, 40, and 60 minutes with the greatest overall absorbance being selected. From the absorbance readings, the approximate amount of glucose, fructose and sucrose could be determined by comparing the absorbance of the microplates before and after adding the sample and calculating the difference to determine the final absorbance. This final absorbance reading was then compared to the glucose standard curve to estimate the level of these sugars within the crown. The glucose standard curve was determined by calculating the slope and y-intercept of the absorbances from the glucose serial dilutions. Final sugar quantification was then determined by using the slope of the curve to calculate the amount of glucose, fructose and sucrose within the strawberry crown in µg/mg of plant material.

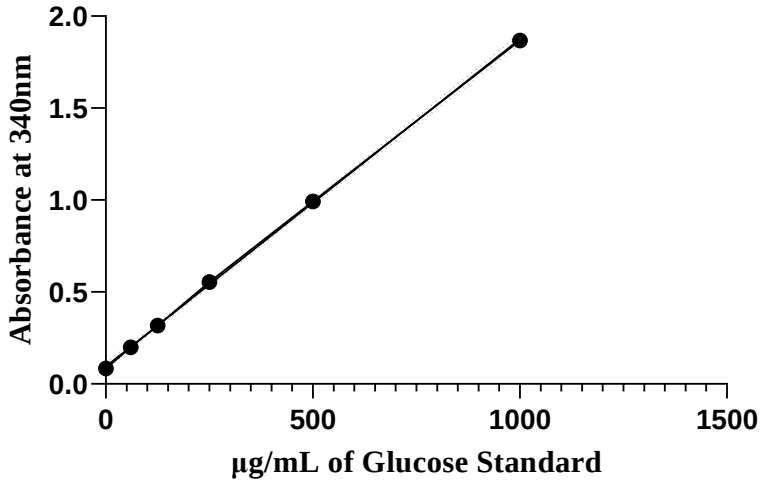

Figure 12. Calculated Glucose standard curve used to determine the relative amount of glucose, fructose, and sucrose present within sampled crown tissue of strawberry transplants. The range of detectable glucose included 1000, 500, 250, 125, 62.5 and 0µg/mL.

Statistical Analysis

All data is expressed by their mean ± standard deviation between each replication. For each data parameter, three replications for each treatment group were collected for phenology, crown dissections and biomass. All data was analyzed using a mixed -model ANOVA to determine the interaction between N source and concentration. A mixed-model was used in order to account for repeat measures of the phenology, crown dissection, biomass and sugar analysis taken during the 6-week experimental period. For all observed values, an alpha value of 0.05 was predetermined to be considered significant. All data for this experiment was analyzed using the SAS statistical package, version 9.4 (SAS Institute Inc., United States).

5.3 Results:

Phenology

In Trial 1, it was observed from the data that there were significant differences observed for both the number of leaves and flower buds produced. Leaves produced among the treatment groups saw significance between the interaction of the N treatment and the sample week and the interaction between N source and concentration, with p-values of <0.0001 for each interaction (Fig. 13). The control treatment produced more leaves than all the other treatments. While the control group allowed for the most significant number of leaves, significance was also seen with the 100 ppm urea treatment producing significantly more leaves than the 150 ppm ammonium treatment. All other treatment groups did not see any significance in weekly leaf production.

Comparing the flower bud production in Trial 1, however, it was observed that there was no significant difference among any of the N treatment groups (Fig. 14). When comparing the interaction between the source and concentration of the N treatments, the p-value was determined to not be significant at a value of 0.073, which marginally exceeds the predetermined alpha value of 0.05.

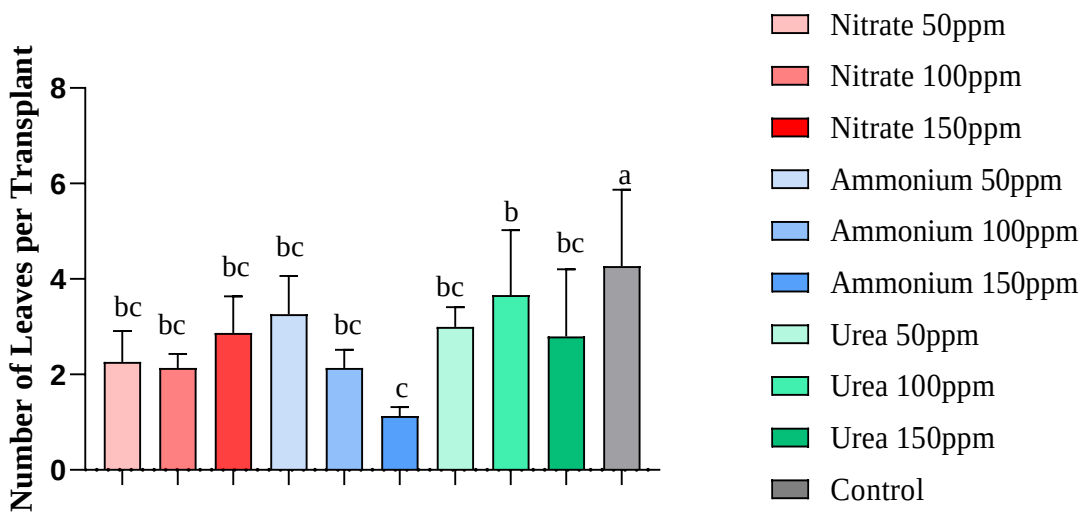

Figure 13. Average number of leaves observed per transplant taken from three transplants from each of the nine N treatments from Trial 1. Leaves were observed once a week for 6 weeks from three transplants from each of the nine nitrogen treatments. Significance between the number of leaves was determined by using a mixed-model ANOVA model.

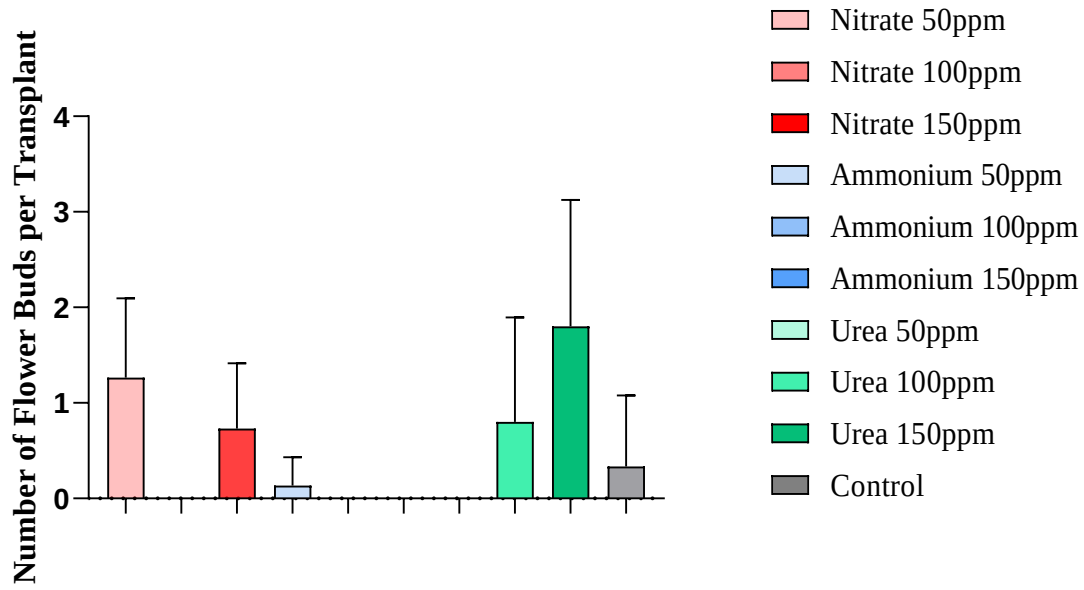

Figure 14. Average number of flower buds observed per transplant taken from three transplants from each of the nine N treatments from Trial 1. Flower buds were observed once a week for 6 weeks from three transplants from each of the nine nitrogen treatments. Significance between the number of flower buds was determined by using a mixed-model ANOVA model.

The phenology data collected from Trial 2 also saw significance for the number of leaves and flower buds produced when comparing the different N:K ratios. First, comparing the number of leaves observed from the treatments, it was determined that the 1:2 ratio of N:K produced significantly more leaves compared to both the 1:1 and 1:4 N:K ratios during the fourth, fifth and sixth week of the trial at a p-value of 0.0154 (Fig. 15). The 1:1 ratio also significantly outperformed the 1:4 ratio with the number of leaves produced for both week 5 and week 6. However, the data collected from weeks 1-3 between the N:K ratios was deemed to have no significance between the treatment groups.

Flower bud production from Trial 2, however, did not see significant differences in the number of flower buds produced between the different N:K ratios (p-value = 0.826) (Fig. 16).

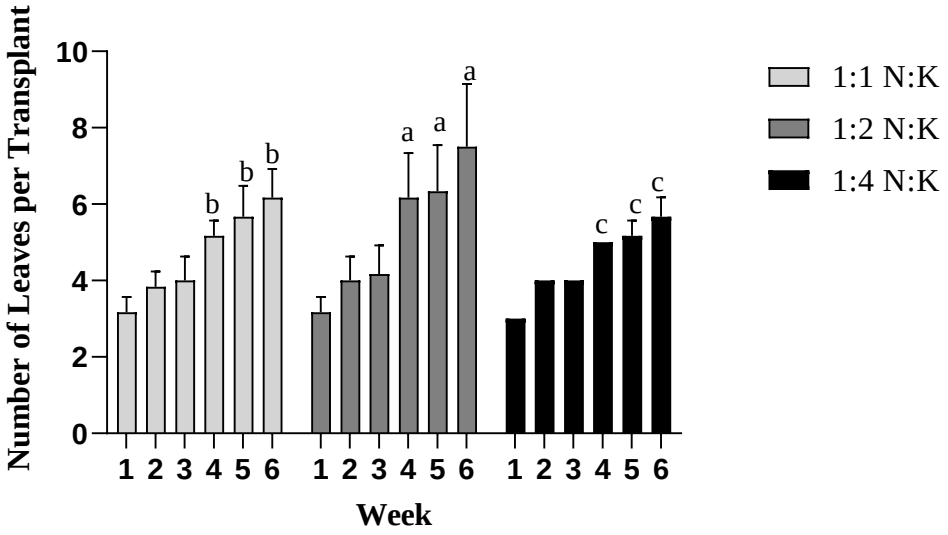

Figure 15. Average number of leaves produced per transplant from each of the N:K ratios each week in Trial 2. Leaves were observed once a week for 6 weeks from 6 transplants from each of the N:K treatments. Significance between the number of leaves was determined by using a mixed-model ANOVA model.

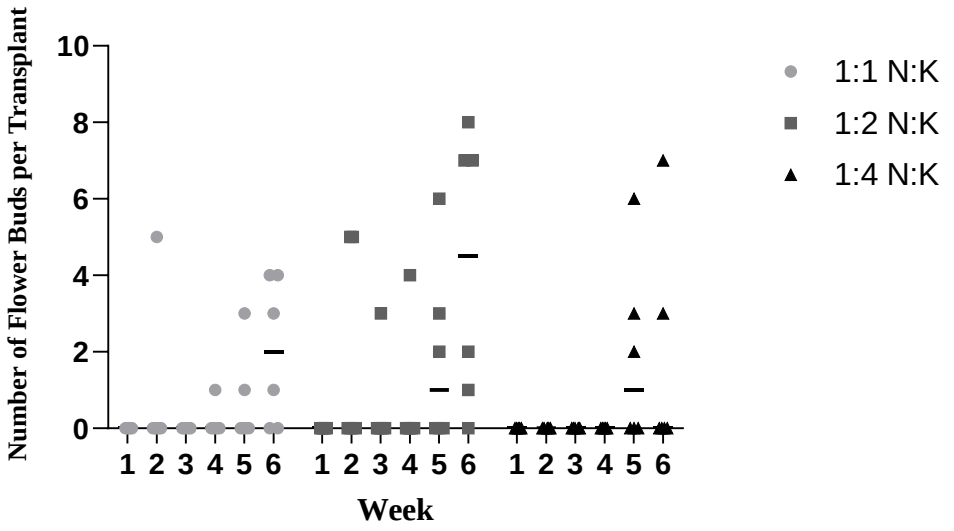

Figure 16. Individual number of flower buds produced from each of the N:K ratios each week in Trial 2. Individual values are shown to better visualize the degree of variation between treatment groups. Flower buds were quantified every week and removed. Average number of flower buds produced per transplant from each of the N:K ratios each week in Trial 2. Flower buds were observed once a week for 6 weeks from 6 transplants from each of the N:K treatments. Significance between the number of flower buds were determined by using a mixed-model ANOVA model

Crown Dissections

The results of the crown dissections taken during Trial 1 saw no significance among any of the treatment groups in either the induction of flower buds within the crown or the number of leaves observed. This was true for both the transplants sampled before dormancy as well as the those sampled once dormancy had been induced in December and January. p-values for the collected data were determined at 0.778 for the number of flower buds and 0.912 for the number of leaves observed when comparing the interaction between N source and concentration before dormancy and 0.433 and 0.592 for the number of flower buds and leaves observed during dormancy, respectively.

Trial 2 also did not see any significance comparing the number of flower buds and leaves observed within the crowns of transplants during the pre-dormancy stage of the experiment. From the transplants sampled during the 6-week pre-dormancy trial, no significance difference in flower

bud induction was noted between N:K ratios, at a p-value of 0.338. There was also no significance in difference leaf production during pre-dormancy among treatment groups, at a p-value of 0.089.

The results of the crown dissections in Trial 2 during dormancy however, found that there were significantly more flower buds induced within the crowns of the 1:2 N:K transplants compared to the 1:1 and 1:4 treatments in the transplants sampled during January and February (Fig. 17). The determined p-value for the data was found to be statistically significant at 0.024. The average number of flower buds per transplant, counted within the crown between treatment groups, was observed at 6.2, 8.6, and 7.1 flower buds for the 1:1, 1:2 and 1:4 N:K treatments in that order. . Furthermore, slight significance was also seen in the number of leaves observed within the crown of the transplants between treatments, with the 1:2 treatment group developing more leaves within the crown than the 1:1 and 1:4 treatments from the transplants sampled in January (Fig. 18). The p-value for leaves was determined at 0.046.

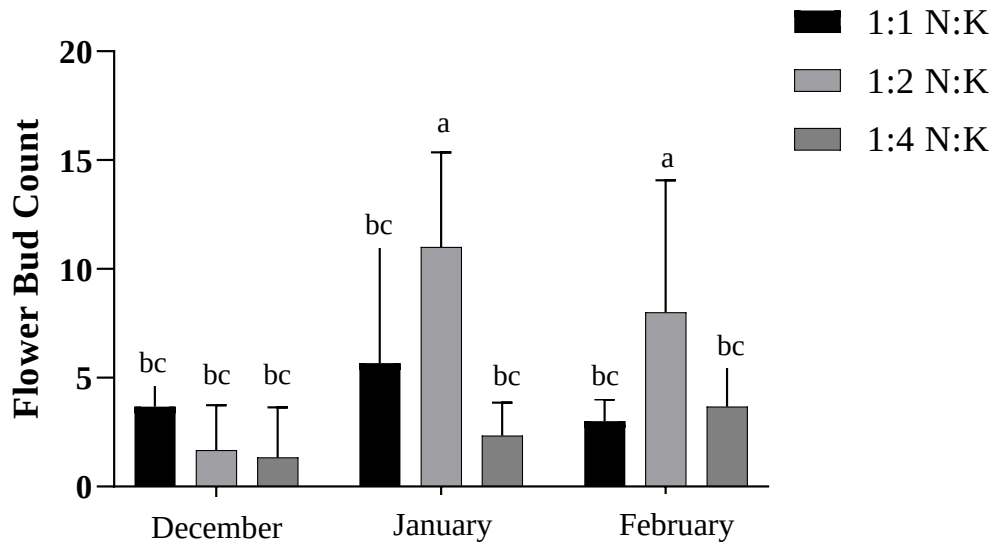

Figure 17. Average number of flower buds per transplant from the three transplant replicates observed within the crowns of dissected transplants from the three N:K treatments in Trial 2. Transplants were collected from cold storage at Ferme Onesime Pouliot once per month from December-February, 2020. Statistical significance was determined using a mixed-model ANOVA.

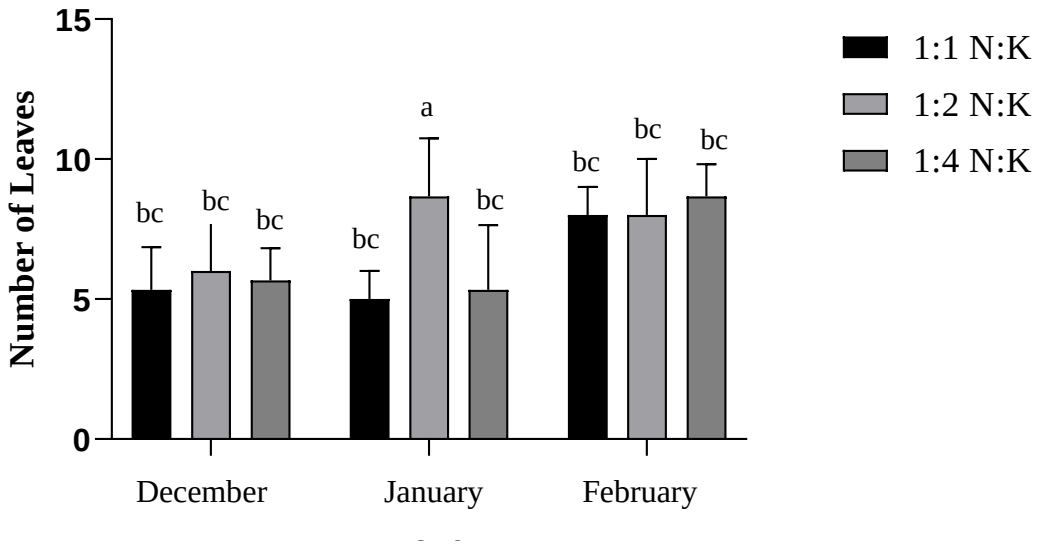

Figure 18. Average number of leaves per transplant from the three transplant replicates observed within the crowns of dissected transplants from the three N:K treatments in Trial 2. Transplants were collected from cold storage at Ferme Onesime Pouliot once per month from December-February, 2020. Statistical significance was determined using a mixed-model ANOVA.

Biomass

The dry biomass results for Trial 1 saw no significance among any of the treatment groups for the total dry weight or individual weights of the separate plant parts. This was the case for transplants sampled before dormancy as well as those sampled during dormancy in December and January. p-values for all transplant parts and the total dry weight were not significant for the interaction of N source and concentration, nor when comparing only the N sources and concentration, for both pre-dormancy and dormancy samples. For pre-dormancy, the recorded p-value for the total dry weight among N treatments was 0.825, whereas it was 0.563 for the samples taken during the dormancy period.

The biomass data for Trial 2 did not show any statistical significance when comparing the total dry weight among treatment groups for both the transplants sampled during pre-dormancy and dormancy, with a p-value of 0.095 and 0.164, respectively. However, significance was noted amongst the individual dry weight of the leaves and flower stalks for the transplants sampled during dormancy. In Figure 20 and 21, the 1:2 ratio of N:K is shown to have significantly greater

leaf (p-value = 0.017) and flower stalk (p-value of 0.020) weights compared to the other treatments.

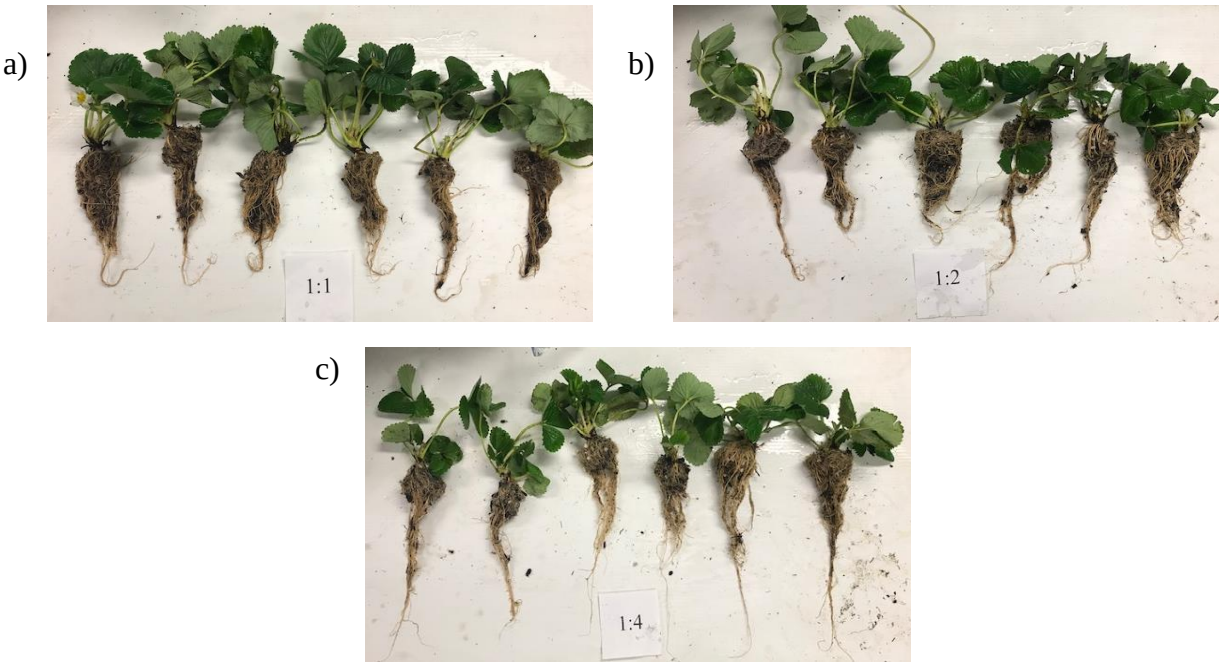

Figure 19. Comparison of transplants from the different N:K treatments. a) 1:1 N:K treatment group, b) 1:2 N:K treatment group, c) 1:4 N:K treatment group. Photo was taken on December 10th, 2020

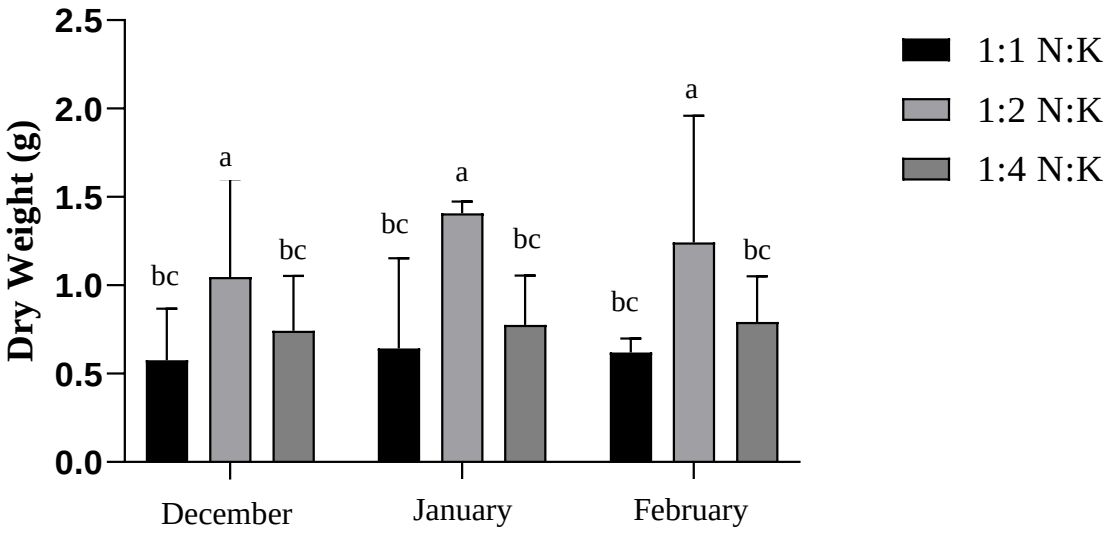

Figure 20. The average dry biomass of leaves per plant from the six transplant replicates from each of the N:K treatments in Trial 2. Transplants were collected from Ferme Onesime Pouliot once per month from December to February 2020. Dry mass was quantified by drying transplants in a 60°C oven for 24 hours. Significance was noted using a mixed-model ANOVA

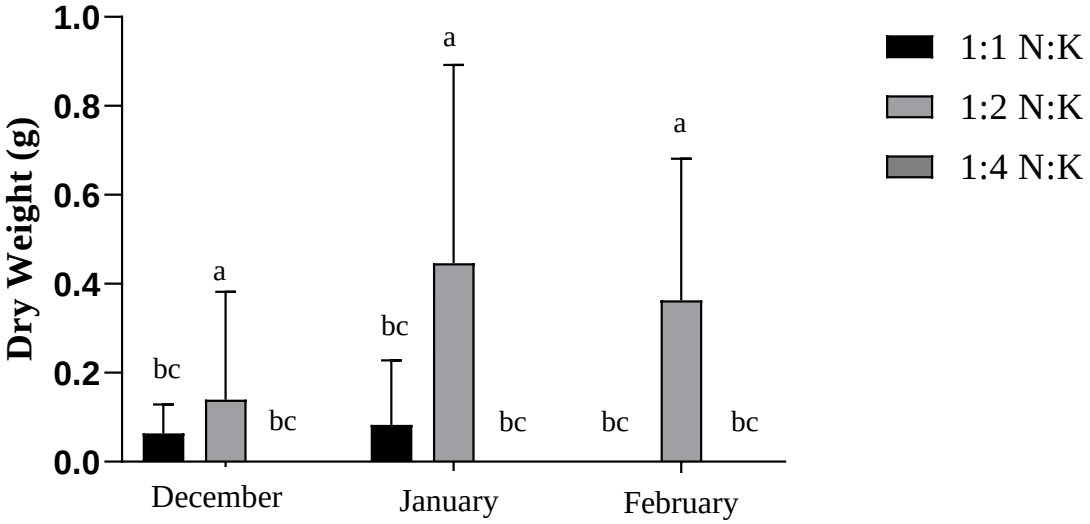

Figure 21. The average dry biomass of flower stalks per plant from the six transplant replicates from each of the N:K treatments in Trial 2. Transplants were collected from Ferme Onesime Pouliot once per month from December to February 2020. Dry mass was quantified by drying transplants in a 60°C oven for 24 hours. Significance was noted using a mixed-model ANOVA

Crown Sugar Analysis

The results of the sugar analysis from the crowns of the transplants in Trial 1 found mixed results when quantifying the amount of sugar observed among the N treatment groups. For the transplant crowns sampled during the pre-dormancy trials, significance was found only in the fructose and sucrose analyses, with fructose seeing a significant difference in both N source and concentration while the sucrose analysis only saw significance when comparing N sources.

For the fructose data in Figure 22, it was observed that the three nitrate treatments and 50 ppm and 100 ppm ammonium treatments had greater production of fructose within the crown compared to the urea treatments, 150 ppm ammonium treatment and control (p-value = <0.0001). The urea treatment at a concentration of 100 ppm also allowed for crowns to produce more fructose compared to crowns subjected to the other urea treatments, 150 ppm ammonium treatment and control (p-value = 0.036). The sucrose analysis of the pre-dormancy trial also showed significance when comparing only the sources of N. From the presented data in Figure 23, strawberry crowns treated with ammonium during pre-dormancy showed a significant increase in production and storage of sucrose within the crown compared to the other N sources (p-value = 0.017). The other N sources displayed no significance when comparing sucrose levels.

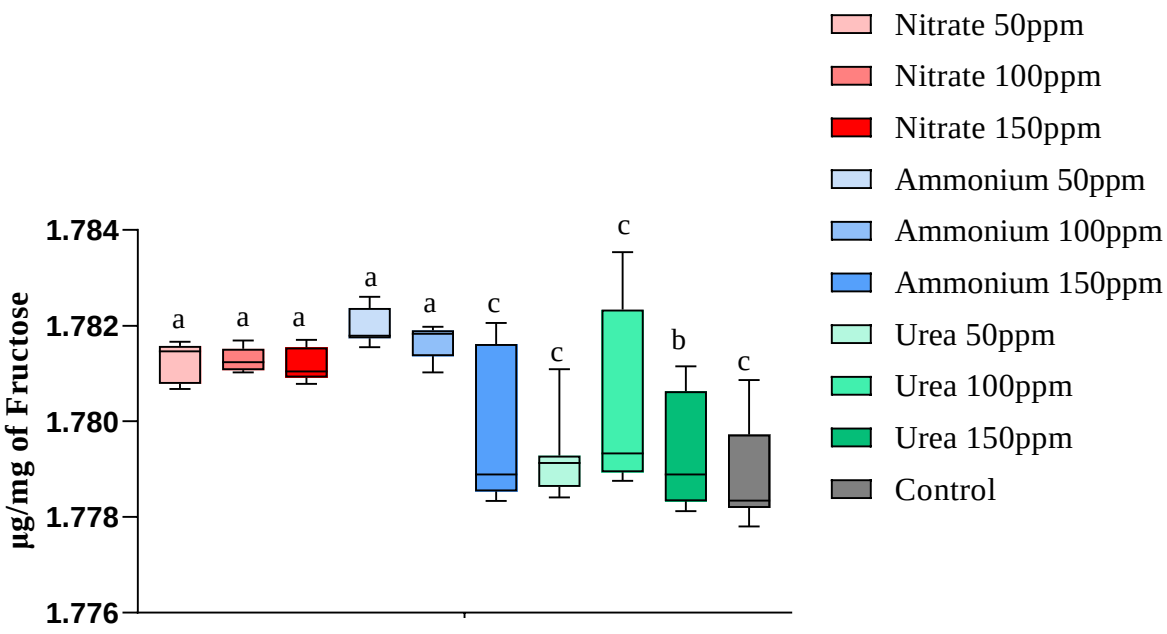

Figure 22. Distribution of fructose values in µg/mg of solution between the interaction of N source and concentration in pre-dormancy transplants. Fructose solutions were sampled from the crowns of three pre-dormancy transplants from each of the nine nitrogen treatments and analyzed at 340nm using a microplate reader. Collected data was compared to a standard curve (Fig. 12) to determine relative sugar concentrations within the crown. These values were analyzed for significance using a mixed-model ANOVA.

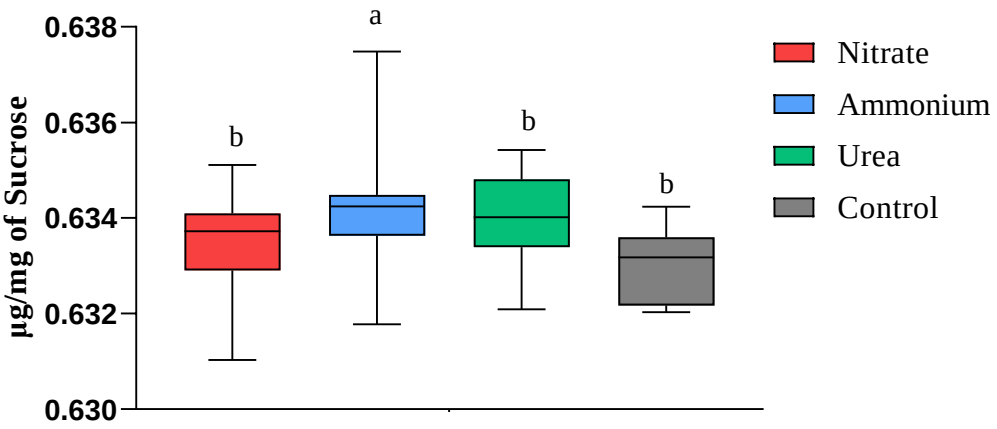

Figure 23. Distribution of sucrose values in µg/mg of solution observed between N sources in pre-dormancy transplants. Sucrose solutions were sampled from the crowns of three pre-dormancy transplants from each of the nine nitrogen treatments and analyzed at 340nm using a microplate reader. Collected data was compared to a standard curve (Fig. 12) to determine relative sugar concentrations within the crown. These values were analyzed for significance using a mixed-model ANOVA.

The results from the sugar analyses taken from the crowns during dormancy differed from the values seen in the pre-dormancy analysis, with shifts in significance of sugar production and storage among the different N treatment groups. For the dormancy transplants, the interaction of N source and concentration was significant for glucose and sucrose. Significance was also observed for the amount of fructose produced; however, this was only among the N sources.

Quantification of the glucose stored within the crown (Fig. 13), showed that ammonium at a concentration of 100 ppm and 150 ppm, as well as all urea treatments and the control treatment produced and stored the most glucose during the dormancy period when compared to the nitrate and ammonium 50 ppm treatments (p-value = 0.001). The 150 ppm nitrate and 50 ppm ammonium treatment also produced significantly more glucose within the crown compared to the 50 ppm and 100 ppm nitrate treatments. The fructose analysis (Fig. 14), on the other hand, saw significant production of fructose only within the transplants treated with nitrate, (p-value = 0.031). Sucrose levels (Fig. 15) also saw differences compared to the results of the pre-dormancy transplants where nitrate at 50 ppm stored more sucrose within the crown compared to all other treatments (p-value = 0.001).

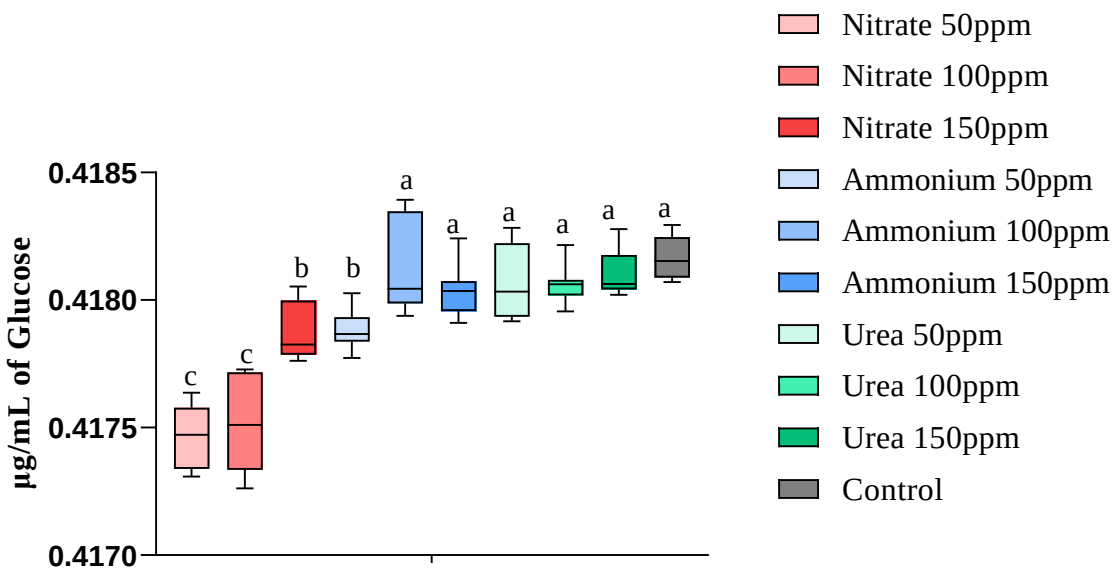

Figure 24. Distribution of glucose values in µg/mg of solution observed between the interaction of N source and concentration in dormancy transplants. Glucose solutions were sampled from the crowns of three pre-dormancy transplants from each of the nine nitrogen treatments and analyzed at 340nm using a microplate reader. Collected data was compared to a standard curve (Fig. 12) to determine relative sugar concentrations within the crown. These values were analyzed for significance using a mixed-model ANOVA.

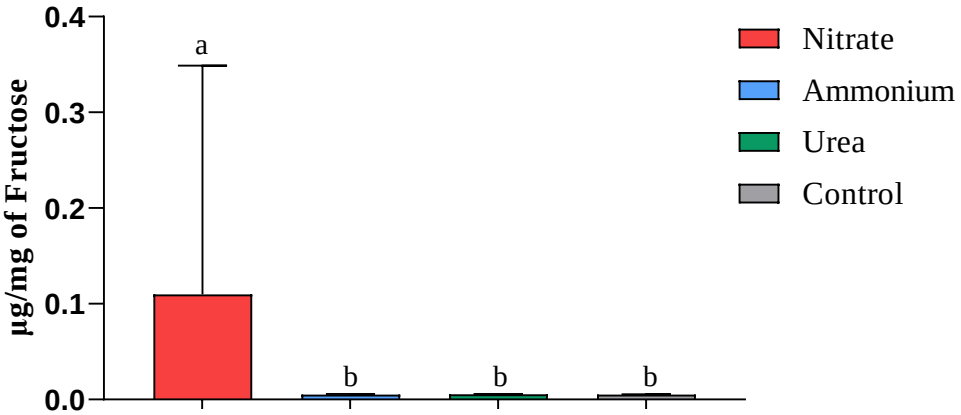

Figure 25. Distribution of fructose values in µg/mg of solution observed between N sources in dormancy transplants. Fructose solutions were sampled from the crowns of three pre-dormancy transplants from each of the nine nitrogen treatments and analyzed at 340nm using a microplate reader. Collected data was compared to a standard curve (Fig. 12) to determine relative sugar concentrations within the crown. These values were analyzed for significance using a mixed-model ANOVA.

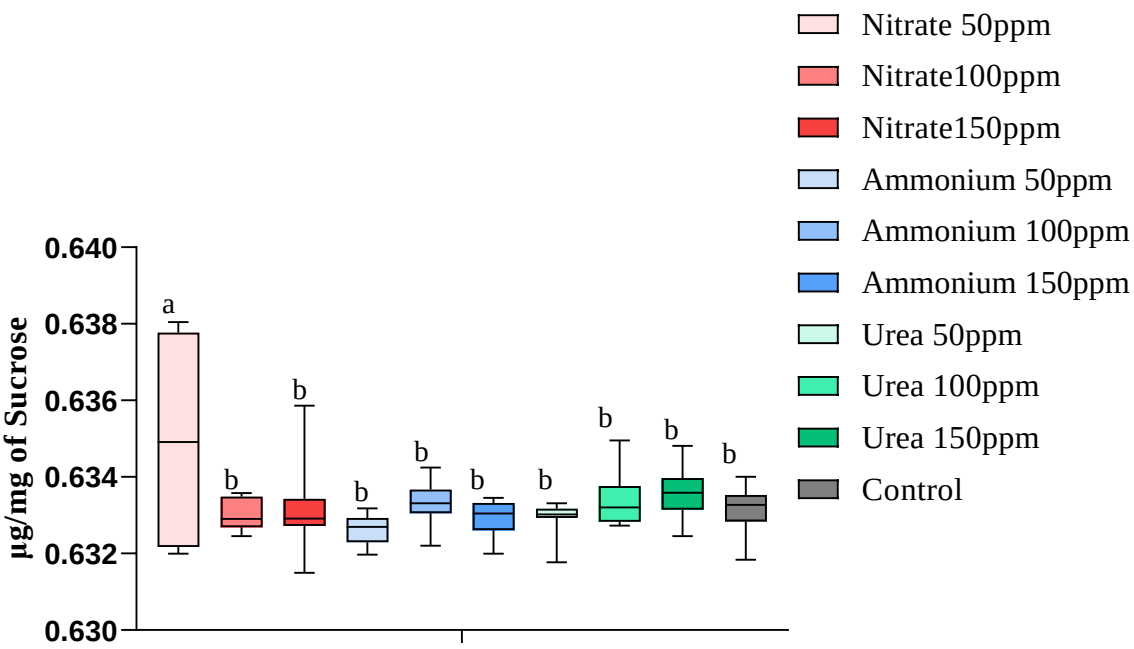

Figure 26. Distribution of sucrose values in µg/mg solution observed between the interaction of N source and concentration in dormancy transplants. Sucrose solutions were sampled from the crowns of three pre-dormancy transplants from each of the nine nitrogen treatments and analyzed at 340nm using a microplate reader. Collected data was compared to a standard curve (Fig. 12) to determine relative sugar concentrations within the crown. These values were analyzed for significance using a mixed-model ANOVA.

5.4 Discussion:

From the conclusion of the two experimental trials, it was observed that the N sources and concentrations that were tested in Trial 1 did not have a significant effect on the phenology, induction of flower buds within the crown, or the overall biomass of DN strawberry transplants. These results suggest that the N source and concentrations tested in this experiment do not significantly affect the growth of transplants at the pre-dormancy stage, suggesting that favoring one N source at this stage will likely not produce any observable differences in plant growth or flower bud induction. It has been observed in previous trials using short-day strawberry that N concentration had no significant effects on transplant phenology and biomass at the transplant stage other than decreasing the crown diameter in transplants at higher N concentrations (Oliviera *et al.,* 2010). Other studies also saw that N applied at the transplant stage, regardless of the source, is often stored within the roots of transplants, resulting in less observable differences in transplant growth (Gagnon *et al.* 1990). This was often more observable in DN varieties than SD varieties as well, with nitrogen primarily sourced for root storage and flower bud differentiation at the transplant stage (Archbold and MacKown, 1995). While phenology and biomass seem to be unaffected at the transplant stage, flower bud production within the crown was more likely to show significant differences among the N treatments. Studies testing N sources at the transplant stage saw that transplants often favored nitrate and urea sources as opposed to ammonium, resulting in greater flower bud differentiation (Wan *et al.*,2017; Neumann and Kafkafi, 1985). Greater N concentrations also allowed for more significant root sequestration and storage of nutrients to allow for more significant flower development (Archbold and MacKown, 1995). In the case of this experiment, since no significance was observed in the number of flower buds produced among the nitrogen treatments, other factors that play a role in the production of flower buds may have affected the results. For instance, temperature of the transplants pre-dormancy, as well as light quality were not factors that were thoroughly investigated in this experiment. Previous research has shown that these factors have significant implications on the induction of flower buds in DN strawberry transplants, with light at lower wavelengths (470-530nm) and a short-day photoperiod (9 hours) producing greater numbers of flower buds than higher wavelengths (640nm) at long-day photoperiods (12+ hours) (Magar *et al.,* 2010; Durner and Poling, 1987). Temperatures leading up to dormancy can also significantly effect flower bud induction, with greater periods of colder temperatures (below 4°C) significantly affecting the production of new flower buds (Durner and

Poling, 1987). Thus, another route that can be explored from this study would be comparing the correlation between N source and concentration alongside light quality and temperature of transplants.

While no significance was observed between the nitrogen treatments of Trial 1 in the phenology, crown dissection, and biomass experiments, the role of nitrogen may still have a significant role in determining flower bud induction for DN strawberries at the pre-dormancy stage. Within this trial, nitrogen treatments were kept at their constant concentrations of either 50 ppm, 100 ppm or 150 ppm for the entire 6-week period to ensure consistency. However, from Trial 2, nitrogen was increased in the week before transplants entered dormancy to boost flower bud induction. While this was omitted from Trial 1, by "boosting" nitrogen right before plants enter the dormant phase, there could likely be more of an effect on flower bud induction within the crown. Soil temperature could have also influenced the uptake of N within Trial 1. Since the trial was conducted within an outdoor greenhouse with open sides, the greenhouse temperature tended to drop by 2-8°C at night. Cooler soil temperatures (<15°C) have been observed to negatively affect N uptake in strawberry plants, particularly at the transplant stage, which could have resulted in some of the discrepancies in the collected data in Trial 1 (Neumann and Kafkafi, 1985). Another factor that moderately affected the data obtained from Trial 1 was that some of the transplants kept in cold storage after February during dormancy were contaminated with mold, presumably from a failure in the cold room. This resulted in temperatures exceeding the intended 1ºC needed to induce dormancy, allowing mold to infiltrate the crowns of the transplants. While samples of dormant Trial 1 transplants were taken for December and January, plants meant to be taken in the following two months needed to be discarded due to the contamination. This limited the amount of data that could be taken observing the effects of the nitrogen treatment on flower bud induction during dormancy as well as not allowing for transplants to be planted in the spring to account for survivability. Whether or not the effects of the nitrogen treatments would have influenced the transplants as they reached the end of the dormant period is uncertain; however, by testing these treatments again, it can be determined if the source and concentration of nitrogen effects flower bud induction near the end of dormancy and if any one treatment allowed for greater survivability of plants.

While Trial 1 showed no significant differences in flower bud induction, there were significant differences in the storage and production of different carbohydrates. The reasoning

behind testing this parameter, was the observation that a greater sugar concentration within strawberry plants leading up to and during dormancy allowed for greater survivability through winter and a greater ability to break dormancy earlier in the spring to begin producing new growth earlier. Furthermore, an optimal balance of sugar within the crown can contribute to greater flower bud induction, as these sugars, particularly sucrose, are observed to be essential in the quantity of flower buds produced (Eshghi and Tafazoli, 2006). Since nitrogen has a key role in the production of these sugars within the plant, it was inferred that with an ideal nitrogen fertilizer treatment, crown sugar levels in the transplants can likewise be optimized for improved yield. From the trial observations, transplants sampled during the pre-dormancy aspect were observed to produce more significant amounts of fructose, at an average of 1.781μg/mg of fructose in solution, within the nitrate and ammonium treatments at all concentrations compared to the urea and control treatment groups, at an average of 1.779μg/mg. A greater sucrose content was also observed among all ammonium treatment groups at this stage with an average 0.001μg/mg higher than all other treatments. These values, however, were reflected differently during the dormancy stage of the experiment, where there was more significant storage of glucose in the urea, control and 150 ppm ammonium treatment than the nitrate and 50 ppm and 100 ppm ammonium treatments. Greater storage of fructose was also observed in all nitrate treatments, and more significant sucrose storage in the 50 ppm nitrate treatment. Within the crown of strawberry plants, glucose is most responsible for growth and development, whereas the role of fructose and sucrose is to signal plant hormones involved in regulating flowering and fruit development (Rolland et al., 2002). Greater quantities of these sugars during dormancy have been shown to allow for earlier development in the spring after breaking dormancy as well as more efficient hormone signaling within transplants (Macias-Rodriguez *et al.*, 2002; Rolland et al., 2002). From the observed N treatments, no one treatment group has been responsible for significantly increasing all three sugars simultaneously during pre-dormancy and dormancy. However, the data observed gives some interesting insight into N source and concentration in sugar composition within the crown. With the knowledge of the obtained data, ammonium sourced treatments show a greater sucrose production during the pre-dormancy experiment. These values are different during dormancy with the 50 ppm nitrate treatment producing more sucrose as well as all nitrate treatments producing more fructose, which suggests that nitrate is important for greater production and storage of these nutrients compared to plants solely given ammonium or urea treatments. Studies examining *Arabidopsis* have suggested that

sucrose and fructose play a crucial role in signaling abscisic acid production needed for plants to undergo dormancy (Jia *et al.* 2013; Rolland *et al.*, 2002). Thus, it could be argued that by optimizing the amount of these sugars within transplants, there could be potential to have better control over the length of dormancy in transplants and could ensure that transplants are equipped with enough nutrients to last throughout the dormant period. While the results of this experiment do not favor any one N treatment group for increasing sugars within the crown of DN transplants, more research into the specific roles of N source and concentration in sugar production leading up to dormancy could be used to fine-tune how DN strawberries survive through winter and the time needed to begin new growth in spring.

In the case of the transplants from Trial 2, significance was noted in the number of flower buds observed during pre-dormancy, with the 1:2 ratio of N:K outperforming the 1:1 and 1:4 ratios in the number of leaves produced during weeks 4, 5 and 6. This trend was also observed in the crown dissection and biomass data taken from Trial 2, where the 1:2 N:K ratio resulted in a greater number of flower buds induced within the crown of the transplant during dormancy as well as an overall greater biomass of leaves and flower stalks compared to the other ratios. Therefore, it can be argued from the data in Trial 2 that a 1:2 ratio of N:K is more favorable for increasing flower bud induction for pre-dormancy DN strawberry and increasing the overall growth of transplants at this stage. These findings coincide with previous studies in SD variety strawberries which have found that increased K in relation to N allows for a significant increase in yield and flower bud production when potassium is greater than the level of nitrogen (Haynes *et al.*, 1987) Thus, increasing the ratio of N:K to 1:2 is arguably a better formula for a DN transplant fertilizer model compared to a 1:1 or 1:4 N:K ratio. To further accept the results observed in Trial 2, it is recommended that the trial be repeated using multiple varieties of DN transplant to confirm if the results are present throughout multiple DN varieties.

5.5 Conclusion:

Day-neutral (DN) strawberry cultivars are favorable varieties of strawberry within Quebec as they offer a longer harvest and greater overall yields. However, these varieties tend to suffer significant transplant loss over winter and delayed new growth in the spring. These setbacks in yield can be reduced by optimizing nitrogen fertilizer and determining the optimal balance between nitrogen and potassium concentrations. The two trials presented in this experiment aimed to

determine a suitable fertilizer model for Quebec farmers to reduce yield accredited losses to transplant storage in winter. From the experiment results, it was discovered that the N treatments in Trial 1 did not result in any significant changes in flower bud induction within the crown nor any significant changes to the weekly phenology of the transplant. Transplant biomass was also unaffected by the N treatments. These results suggests that other factors could effect flower bud induction aside from N source and concentration, such as a the temperature or light quality provided. Within the crown, the N treatments significantly impacted the production and storage of simple sugars needed for breaking dormancy, which could be further explored to create a fertilizer model targeting specific sugar production. Trial 2 also discovered that a 1:2 ratio of N:K allows for greater production of leaves during pre-dormancy, greater overall plant biomass, and greater flower bud induction within the crown during dormancy compared to a 1:1 and 1:4 N:K ratio. This discovery can be incorporated into Quebec pre-dormancy fertilizer models to boost flower bud induction for greater yields in spring.

6. Final Discussion:

Strawberry is an important economic crop within Quebec, making up nearly half of all strawberry production within Canada, however, exports of the crop are still lagging far behind the import demand from outside the country. The introduction of day-neutral strawberry in 1990 has helped expand the potential of Quebec strawberry production significantly by increasing the harvest season by 14 weeks (Petran *et al.*, 2016). Even still, farmers have had poor results implementing day-neutral varieties in Canada and other northeastern climates, as these varieties are more susceptible to temperature fluctuations in both the summer months and are less hardy survivors of cold storage in winter (Weebadde *et al.*,2008). As such, yields have failed to reach the potentials they have displayed in controlled greenhouse research (Gagnon *et al.*, 1990). Crossbreeding of day-neutral and wild-type strawberries to create cultivars more accustomed to northeastern climates is a viable option for addressing these yield discrepancies. However, these efforts can come with trade-offs in terms of other day-neutral characteristics such as the response to photoperiod as well as disease resistance. Thus, optimizing fertilization of these varieties within Quebec is a proposed alternative way to boost yield and get the best performance out of day-neutral strawberry plants while implementing well-researched cultivars.

Field research into proper fertilization of day-neutral strawberry outside of controlled studies is severely lacking and, as such, could be contributing to the yield shortages seen in day-neutral strawberry, particularly in the transplant stages both at the beginning and end of the harvest season when the strawberry plant is arguably the most susceptible to fertilization practices. Thus, the two experiments presented within this paper attempt to address some of the concerns around day-neutral strawberry yield in Quebec by testing how to optimize nitrogen at the nursery stage until production and then from transplanted runner tips until winter dormancy. It was expected that the practices that best stimulate flower bud induction, growth of inflorescences, increase the photosynthetic rate and increase sugar content within crowns over winter would be determined and able to be implemented into Quebec day-neutral strawberry fertilizer plans. The results of these experiments found that within the production trial of the first experiment that urea had the best luck at improving flower bud growth at a rate of 150mg/L per plant compared to ammonium at the same concentration, which coincides with past research determining urea to be an excellent nitrogen source for increasing flowering compared to ammonium which promotes vegetative growth. Furthermore, urea was seen to improve the transplants' overall photosynthetic rate compared to both nitrate and urea, allowing for more efficient uptake and expenditure of nutrients. From this experiment, it can be arguably stated that farmers who implement more urea into their fertilizer plants will likely see some improvement in their transplants' flowering, which could affect annual yields.

The second experiment followed up with the same treatments of the first while also considering if potassium also contributes to flower bud induction and yield leading up to dormancy in plug transplants. The nitrogen trial only saw any significance from the two trials when analyzing sugar content within the crown. The data obtained was slightly affected by lack of results caused by malfunctions in winter storage. However, the sugar data that was collected did give intriguing insights into how nitrogen can acutely affect the levels of certain sugars within the crown. Thus, this data could be further expanded upon to create precise fertilizer practices that exploit the signaling qualities of each sugar to break dormancy earlier and begin flower development earlier in the season. The second trial results within the experiment also suggested that a 1:2 ratio of N:K is more desirable for improving flower bud induction over dormancy and increasing the overall biomass of the transplants. This data highlights the critical role that potassium also plays in flower

bud induction within day-neutral strawberries and thus could be increased within fertilizer models to increase yields.

While the results of these experiments introduce several practices that can increase yield within day-neutral strawberries in Quebec, a continuation of these studies will likely allow for these ideas to be implemented within Quebec fertilizer plans. For example, both experiments should be repeated to truly test the efficacy of each of the nitrogen treatments as well as testing multiple cultivars of day-neutral strawberry to determine if the results are universal among all day-neutral varieties or are unique to cv. Albion. Another area of study that could be observed is the activity within the soilless coco coir substrate used to determine how rhizospheric communities respond to the nitrogen and potassium treatments. Research into soilless strawberry transplant production has found that these communities often significantly respond nutrient application within soilless substrates, since these substrates are naturally inert, which could ultimately affect how efficiently these nutrients are used (Martinez *et al.,* 2013; Othman *et al*, 2019). The potential of controlling sugar storage and use within the crown also offers potential for having precise control over breaking dormancy, inducing flower bud development, and improving metabolic efficiency in transplants through signaling of certain plant hormones and metabolic pathways (Jia *et al.* 2013). Therefore, a closer look into several fertilizer concentrations within the nitrogen sources used in the experiments could prove significant in optimizing fertilizer within DN strawberry transplants.

Overall, these experiments have introduced interesting fertilizer optimization concepts within day-neutral strawberries, which can be expanded upon to increase yield for Quebec strawberry farmers. A repeat study using multiple day-neutral cultivars will further confirm or reject the results of this study which then can be implemented into fertilizer plans for transplants. With the knowledge obtained from this project, it is hoped that day-neutral strawberries will overcome yield shortages within northeastern climates, and strides can be made towards improving the import/export gap of strawberries within Canada.

7. Conclusion:

Strawberry has been an increasingly valuable crop worldwide, where high demand has been affecting the need for significant increases in production and yield within Quebec. Day-neutral strawberry varieties offer the potential to increase annual yield by extending the harvest season significantly. However, these varieties of strawberries yield below their potential within northeastern climates throughout production and suffering losses over winter. This study aimed to determine ideal nitrogen and potassium fertilization during the transplant stages before production and before dormancy in day-neutral strawberries to mitigate these yield losses. It was discovered that the source and concentration of nitrogen significantly impacted the number of flower buds induced during the production stage of transplants, with a high concentration of urea (150mg/L) producing significantly more flower buds than 150mg/L of ammonium. Photosynthesis readings also saw a significantly greater rate of CO2 gas exchange in urea treatments suggesting a more efficient use of nitrogen within the transplant. No significance was observed in the growth or induction of flower buds from the nitrogen treatments within the dormancy trials, however, a 1:2 ratio of N:K did allow for greater flower bud induction and growth of the transplant leading up to dormancy. Analysis of sugars produced within the crowns of the transplants also revealed that the source and concentration of nitrogen could significantly affect the concentration of sugar produced by the plant. This data is useful for increasing certain sugars for earlier breaking of dormancy or increased flower bud induction. Overall, the outcome of these experiments allows for a greater insight into how optimizing fertilizer can positively affect yield and growth of day-neutral transplants for Quebec fertilizer models.

8. Appendix

1. Optimal ppm of Nutrients per Strawberry Transplant

Macronutrients	**Optimal ppm**
Potassium (K)	200 ppm
Calcium (Ca)	140 ppm
Sulfur (S)	56ppm
Phosphorus (P)	50 ppm
Magnesium (Mg)	48ppm
Iron (Fe)	2.8ppm
Micronutrients	**Optimal ppm**
Manganese (Mn)	0.4ppm
Boron (B)	0.3ppm
Zinc (Zn)	0.2ppm
Copper (Cu)	0.1ppm
Molybdenum (Mo)	0.05ppm

Source: Cantliffe *et al.*, (2007), Paranjpe *et al.*, (2003)

Optimal ppm values for strawberry transplants used in the first manuscript were founded on the values obtained by Cantliffe *et al.*, (2007) and Paranjpe *et al.*, (2003). These values represent the optimal ppm of nutrients delivered per transplant in strawberry cultivars. These values were used as a reference for determining the concentration of macronutrients and micronutrients used in fertilizing the transplants grown in the nitrogen experiments.

2. Fertilizer Calculations from Experiment 1

2.1 Calculations for nutrients applied by hand watering each week in grams per liter

Fertilizer	Desired ppm	Percent N	Calculation	N needed g/L
Nitrate (KNO_3)	50 ppm	13.5% N	50mg/L / .135% N	**3.84 g/L**
	100 ppm		100mg/L / .135% N	**7.69 g/L**
	150 ppm		150mg/L / .135% N	**11.54 g/L**
Ammonium (NH_4SO_4)	50 ppm	21% N	50mg/ L / .21% N	**2.38g/L**
	100 ppm		100mg/ L / .21% N	**4.76 g/L**
	150 ppm		150mg/ L / .21% N	**7.14 g/L**
Urea	50 ppm	46% N	50mg/ L / .46% N	**1.09 g/L**
	100 ppm		100mg/ L / .46% N	**2.17 g/L**
	150 ppm		150mg/ L / .46% N	**3.26 g/L**

Fertilizer	Nitrate ppm	K ppm to match	Percent K	Calculation	K needed mg/L
K_2SO_4	50 ppm	149ppm	48%	149mg/L / .48% K	**3.10 g/L**
	100 ppm	292ppm		292mg/L / .48% K	**6.08 g/L**
	150 ppm	446ppm		446mg/L / .48% K	**9.29 g/L**

Fertilizer	Desired ppm	Percent Ca	Calculation	Ca needed mg/L
$CaCO_3$	140 ppm	40% Ca	140 mg/L / .40 % Ca	**3.50 g/L**

2.2 Calculations used for drip irrigation stock solutions at a 1:80 dilution rate

Solution	**Fertilizer(s)**	**ppm Needed**	**Percent Nutrient**	**Calculation** **ppm x 80 / % nutrient x 10**	**Amount needed g/L**
A	Fe(EDTA)	2.8ppm	Fe 10%	2.8 mg/L x 80 / 10% x 10	**2.24 g/L**
B	MPK	50 ppm	P 34%	50 mg/L x 80 / 34% x 10	**11.7 g/L**
	$MgSO_4$	48ppm	Mg 20%	48 mg/L x 80 / 20% x 10	**19.2 g/L**
C	$Mn(SO_4)_2$	0.4ppm	Mn 36%	0.4 mg/L x 80 / 36% x 10	**0.09 g/L**
	$ZnSO_4$	0.2ppm	Zn 36%	0.2 mg/L x 80 / 36% x 10	**0.04 g/L**
	$CuSO_4$	0.1ppm	Cu 25%	0.1 mg/L x 80/ 25% x 10	**0.03 g/L**
	B	0.3ppm	B 17.5%	0.3 mg/L x 80 /17.5% x 10	**0.14 g/L**
	Mo	0.05ppm	Mo 60%	0.05 mg/L x 80/ 60% x 10	**0.007 g/L**

The above calculations were used in Experiment 1 to determine the amount of fertilizer needed to be added in grams per 1L of solution to fulfill the nutrient ppm requirement in Appendix 1. For the nutrients applied by hand-watering, 1L stock solutions were made on Mondays, Wednesdays and Fridays and applied to individual transplants at 50mL of nitrogen treatment followed by 50mL of calcium solution.

The stock solutions in Appendix 2.2 were made once a week by adding fertilizer to 3L of water. Nutrients were separated into three stock solutions to avoid precipitation. Each of the stock solutions were then taken up by separate fertilizer injectors and diluted with water in a 1:80mL dilution before being delivered to the transplants within the experiment.

3. Fertilizer Calculations and Procedure from Experiment 2

3.1 Calculations for nitrogen and potassium applied by hand-watering each week in grams per liter

Fertilizer	**Desired ppm**	**Percent N**	**Calculation**	**N needed g/L**
Nitrate (KNO_3)	50 ppm	13.5% N	50 mg/L / .135% N	**3.84 g/L**
	100 ppm		100 mg/L / .135% N	**7.69 g/L**
	150 ppm		150 mg/L / .135% N	**11.53 g/L**
Ammonium (NH_4SO_4)	50 ppm	21% N	50 mg/ L / .21% N	**2.38 g/L**
	100 ppm		100 mg/ L / .21% N	**4.76 g/L**
	150 ppm		150 mg/ L / .21% N	**7.14 g/L**
Urea	50 ppm	46% N	50 mg/ L / .46% N	**1.08 g/L**
	100 ppm		100 mg/ L / .46% N	**2.17 g/L**
	150 ppm		150 mg/ L / .46% N	**3.26 g/L**

Fertilizer	**Nitrate ppm**	**K ppm to match**	**Percent K**	**Calculation**	**K needed g/L**
K_2SO_4	50 ppm	149 ppm	48%	149 ppm / .48% K	**3.10 g/L**
	100 ppm	292 ppm		292 ppm / .48% K	**6.08 g/L**
	150 ppm	446 ppm		446 ppm / .48% K	**9.29 g/L**

3.2 Calculations for macronutrients and micronutrients applied by hand-watering

Solution	Fertilizer(s)	ppm Needed	Percent Nutrient	Calculation	Amount needed g/L
A	Fe(EDTA)	2.8 ppm	Fe 10%	2.8 mg/L x 80 / 10% x 10	**2.24 g/L**
B	MPK	50 ppm	P 34%	50 mg/L x 80 / 34% x 10	**11.7 g/L**
	$MgSO_4$	48 ppm	Mg 20%	48 mg/L x 80 / 20% x 10	**19.2 g/L**
C	$Mn(SO_4)_2$	0.4 ppm	Mn 36%	0.4 mg/L x 80 / 36% x 10	**0.009 g/L**
	$ZnSO_4$	0.2 ppm	Zn 36%	0.2 mg/L x 80 / 36% x 10	**0.004 g/L**
	$CuSO_4$	0.1 ppm	Cu 25%	0.1 mg/L x 80/ 25% x 10	**0.003 g/L**
	B	0.3 ppm	B 17.5%	0.3 mg/L x 80 /17.5% x 10	**0.014 g/L**
	Mo	0.05 ppm	Mo 60%	0.05mg/L 80/ 60% x 10	**0.0007g/L**

The above calculations were used in Experiment 2 to determine the amount of fertilizer needed to be added in grams per 1L of solution to fulfill the nutrient ppm. For the nutrients applied by hand-watering, 1L stock solutions were made on Mondays, Wednesdays and Fridays and applied to individual transplants at 50mL of nitrogen treatment followed by 50mL of calcium solution.

The stock solutions in Appendix 3.2 were made once a week using the macronutrient and micronutrient guideline used adding a week's value of fertilizers from solutions A, B and, C to separate 1L containers and dissolving in 1L of water to make concentrate solutions. Half of each of the concentrate solutions were then mixed, along with 7.8L of water, immediately before watering transplants to avoid precipitation. Transplants were then each given 30mL of the nutrient solution by hand twice a week.

4. Pouliot Farm Fertilizer Model for use in Experiment 2 and Control

For Trial 2 of the second experiment, transplants were supplied fertilizer following the weekly schedule used by Ferme Onesime Pouliot Inc. for day-neutral strawberry transplants leading up to dormancy. This model was used for Trial 2 of Experiment 2 to determine the N:K ratio that allowed for the most favorable yield and flower bud induction before dormancy.

For the experiment, all macronutrients and micronutrients were supplied using the Ferme Onesime Pouliot schedule at a 1:1 ratio of N:K. Adjustments were then made only to the nitrogen and potassium fertilizers to provide the 1:2 and 1:4 ratios of N:K shown in Appendix 4.1, 4.2 and, 4.3. Fertilizers were separated into three stock concentrates labeled A, B, and C to avoid precipitation but were combined immediately before irrigating transplants. Fertilizer solutions were supplied to transplants in the form of an overhead sprinkler system which would output 8 liters of fertilizer solution per minute for a total of 5.5 minutes each day.

Over the six-week experimental period, this fertilizer model was adjusted based on fertilizer recommendations for pre-dormancy transplants to boost growth. The fertilizer model changed every two weeks based on Ferme Onesime Pouliot guidelines. The first model, depicted in Appendix 4.1, was used for the first two weeks of the experiment with a nitrogen target of 120mg/L of N per transplant. The model was then adjusted during weeks 3 and 4 (Appendix 4.2) to reduce nutrient levels by half, with a target of 60mg/L of N per transplant. The model was adjusted one last time for the final two weeks by boosting nitrogen levels again for a target of 180mg/L of N per transplant, shown in Appendix 4.3. For all three models, the K level adjusted accordingly in order to reflect a 1:1, 1:2, or 1:4 ratio of nutrients.

Aside from the fertilizer model being used for Trial 2 of Experiment 2, it was also used in Trial 1 of experiment 2 as the control treatment group for the different N treatments. As a control group, nutrients were supplied following the model guidelines; however, only the 1:1 ratio of N:K was used as the control. Nutrient values for the control group in Trial 1 also followed the biweekly adjustments to stay consistent with Ferme Onesime Pouliot's practices.

Weeks 1&2

	200 L		1:1
A	Calcium Nitrate	1.90	kg
	Ammonium Nitrate 34%	0.55	kg
	Magnesium Nitrate	1.00	kg
	Iron Chelate (EDTA) 13%	59.6	g
B	Mono potassium phosphate	0.50	kg
	Potassium Sulphate	1.04	kg
	Magnesium Sulphate	0.00	kg
	Potassium Nitrate	0.00	kg
Oligo	Manganese Chelate 13%	12	g
	Zinc 15%	5.30	g
	Borax 15%	4.10	g
	Copper Sulphate 25.2%	0.50	g
	Molybdate 46%	0.300	g

	Concentrations of Elements in Solution							Quantity in kg						
	% N	% Ca	% P	% K	% Mg	% ammonium	% S	N	Ca	P	K	Mg	Ammonium	S
Calcium Nitrate	0.145	0.190				0.01		0.276	0.361	0	0	0	0.019	0
Ammonium Nitrate	0.170					0.17		0.094	0	0	0	0	0.0935	0
Magnesium Nitrate	0.11				0.093			0.11	0	0	0	0.093	0	0
Mono potassium phosphate			0.227	0.287				0	0	0.114	0.144	0	0	0
Potassium Sulphate				0.43			0.18	0	0	0	0.447	0	0	0.1872
Magnesium Sulphate					0.098		0.13	0	0	0	0	0	0	0
Potassium Nitrate	0.135			0.384				0	0	0	0	0	0	0
TOTAL								0.479	0.361	0.114	0.591	0.093	0.1125	0.1872
N:K	1.001													

	200 L		1:2
	Calcium Nitrate	1.90	kg
	Ammonium Nitrate 34%	0.550	kg
	Magnesium Nitrate	1	kg
	Iron Chelate (EDTA) 13%	60	g
	Mono potassium phosphate	0.50	kg
	Potassium Sulphate	2.42	kg
	Magnesium Sulphate	0.0	kg
	Potassium Nitrate	0.0	kg
	Manganese Chelate 13%	12	g
	Zinc 15%	5.30	g
	Borax 15%	4.10	g
	Copper Sulphate 25.2%	0.50	g
	Molybdate 46%	0.30	g

	Concentrations of Elements in Solution							Quantity in kg						
	% N	% Ca	% P	% K	% Mg	% ammonium	% S	N	Ca	P	K	Mg	Ammonium	S
Calcium Nitrate	0.145	0.190				0.01		0.276	0.361	0	0	0	0.019	0
Ammonium Nitrate	0.17					0.17		0.094	0	0	0	0	0.0935	0
Magnesium Nitrate	0.11				0.093			0.11	0	0	0	0.093	0	0
Mono potassium phosphate			0.227	0.287				0	0	0.114	0.144	0	0	0
Potassium Sulphate				0.43			0.18	0	0	0	1.038	0	0	0.4347
Magnesium Sulphate					0.098		0.13	0	0	0	0	0	0	0
Potassium Nitrate	0.135			0.384				0	0	0	0	0	0	0
TOTAL								0.479	0.361	0.114	1.182	0.093	0.1125	0.4347
N:K	0.5													

	200 L		1:4
	Calcium Nitrate	1.9	kg
	Ammonium Nitrate 34%	0.55	kg
	Magnesium Nitrate	1.0	kg
	Iron Chelate (EDTA) 13%	60	g
	Mono potassium phosphate	1	kg
	Potassium Sulphate	5	kg
	Magnesium Sulphate	0.0	kg
	Potassium Nitrate	0.0	kg
	Manganese Chelate 13%	12	g
	Zinc 15%	5	g
	Borax 15%	4.1	g
	Copper Sulphate 25.2%	0.5	g
	Molybdate 46%	0.3	g

	Concentrations of Elements in Solution							Quantity in kg						
	% N	% Ca	% P	% K	% Mg	% ammonium	% S	N	Ca	P	K	Mg	Ammonium	S
Calcium Nitrate	0.145	0.190				0.01		0.276	0.361	0	0	0	0.019	0
Ammonium Nitrate	0.17					0.17		0.094	0	0	0	0	0.0935	0
Magnesium Nitrate	0.11				0.093			0.11	0	0	0	0.093	0	0
Mono potassium phosphate			0.227	0.287				0	0	0.114	0.144	0	0	0
Potassium Sulphate				0.43			0.18	0	0	0	2.219	0	0	0.9288
Magnesium Sulphate					0.098		0.13	0	0	0	0	0	0	0
Potassium Nitrate	0.135			0.384				0	0	0	0	0	0	0
TOTAL								0.479	0.361	0.114	2.362	0.093	0.1125	0.9288
N:K	0.25													

Weeks 3&4

	200 L		1:1
A	Calcium Nitrate	1.03	kg
	Ammonium Nitrate 34%	0.05	kg
	Magnesium Nitrate	0.53	kg
	Iron Chelate (EDTA) 13%	31.7	g
B	Mono potassium phosphate	0.20	kg
	Potassium Sulphate	0.40	kg
	Magnesium Sulphate	0.00	kg
	Potassium Nitrate	0.01	kg
Oligo	Manganese Chelate 13%	7	g
	Zinc 15%	3.00	g
	Borax 15%	2.20	g
	Copper Sulphate 25.2%	0.30	g
	Molybdate 46%	0.100	g

	Concentrations of Elements in Solution							Quantity in kg						
	% N	% Ca	% P	% K	% Mg	% ammonium	% S	N	Ca	P	K	Mg	Ammonium	S
Calcium Nitrate	0.145	0.190				0.01		0.149	0.196	0	0	0	0.0103	0
Ammonium Nitrate	0.170					0.17		0.009	0	0	0	0	0.0085	0
Magnesium Nitrate	0.11				0.093			0.058	0	0	0	0.049	0	0
Mono potassium phosphate			0.227	0.287				0	0	0.045	0.057	0	0	0
Potassium Sulphate				0.43			0.18	0	0	0	0.172	0	0	0.072
Magnesium Sulphate					0.098		0.13	0	0	0	0	0	0	0
Potassium Nitrate	0.135			0.384				0.001	0	0	0.004	0	0	0
TOTAL								0.218	0.196	0.045	0.233	0.049	0.0188	0.072
N:K	1.013													

	200 L		1:2
A	Calcium Nitrate	1.03	kg
	Ammonium Nitrate 34%	0.050	kg
	Magnesium Nitrate	1	kg
	Iron Chelate (EDTA) 13%	32	g
B	Mono potassium phosphate	0.20	kg
	Potassium Sulphate	0.95	kg
	Magnesium Sulphate	0.0	kg
	Potassium Nitrate	0.0	kg
Oligo	Manganese Chelate 13%	7	g
	Zinc 15%	3.00	g
	Borax 15%	2.20	g
	Copper Sulphate 25.2%	0.30	g
	Molybdate 46%	0.10	g

	Concentrations of Elements in Solution							Quantity in kg						
	% N	% Ca	% P	% K	% Mg	% ammonium	% S	N	Ca	P	K	Mg	Ammonium	S
Calcium Nitrate	0.145	0.190				0.01		0.149	0.196	0	0	0	0.0103	0
Ammonium Nitrate	0.17					0.17		0.009	0	0	0	0	0.0085	0
Magnesium Nitrate	0.11				0.093			0.058	0	0	0	0.049	0	0
Mono potassium phosphate			0.227	0.287				0	0	0.045	0.057	0	0	0
Potassium Sulphate				0.43			0.18	0	0	0	0.409	0	0	0.171
Magnesium Sulphate					0.098		0.13	0	0	0	0	0	0	0
Potassium Nitrate	0.135			0.384				0.001	0	0	0.004	0	0	0
TOTAL								0.218	0.196	0.045	0.47	0.049	0.0188	0.171
N:K	0.503													

	200 L		1:4
A	Calcium Nitrate	1.0	kg
	Ammonium Nitrate 34%	0.05	kg
	Magnesium Nitrate	0.5	kg
	Iron Chelate (EDTA) 13%	32	g
B	Mono potassium phosphate	0	kg
	Potassium Sulphate	2	kg
	Magnesium Sulphate	0.0	kg
	Potassium Nitrate	0.0	kg
Oligo	Manganese Chelate 13%	7	g
	Zinc 15%	3	g
	Borax 15%	2.2	g
	Copper Sulphate 25.2%	0.3	g
	Molybdate 46%	0.1	g

	Concentrations of Elements in Solution							Quantity in kg						
	% N	% Ca	% P	% K	% Mg	% ammonium	% S	N	Ca	P	K	Mg	Ammonium	S
Calcium Nitrate	0.145	0.190				0.01		0.149	0.196	0	0	0	0.0103	0
Ammonium Nitrate	0.17					0.17		0.009	0	0	0	0	0.0085	0
Magnesium Nitrate	0.11				0.093			0.058	0	0	0	0.049	0	0
Mono potassium phosphate			0.227	0.287				0	0	0.045	0.057	0	0	0
Potassium Sulphate				0.43			0.18	0	0	0	0.882	0	0	0.369
Magnesium Sulphate					0.098		0.13	0	0	0	0	0	0	0
Potassium Nitrate	0.135			0.384				0.001	0	0	0.004	0	0	0
TOTAL								0.218	0.196	0.045	0.943	0.049	0.0188	0.369
N:K	0.251													

Weeks 5&6

	200 L		1:1
A	Calcium Nitrate	1.03	kg
	Ammonium Nitrate 34%	0.80	kg
	Magnesium Nitrate	0.53	kg
	Iron Chelate (EDTA) 13%	31.7	g
B	Mono potassium phosphate	0.20	kg
	Potassium Sulphate	0.06	kg
	Magnesium Sulphate	0.00	kg
	Potassium Nitrate	1.64	kg
Oligo	Manganese Chelate 13%	7	g
	Zinc 15%	3.00	g
	Borax 15%	2.20	g
	Copper Sulphate 25.2%	0.30	g
	Molybdate 46%	0.100	g

	Concentrations of Elements in Solution							Quantity in kg						
	% N	% Ca	% P	% K	% Mg	% ammonium	% S	N	Ca	P	K	Mg	Ammonium	S
Calcium Nitrate	0.145	0.190				0.01		0.149	0.196	0	0	0	0.0103	0
Ammonium Nitrate	0.170					0.17		0.136	0	0	0	0	0.136	0
Magnesium Nitrate	0.11				0.093			0.058	0	0	0	0.049	0	0
Mono potassium phosphate			0.227	0.287				0	0	0.045	0.057	0	0	0
Potassium Sulphate				0.43			0.18	0	0	0	0.025	0	0	0.0103
Magnesium Sulphate					0.098		0.13	0	0	0	0	0	0	0
Potassium Nitrate	0.135			0.384				0.221	0	0	0.629	0	0	0
							TOTAL	0.565	0.196	0.045	0.711	0.049	0.1463	0.0103
							N:K	1						

	200 L		1:2
	Calcium Nitrate	1.03	kg
	Ammonium Nitrate 34%	0.800	kg
	Magnesium Nitrate	1	kg
	Iron Chelate (EDTA) 13%	32	g
	Mono potassium phosphate	0.20	kg
	Potassium Sulphate	1.71	kg
	Magnesium Sulphate	0.0	kg
	Potassium Nitrate	1.6	kg
	Manganese Chelate 13%	7	g
	Zinc 15%	3.00	g
	Borax 15%	2.20	g
	Copper Sulphate 25.2%	0.30	g
	Molybdate 46%	0.10	g

	Concentrations of Elements in Solution							Quantity in kg						
	% N	% Ca	% P	% K	% Mg	% ammonium	% S	N	Ca	P	K	Mg	Ammonium	S
Calcium Nitrate	0.145	0.190				0.01		0.149	0.196	0	0	0	0.0103	0
Ammonium Nitrate	0.17					0.17		0.136	0	0	0	0	0.136	0
Magnesium Nitrate	0.11				0.093			0.058	0	0	0	0.049	0	0
Mono potassium phosphate			0.227	0.287				0	0	0.045	0.057	0	0	0
Potassium Sulphate				0.43			0.18	0	0	0	0.736	0	0	0.308
Magnesium Sulphate					0.098		0.13	0	0	0	0	0	0	0
Potassium Nitrate	0.135			0.384				0.221	0	0	0.629	0	0	0
							TOTAL	0.565	0.196	0.045	1.422	0.049	0.1463	0.308
							N:K	0.5						

	200 L		1:4
	Calcium Nitrate	1.0	kg
	Ammonium Nitrate 34%	0.80	kg
	Magnesium Nitrate	0.5	kg
	Iron Chelate (EDTA) 13%	32	g
	Mono potassium phosphate	0	kg
	Potassium Sulphate	5	kg
	Magnesium Sulphate	0.0	kg
	Potassium Nitrate	1.6	kg
	Manganese Chelate 13%	7	g
	Zinc 15%	3	g
	Borax 15%	2.2	g
	Copper Sulphate 25.2%	0.3	g
	Molybdate 46%	0.1	g

	Concentrations of Elements in Solution							Quantity in kg						
	% N	% Ca	% P	% K	% Mg	% ammonium	% S	N	Ca	P	K	Mg	Ammonium	S
Calcium Nitrate	0.145	0.190				0.01		0.149	0.196	0	0	0	0.0103	0
Ammonium Nitrate	0.17					0.17		0.136	0	0	0	0	0.136	0
Magnesium Nitrate	0.11				0.093			0.058	0	0	0	0.049	0	0
Mono potassium phosphate			0.227	0.287				0	0	0.045	0.057	0	0	0
Potassium Sulphate				0.43			0.18	0	0	0	2.15	0	0	0.9
Magnesium Sulphate					0.098		0.13	0	0	0	0	0	0	0
Potassium Nitrate	0.135			0.384				0.221	0	0	0.629	0	0	0
							TOTAL	0.565	0.196	0.045	2.836	0.049	0.1463	0.9
							N:K	0.251						

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